OC 1: Vascular biology

OC 1.1 Evidence for functional PAR4 thrombin receptor expression in cardiac fibroblasts and its regulation by glucose

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Objectives: Thrombin inhibition was recently reported to protect against diabetic cardiomyopathy in mice, in part by downregulating protease-activated receptors PAR1 and PAR4. PAR1 is highly abundant in cardiac fibroblasts, PAR4 is reportedly absent. In smooth muscle cells, PAR4 expression is also low but rises markedly with hyperglycemia, contributing to vascular remodelling in diabetic mice. We here examined if PAR4 is a glucose-responsive gene with remodelling-related functions in cardiac fibroblasts.
Methods: Diabetes was induced in mice with streptozotocin or diabetogenic diet. Cardiac fibroblasts were isolated by enzymatic digestion and cultured in low (LG, 5.5. mM) or high glucose (HG, 25 mM) medium. Gene and protein expression were assayed by realtime PCR and western blot, redox stress with dihydroethidium fluorescence, migration by wound-scratch assay.
Results: PAR4 expression was higher in whole hearts and cardiac fibroblasts from diabetic mice and in cardiac fibroblasts cultured in HG. PAR4 mRNA adapted rapidly to incremental changes in extracellular glucose. HG fibroblasts exhibited increased basal redox stress and cytokine gene expression but less migration than LG cells, and responded more strongly to thrombin. This functional augmentation was absent in PAR4$^{-/-}$ fibroblasts.
Conclusion: PAR4 is expressed in cardiac fibroblasts and dynamically regulated by hyperglycemia *in vitro* and *in vivo*, impacting on thrombin-driven migration, inflammation and oxidant stress. Since the heart is a major target for end-organ damage in diabetes, to which cardiac fibroblasts critically contribute, these findings might add further evidence for the usefulness of the recently developed PAR4 antagonists in clinical settings.
Disclosure: No significant relationships.

OC 1.2 CLIC1 promotes thrombus formation and angiogenesis through interaction with integrins

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Objectives: Chloride intracellular channel 1 (CLIC1) has been shown to be involved in thrombus formation as well as angiogenesis but its role in these processes is largely unknown. Here, we tested if CLIC1 supports cell adhesive processes relevant for endothelial and platelet function.
Methods: Human umbilical venous endothelial cells (HUVEC) were probed for cell proliferation on plastic and cell invasion/survival after embedding in fibrin and transfection with siCLIC1. The subcellular localization of CLIC1 in HUVEC as well as platelets was analyzed with fluorescence microscopy following treatment with the synthetic CLIC1 inhibitor IAA94, which was also used to assess the effect of CLIC1 on integrin activation and platelet aggregation. The role of CLIC1 on thrombus formation *in vivo* was assessed by intravital fluorescence microscopy in a mouse dorsal skin fold chamber model.
Results: Knocking down endothelial CLIC1 with siRNA caused a defect in cell spreading that was associated with decreased cell proliferation, invasion and survival. The effect of CLIC1 inhibition could be traced back to diminished CLIC1 cell membrane expression, which, in turn, resulted in disorganized lamellipodia formation. Paralleling these results, we detected integrin-dependent CLIC1 membrane relocation in platelets. Treatment of platelets with the synthetic CLIC1 antagonist IAA94 targeted membrane CLIC1 and, in the process, reduced integrin αIIbβ3 activation. As a consequence, inhibition of CLIC1 impaired platelet aggregation *in vitro* and vaso-occlusion in a mouse model of photo-chemical thrombus formation *in vivo*.
Conclusion: CLIC1 cooperates with integrins during cell adhesion and as such mediates functions related to thrombus formation, endothelial homeostasis and angiogenesis.
Disclosure: No significant relationships.

OC 1.3 Sublingual functional capillary rarefaction in chronic heart failure

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Objectives: Microcirculatory changes contribute to clinical symptoms and disease progression in chronic heart failure (CHF). A depression of coronary flow reserve is associated with a lower myocardial capillary density in biopsies. We hypothesized that changes in cardiac microcirculation might also be reflected by a systemic reduction of capillaries and visualized by sublingual videomicroscopy. The aim was to study *in vivo* capillary density and glycocalyx dimensions in patients with CHF vs. healthy controls.
Methods: Fifty-three patients with ischemic and non-ischemic cardiomyopathy and conservative treatment were compared to thirty-five healthy age-matched subjects in a prospective cross-sectional study. Sublingual microcirculation was visualized using a Sidestream Darkfield videomicroscope. Functional and perfused total capillary densities were compared between patients and controls. A reduced glycocalyx thickness was measured by an increased perfused boundary region (PBR).
Results: Median functional and total perfused capillary densities were 31% and 46% lower in patients with cardiomyopathy (both $p<0.001$). Intake of oral vitamin K antagonists was associated with significantly lower capillary densities ($p<0.05$), but not independent of NT-proBNP. Dimensions of the glycocalyx were marginally lower in CHF patients than in healthy controls (<7% difference). However, PBR correlated significantly with inflammation markers (fibrinogen: r=0.54; C-reactive protein: r=0.36), platelet counts (r=0.38), and inversely with measures of liver/renal function such as bilirubin (r=-0.39) and albumin (r=-0.30) or estimated glomerular filtration rate (r=-0.34) in CHF patients.
Conclusion: CHF patients have got a markedly lower functional and total perfused capillary density in sublingual microvasculature when compared to controls, indicating a systemic decrease in microcirculation.
Disclosure: No significant relationships.

OC 1.4 FasR-FasL-mediated signaling of red blood cells (RBCs) and platelets leads to phosphatidylserine exposure of RBCs and is substantial for thrombus formation and hemostasis.

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Objectives: A critical step in thrombus growth and stability is the contribution of platelets to coagulation and thrombin generation by providing a procoagulant surface. RBCs externalize phosphatidylserine (PS) on their membrane as well and might contribute to thrombin generation. First results revealed a direct interaction of platelets and RBCs, important for thrombin generation and stable thrombus formation, suggesting an impact of RBCs in hemostasis and thrombosis beside their passive impact in rheology.
Methods: Analysis of RBCs and RBC-platelet interactions upon thrombus formation were performed using platelets from different knock-out mice and healthy volunteers.
Results: A small population of RBCs via direct cell-cell contact with platelets mediated by the FasL-FasR signaling pathway enhanced platelet activation. Activated platelets externalized FasL on the membrane that activated FasR on RBCs leading to PS exposure on the RBC membrane. Accordingly, inhibition or genetic deletion of FasR on RBCs strongly reduced PS exposure followed by significantly reduced the formation of three-dimensional thrombi *in vitro* and *in vivo*. Decreased PS exposure of RBCs and reduced adhesion to recombinant FasR after treatment of platelets with Abciximab suggested that integrin aIIbb3 serves as another ligand on the platelet membrane important for FasR activation on RBCs. First results of dynamic adhesion and thrombus formation using a-CD36 antibody indicated a contribution of this adhesion molecule to the active recruitment of RBCs into the growing thrombus.
Conclusion: RBCs play an active role in platelet thrombus formation upon FasR-FasL/integrin-mediated signaling. However, the active recruitment of RBCs to the growing thrombus is under investigation.
Disclosure: No significant relationships.

OC 1.5 The haemostatic properties of thrombospondin-1 are enhanced by neutrophil-mediated proteolysis

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Objectives: Thrombospondin-1 (TSP-1) is a protein that is primarily expressed by platelets and endothelial cells and rapidly released upon their activation. It functions in haemostasis as bridging molecule in platelet aggregation, by promoting platelet adhesion to collagen and by protecting von Willebrand factor strings from degradation. In co-cultures of platelets or endothelial cells with neutrophils we observed proteolysis of the 185 kDa full-length TSP-1 to a 160 kDa isoform. We hypothesized that TSP-1 processing may alter its haemostatic properties.

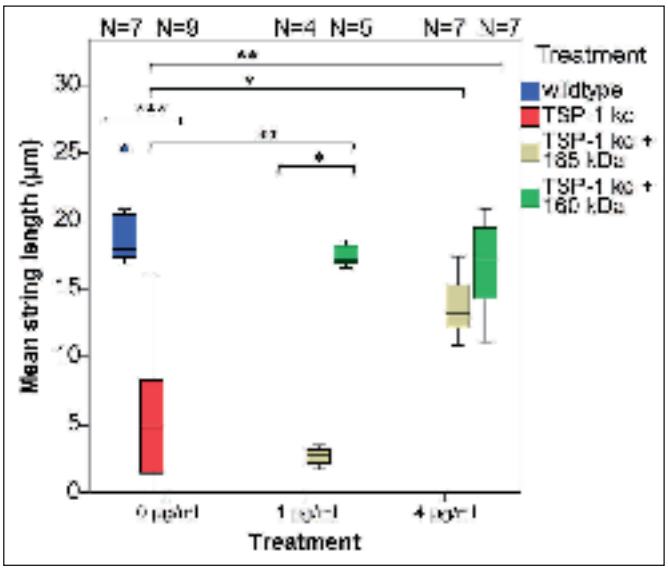

Fig. 1 Blood from wildtype or TSP-1 ko mice with or without the addition of purified 160 kDa or 185 kDa TSP-1 (at 1 or 4 µg/ml) was perfused over collagen at a shear rate of 7 dyne/cm2 (700 s-1) for 7 min. Images were then taken under flow conditions and the mean length of adherent platelet strings was calculated.

Methods: The proteases were identified by inhibitors and the cut site in TSP-1 was mapped by Edman sequencing. Both isoforms were recombinantly generated and purified. Haemostatic properties were tested in assays of platelet aggregation (light-transmission aggregometry, plate-and-cone analyser), whole blood coagulation (ROTEM) as well as platelet adhesion and string formation on collagen under flow (ibidi system). Platelets from TSP-1 ko mice (compared to wildtype) were reconstituted with purified 160 kDa or 185 kDa isoform.
Results: By applying selective inhibitors the neutrophil proteases elastase and cathepsin G were identified to mediate TSP-1 processing. The cut site of cathepsin G was mapped to amino acids R255/T256. TSP-1 ko platelets did not differ from wildtype in platelet aggregation but showed severe impairment of platelet adhesion and string formation under flow. The 160 kDa TSP-1 isoform was found to be markedly more potent than the 185 kDa full-length molecule in restoring function (▶Fig.1).
Conclusion: TSP-1 processing by neutrophil proteases yields a 160 kDa isoform which shows enhanced potency to promote platelet adhesion and string formation.
Disclosure: No significant relationships.

OC 2: Laboratory tests I

OC 2.1 Simple and easy ADAMTS13 activity quantification based on a VWF activity assay reducing turn-around-time

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Objectives: The detection of ADAMTS13 can be time-consuming and cumbersome with some assays. Although there are some commercially available ELISA based assays, ADAMTS13 activity measurements are not available in most laboratories. Based on Furlan's concept of degrading ultralarge von Willebrand factor (VWF) multimers, acquired and heritated ADAMTS13 deficiency can easily be detected with modern von VWF activity assays based on recombinant GP Ib fragments.

Tab. 1 Results and statistical characteristics for both methods

	ADAMTS13 ELISA based (reference)	ADAMTS13 VWF based (new method)
Mean+/-SD	54+/-41%	48+/-31%
Correlation r^2		0,942
Range		0–150%
Sensitivity <10% ADAMTS13 (95% CI)		100% (63.06% to 100.00%)
Specificity <10% ADAMTS13 (95% CI)		100% (85.18% to 100.00%)
inter-/intra-assay CVs		<8%

Methods: In this study, we compared the results from a commercially avialable kit (Technoclone ADAMTS13 activity ELISA, Technoclone GmbH, Vienna) with results obtained with a commercially available von Willebrand Activity assay (Siemens) to quantify ADAMTS13 acitivty. TRIS diluted plasma samples and controls (100%, 50%, and 0% AMATS13 activity) were mixed with VWF (200U/ml) and incubated for 50 minutes at 37°C . The residual VWF activity in the samples were then automatically analysed and the corresponding ADAMTS13 activity calculated based on the results of the controls.
Results: In a total of 31 plasma samples of patients with Thrombotic Thrombocytopenic Purpura (TTP) in various sates of disease ADAMTS 13 activity was measured with both methods (▶Table 1).
Conclusion: The results of this study demonstrate the VWF-activity based ADAMTS13 can provide accurate and fast ADAMTS13 activity measurements. It can be used to faster diagnose and treat TTP, a life-threatening disease.
Disclosure: No significant relationships.

OC 2.2 Residual activity of ADAMTS13 mutants under flow conditions

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Objectives: Prothrombotic high molecular weight multimers of von Willebrand factor (VWF) are size-regulated by the metalloprotease ADAMTS13 by shear force-activated proteolytic cleavage. A lot of effort has previously been put into the characterization of ADAMTS13 mutants but they mostly have been investigated with respect to defects in biosynthesis or secretion. State-of-the-art diagnostic tests further measure residual activity employing unphysiological static approaches. We have developed a shear flow assay to provide a method for assessing ADAMTS13 activity under physiological laminar flow conditions.
Methods: VWF string formation is induced by histamine stimulation of HUVEC cells under unidirectional flow at 5 dyne/cm^2 shear stress, and VWF strings are detected employing the VWF binding peptide of platelet glycoprotein GPIbα coupled to latex beads. VWF strings are then used as substrate for kinetic studies of recombinant ADAMTS13 mutants identified in patients with congenital Thrombotic Thrombocytopenic Purpura (cTTP). Analysis of time-lapse images allows quantification of VWF cleavage.
Results: We measured the activity of ADAMTS13 mutants, which exhibit residual secretion upon expression in HEK293 cells. For example, we found that mutants p.Aps235Tyr and p.Gly702Arg possess significant residual proteolytic activity.
Conclusion: There is considerable phenotypical variation in patients with cTTP. It has previously been shown that some ADAMTS13 mutations result in low protein expression levels of mutants without residual activity under static conditions. We have shown here that some of these mutants do exhibit residual activity under physiological flow conditions. Our data might explain a milder phenotype in patients carrying such mutations.
Disclosure: No significant relationships.

OC 2.3 Differential sensitivity of von Willebrand factor activity assays to reduced VWF molecular weight forms: a large international cross-laboratory study.

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Objectives: von Willebrand disease (VWD), the most common inherited bleeding disorder, is due to deficiencies/defects in von Willebrand factor (VWF). Effective diagnosis requires testing for FVIII, VWF antigen and one or more VWF 'activity' assays, as classically assessed using ristocetin cofactor (VWF:RCo). However, collagen binding (VWF:CB) and/or other VWF 'activity' assays are used by many laboratories. This extensive international cross-laboratory study has specifically evaluated contemporary VWF activity assays for comparative sensitivity to reduction in high molecular weight (HMW) VWF, and their ability to differentiate type 1 vs 2A VWD-like samples.
Methods: A set of four samples representing step wise reduction in HMW VWF were tested by over 400 laboratories worldwide using various assays. A second set of two samples representing type 1 or type 2A VWD-like plasma was tested by a subset of 251 laboratories.
Results: Combined data identified some differences between VWF activity assays, with sensitivity for reduction of HMW being highest for VWF:CB and VWF:GPIbM, intermediate for VWF:RCo and VWF:GPIbR, and lowest for VWF:Ab. 'Within' method analysis identified the Stago method as the most sensitive VWF:CB assay. Furthermore, a large variation in inter-laboratory CV was demonstrated for various methods (8 – > 40%). Although the performance of various methods differed significantly, most laboratories correctly differentiated between type 1 and 2 samples, irrespective of the VWF activity assay employed.
Conclusion: These results hold significant clinical implications for diagnosis and therapy monitoring of VWD, as well as potential future diagnosis and therapy monitoring of thrombotic thrombocytopenic purpura (TTP).
Disclosure: No significant relationships.

OC 2.4 Automated platelet aggregation testing is feasible in a routine setting

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Objectives: Automated platelet aggregation has the potential to skirt some of the disadvantages of classical LTA such as manual pipetting. Or aim was to test diagnostic accuracy of automated platelet aggregation testing in a routine setting.

Methods: Patients being referred to our center for bleeding disorder work up were included prospectively in this study. Platelet rich plasma samples (PRP) adjusted to a standardized platelet count between 200 and 300G/l were prepared from every patient. LTA was performed according to our routine protocol on an APACT analyzer (Haemochrom diagnostica, Essen, Germany) using the agonists ADP, collagen, epinephrine, and ristocetin in different concentrations. In parallel, platelet aggregation was tested from the same PRP samples on a CS 2500i analyzer (Sysmex Suisse AG, Horgen, Switzerland) using an identical set of agonists. Additionally, aggregation imprecision using collagen and ADP as agonists was examined on the CS 2500i analyzer.
Results: Platelet function testing was performed for 28 patients on both platforms. Both methods showed identical aggregation patterns in every study participant with an at least acceptable correlation of maximal aggregations responses for all agonists (correlation coefficients 0.4–0.7, $p<0.05$). On CS2500i, mean variation coefficient was 2.6% using collagen as an agonist and 3.8% for ADP.
Conclusion: In a routine setting, aggregation testing on CS2500i showed an excellent correlation with LTA performed on APACT 4 with a very low imprecision. Automated aggregometry is a promising technology offering the possibility of standardized platelet function testing in a high throughput setting.
Disclosure: No significant relationships.

OC 2.5 Use of next-generation sequencing to identify underlying defects in patients with inherited platelet disorders

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Objectives: Background: Platelet function disorders (PFDs) arise as a result of defects in platelet function and number and lead to a bleeding diathesis of varying severity. Because of their heterogeneity identification of the underlying genetic defect is difficult. The use of next-generation sequencing (NGS) technologies has facilitated the identification of novel genetic defects.
Methods: Materials and Methods: We investigated 39 PFDs samples with NGS – target panel for PFDs, including 70 genes corresponding to the exons and splice sites in candidate genes.
Results: Results: In 19 samples the genetic alterations corresponding to clinical picture and laboratory data were identified. In the remaining 20 samples no suitable to the clinical picture genetic changes were found. In 9 patients with Glanzmann thrombasthenia (GT), mutations were detected either in *ITGB3* or *ITGA2B* genes. Interestingly, in one family a novel mutation Leu738Arg in *ITGB3* lead to autosomal dominant inheritance corresponding to GT like phenotype. In the remaining 10 patients following genetic defects were detected corresponding to specific phenotype: 1 in *GP9* gene – Bernard–Soullier syndrome; 1 in *MHY9* – May-Hegglin-Anomaly; 3 in *TBXAS1* – the thromboxane synthase deffect; 1 in *ACTN1*- bleeding disorder platelet-type 15; 1 in *PLA2G7* – ASS-Like syndrome; 1 in *Gp6* and 1 in *GFI1B*.
Conclusion: Conclusion: Due to the vast amount of genetic information received with NGS the interpretation of the data require a careful analysis. The use of NGS will continue to identify novel genetic alteration in association with PFDs and unravel mechanisms involved in platelet formation and function.
Disclosure: No significant relationships.

OC 3: Inherited bleeding disorders: clinical

OC 3.1 Stable elevations in FIX activity and reductions in annualized bleeding rate over up to 2 years of follow-up of adults with severe or moderate-severe hemophilia B treated with AMT-060 (AAV5-hFIX)

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Objectives: Gene transfer for hemophilia offers the potential to convert the disease from a severe to mild phenotype with a single treatment. AMT-060 consists of an adeno-associated virus serotype 5 (AAV5) vector containing a codon-optimized wildtype human factor IX (FIX) gene under control of a liver-specific promoter.
Methods: Multi-national, open-label, dose-escalating Phase 1/2 study investigating the safety and efficacy of AMT-060 in adult males with factor IX [FIX] activity ≤2% of normal and a severe bleeding phenotype (prophylactic exogenous FIX; or on-demand exogenous FIX, plus ≥4 bleeds/year or hemophilic arthropathy). Patients received either $5x10^{12}$ gc/kg (Cohort 1; n=5) or $2×10^{13}$ gc/kg (Cohort 2; n=5) of AMT-060 iv. Efficacy assessments include endogenous FIX activity (measured ≥10 days after last exogenous FIX); exogenous FIX use; and annualized bleeding rates (ABR). Safety assessments include treatment-related adverse events, immunological and inflammatory biomarkers. Results up to 2 years will be presented.

Tab. 1 Key efficacy outcomes following a single infusion of AMT-060

Mean	Cohort 1 ($5x10^{12}$ gc/kg)[a]		Cohort 2 ($2x10^{13}$ gc/kg)[b]	
	Year Prior to AMT-060	Post AMT-060c	Year Prior to AMT-060	Post AMT-060c
FIX activity (95% confidence interval)	--	4.6 IU/dL (1.6–7.6)	--	7.1 IU/dl (3.2–11.1)
Annualized total bleeds	14.4	6.6 (↓54%)	4.0	1.5 (↓64%)
Annualized spontaneous bleeds	9.8	4.2 (↓58%)	3.0	0.5 (↓84%)
Cumulative annualized FIX consumption, IU [d]	1,774,000	267,055 (↓85%)	866,000	277,687 (↓68%)

FIX, factor IX. [a] 1.5 years of follow up was available at submission for Cohort 1. [b] 1 year of follow up was available at submission for Cohort 2.[c] Excludes prophylaxis tapering period. [d] Excluding FIX replacement used for surgeries

Results: A dose-dependent increase in FIX activity was observed, accompanied by decreased exogenous FIX consumption and declines in both total and spontaneous bleed rates in both cohorts (see ▶Table 1). 8/9 patients on FIX prophylaxis discontinued use. 3 patients experienced mild, temporary elevations in alanine transaminase (ALT) levels and received a tapering course of prednisolone. ALT elevations were not associated with changes in FIX activity or capsid-specific T-cell responses.

Conclusion: Patients continue to show sustained clinical benefit and endogenous FIX activity with no T-cell activation ≥1 year after a single infusion of AMT-060.

Disclosure: W. Miesbach reported consultant fees from UniQure. B.V. during the conduct of the study; grants and personal fees from Novo-Nordisk, personal fees from Bayer, Shire, Biotest, Pfizer, Octapharma, LFB, CSL Behring, SOBI, Biogen, and BPL outside the submitte

OC 3.2 Efficacy, safety and pharmacokinetics (PK) of emicizumab (ACE910) prophylaxis (Px) in persons with haemophilia A with inhibitors (PwHAwI): randomized, multicenter, open-label, phase 3 study (HAVEN 1)

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Objectives: Emicizumab, a novel, subcutaneously administered, bispecific humanized monoclonal antibody bridges FIXa and FX to replace missing FVIII function, resulting in thrombin-generation and coagulation in PwHAwI.

Methods: Study (NCT02622321) included PwHAwI ≥12 y.o. (arms A–D). Patients without prior bypassing agent (BPA) Px were randomized (2:1) to emicizumab Px (A) or no Px (B). PwHAwI with prior BPA Px received emicizumab Px (C). Patients of the non-interventional study BH29768 unable to be enrolled into A/B received emicizumab (D). Primary endpoint compared treated bleed rates in Arm A vs B (24 weeks).

Results: 109 PwHAwI were enrolled, median age 28 (range 12–75) years. All primary/secondary endpoints were statistically significant, with clinically meaningful reductions in treated (87%), all, spontaneous, joint, and target joint bleeds in Arm A vs B (▶Table 1). Treated bleeds in Arm C were reduced by 79% vs previous BPA Px (intraperson comparison). Arm A had significantly better health-related (HR) QoL and health status than Arm B. Emicizumab safety was acceptable (most common AE: injection site reaction, 15%). Two thrombotic SAEs and three thrombotic microangiopathy SAEs occurred after patients received average cumulative doses of >100 U/kg/day emicizumab for 24 hours or more. Two persons resumed emicizumab without further sequelae. No antidrug antibodies were reported. Mean trough emicizumab concentrations >50 µg/mL were achieved after 4 weeks and sustained.

Conclusion: Emicizumab Px safely prevented/reduced bleeds and improved HRQoL in PwHAwI. In the absence of concomitant aPCC PwHAwI sustained PK-levels without excess thrombotic risk. These data may support a potential new standard of care in HAwI management.

Disclosure: Participation in a company sponsored speaker's bureau: Baxter, Bayer, Biogen Idec, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche, Shire, Swedish Orphan Biovitrum Receipt of honoraria or consultation fees: Baxter, Bayer, Biogen I

OC 3.3 Safety and efficacy of recombinant von Willebrand factor (rVWF) in patients with severe von Willebrand disease (VWD) undergoing major and minor elective surgical procedures: a prospective clinical trial

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Bleeds[a]	Arm A (emicizumab Px; n=35)	Arm B (no emicizumab Px; n=18	Arm C (emicizumab Px, prior Px with BPA; n=49)
Bleeds: Treated (with BPAs), ABR[b]	2.9[c]	23.3[c]	5.1
Bleeds: All (Treated & Not treated with BPAs), ABR[b]	5.5[d]	28.3[d]	6.5
Treated spontaneous bleeds, ABR[b]	1.3[e]	16.8[e]	3.1
Treated joint bleeds, ABR[b]	0.8[f]	6.7[f]	0.6
Treated target joint bleeds, ABR[b]	0.1[g]	3.0[g]	0.3
Participants with zero treated bleeds, %	62.9	5.6	69.4
ABR, median (treated bleeds)	0.0	18.8	0.0

Tab. 1 Bleeding events in HAVEN 1 study

[a]Bleed/medication questionnaire was completed by participant/caregiver via electronic handheld device. Bleed definitions were based on ISTH criteria (Banchette VS, et al. J Thromb Haemost 2014; 12: 193539). [b]Negative binominal model for Arm A vs Arm B: [c]RR=0.13, P<0.0001; [d]RR=0.20, P<0.0001; [e]RR=0.08, P<0.0001; [f]RR=0.11, P=0.0050; [g]RR=0.05, P=0.0002

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Objectives: Surgical hemostatic management in VWD is critical for patient safety. We evaluated hemostatic efficacy and safety of rVWF (VONVENDI)±rFVIII in patients with severe VWD who were undergoing major and minor elective surgical procedures.
Methods: Intraoperative and overall hemostatic efficacy ratings were assessed using a 4-point nominal scale; overall efficacy was assessed 24 hrs after the last rVWF infusion or at day 14, whichever occurred earlier. An initial dose of rVWF was given 12–24 hrs pre-surgery to raise endogenous FVIII:C; if target FVIII:C was not reached, a preoperative dose of rVWF and rFVIII was given to raise FVIII:C to recommended levels. Peri- and postoperative rVWF and rFVIII were infused to maintain target trough levels.
Results: All 15 subjects (▶Table 1) treated with rVWF±rFVIII had overall hemostatic efficacy ratings of excellent (73.3%) or good (26.7%). Intraoperative hemostatic efficacy ratings were also excellent (86.7%) or good (13.3%) for all subjects. Subjects received 121 infusions of rVWF±rFVIII, with the majority of subjects receiving rVWF alone: 100% (15/15) for the priming dose, 80% (12/15) for the loading dose, 80% (12/15) postoperatively, and 2 subject received no additional postoperative rVWF or rFVIII. The median overall surgical dose of rVWF was 220.4 IU/kg (63.8 – 648.4 IU/kg). No treatment-related AEs occurred, and none were due to a severe allergic reaction. No subjects developed neutralizing antibodies to rFVIII or rVWF.
Conclusion: These data support the safe and effective use of rVWF±rFVIII in achieving peri- and postoperative hemostasis in subjects with VWD undergoing major, minor, and oral elective surgery.
Disclosure: consultancy (Freeline, Kedrion, Biopharma, LFB, Octapharma); honoraria (Ablynx, Bayer, Grifols, Novo Nordisk, Sobi), member of advisory board (Ablynx, F. Hoffmann-La Roche, Shire)

Tab. 1 Demographics

Total number of subjects exposed	**15**	
Age, years (range)	Median (range)	40 (20–70)
Sex, n (%)	Male	7 (46.7)
	Female	8 (53.3)
VWD type, n (%)	1	3 (20)
	2A	2 (13.3)
	2B	1 (6.7)
	2M	1 (6.7)
	3	8 (53.3)
Weight, kg	Median (range)	73.5 (52.0–127.2)
Surgical Procedure Classification, n (%)	Major	10 (66.7)
	Minor	4 (26.7)
	Oral	1 (6.7)

OC 3.4 Altered expression of angiogenesis factors in von Willebrand disease

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Objectives: Besides coagulation von Willebrand Factor (VWF) seems to play an important role in angiogenesis. A lack of VWF may result in angiodysplasia altering the function of affected organs. We, therefore, analysed different tissues in a pig model for VWD comprising all genotypes (VWD type 3, type 1, and wildtype) for their expression of Integrin αVβ3 (ITG) and VEGFR2 focussing on female reproductive organs.
Methods: Uterus and oviduct tissue samples were collected from 6 pigs (two with VWD type 3, two with type 1, two controls). The expression of ITG and VEGFR2 was compared among the different types using immunohistochemistry.
Results: The immunohistochemical analysis for ITG showed a strong staining of the apical epithelial cell membrane in wildtype compared with cytoplasmic staining in VWD animals. Regarding VEGFR2, a stronger cytoplasmic staining of the glandular epithelium and epithelial cells was seen in VWD animals compared with controls. Furthermore, angiodysplasia is visible in the uterine wall.
Conclusion: Our study confirms effects of VWF on angiogenesis as ITG and VEGFR2 are altered in VWD suggesting that VWF stabilising ITG in the apical membranes. VWD causes a stronger cytoplasmic staining, probably due to an increased internalization of ITG. This might also contribute to the changes seen in VEGFR2 expression, as ITG is known to inhibit VEGFR2. Therefore, the diminished inhibition of ITG in VWD animals might explain the increased VEGFR2 expression resulting in angiodysplasia. These alterations might impact female reproductive function.
Disclosure: No significant relationships.

OC 3.5 Intramolecular disulfide bond disruption by a novel Cys to Trp mutation in the integrin β3 of a GT patient

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Objectives: The integrin αIIbβ3 is an essential receptor which cross-links platelets by binding of fibrinogen. Patients with quantitative or qualitative abnormalities in the integrin αIIbβ3 show an absence of platelet aggregation to all physiological agonists except ristocetin. This autosomal recessive bleeding disorder, Glanzmann thrombasthenia (GT), is caused by mutations in the *ITGA2B* (αIIb) or *ITGB3* (β3) gene. Routine diagnostics and functional analysis by light transmission aggregometry suffice for distinct diagnosis. Additionally, flow cytometry and DNA sequencing are the methods of choice for more detailed analysis.
Methods: We analyzed six patients with GT. For molecular genetic analysis we isolated genomic DNA from EDTA-anticoagulated whole blood. The respective exon regions from the *ITGA2B* and *ITGB3* gene were amplified by PCR using HotStar Taq DNA polymerase. For DNA sequencing we used the Sanger method. Sequencing was performed in an Applied Biosystem sequencer.

Results: We found two already described mutations. Further, we identified a novel homozygous C to G transversion in the exon 10 of *ITGB3* gene. This mutation resulted in a Cys to Trp amino acid substitution. The normally expressed Cys486 is localized in a cysteine rich, proteinase-resistant core of the integrin β3 and usually forms an intramolecular disulfide bond with the Cys473. The patient with the Cys486Trp mutation showed the pathognomonic aggregation typical for GT.
Conclusion: In our GT sequence analyses we found a novel mutation in the integrin β3, which leads to disruption of an intramolecular disulfide bond. We suggest that the GT phenotype is the result of this Cys486Trp mutation.
Disclosure: No significant relationships.

OC 4: Antithrombotics

OC 4.1 Coagulation Activation Parameters F1+2 and hsD-dimers in patients under different anticoagulant regimes after diagnosis of Venous Thromboembolism (VTE) and under VTE-prophylaxis after Knee-Prothesis (KTP)

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Objectives: The Prothrombin-fragment-F1+2 (F1+2) is a sensitive marker of thrombin generation and results after cleavage of prothrombin by FactorXa into thrombin. D-dimer results through degradation of the cross-linked fibrin. Two new and pre-commercial assays, INNOVANCE®-LOCI®-F1+2– and INNOVANCE®-LOCI®-high-sensitivity-D-dimer-Assays (hsD-dimer®, Siemens-Healthineers, Marburg, Germany), that seem to have the potential to reliably assess the activity of the coagulation system. This may be important to assess the efficacy and duration of anticoagulation.
Methods: First, we analyzed 130 samples from hospitalized patients under Phenprocoumon®-therapy with different INR-Ranges (<2.0, 2.0–3.0, >3.0–4.5, >4.5). Second, 10 patients on 20mg-Rivaroxaban® (VTE-Therapy) were included. Third, 10 patients on 10mg Rivaroxaban® (VTE-prophylaxis) after KPT were enrolled. In order to investigate the impact of a diurnal Rivaroxaban® administration on the coagulation activation profile three blood samples were done: before, 3h and 6–8h after drug intake. F1+2 and hsD-dimers were measured using the INNOVANCE®-LOCI®-F1+2– and the INNOVANCE®-LOCI®-high-sensitive-D-dimer-Assays (hsD-dimer®) on Atellica-COAG-360-System, Siemens-Healthineers, Marburg, Germany. Statistical analysis was calculated using the MedCalc-Program v.15.11.4.
Results: In patients on Phenprocoumon®-therapy F1+2 and hsD-dimers significantly decreased with increasing INR up to 4.5 ($p<0.05$). Patients on Rivaroxaban®-prophylaxis (10mg) and Rivaroxaban®-therapy for VTE (20mg) showed no statistically significant difference between the different time points of F1+2 or hsD-dimers, but the prophylaxis group was significantly different with higher values than the therapy group ($p<0.05$).
Conclusion: Rivaroxaban®-therapy- and -prophylaxis seem to suppress the activation coagulation parameters F1+2 and hsD-dimers in a dose-dependent-way and stronger than Phenprocoumon ($p<0.05$).
Disclosure: No significant relationships.

OC 4.2 Monitoring of unfractionated heparin in clinical practice

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Objectives: Monitoring of unfractionated heparin (UFH) is recommended by all scientific guidelines. There is however no consensus about the optimal laboratory test to be used in clinical practice. We aimed to investigate the accuracy, reproducibility, and costs of different laboratory assays for monitoring of UFH in clinical practice and to study test utilisation in Switzerland.
Methods: Samples of 254 consecutive patients referred for UFH monitoring in a primary care hospital were analysed with activated partial thromboplastin time (aPTT), thrombin time (TT; high and low thrombin concentration), prothrombinase-induced clotting time (PiCT), and anti-Xa activity. A survey among Swiss hospitals and laboratories was conducted.
Results: In relation to anti-Xa activity, spearman's correlation coefficient was 0.68 (95%CI 0.60, 0.75) for aPTT, 0.79 (0.69, 0.86) for TT, and 0.94 (0.93, 0.95) for PiCT. Correlation between anti-Xa activity and heparin concentration as determined by spiking plasma samples was 1.0 (1.0, 1.0). Coefficient of variation was at most 5% for PiCT and anti-Xa activity. Total costs per test were CHF 23.40 for aPTT, CHF 33.30 for TT, CHF 15.70 for PiCT, and CHF 24.15 for anti-Xa activity. Swiss institutions implemented aPTT in 53.2%, TT in 21.6%, anti-Xa activity in 7.2%, PiCT in 1.4%, and more than one test in 16.6%.
Conclusion: Accuracy and reproducibility of PiCT and anti-Xa activity for monitoring of UFH was superior and analytical costs were lower or equal to aPTT and TT. Widespread implementation of PiCT and anti-Xa activity in clinical practice has the potential to improve patient care and reduce health care costs.
Disclosure: No significant relationships.

OC 4.3 Evaluation of DOAC measurement on the CS-5100 using the INNOVANCE Heparin und INNOVANCE DTI reagents

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Objectives: The direct oral anticoagulants (DOAC) dabigatran, rivaroxaban, apixaban and edoxaban are well established in clinical practice, as is argatroban. Monitoring of DOACs is not required, but with the wider distribution, more patients under DOAC therapy are getting into situations where emergency surgery is unavoidable. In such situations, the measurement of DOAC concentration is helpful. Therefore, we evaluated the measurement of DOACs on the routine coagulation analyzer CS-5100 with the appropriate reagents from Siemens Healthcare.
Methods: All measurements were performed on a Sysmex CS-5100 analyzer using INNOVANCE Heparin and INNOVANCE DTI reagents. Controls and calibrators were from Technoclone (apixaban, rivaroxaban), coachrom (argatroban), STA (edoxaban) and Siemens Healthcare (dabigatran). Additionally, the DOAC concentrations were measured with LC MS/MS. We evaluated the CV, cut-off and recoveries for the assays. For method comparison, we measured patient samples with LC MS/MS, the assays used until that time, and the assays using the Siemens Healthcare reagent.
Results: The CV for the assays was between 1.1 and 4.7% and cut-off was <10 ng/ml for the DOAC assays and <0.005 µg/ml for the argatroban assay. R^2 between the DOAC measurement on the CS-5100 and the LC MS/MS from the patient samples was >0.95. The recoveries had a mean deviation of <15 % and < 17 ng/ml or 0.03 µg/ml, respectively.

Conclusion: For laboratories using a CS-5100, the reagents INNOVANCE Heparin and INNOVANCE DTI are fast and stable alternatives for DOAC and argatroban measurement using only one reagent for all FXa-inhibiting drugs and one for FIIa-inhibiting drugs.
Disclosure: No significant relationships.

OC 4.4 Performance characteristics of a point of care test from urine for direct oral anticoagulants

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Objectives: Direct oral anticoagulants (DOAC) are metabolized predominantly by excretion into urine. A specific and rapid detection indication by a point of care test may support diagnosis of anticoagulant therapy with DOACs especially in emergency medicine.
Methods: The diagnostic test strip DOAC Dipstick determines qualitatively the absence or presence of DOACs in human urine by visual identification of colours. The test consists of a change of colour upon reaction of Factor Xa or Thrombin with a Factor Xa or Thrombin chromogenic substrate in relation to the amount of DOAC present in the urine sample. The colours allow the detection of DOAC in a urine sample by naked eye, with interpretation as "negative" in the absence of a DOAC and as "positive" in the presence of a DOAC.
Results: Sensitivity and specificity of detection was 0.92 and 0.93 (rivaroxaban) and 0.90 and 0.93 (dabigatran, 100ng/ml each) in normal human urine spiked with 0 to 1500 ng/ml of each DOAC. Using urine samples of patients treated with DOACs, sensitivities and specificities were all 1.0. Concentrations of DOACs in urine (mass spectrometry) were higher due to a smaller distribution volume compared to the blood volume (mean 5.600ng/ml Dabigatran, 2.700ng/ml Rivaroxaban, 1.800ng/ml Apixaban, n=29 each, normal <5ng/ml). Heparins did not interact with the test. Bilirubin, urobilinogen, macroproteinuria and macrohaematuria interfered with the colours of the test results.
Conclusion: The DOAC Dipstick test from urine samples of patients offers a rapid, reliable and valid detection of DOACs in medical emergency situations.
Disclosure: No significant relationships.

OC 4.5 NOAC use in transplant recipients – A Case Series from the Prospective Dresden NOAC Registry (NCT01588119)

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Objectives: Although NOACs may have significant drug-drug interactions with immunosuppressive drugs and other co-medications, they may offer advantages also for organ and hematologic transplant recipients but data on this approach are scarce.
Methods: Using data from the prospective, non-interventional *Dresden NOAC Registry* we evaluated effectiveness and safety of NOACs in transplant recipients. All registry patients undergo long-term follow-up by quarterly phone calls and outcome events are centrally adjudicated using standard definitions.

Tab. 1 Patient's characteristics (OC 4.5)

#	age (years), gender	NOAC Indication	Transplant type	Relevant co-medications with potential for CYP3A4 or p-GP interactions	NOAC therapy	follow-up duration (months)	exposure time (months)	major vascular event	first ISTH major bleeding (on treatment)	first ISTH CRNM bleeding (on treatment)
1	45, female	VTE	Liver	tacrolimus	Rivaroxaban 20 mg OD	33.6	5.7	---	---	---
2	56, female	VTE	Kidney	ciclosporin	Apixaban 5 mg BID	15.1	15.1	---	---	---
3	62, female	VTE	Kidney	tacrolimus	Rivaroxaban 15 mg OD	51.9	12.9	---	---	perirenal hematoma & macrohematuria; after 2.1 months
4	62, female	SPAF	Liver	ciclosporin	Apixaban 2.5 mg BID	24.4	24.4	---	postoperative bleeding with anemia after 2.8 months	---
5	68, male	VTE	Bone marrow	none	Rivaroxaban 10 mg OD	45.8	40.9	DVT after 40.9 months	---	macrohematuria; 18.3 months
6	74, male	SPAF	Stem cell Tx	none	Rivaroxaban 20 mg OD	60.9	60.9	---	---	varices bleeding, after 53.8 months
7	74, male	SPAF	Bone marrow & stem cell Tx	none	Rivaroxaban 15 mg OD	36.2	36.2	---	---	bleeding after dental extraction, after 21 months
8	76, female	SPAF	Kidney	tacrolimus	Apixaban 5 mg BID	24.3	10.9	---	---	---

Results: Until September 20th 2017, 8 transplant patients receiving NOAC were enrolled (▶Table 1). Mean exposure to NOAC was 23.9 months (5–61 months). During NOAC treatment, one VTE event occurred and 6 patients had a total of 12 bleeding complications, which consisted of ISTH minor bleeding in 5 cases, clinically relevant non-major bleeding in 5 and ISTH major bleeding in 2 cases (wound bleeding post hip surgery; bleeding from iliac artery lesion 7 days later in the same patient). During follow-up (mean 37 months; 15–61 months), 2 patients had a scheduled end of NOAC treatment (completed VTE therapy) and 2 patients were switched to other oral anticoagulation (onset of DVT; fear for drug interactions). No patient died during follow-up.
Conclusion: Although transplant recipients receive complex co-medications, few of them have a potential for relevant drug-drug interactions via CYP3A4 or p-GP. NOACs are being used in this setting to treat for VTE and atrial fibrillation and our small case series indicates that this approach may be sufficiently effective and safe, but dedicated studies need to evaluate this further before recommendations can be made.
Disclosure: J.B.-W.: honoraria and research support from Bayer HealthCare, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer and Daiichi Sankyo. S.M.: honoraria from Bayer HealthCare. L.T.: nothing to declare

OC 5: Laboratory tests II

OC 5.1 Accuracy of heparin-induced platelet aggregometry (PAT) for the diagnosis of heparin-induced thrombocytopenia

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Objectives: Whereas the utility of platelet washed assays such as the heparin-induced platelet activation assay (HIPA) for the diagnosis of heparin-induced thrombocytopenia (HIT) is regarded as high, is the performance of simpler assays such as the heparin-induced platelet aggregometry (PAT) still elusive. We aimed to assess the accuracy of PAT for the diagnosis of HIT.
Methods: Frozen samples of a well-characterized cohort were further analyzed with HIPA. In this previously conducted single-center cohort study, 1291 consecutive patients with suspected HIT were included and samples were analyzed with PAT. The study population was mixed, median age was 67.9 years and 44% of the patients were female. Out of this cohort, 125 consecutive serum samples with a positive polyspecific PF4/heparin ELISA result were examined. HIPA was implemented as previously described and diagnosis of HIT was defined as a positive HIPA, that is a positive reaction in 2/4 donor platelets within 30 minutes.
Results: HIPA was positive in 40 out of 125 patients corresponding to a prevalence of 32%. Median OD (polyspecific ELISA) was 2.87 (IQR 2.4, 3.0) in patients with HIT and 0.79 (IQR 0.68, 2.37) in patients without HIT. The number of true positives was 28, the number of true negatives 85, the number of false-negatives was 12 and the number of false-positives 0. Thus, the sensitivity of PAT for the diagnosis of HIT was 70% and the specificity 100%.
Conclusion: Our investigation suggests that PAT is a valuable test to confirm HIT but it provides limited benefit in ruling-out HIT.
Disclosure: No significant relationships.

OC 5.2 Distribution and cardiovascular determinants of thrombin generation assessed in plasma of Gutenberg Health Study participants

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Objectives: The thrombin generation (TG) assay may be a potential tool to improve risk stratification for cardiovascular diseases (CVDs). This study aims to explore the relation between TG and cardiovascular risk factors (CVRFs), CVDs, and total mortality.
Methods: For this study, N=5000 subjects from the population-based Gutenberg Health Study were analyzed in a highly standardized setting. TG was assessed by Calibrated Automated Thrombogram method at 1 and 5 pM tissue factor (TF) trigger in platelet poor plasma. Lag time, endogenous thrombin potential (ETP), and peak height were derived from the TG curve. Multivariable linear and cox regression analysis were used to assess clinical determinants of TG and the association between TG parameters and total mortality.
Results: Lag time (at 1 and 5 pM TF) was positively associated with obesity and dyslipidemia for both sexes ($p<0.0001$). Obesity was also a positive determinant of ETP (at 1pM and 5 pM TF) in both sexes ($p<0.0001$) and peak height in males (1 pM TF, $p=0.0048$) and females (1 pM TF and 5 pM TF, $p<0.0001$). Cox regression models showed an increased mortality in individuals with lag time (1 pM TF, HR=1.46, [95% CI: 1.07; 2.00], $p=0.018$) and ETP (5 pM TF, HR = 1.50, [1.06; 2.13], $p=0.023$) above the 95 percentile of the reference group, independent of the cardiovascular risk profile.
Conclusion: This large-scale study demonstrates traditional CVRFs, particularly obesity as relevant determinants of TG. Lag time and ETP were found as potentially relevant predictors of increased mortality, which deserves further investigation.
Disclosure: No significant relationships.

OC 5.3 Laboratory Reclassification of von-Willebrand disease type 1 in the Elderly

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Objectives: In this two-center cohort study we re-evaluated patients previously diagnosed with von-Willebrand disease (VWD) type 1 with respect to their bleeding phenotype and VWF levels based on age-and blood group (BG)-dependent reference values.

Methods: 969 consecutively admitted patients presenting with VWD one year before laboratory re-classification were enrolled. Reclassification was performed on the basis of age- and BG-dependent reference values with respect to the presence or absence of VWD. 174 of 504 patients aged 1 to 86 years (median 35) were diagnosed with VWD type 1 (n=154), type 2 (n=17) or 3 (n=3) within the last two decades prior re-classification based on VWF levels including CBA, multimeric pattern and/or genotyping.
Results: In the present patient follow-up visits in patients aged 20 to ≤ 49 years 42 of 230 individuals (18,3%) compared to 55 of 131 older patients aged 50 to 86 years (median 66: 42%) the formerly classification of VWD type 1 would have been missed (p<0,001) without using age- and BG-dependent cut-off values. In patients < 20 years of age previous diagnosis were confirmed in all cases (n=39). Of note, inclusion of the variable blood group "0" versus "non-0" in our model has improved the detection rate of previously diagnosed VWD by 5%.
Conclusion: In prediagnosed VWD the use of age- and BG-dependent reference values is suggest in order not to miss patients prone to possible bleeding episodes during emergency or elective interventions.
Disclosure: No significant relationships.

OC 5.4 Effects and interferences of emicizumab – a humanized bispecific antibody mimicking activated factor VIII cofactor function – on coagulation assays

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Objectives: Emicizumab bridges activated FIX (FIXa) and FX to restore the missing function of activated FVIII in persons with hemophilia A (PwHA). The efficacy of emicizumab in PwHA with inhibitors was recently described (Oldenburg J, *NEJM* 2017). Unlike FVIII, emicizumab does not require activation; thus, in coagulation assays the behavior of emicizumab may differ from that of FVIII.
Methods: The effect of emicizumab on multiple coagulations assays, including potential interference was assessed.
Results: Emicizumab did not interfere with the following assays: single factor assays triggered by prothrombin time (PT) reagents; immunoassays for von Willebrand factor, D-Dimer, plasminogen, or FXIII; chromogenic assays for FIX, antithrombin, protein C, anti-Xa, or plasminogen; and clotting assays triggered by a prothrombin activator (APC resistance) or thrombin (thrombin time and Clauss fibrinogen). However, emicizumab did have a small but detectable effect on assays for PT and derived fibrinogen. As expected based on its mechanism and species specificity, emicizumab showed concentration-dependent activity in a FVIII chromogenic assay using human FIXa and FX, but exhibited no activity in a FVIII chromogenic assay using bovine FIXa and FX. Emicizumab had an overly strong effect on the activated partial thromboplastin time (aPTT), which resulted in interference with several aPTT-based assays including single factor assays (FVIII, FIX, FXI, FXII), protein C activity, protein S activity, and APC resistance.
Conclusion: The observed effects are consistent with the novel mode of action of emicizumab. Potential assay interferences should be taken into account in the selection and interpretation of test results for PwHA receiving emicizumab therapy.
Disclosure: Andreas Calatzis is a consultant to Genentech, Inc. David C. Chen, Ido Paz-Priel, and Joanne I. Adamkewicz are employees of Genentech, Inc.

OC 5.5 Monitoring of emicizumab using a two-stage chromogenic factor Xa (FXa) generation assay

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Objectives: Emicizumab is a factor VIII-(FVIII)-mimetic antibody that simultaneously binds to activated factor IX (FIXa) and factor X (FX) inducing the formation of FXa. Plasma levels of emicizumab ranging between 10 and 100 µg/ml have been shown to be effective in the treatment of hemophilia A patients. The objective of the present study was to develop a chromogenic assay for measurement of emicizumab in plasma.
Methods: A two-stage assay format was used to monitor emicizumab-induced FXa generation. First, diluted plasma is mixed with FIXa and FX, 25 µl each, in the presence of calcium and phospholipids. Second, 25 µl of the chromogenic FXa-substrate is added and FXa-mediated substrate conversion was measured. The limit of detection (LOD), the lower limit of quantification (LLOD), precision, and accuracy were tested using emicizumab-spiked heat-inactivated plasma. Emicizumab was a kind gift of Roche pharmaceuticals.
Results: Using the optimum concentration of reagents, emicizumab plasma concentrations ranging from 3.5 to 100 µg/ml were found to be within the dynamic range of the assay. The LOD and LLOQ were determined as 0.32 and 3.45 µg/ml, respectively. Using emicizumab plasma concentrations of 50 and of 15 µg/ml, intra- and interassay CV values were ≤ 6.8% while the relative error of absolute quantification did not exceed 33.4 %.
Conclusion: We introduced a two-stage FXa-generation assay allowing the determination of clinically relevant plasma levels of emicizumab. The use of standard reagents as well as a chromogenic substrate will allow the widespread adaption of the assay on automated test systems.
Disclosure: Emicizumab was kindly provided by Roche pharmaceuticals

OC 6: Platelets

OC 6.1 Fluoxetine amplifies ticagrelor mediated cardioprotection after acute MI

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Objectives: Restoration of blood flow after acute myocardial infarction (MI) comes at the cost of reperfusion injury, a multifactorial process that comes with an inflammatory response. Platelet serotonin amplifies acute inflammation and it is unclear how this affects reperfusion injury. Utilizing fluoxetine (Flx), we depleted peripheral serotonin in mice and additionally treated animals with ticagrelor prior to MI. The goal was to evaluate if and how excessive serotonin levels interfere with P2Y12 mediated cardioprotection.
Methods: Mice were put on a Flx containing diet for 3 weeks. After administration of ticagrelor, MI was induced for 30 minutes, followed by 24 hours of reperfusion. Heart function, infarct size and integrin expression was evaluated.
Results: Serotonin peaked 24 hours after MI in WT mice (150 ng/mL) after MI. Heart function in Flx treated compared to WT was improved and infarct size was reduced (38 in Flx, 53 in WT; % area at risk (AAR)). WT mice revealed increased MPO levels in the heart and elevated neutrophil counts in the AAR (14 vs. 28 in WT per mm^2 tissue). Neutrophils had decreased expression of CD11b in Flx (70%) treated mice compared to WT. ISurprisingly, the protective effect in 5-HT deficient mice was additive to ticagrelor (100mg/kg loading; 50mg/kg twice afterwards) mediated cardio protection in terms of infarct size and integrin expression.

Conclusion: Serotonin directly mediates neutrophil migration during myocardial reperfusion injury by inducing degranulation and subsequent upregulation of CD11b. Since this effect was also present after treatment with ticagrelor, intervening in serotonin-neutrophil crosstalk might provide novel anti-thromboinflammatory treatment options.
Disclosure: No significant relationships.

OC 6.2 IκB Kinase 2 impairs Platelet Activation

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Objectives: Megakaryocytes can sense inflammatory signals, but little is known how this might change platelet function. Most inflammatory signalling pathways converge at the kinase IKK2 (IκB kinase 2) activating the transcription factor NF-κB. Our aim was to determine the effect of persistent inflammation on platelet function, by NF-κB activity in megakaryocytes with a constitutively active IKK2.
Methods: Mice with a megakaryocyte-specific constitutively active IKK2 (caIKK2) were compared to littermate controls. Platelet count and lifespan was determined and function was tested *in vitro* by agonist-induced degranulation and aggregation and *in vivo* by tail bleeding, intra vital microscopy of ferric chloride induced thrombus formation and mechanical aorta injury.
Results: Platelet count and lifespan is unaltered, however platelet aggregation, degranulation and GPIIb/IIIa activation were decreased in platelets with caIKK2 upon stimulation with ADP and PAR4 receptor agonist. Consistently, *in vivo* thrombus formation is impaired in both, in the ferric chloride and mechanical injury model and bleeding time is increased in caIKK2 platelets. Furthermore the G-protein $G_{\alpha}q$ is downregulated.
Conclusion: Platelets of mice with megakaryocyte-specific caIKK2 exhibit decreased activation, degranulation and aggregation *in vitro*. This is in line with the remarkable reduction of thrombus formation *in vivo* and increased bleeding time. This may be partially explained by the downregulation of $G_{\alpha}q$, which results in decreased signaling strength and therefore platelet activation. Taken together our data indicates that active IKK2 or NF-κB interferes with signaling mediated platelet activation, either directly through kinase activity in platelets or via constitutively active NF-κB signaling in megakaryocytes.
Disclosure: No significant relationships.

OC 6.3 Deformable Nano and Microstructures for Quantifying Platelet Contractile Forces and Platelet Cytoskeletal Mechanics

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Objectives: While the role of platelets in preventing blood loss is well characterized from a biological perspective, the mechanobiological aspects are only poorly understood. Platelet biomechanics regulated through platelet cytoskeletal elements are a fundamental feature of platelet function. Using direct 3D laser writing, we have developed deformable nano/-microstructures to investigate how changes in the platelet cytoskeleton impair platelet adhesion, spreading and activation dynamics.
Methods: *In silico* finite element mechanics (FEM) simulation was performed to determine optimal material parameters for physical dimensions of deformable structures (▶Figure 1 & ▶Figure 2).3D micropillar arrays (700nm diammeter) and microbeam arrays (140nm in diameter) with different heights and lengths were fabricated using two photon direct laser writing system. Femtosecond-pulsed laser at 780 nm and IP-L photoresist (Nanoscribe GmbH) by means of a high-numerical aperture oil-immersion objective (N.A. = 1.4) was used to fabricate these strcutures. Washed platelets were incubated with nano/-microstrctures functionalized with fibrinogen and platelet dynamics was followed by live imaging and confocal fluorescence microscopy (▶Figure 3 & ▶Figure 4).
Results: On micropost arrays platelets generated contractile forces in the range from 9.2 to 87 nN (mean: 23 ± 4.1 nN, n = 25 single platelets) as measured by post deflection. On microbeams platelets were able to contract the beams with forces ranging from 15.1 to 110.8 nN (mean: 31 ± 2.9 nN, n = 25 single platelets).
Conclusion: Using defromable 3D nano and micro structures we can measure contratile forces produced by platelet cytoskeleton during adhesion and spreading thus provoiding insights into biophysical mechanisms involved in the physiology of clot formation and plug stability.
Disclosure: No significant relationships.

OC 6.4 Defective Mg2+ transport enhances Ca2+ responses in B cells and platelets thereby accelerating immuno-thrombotic effects in mice

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Objectives: Beside calcium (Ca^{2+}), magnesium (Mg^{2+}) is one of the crucial divalent cations mediating numerous physiological processes in immune cells and platelets. Magnesium infusion or supplementation is practiced in the treatment course of various cardio- and cerebrovascular pathologies, but the molecular mechanisms of Mg^{2+} influx/efflux are poorly investigated in platelets. In human XMEN syndrome, abolished function of MAGT1 accounts for a dysregulated Mg^{2+} homeostasis in T and NK cells.
Methods: Using non-infected healthy *Magt1* knockout mice (*Magt1*$^{-/y}$) we analysed cation homeostasis and physiological responses of B cells and platelets.
Results: Our study reveals an important contribution of MAGT1 to the regulation of Mg^{2+} homeostasis in B cells, associated with an altered BCR-induced Ca^{2+} responses and PKC activation, which resulted in a disturbed marginal zone B and plasma cells development. In addition, our report gives the first *in vivo* evidence, that interference with MAGT1 function also results in imbalanced Mg^{2+} homeostasis in platelets. This accounts for an increased Ca^{2+} influx, which strongly modulates platelet aggregation and thrombus formation, and accelerates occlusive arterial thrombus formation *in vivo*, shorter bleeding time, and a dramatically worsened brain damage after focal cerebral ischemia. Surprisingly, the aberrant Ca^{2+} influx is independent of SOCE, but strongly dependent on P2X1 and TRPC6 channel activation. Consequently, haploinsufficiency of either P2X1 or TRPC6, or Mg^{2+} supplementation could revert the observed prothrombotic phenotype in *Magt*$^{-/y}$ mice.
Conclusion: These results reveal that decreased intracellular Mg^{2+} concentrations by altering Mg^{2+} transport activity may be a risk factor for B cell related immune and platelet dependent thrombo-inflammatory diseases.
Disclosure: No significant relationships.

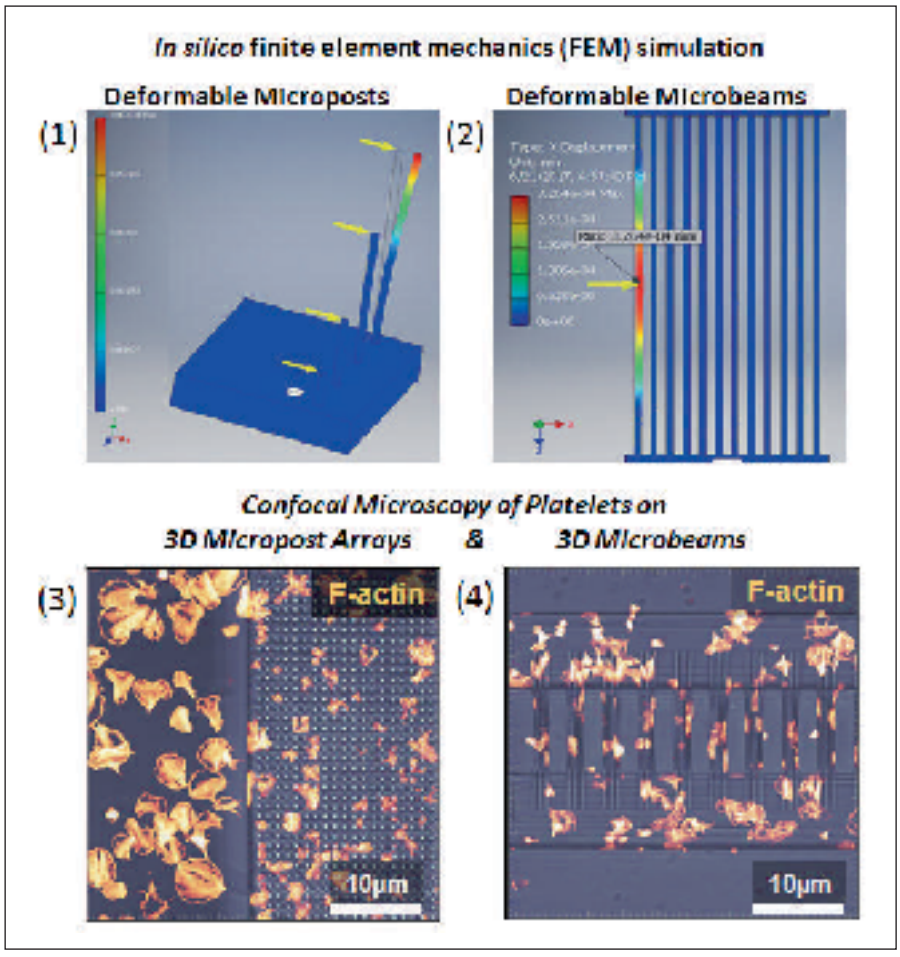

Fig. 1
In silico finite element mechanics (FEM) simulation. Deformable Microposts (OC 6.3)

Fig. 2
In silico finite element mechanics (FEM) simulation. Deformable Microbeams (OC 6.3)

Fig. 3
Confocal Microscopy of Platelets on 3D Micropost Arrays (OC 6.3)

Fig. 4
Confocal Microscopy of Platelets on 3D Microbeams (OC 6.3)

OC 6.5 Differential regulation of GPIbα- and GPVI-specific signaling pathways by cAMP- and cGMP-elevating inhibitors of human platelets

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Objectives: Von Willebrand factor and collagen are main ligands of the platelet adhesive receptors GPIb-IX-V and GPVI, respectively, but also bind to the integrin $\alpha_{IIb}\beta_3$ and $\alpha_2\beta_1$, respectively. C-type lectin snake toxins are widely used as specific platelet receptor ligands. Whereas convulxin induces GPVI-specific signaling, echicetin is a selective GPIbα agonist when coated to polystyrene beads (Navdaev A., PLoS One, 2014). We aimed to elucidate the effects of cAMP-/cGMP-elevating inhibitors of human platelets on their selective GPIbα- or GPVI-mediated activation and signaling.
Methods: Echicetin was purified from Echis carinatus venom, validated by mass spectrometry, and used to prepare echicetin beads (EB) as described (Navdaev A., PLoS One, 2014.) Platelet aggregation was analyzed by light transmission aggregometry, platelet signaling by Western blot analysis of phosphoproteins.
Results: EB and convulxin induced full platelet aggregation and strong phosphorylation of tyrosine and serine/threonine protein kinases such as Syk, FAK, Akt, p38 and of $PLC\gamma_2$, a Syk substrate. Activation of cAMP or cGMP pathways by Iloprost or Riociguat, respectively, strongly inhibited platelet aggregation and Akt^{S473}, FAK^{Y397} and $p38^{Y180/T182}$ phosphorylation but not phosphorylation of Syk^{Y352}, $Syk^{Y525/526}$ and $PLC\gamma_2{}^{Y759}$.
Conclusion: Both GPIbα- and GPVI-specific signaling include phosphorylation of Syk^{Y352}, $Syk^{Y525/526}$, FAK^{Y397}, Akt^{S473}, $p38^{Y180/T182}$ and $PLC\gamma_2{}^{Y759}$. In contrast to Akt, FAK and p38, phosphorylation/activation of Syk and $PLC\gamma_2$ was not impaired by cAMP- or cGMP-elevating platelet inhibitors. This suggests that distinct Syk-dependent responses mediated by GPIbα or GPVI are not blocked by cAMP/cGMP pathways in human platelets.
Disclosure: No significant relationships.

OC 7: Acquired coagulation disorders

OC 7.1 Effects of an awareness campaign towards hospital cases of acquired hemophilia A (AHA) 2010–2015 in Germany – Updated

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Objectives: Acquired hemophilia A (AHA) is a rare bleeding disorder, caused by the development of autoantibodies against human coagulation factor VIII. AHA may lead to spontaneous or trauma induced bleeds that are treated with bypassing agents. The incidence of the disease was estimated to about 1.4 per million per year in the UK. Data from Germany are not available. Since 2010 an intense awareness camping informed physicians how to detect AHA. A specific ICD 10 code for AHA exists since 2010, allowing epidemiological insights. We examined the frequency of AHA based on these data, updating the findings until 2014 and compared it to results from the recent GTH-AH 01/2010 study.
Methods: The reports from German DRG Institute (InEK), Statistical Office (DESTATIS) and the hospital quality reports for 2010–2015 were analyzed for cases of AHA and treatments with high amounts of bypassing agents (APCC > 150.000 units, rFVIIa > 500 mg). Statistical analysis was performed using Microsoft-Excel and Access version 2016.
Results: The number of cases with a main diagnosis of AHA (D68.31, ICD10-GM) increased from 29 (2010) to 142 (2015, +390%). The mean age of patients (71.5 +/- 18.1 years) and the gender distribution (58% male) remained stable over time and were very similar to data from the GTH-AH study. 60% of patients were aged 70–85. The average length of hospital stay of male patients (25.2 days) was significantly longer than for females (18.4 days), possibly reflecting the trend towards a less favorable prognosis of AHA in male patients as seen in the GTH study. The number of cases with a secondary diagnosis of AHA increased from 186 (2010) to 491 (2015, +164%). The total number of cases in 2014 was 633 (~8 per mio. per year), indicating a much higher incidence than suggested from UK data. The increase in cases was not associated with an equivalent growth in treatments with high doses of rFVIIa (108 in 2010, 127 in 2015; +18%) or APCC (66 in 2010, 75 in 2015; +14%).
Conclusion: We found a further increase in documented hospital cases with AHA from 2010 to 2015. The overall number exceeds the expected number of patients based on previously reported incidence. This may reflect a growing awareness towards AHA, under-diagnosis in previous studies, or both. Remarkably, the number of patients intensively treated with bypassing agents decreased, suggesting that higher awareness may lead to earlier diagnosis and prevention of high costs due to bleeding.
Disclosure: No significant relationships.

OC 7.2 GTH-Registry: Treatment and Outcome in Patients with Haemophilia B and Inhibitors

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Objectives: Immuntolerance induction (ITI) for inhibitors in haemophilia B (HB) with high dose factor IX may be complicated by anaphylaxis or nephrotic syndrome. Additionally, efficacy seems to be limited (13–25% success rate). Registry data and published case reports suggest that addition of immunosuppression may improve outcome.
Methods: The GTH-Factor IX inhibitor registry was set up with the aim to collect data of all patients with HB and FIX-inhibitors in Germany regardless whether they had had an ITI-attempt or not. Information on Symptoms at time of inhibitor development, treatment regimens and success rates as well as complications are collected from patient charts.
Results: We report data on eleven inhibitor patients with severe haemophilia B from 9 Haemophilia Centres in Germany. All patients underwent ITI, 10 got two or more ITI´s. Regimens included high dose factor IX, mostly in combination with immunomodulation using various regimens (i.e. Steroids, mycofenolate mofetil, Cyclosporin, iv-Immunoglobulins and/or Rituximab). In two patients we observed complications like allergic reaction or nephrotic syndrome. A Cyclosporin induced hypertension occurred in another patient. In 8 patients remission of the inhibitor was achieved, 3 patients are on ongoing ITI for a persistent inhibitor.
Conclusion: Preliminary data of this registry support the hypothesis, that addition of immunosuppression can improve treatment outcome of patients with haemophilia B and inhibitors. In a small patient population with a rare complication of a rare disease registries are a valuable tool to develop evidence. To avoid reporting bias, we aim for documentation of all HB patients with inhibitors.
Disclosure: This study was supported by PFIZER Pharma GmbH

OC 7.3 Relevance of lysine residues in the conformational change of beta 2 glycoprotein I from closed to open involved in antiphospholipid syndrome (APS) disease

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Objectives: Beta 2 glycoprotein I (beta2GPI) is the main antigen present in autoimmune disease antiphospholipid syndrome (APS). The closed (circular) beta2GPI conformation circulates in blood, while the open (linear) conformation exposes a cryptic epitope and is potentially antigenic. It may lead to formation of antibody-protein complexes, resulting in disease progression. In the present study we investigate the relevance of lysine residues in switching beta2GPI conformation by acetylation.
Methods: We prepared acetylated beta2GPI by reaction of lysine residues with acetyl acid N-hydroxysuccinimide ester (NHS-Ac) and compared its structure to untreated beta2GPI using atomic force microscopy (AFM), circular dichroism (CD) spectroscopy and size determination by dynamic light scattering (DLS). Proper acetylation of lysine residues was demonstrated by fluoraldehyde o-phthalaldehyde (OPA) reagent and anti-lysine Western blot.
Results: OPA reagent showed acetylation of lysine residues at a ratio of 1.000 mol NHS-Ac / mol lysine. Anti-lysine Western blot supported this finding showing bands of acetylated lysine at ratios of 100 and 1.000. AFM indicated an increased amount of open beta2GPI with increasing ratio of acetylation agent. These results were further confirmed by a smaller hydrodynamic radius of fully acetylated beta2GPI in comparison to untreated beta2GPI. Interestingly, slight changes of CD spectra were visible between acetylated and untreated beta2GPI indicating a hardly altered protein secondary structure.
Conclusion: Lysine residues play a role in shifting beta2GPI from closed to open conformation. Beta2GPI structure could be altered *in vivo* by acetylation of lysine residues as a posttranslational modification causing beta2GPI to adopt open conformation, allowing APS antibody to bind.
Disclosure: No significant relationships.

OC 7.4 Antibody mediated glycan modification on platelets and megakaryocytes: a potential role in platelet destruction in autoimmune thrombocytopenia

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Objectives: Introduction: Immune thrombocytopenia (ITP) is a bleeding disease caused by autoantibodies (AAbs) directed against platelet glycoproteins. Fc-independent platelet clearance via Ashwell-Morell receptors, which recognize glycan changes on platelet surface, has been proposed as a new mechanism of ITP in mice. In this study, we investigated the impact of AAbs from ITP patients on glycan pattern of megakaryocytes and platelet.
Methods: Methods: Megakaryocytes were differentiated from CD34+ cells after cultivation with thrombopoietin. Megakaryocytes and platelets were incubated with ITP-sera and the modification in glycan pattern was assessed by flow cytometry using two lectins: RCA and ECL. NOD/SCID mouse model was used to analyze the impact of glycan patterns on survival of human platelets.
Results: Results: Different patterns of glycan modification were observed on platelet surface after incubation with ITP-sera: 9/37 sera induced high ECL binding, while 8/37 sera showed decrease in RCA binding. Interestingly, a subgroup of ITP-sera was able to modify glycan pattern on megakaryocytes surface (3 fold increase in RCA-binding compared to control). The injection of AAbs accelerated platelet clearance *in vivo*. The destruction of human platelets by ITP-AAbs was largely decreased by a neuraminidase inhibitor that prevents glycan changes on platelet surface (platelet survival after 5h: 60% vs 40%).
Conclusion: Conclusion: Our results demonstrate that AAbs from ITP patients are able to induce changes in glycan patterns on both megakaryocytes and platelets surfaces. The mechanism of antibody-mediated modification of glycan patterns seems to contribute to platelets destruction as well as to interfere with platelets production from megakaryocytes.
Disclosure: No significant relationships.

OC 7.5 Differences in the platelet proteome between healthy controls and patients with cancer at high risk of venous thromboembolism

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Objectives: Patients with lung, brain and pancreatic cancer are at high risk to develop venous thromboembolism (VTE). However, the exact mechanisms of VTE in the cancer types are incompletely understood. Platelets have been recently suggested to contribute to increased risk of cancer-associated VTE.
Methods: We investigated the platelet proteome of patients with brain (BC; n=19), lung (LC; n=20) and pancreatic (PC; n=6) cancer and matched healthy controls (n = 45). To analyse the platelet proteome we have applied two-dimensional differential in-gel electrophoresis (2D-DIGE) and mass spectrometry to investigate differences in platelet proteomics.
Results: Significant changes in the abundance of 16 proteins were observed between patients with cancer (n=45) and healthy controls (▶Table 1). Most identified proteins correspond to cytoskeleton arrangements but also platelet interaction, as Integrin-alpha-IIb, and Integrin-alpha-6 were differentially expressed. Furthermore protein-disulfide-isomerase (PDI) and glucose-regulated-protein-78, which are associated with thrombus formation and aggregation, are differentially expressed. Interestingly, different tumour entities show significant differences in the pattern of proteins. Integrin-alpha-IIb was significantly lower expressed in all patients however PDI was upregulated in LC but downregulated in BC and PC. A connection between thrombosis and the immune system could also be hypothesized as leucocyte-elastase-inhibitor is downregulated in all cancer types.

Tab. 1 Differentially expressed proteins between all included CATS patients and healthy controls. Protein abundance ratios and unadjusted p-values were calculated using all tumour entities together of single tumour entities versus healthy controls. Proteins with a p-value <0.05 are highlighted.

Protein name (Uniprot)	CATS vs HD n = 45 unadjusted Student tTest		Brain Cancer (BC) n = 19 unadjusted Student tTest		Lung Cancer (LC) n= 20 unadjusted Student tTest		Pancreas Cancer (PC) n = 6 unadjusted Student tTest	
	ratio CATS/HD	p-value	ratio BC / HD	p-value	ratio LC / HD	p-value	ratio PC / HD	p-value
Integrin-alpha-IIb (P08514)	0.89	<0,001	**0.93**	**0.032**	**0.87**	**<0.001**	**0.79**	**<0.001**
Nucleosome assembly protein (P55209)	0.89	<0,001	**0.9**	**0.007**	**0.88**	**0.002**	0.91	0.1
Zyxin (Q15942)	0.84	0.002	0.91	0.08	**0.85**	**0.009**	**0.58**	**<0.001**
Calreticulin (P27797)	1.09	0.008	1.01	0.76	**1.15**	**<0.001**	**1.14**	**0.046**
14–3–3 protein eta (Q04917)	1.06	0.009	1.06	0.069	**1.07**	**0.02**	1.05	0.29
Gamma-enolase (P09104)	0.88	0.01	**0.87**	**0.025**	0.93	0.2	**0.72**	**0.006**
Tropomyosin 1 (P09493–1)	1.24	0.014	1.12	0.46	**1.32**	**0.007**	**1.37**	**0.015**
Fibrinogen beta chain (P02675)	1.16	0.015	1.12	0.12	1.15	0.07	1.35	0.08
Leukocyte elastase inhibitor (P30740)	0.91	0.016	0.95	0.4	0.92	0.11	**0.83**	**0.04**
Integrin alpha-6 (P23229)	0.91	0.018	0.95	0.4	**0.87**	**0.016**	0.85	0.076
Protein disulfide isomerase (P07237)	1.07	0.024	1	0.87	**1.13**	**<0.001**	0.89	0.063
Glucose-regulated protein 78 (P11021)	1.1	0.025	1.02	0.85	**0.83**	**<0.001**	0.88	0.054
Tubulin beta-1 chain (Q9H4B7)	1.19	0.027	1.03	0.98	**1.29**	**<0.001**	**0.65**	**0.034**
Fibrinogen	1.24	0.037	1.05	0.59	**1.34**	**0.022**	1.53	0.065
Histamine-releasing factor (P13693)	1.06	0.041	1.03	0.33	1.07	0.069	1.14	0.09
Peroxiredoxin 6 (P30041)	0.96	0.046	0.97	0.31	0.96	0.14	**0.87**	**0.01**

Conclusion: This exploratory approach is the first attempt to show alterations in the platelet proteome of patients with cancer versus healthy controls. Furthermore a quite different picture of differentially expressed proteins in various tumour entities was observed. Further investigation with ELISA and Western Blot will be necessary to validate these findings.
Disclosure: No significant relationships.

OC 8: Venous thromboembolism

OC 8.1 Coagulation factors 11 and 12 and the risk of recurrent venous thromboembolism: a prospective cohort study

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Objectives: Factors 11 (F11) and 12 (F12) play a major role in the activation of the contact system. F11 is associated with first venous thromboembolism (VTE), recurrent VTE in women and ischemic stroke. High F12 confers an increased risk of ischemic stroke in young women. F11 and F12 are considered promising targets for future anticoagulant strategies. We aimed to assess the relationship between F11 and F12 and the risk of VTE in high risk patients.
Methods: We followed patients with a first, unprovoked VTE after anticoagulation withdrawal for an average of 6 years. We excluded patients with major thrombophilia, cancer or requirement for indefinite anticoagulation for other reasons. Study endpoint was symptomatic recurrent VTE.
Results: We included 815 patients (66% men, mean age 53 years). 265 patients (32%) had recurrence. After 10 years the cumulative incidence of recurrence was 33% (95% CI:26–40) in patients with F11 levels below the 33rd percentile and 41% (95% CI:36–46), in those with higher levels, p = 0,03 (▶Figure 1). The hazard ratio (HR), adjusted for age and sex, was 0.74 (95% CI 0.60–0.97). The cumulative incidence of recurrence was similar when patients were compared according to F12 terziles. No risk reductions in patients with F12 levels below the 33rd percentile compared to those with higher levels (adjusted HR – 1.0, 95 CI 0.8–1.3) was found.
Conclusion: Low levels of F11 are associated with a lower risk of recurrence, whereas F12 levels are not related to the risk of recurrent VTE.
Disclosure: No significant relationships.

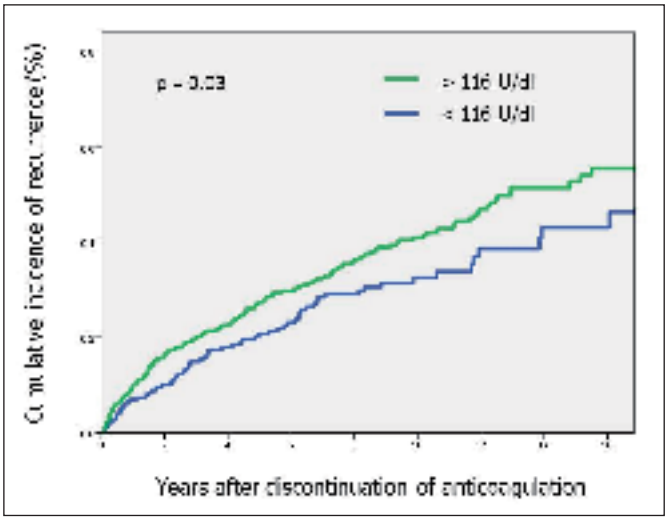

Fig. 1 Cumulative incidence of recurrent VTE according to 33rd perzentile of F11

OC 8.2 A genome-wide association study implicates ADAMTS13 as a universal susceptibility locus for thrombotic diseases

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Objectives: Deep Venous Thrombosis (DVT) is a common multifactorial disease that is influenced by environmental and genetic factors. Previous genome-wide association studies (GWAS) identified several thrombophilic mutations in coagulation factors as well as genetic variants in fibrinogen alpha and gamma chains (FGA/FGG) and the ABO locus. Further susceptibility loci i.e. ZFPM2, TSPAN15, SLC44A2, GP6, KNG1, STXBP5, HIVEP1 and NME7 have been suggested. However, a large proportion of missing heritability remains to be accounted for.
Methods: Here we present a GWAS on adult DVT comprising 962 cases and 902 healthy controls of Caucasian descent. DNA isolated from blood samples was hybridized to Illumina Infinium Global Screening Arrays MD24 v1.0. Logistic regression assuming an additive model adjusted for sex, age and the first 5 principal components was used for association analysis. Genomic inflation factor was estimated as 1.0499 indicating no relevant population stratification.
Results: The previously described loci FV^{Leiden} ($p=9.35e^{-29}$), ABO ($p=6.14e^{-16}$), FGA/FGG ($p=3.48e^{-09}$) and NME7 ($p=3.66e^{-19}$) were confirmed in our study. Additionally, rs4962153 located in the von Willebrand cleavage protease ADAMTS13 on chromosome 9 ($p= 6.36e^{-08}$) and rs17474001 located in the neighboring gene serine/threonine kinase like domain containing 1 (STKLD1, $p=1.78e^{-08}$) were associated with genome-wide significance.
Conclusion: ADAMTS13 is an established risk factor for ischemic stroke and arterial thrombosis. While recent studies failed to provide evidence for an association of ADAMTS13 variants and DVT at genome-wide significant levels, we here present the first GWAS showing such an association stressing the universal impact of ADAMTS13 on thrombotic diseases.
Disclosure: No significant relationships.

OC 8.3 Role of the prothrombin 19911 A>G polymorphism in adult patients with VTE: a German case-control study

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Objectives: The A>G polymorphism at position 19911 of the prothrombin gene (rs3136516) is associated with increased plasma prothrombin levels but its role as a risk factor for venous thromboembolism (VTE) is still unclear. The presence of the prothrombin 19911 A>G polymorphism in adult Caucasian VTE patients compared to healthy blood donors was evaluated.
Methods: In the present case-control study 1012 patients aged 18 to 60 years with > 70% of provoked VTE derived from the north-eastern part from Germany were investigated for the presence of prothrombin 19911 A>G (rs3136516) polymorphism and compared with 902 healthy individuals from the same catchment area. Odds ratios and 95% confidence intervals (OR/CI) were calculated for the *i)* homozygous GG genotype (minor allele), *ii)* the heterozygous AG variant and *iii)* i.e. the combination of GG&AG genotypes compared to the AA wildtype. The latter additive model was adjusted for age and sex.

Results: Compared to the AA wildtype the GG genotype at position 19911 modestly increase the risk of VTE with an OR of 1,32 (CI:1,02–1,7; p=0.03) and 1,2 (CI: 0,96–1,5; p=0,09) for carriers of the AG variant. The additive model showed an OR of 1,24 (CI: 1,007–1,5; p=0,042). Following adjustment for age and gender the OR was still significant: 1,16 (p=0,027). Of note: in the present case-control study the OR of the prothrombin G20210A variant (rs51799963) was 2,52 (p=0001).
Conclusion: In univariate analysis the homozygous prothrombin 19911 A>G polymorphism was significantly associated with a 1.32-fold increased risk of VTE in the cohort investigated.
Disclosure: No significant relationships.

OC 8.4 Splanchnic Vein Thrombosis Treatment with Rivaroxaban – A Case Series from the Prospective Dresden NOAC Registry (NCT01588119)

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Objectives: Although not specifically approved, rivaroxaban may offer advantages in the treatment of splanchnic vein thrombosis (SVT), but data for this approach are scarce.
Methods: Using data from the prospective, non-interventional *Dresden NOAC Registry* we evaluated effectiveness and safety of rivaroxaban in acute or chronic SVT treatment. All patients in the registry undergo long-term follow-up by quarterly phone calls and suspected outcome events are centrally adjudicated.
Results: Until September 20th 2017, 26 registry patients received rivaroxaban for SVT treatment: isolated portal SVT in 14 cases (53.8%), splenic SVT in 4 (15.4%) and multiple SVT locations in 8 (30.8%) cases. SVTs were unprovoked in 38.5% of cases and provoked by intra-abdominal infections in 34.6%, by advanced stages of liver cirrhosis in 23.1% and by cancer in 15.4% of cases, respectively. Median time between SVT diagnosis and rivaroxaban initiation was 28 days (25th/75th percentile 5.5–82 days). Mean exposure time of rivaroxaban was 14 months (range 1–46 months), during which no thromboembolic event occurred. A total of 21 bleeding complications occurred in 11 patients during treatment with rivaroxaban, which were ISTH minor bleeding in 9 cases, clinically relevant non-major bleeding in 7 and ISTH major bleeding in 5 cases (3 x gastrointestinal, 1 x hemorrhoid; 1 x traumatic intracranial bleeding) (▶ Table 1).
Conclusion: Our cohort of 26 consecutive patients currently represents the largest case series of rivaroxaban treatment for SVT and demonstrated high effectiveness to prevent thromboembolic complications but bleeding complications are common, which is known for SVT patients.
Disclosure: J.B.-W.: honoraria and research support from Bayer HealthCare, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer and Daiichi Sankyo. S.P. and S.M.: honoraria from Bayer HealthCare. L.T.: nothing to declare

Tab. 1 Individual patients (ranked by time between diagnosis and start rivaroxaban), outcome events and SVT thrombus burden during follow-up (pt. order according to time between SVT diagnosis and start rivaroxaban; pts. above the bold line initiated rivaroxaban within 30d of SVT diagnosis)

#	age (years), gender	Time between diagnosis and start rivaroxaban (days); initial therapy	follow-up duration (months)	exposure time (months)	VTE (incl. recurrent SVT)	major bleed	CRNM bleed	cause of death; anticoagulation at event	SVT thrombus burden during follow-up
1	43, male	0	3.1	3.1, ongoing	0	0	0	0	resolution
2	51, female	0	27.8	19.4	0	0	Skin bleeding, day 569, during rivaroxaban 10	0	resolution
3	65, male	0	49	27.2	0	0	0	0	resolution
4	66, female	0	18.7	18.7, ongoing	0	0	0	0	resolution
5	28, male	1; LMWH	1	1, ongoing	0	0	0	0	improved
6	56, male	4; LMWH	9.2	8.9	0	0	0	0	resolution
7	70, male	5; LMWH	6.1	6.1, ongoing	0	Fatal traumatic ICH day 172; during rivaroxaban 20	Macrohematuria, day 9; during rivaroxaban 20	Fatal traumatic ICH	not available
8	68, male	7; LMWH	24.8	24.8, ongoing	0	0	0	0	resolution
9	75, male	14; LMWH	9.5	6.9, switch to LMWH	0	GI bleed, day 190, during rivaroxaban 15	hematemesis, day 202; during rivaroxaban 15	terminal malignant disease	unchanged
10	72, male	16; LMWH	18.2	18.2, ongoing	0	0	0	0	not available
11	45, female	20; LMWH	33.6	5.7	0	0	0	0	resolution
12	48, female	26; LMWH	45.8	45.8, ongoing	0	0	Hypermenorrhoea, day 750; during rivaroxaban 20	0	resolution

Tab. 1 continued.

#	age (years), gender	Time between diagnosis and start rivaroxaban (days); initial therapy	follow-up duration (months)	exposure time (months)	VTE (incl. recurrent SVT)	major bleed	CRNM bleed	cause of death; anticoagulation at event	SVT thrombus burden during follow-up
13	81, female	26; LMWH	9.1	9.1, ongoing	0	0	0	0	not available
14	48, male	30; LMWH	21.4	6.8	0	0	0	0	resolution
15	52, female	31; LMWH	30.9	30.9, ongoing	0	0	Hemoptysis, day156; during rivaroxaban 20	0	not available
16	49, female	32; LMWH	32.1	32.1, ongoing	0	0	0	0	resolution
17	81, male	33; LMWH	21.5	21.5, ongoing	0	0	0	0	not available
18	42, female	48; LMWH	12.7	1.8	0	0	0	0	unchanged
19	54, male	52; LMWH	40	4.2, switch to LMWH	0	GI bleed, day 126, during rivaroxaban 20	recurrent GI bleed, day 615, during apixaban 2.5 BID	0	unchanged
20	79, male	92; LMWH	16.5	3	0	Fatal traumatic ICH, day 495, during VKA	0	Fatal traumatic ICH during VKA	not available
21	66, male	157; LMWH + VKA	3.3	3.3, ongoing	0	0	0	0	not available
22	45, male	170; LMWH	39.7	9.7	0	0	0	0	unchanged
23	48, female	214; LMWH	58.5	2.1, switch to LMWH	0	GIB day 65, during rivaroxaban 10,	recurrent GI bleeding events after day 65 during LMWH and after stopping any anticoagulation	terminal liver disease/ organ failure; no anticoagulation	unchanged
24	61, male	263; LMWH + VKA	31	10.3, switch to other OAC	0	GI bleed on day 811, during apixaban 5 BID	0	0	improved
25	73, male	328; LMWH	17.2	17.2, ongoing	0	0	rectal bleeding, day 141, during rivaroxaban 10	liver failure in terminal liver cirrhosis	unchanged
26	45, male	1070; LMWH	30.6	18.5, switch to other OAC	0	Hemorrhoid bleed, day 435, during rivaroxaban 20	Hemorrhoid bleed, day 305, during rivaroxaban 20	0	unchanged

OC 8.5 Risk of venous thromboembolism during rehabilitation of patients with spinal cord injury

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Objectives: Patients with spinal cord injury (SCI) are at risk of thrombosis and bleeding. Data on the risks during rehabilitation are inconsistent, and thromboprophylactic strategies are heterogeneous. We aimed to evaluate the thrombotic risk and bleeding events of SCI patients during rehabilitation.
Methods: We retrospectively collected hospital record data of 263 consecutive SCI patients admitted at a rehabilitation center between 2007–2017. Patients with an acute venous thromboembolism (VTE) at the primary center, without acute trauma or lower extremity paresis, with late admission after the acute trauma, <1 month rehabilitation, or reasons for long-term therapeutic anticoagulation, were excluded. All patients received pharmacologic thromboprophylaxis throughout rehabilitation. Primary endpoint was objectively diagnosed deep vein thrombosis or pulmonary embolism; secondary endpoint was bleeding.
Results: Of 185 patients, 162 (88%) were men and the mean age was 47.8 years. 94 patients were tetraplegic and 91 paraplegic. During a mean (+SD) rehabilitation time of 5.1+2.1 months, 8 patients had VTE. The probability of VTE after 6 months was 4.7% (95% CI 1.4–7.9%). Only high D-Dimer upon admission was significantly associated with risk of VTE (adjusted HR 2.3, 95% CI 1.4–4.1). Of 24 bleedings, 14 (64%) occurred at the heparin injection site. Six patients had bleedings at other sites and two had major bleedings.
Conclusion: Patients with SCI are at risk of VTE during rehabilitation despite heparin thromboprophylaxis, but also have an increased bleeding risk. Direct oral anticoagulants, which have a favourable risk-benefit profile and are convenient, should be explored for thromboprophylaxis in these patients.
Disclosure: No significant relationships.

OC 9: Inherited bleeding disorders: basic

OC 9.1 Effects of von Willebrand Factor Fragments on Pharmacokinetics of Subcutaneously Administered Simoctocog Alfa in a Minipig Model

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Objectives: Factor VIII (FVIII) bioavailability is a major limitation to the use of subcutaneous (SC) administration for hemophilia A therapy. We evaluated the effects of two von Willebrand factor (VWF) fragments on bioavailability of SC administered FVIII in a minipig model.
Methods: OCTA12 and OCTA13 are VWF fragments containing the D'D3 and D'D3-A1-A2-A3 domains respectively. After biochemical characterization of the fragments, Aachener minipigs were administered with recombinant human FVIII (rhFVIII; simoctocog alfa) SC either alone or in combination with OCTA12 or OCTA13.
Results: FVIII binding affinity (K_D) of the OCTA12 and OCTA13 fragments was 0.93 nM and 1.81 nM respectively. No inhibition of full-length VWF activity was observed. After administration in the minipig, FVIII peak concentrations were 2 IU/ml (rhFVIII, intravenous [IV]), 0.02 IU/ml (rhFVIII, SC) and 0.23 IU/ml (rhFVIII/OCTA12, SC). Peak levels of VWF fragment were 1.17 nM and 0.8 nM for OCTA12 and OCTA13, respectively. FVIII bioavailability increased with the VWF fragments from 2% to 41% (rhFVIII/OCTA12) and 32.9% (rhFVIII/OCTA13). rhFVIII/OCTA12 increased the time to 1% FVIII trough level 2.3-fold, compared with IV rhFVIII. At 120 h post administration, antigen levels were 0.14 nM (OCTA13) and 0.83 nM (OCTA12), indicating a long half-life and/or slow release into the circulation.
Conclusion: The two novel VWF fragments OCTA12 and OCTA13 significantly increased the bioavailability of FVIII after SC administration. The slow release and/or prolonged circulatory half-life of OCTA12 contributed to significantly prolonged FVIII antigen levels in the circulation. OCTA12 is a promising FVIII chaperone for SC administration in hemophilia A.
Disclosure: C.K. and B.S. are employees of Octapharma Biopharmaceuticals GmbH, Berlin, Germany. S.W. is an employee of Octapharma Pharmazeutika Produktionsges.m.b.H., Vienna, Austria.

OC 9.2 Recombinant human von Willebrand factor has a unique pattern of ultra large multimers: results from physicochemical, biochemical and in vivo studies

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Objectives: Ultra-large multimers (ULM) of VWF are the most active and therefore are of critical importance for the function of VWF in stabilizing the primary hemostatic plug. In contrast to plasma-derived FVIII-VWF, human rVWF obtained from mammalian cell culture retains the full-spectrum of intact multimers, including ULM.
Methods: Tapping mode atomic force microscopy (AFM) was used for imaging the structure of rVWF and rVWF-rFVIII molecular interactions and for visualization of cleavage by ADAMTS13. Contribution of multimer size was investigated by fractions containing distinct portions of VWF multimers that were generated from rVWF and analyzed for their ability to mediate platelet adhesion under shear stress. Pharmacokinetics and efficacy of rVWF versus a pdVWF-FVIII preparation was evaluated in VWF knock-out mice.
Results: In AFM, large multimeric filaments were more prominent in the images of rVWF than in pdVWF. Spiking blood with rVWF increased platelet adhesion to collagen type I. Use of different rVWF fractions to promote platelet adhesion showed that multimer size of rVWF contributed to platelet adhesion. Terminal half-life of rVWF was longer than that of pdVWF. rVWF stabilized endogenous FVIII in VWD mice as seen by a secondary rise in murine FVIII which lasted longer in mice treated with rVWF than with pdVWF. Only rVWF in combination with rFVIII was able to reduce blood loss upon tail cutting.
Conclusion: rVWF exhibited superior structure and function compared to pdVWF. Most of these effects correlated with the multimer size and therefore could be attributed to the presence of ULM in rVWF preparations.
Disclosure: Employee of Shire

OC 9.3 Conventional but also alternative secretory pathways regulate the secretion of F8.

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Objectives: Few proteins are known to be involved in the conventional secretion of F8. Calnexin and calreticulin (CANX/CALR) control the correct folding of F8, while LMAN1 and MCFD2 (ERGIC) transport the protein in COPII-coated vesicles to the Golgi. Recently, we identified GABARAP as potential F8 interacting partner in the Golgi. The later belongs to the Atg8-like molecules that play a key function in the autophagy process. This suggested that also alternative pathways could play a role in fine-tuning/modulating the secretion of F8. In this study we generated single (SKO) and double endogenous knockouts (DKO) targeting key proteins involved in the conventional and putative alternative secretory pathway of F8.
Methods: Endogenous KOs were generated using CRISPR/Cas9 in a stable F8 secreting HEK clone. F8 activity was measured and compared to mock.
Results: *Conventional Secretory Pathway:* Our results show the expected decrease in F8 secretion for SKO and DKO of LMAN1/MCFD2 (SKO 70% and 50%, DKO 50% decrease). The SKOs of CANX/CALR show effects going in opposite directions (CANX 90% increase, CALR 40% decrease, DKO 40% decrease). *Potential Alternative Secretory Pathway:* SKOs of GABARAP-L1 and ATG7 both increase F8 activity (100% and 60%), while GABARAP decrease F8 activity (40%). DKO of GABARAP&CALR (putative interaction partners) decrease F8 activity (40%).
Conclusion: Nearly all protein KOs involved in degradative functions increase F8 activity, while protein KOs involved in trafficking functions decrease F8 activity. GABARAP and Calreticulin show similar effect of decrease in F8 activity, suggesting that both proteins might work in the same "trafficking" pathway.
Disclosure: No significant relationships.

OC 9.4 Selective elimination of FVIII-specific B cells by immunotoxins

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Objectives: The development of inhibitory antibodies is the most serious complication for patients with hemophilia A undergoing factor VIII (FVIII) replacement therapy. Eradication of inhibitors is only achieved in about 70% of the patients undergoing immune tolerance induction. We explored the potential use of immunotoxins comprised of a single FVIII domain fused to the Exotoxin A (ETA) from *Pseudomonas aeruginosa* for elimination of FVIII-specific B cells.
Methods: For proof of concept studies we focused on the immunodominant C2 domain and bacterially produced a C2 domain-ETA fusion protein (hC2-ETA). To test elimination capacity, FVIII knockout mice were immunized with FVIII and i) splenocytes were incubated *ex vivo* with hC2-ETA and controls or ii) mice received injections of hC2-ETA and controls for *in vivo* analysis. Elimination of memory B cells was analyzed by re-stimulation of antibody secreting cells (ASCs) in an enzyme linked immunospot (ELISPOT) assay.
Results: Differentiation of C2-specific ASCs was completely blocked in a dose-dependent manner by *ex vivo* incubation with hC2-ETA suggesting the complete elimination of C2-specific memory B cells. Further, application of hC2-ETA *in vivo* resulted in complete elimination of C2-specific B cells in three out of six mice. Limited elimination capacity in vivo was most likely due to present C2-specific antibodies, which partially prevented hC2-ETA from reaching the target cells.
Conclusion: Our results show that hC2-ETA selectively and efficiently eliminated C2-specific B cells. The application of FVIII domain immunotoxins is therefore a potential new strategy for the elimination of FVIII specific B cells in inhibitor patients.
Disclosure: This project was funded by the Excellence Cluster for Individualised Immune Intervention (Ci3) supported by the German Ministry for Education and Research (BMBF) and Bayer Vital GmbH

OC 9.5 Defective Zn2+ homeostasis of human and mouse platelets in storage pool disorders

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Objectives: Zinc (Zn^{2+}) can modulate intra- and extracellular signalling pathways of platelets and act as an important cofactor in metabolic pathways. However, the localization of platelet Zn^{2+} store and the molecular mechanism of Zn^{2+} influx/efflux are unknown.
Methods: To detect the Zn^{2+} store in human and mouse platelets, accumulation of the Zn^{2+} specific fluorescent dye FluoZin-3 was visualized in patients of storage pool disorders (SPD) and Nbeal2 and Unc13d knockout mice.
Results: Transient accumulation of the dye was observed into distinct cytosolic puncta in resting state, which was lost in activated platelets upon spreading. Flow cytometry indicated that approximately half of the labelled Zn^{2+} was released upon platelet activation. Granule release defective SPD platelets isolated from *Unc13d*$^{-/-}$ mice showed slightly reduced basal Zn^{2+} levels in the cytoplasm and strongly impaired Zn^{2+} efflux upon platelet activation. In contrast, alpha-granule absent SPD platelets from *Nbeal2*$^{-/-}$ platelets showed strongly impaired Zn^{2+} homeostasis in both resting and activated conditions. In flow based assays, platelet-dependent fibrin formation was impaired in *Unc13d*$^{-/-}$ blood samples, which was restored with extracellular Zn^{2+}. Moreover, Zn^{2+} homeostatic defects of SPD mice were compaired to human patients with SPD, Hermansky Pudlack and Gray platelet syndromes.
Conclusion: We conclude that the ionic form of functional Zn^{2+} is mainly stored in platelet alpha-granules in human and mice. In addition, abnormal granule biogenesis or release dysregulates platelet Zn^{2+} homeostasis and platelet Zn^{2+} dependent fibrin clotting.
Disclosure: No significant relationships.

OC 10: Extracellular vesicles

OC 10.1 Mitochondria of parental cells control the capacity of monocyte-derived microvesicles to activate endothelial cells.

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Objectives: Microvesicles (MVs) are recognized as mediators of inflammation and increased numbers of MVs have been described in cardiovascular diseases. Mitochondrial content has been shown to be present in MVs and to promote pro-inflammatory responses. However, the role of mitochondria in the MV-mediated pro-inflammatory communication between monocytes and endothelial cells is unknown. The aim was to elucidate the contribution of mitochondria to the biological activity of monocyte-derived MVs on endothelial cells.
Methods: MVs were isolated from conditioned media of THP-1 monocytes by differential centrifugation, characterized by flow-cytometry and their ability to activate human umbilical vein endothelial cells (HUVECs) was tested.
Results: LPS-stimulation of monocytes induced the release of MVs enriched in mitochondrial content as demonstrated by flow cytometry and quantitative PCR. Stimulation of endothelial cells with these MVs potently induced the expression of VCAM-1 and ICAM mRNA and IL-8 protein in endothelial cells. Disintegration of MVs by sonication did not alter this activity, indicating that MV integrity is not required to mediate these effects. However, the pro-inflammatory potential of MVs was drastically reduced when MVs were derived from monocytes with non-respiring mitochondria or monocytes cultured in the presence of pyruvate. MVs released by cells treated with the mitochondrial ROS scavenger MitoTEMPO had a reduced pro-inflammatory potential. Moreover, even mitochondria alone isolated from LPS-stimulated, but not from unstimulated cells, were able to activate endothelial cells.
Conclusion: Mitochondrial activity of parental cells defines the content and ability of MVs released by LPS-stimulated monocytes to induce pro-inflammatory responses in endothelial cells.
Disclosure: No significant relationships.

OC 10.2 Inflammatory functions of ribosomal RNA-containing microvesicles from mast cells

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Objectives: Mast cells (MC) are well known for their role in inflammatory responses, allergic and anaphylactic reactions. Upon stimulation, MC release mediators including pre-stored histamine as well as lipid mediators, cytokines and chemokines. Although ribosomes and cytosolic RNAs are located around and attached to secretory granules in mature MC, their role in MC responses and degranulation are still unknown. Extracellular ribosomal RNA as such was shown to promote inflammatory and procoagulant activities *in vitro* and *in vivo*. The release of RNA during the degranulation of MC and its role in inflammatory responses on endothelial cells was investigated.
Methods: Degranulation of MC was followed by the release of histamine and b-hexosaminidase activity. Microvesicles were characterized by Annexin staining and FACS analysis.
Results: Stimulation of MC by various degranulating agonists resulted in the release of RNA (predominantly ribosomal RNA), which was associated with the microvesicle fraction. The liberation of extracellular RNA from MC was abolished by MC stabilizers such as cromolyn and by preventing the increase of intracellular Ca^{2+} level. rRNA was found mainly inside microvesicles (about 500 nm diameter) as demonstrated by electron-microscopy and immunocytochemistry. Following uptake of MC-released microvesicles by human umbilical vein endothelial cells in a dose-dependent manner, the according release of von-Willebrand-factor as well as the expression of cytokines such as monocyte chemoattractant protein or interleukin-6 was observed.
Conclusion: Results indicate that MC-derived RNA-containing microvesicles play a role in amplifying the initial cytokine storm during the inflammatory response and may modulate the haemostatic properties of the endothelium.
Disclosure: No significant relationships.

OC 10.3 The regulation of miR-143 in collateral vessel growth

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Objectives: Collateral vessel growth (arteriogenesis) describes the formation of collateral arteries from preexisting small vessels at sites of occlusion and is induced by fluid shear stress (FSS) as well as infiltrating leukocytes and cytokines. Several microRNAs were shown to be highly expressed in arteriogenesis including miR143. Since knockdown of miR-143 inhibited the growth of collateral arteries, a decisive role for miR-143 in arteriogenesis was proposed. Here, the cellular mechanism of FSS-induced activation of miR-143 expression in collateral vessels and the influence of different regulatory factors, such as transforming growth factor β (TGF-β), were examined.
Methods: The mRNA and miRNA expression of FSS-treated endothelial cells (HUAEC) and TGF-β stimulated smooth muscle cells (MVSMC) were analyzed by qPCR; the release of TGF-β was detected by ELISA. Transfection with siRNA was used for target gene silencing.
Results: The expression of miR-143 was significantly increased in FSS-treated HUAEC, accompanied by a significantly higher mRNA expression and release of TGF-β protein. TGF-β, known to induce smooth muscle cell differentiation, increased the expression of myocardin, the transcriptional coactivator of serum response factor (SRF) to regulate miR-143 expression, in MVSMC. TGF-β further induced the expression of miR-143, but did not affect SRF expression. Accordingly, knockdown of myocardin significantly decreased TGF-β-induced miR-143 expression, while the expression of miR-143 was significantly decreased by knockdown of SRF in MVSMC.
Conclusion: These findings identify TGF-β, which is upregulated in endothelial cells by FSS, as a regulatory factor for miR-143 in collateral vessel growth by activating the expression of myocardin.
Disclosure: No significant relationships.

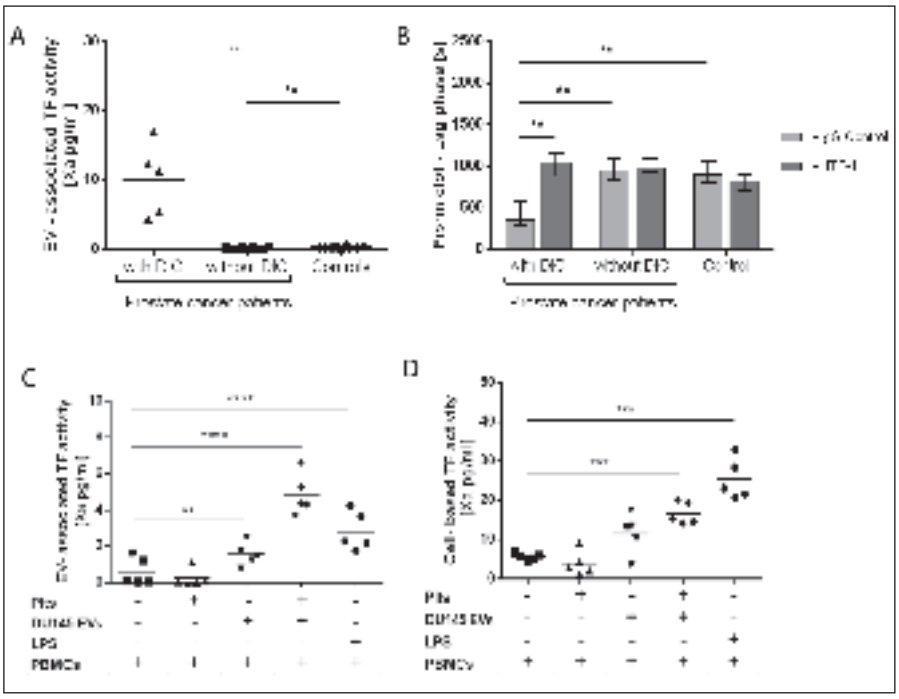

Fig. 1

OC 10.4 Extracellular vesicle-associated tissue factor activity is increased in prostate cancer patients with disseminated intravascular coagulation and induced by cellular interactions in vitro

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Objectives: Tissue factor (TF) might play an important role in prostate cancer-related disseminated intravascular coagulation (DIC). Elevated plasma EV-associated TF activity might be present in prostate cancer patients with DIC and could be induced by the interplay between prostate cancer cells, immune cells and platelets. Therefore, we investigated EV-associated TF activity in prostate cancer patients with DIC and in co-culture experiments.
Methods: Five prostate cancer patients with DIC, 10 matched prostate cancer controls without DIC and 10 matched healthy individuals were included. *In vitro* we co-cultured EVs derived from the TF positive DU145 or the TF negative LNCaP prostate cancer cell line with peripheral blood mononuclear cells (PBMCs), polymorph nuclear leukocytes (PMNs), and platelets. TF activity and TF expression were determined.
Results: EV-associated TF activity was highly increased in plasma from prostate cancer patients with DIC. Only EVs from DIC patients reduced the fibrin clot formation time of normal pooled plasma in a TF dependent manner (▶Figure 1A+1B). *In vitro* EVs derived from the DU145 prostate cancer cell line (but not LNCaP) increased EV-associated TF activity and cell based TF activity on PBMCs in the presence of platelets (▶Figure 1C+1D). No effect on PMNs was seen. Applying flow cytometry in this co-culture approach we found increased TF expression on monocytes but no change on granulocytes or lymphocytes
Conclusion: This study indicates that elevated plasma EV-associated TF activity leads to coagulation activation in prostate cancer patients with overt DIC and results from interactions between prostate cancer cells, monocytes and platelets.
Disclosure: No significant relationships.

OC 10.5 Effects of Chemotherapy on Extracellular Vesicles and Coagulation Activation in Advanced Colorectal Cancer Patients

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Objectives: Extracellular vesicles (EV) are elevated in colorectal cancer (CRC) patients and affect coagulation activation by exposing tissue factor (TF) and phospholipids (Hron, Thromb Haemost 2007). We aimed to evaluate the long-term effects of chemotherapy on number and origin of EV and coagulation activation.
Methods: Advanced CRC patients receiving 5-fluorouracil based chemotherapy were eligible. The number of EV was assessed by flow cytometry in fresh platelet poor plasma obtained from venous blood collected immediately before chemotherapy. EV were defined by size (forward scatter, <1 µm) and annexin V binding and labeled using antibodies to categorize EV origin. D-Dimer was assessed by ELISA. The paired t-test was used to compare baseline levels with follow up levels. Data are given in absolute numbers (median [quartiles]).
Results: 46 patients (mean age 64 years, 74% men) were enrolled. ▶Table 1 shows the number of EV, EV sub-species and levels of D-dimer at baseline and before 2nd, 3rd, 4th and 5th chemotherapy, respectively. EV significantly decreased from 386 (268; 578)x10^3 mL^{-1} at baseline to 239 (172; 450)x10^3 mL^{-1} before the 5th cycle. PLT^+EV significantly decreased from 176 (126; 287)x10^3 mL^{-1} at baseline to 94 (60; 205)x10^3 mL^{-1} before the 5th cycle. The proportion of PLT^+EV was about 40% throughout chemotherapy. Number and proportion of TF^+EV were small at all time points. D-dimer levels were 1.01 (0.49; 2.31) µg mL^{-1} at baseline and remained stable.

Tab. 1 Number of extracellular vesicles (EV) and levels of D-dimer in venous blood of colorectal cancer patients before and during chemotherapy.

	Before 1st ChTx	Before 2nd ChTx	*p*-value 1st vs 2nd	Before 3rd ChTx	*p*-value 1st vs 3rd	Before 4th ChTx	*p*-value 1st vs 4th	Before 5th ChTx	*p*-value 1st vs 5th
EV ($x\ 10^3\ mL^{-1}$)	386 (268; 578)	331 (230; 515)	0.13	273 (213; 474)	0.13	318 (201; 491)	0.25	239 (172; 450)	0.013
PLT^+EV ($x\ 10^3\ mL^{-1}$)	176 (126; 287)	148 (97; 254)	0.026	117 (75; 180)	<0.001	133 (92; 222)	0.014	94 (60; 205)	0.005
Proportion (%) of EV	46	45		43		42		39	
TF^+EV ($x\ 10^3\ mL^{-1}$)	16 (10; 37)	15 (9; 31)	0.69	12 (8; 26)	0.33	12 (8; 27)	0.001	11 (8; 15)	0.009
Proportio (%) of EV	6	7		6		7		5	
D-Dimer ($\mu g\ mL^{-1}$)	1.01 (0.49; 2.31)	0.95 (0.55; 2.8)	0.61	0.95 (0.52; 1.63)	0.75	0.78 (0.49; 2.21)	0.39	0.71 (0.45; 1.81)	0.82

Conclusion: In patients with advanced CRC, chemotherapy attenuates coagulation activation as indicated by a decline of the number of EV.
Disclosure: No significant relationships.

OC 11: Best abstracts

OC 11.1 A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Study of Caplacizumab in Patients with Acquired Thrombotic Thrombocytopenic Purpura

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Objectives: We assessed the efficacy and safety of caplacizumab in patients with acquired thrombotic thrombocytopenic purpura (aTTP).
Methods: Patients received placebo or caplacizumab daily during the PE period and for at least 30 days thereafter (▶Figure 1).

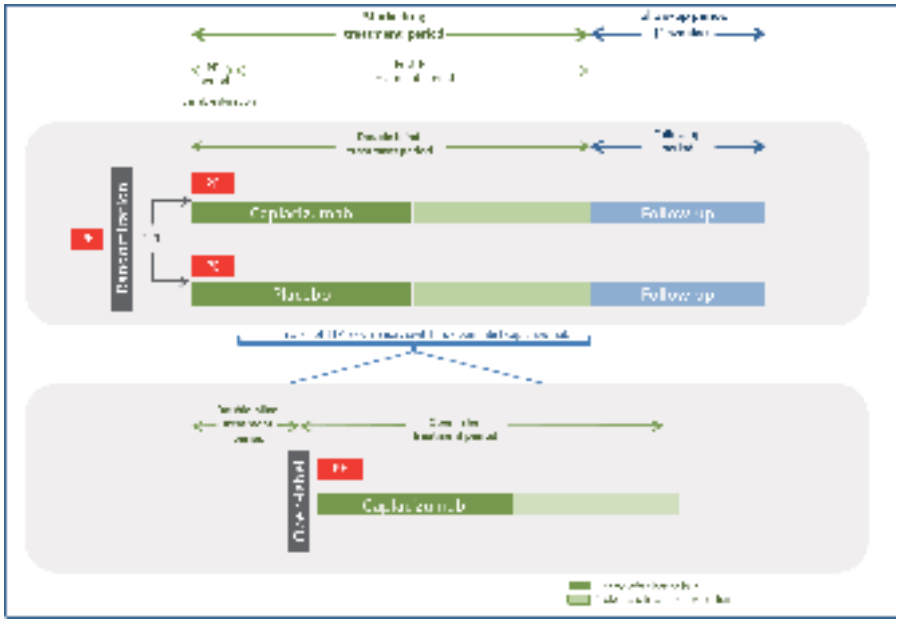

Fig. 1
Study design. Patients with an episode of aTTP who had received one PE treatment were randomized 1:1 to placebo or 10 mg caplacizumab, in addition to daily PE and corticosteroids. A single IV dose of study drug was given before the first on-study PE and a SC dose was given daily during the PE period and 30 days therafter. If at the end of this period there was evidence of ongoing disease, such as suppressed ADAMTS13 activity, investigators were encouraged to extend the blinded treatment for a maximum of 4 weeks together with optimization of immunosuppression. All patients entered 28-day treatment-free follow up period after the last dose of study drug. In case of a first recurrence during study drug treatment period patients were corssed-over to open-label caplacizumab. (clinicaltrieals.gov: NCT02553317)

Tab. 1 Safety summary

Number of patients (%) with	Placebo N = 73	Caplacizumab N = 71
At least one TEAE	71 (97.3)	69 (97.2)
At least one study drug-related TEAE	32 (43.8)	41 (57.7)
At least one TEAE leading to study drug discontinuation	9 (12.3)	5 (7.0)
At least one SAE	39 (53.4)	28 (39.4)
At least one study drug-related SAE	4 (5.5)	10 (14.1)
Death	3 (4.1)	1 (1.4)*

*Occurred during the follow-up period of the study and was assessed by the investigator as not related to study drug treatment

Results: 145 patients were randomized (73 placebo, 72 caplacizumab). Compared to placebo, caplacizumab-treated patients were >50% more likely to achieve a platelet count response (platelet count normalization rate 1.55, 95% CI 1.10 – 2.20, p<0.01). During the study drug treatment period, treatment with caplacizumab resulted in a 74% reduction in TTP-related death, recurrence of TTP, or a major thromboembolic event (p<0.0001). During the overall study period, patients administered caplacizumab had a 67% reduction in disease recurrence (p<0.001). No caplacizumab-treated patients were refractory to therapy versus 3 on placebo (p=0.057). Treatment with caplacizumab was associated with a trend toward faster normalization of organ damage markers. Safety is summarized in ▶Table 1. The most common caplacizumab-related TEAEs were epistaxis, gingival bleeding, and bruising. During the study drug treatment period, 3 patients on placebo died. One caplacizumab-treated patient died during the follow-up period (investigator assessed as not related to study drug).

Conclusion: Treatment with caplacizumab reduced the time to platelet count response and resulted in a clinically meaningful reduction in aTTP-related death, recurrence of aTTP, or a major thromboembolic event during study drug treatment, as well as recurrences during the overall study period. The safety profile was favorable, with mucocutaneous bleeding the most frequently reported AE. Caplacizumab represents a novel treatment option for patients with aTTP.

Disclosure: Ablynx employees have a conflict of interest as they have an employment relationship with Ablynx. All other authors have received consultancy fees from Ablynx (all members of the Steering Committee of this study).

OC 11.2 Analysis of acute phase serotonin mediated neutrophil trafficking

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Objectives: Platelet serotonin (5-HT) affects neutrophil-endothelial interactions in models of acute inflammation. Mice lacking tryptophan hydroxylase 1 (Tph1$^{-/-}$) show attenuated inflammatory neutrophil recruitment. We aimed to understand 5-HT mediated neutrophil-endothelial interactions in models of acute inflammation.

Methods: Expression of endothelial- and neutrophil derived adhesion molecules and leukocyte transmigration was analyzed after endotoxic shock and mesenteric I/R by flow cytometry, histology and intravital microscopy.

Results: Blood neutrophil kinetics after endotoxic shock were similar in WT and Tph1$^{-/-}$ mice (2,4 vs. 2,7 x10^5 cells/mL; n.s.) whereas peritoneal neutrophils were reduced by 40% in Tph1$^{-/-}$ mice (▶Fig. 1A). Flow cytometry revealed similar levels of ICAM, VCAM, E- and P-selectin expression on endothelial cells (EC). Neutrophil adhesion was reduced in Tph1$^{-/-}$ mice by 50% after 30 min of ische-

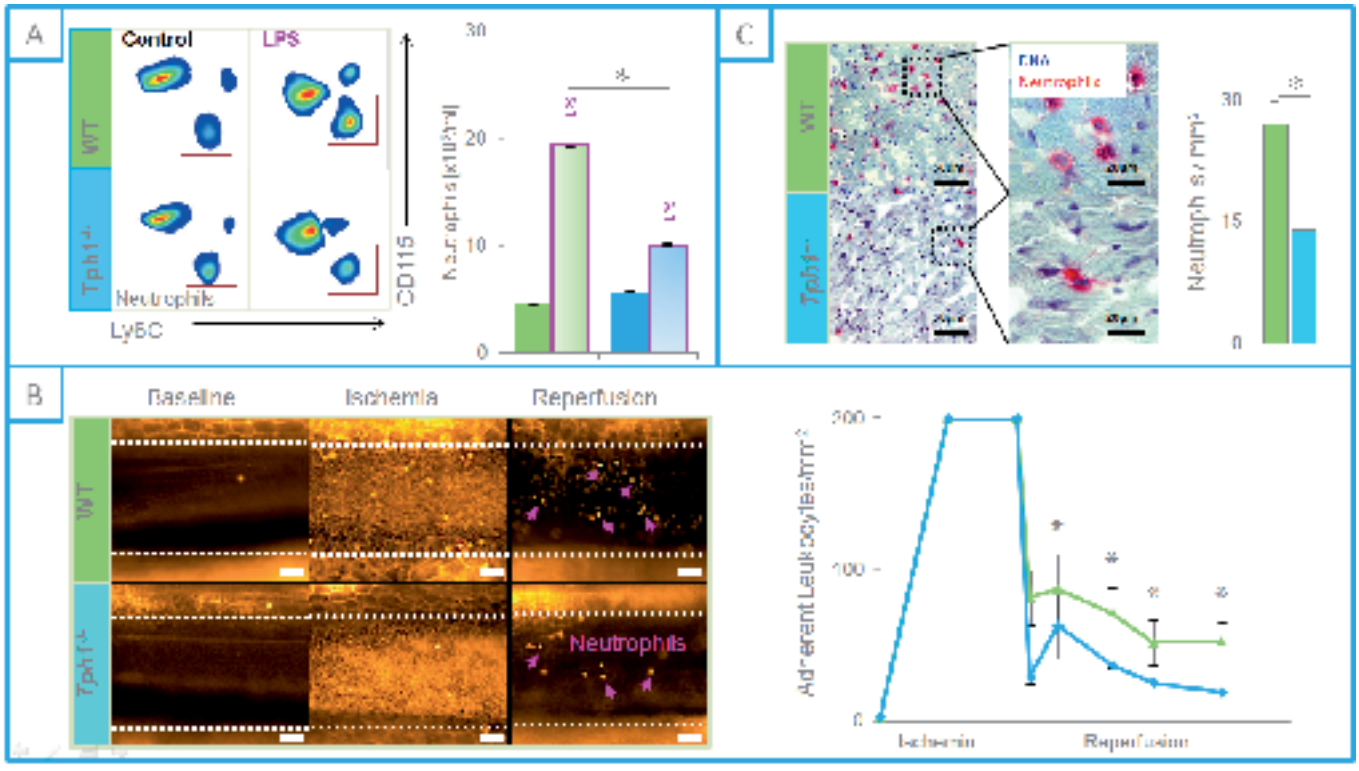

Fig. 1

mia (▶Fig. 1B). Tph1$^{-/-}$ mice showed less neutrophil infiltration into the infarcted tissue after myocardial I/R (14 vs. 28 cells/mm^2 tissue in WT; ▶Fig. 1C). Circulating neutrophils showed a 50% reduction of surface-CD11b in Tph1$^{-/-}$ mice, whereas other adhesion molecules were equally expressed in both genotypes. QPCR analysis revealed 5-HT receptor transcripts in isolated neutrophils. Stimulation of neutrophils with 5-HT results in enhanced CD11b levels on the cell surface, which could be inhibited by specific 5-HT receptor antagonism.
Conclusion: 5-HT amplifies neutrophil attachment and subsequent transmigration at sites of acute inflammation by upregulation of adhesion molecule CD11b on neutrophils. Our in vitro data suggests that targeting neutrophil 5-HT receptors might provide new possible treatments for thrombo-inflammatory diseases.
Disclosure: No significant relationships.

OC 11.3 Anti-idiotypic antibodies against ADAMTS13 autoantibodies are present in patients after acute episodes of immune-mediated Thrombotic Thrombocytopenic Purpura (iTTP)

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Objectives: A severe ADAMTS13 deficiency caused by ADAMTS13 autoantibodies is the hallmark of iTTP. The presence of ADAMTS13 immune complexes years after acute episodes point at an ongoing autoimmune process balanced by as yet unknown adaptive mechanisms. Failure to uphold these mechanisms might result in relapses, which are frequently seen in iTTP patients. We hypothesized that anti-idiotypic antibodies against ADAMTS13 autoantibodies might constitute such an adaptive mechanism.
Methods: Splenic mononuclear cells of two iTTP patients, splenectomized after a relapsing disease course, were used to generate IgG_1 Fab κ/λ libraries by phage display. Expressed phages were screened for binding to two separate pools of anti-ADAMTS13 Fabs (Schaller et al., Blood 2014; pooled according to their CDR3 motifs). ADAMTS13 autoantibody-specific anti-idiotypic phages were sequenced to determine their immunoglobulin variable heavy (IGV_H) and light (IGV_L) chain genes.
Results: A total of 15 sequenced anti-idiotypic Fabs, yielded 14 productive IGV_H chains, with IGHV3–23*01 shared by both patients (3/14 Fabs). Four of the 14 IGV_H were paired with a productive IGV_L chain (three κ, with IGKV1–39*01 shared by both patients, and one λ). Anti-idiotypic Fabs with paired productive IGV_H and IGV_L chains were detected only for the pool of ADAMTS13 autoantibodies of CDR3 motifs 1/2.
Conclusion: Our results indicate, that in both iTTP patients analyzed, the IgG_1 repertoire contains a distinct set of ADAMTS13 autoantibody-specific anti-idiotypic antibodies. Functional analysis of these anti-idiotypic Fabs as well as screening of patients' plasmas for the presence of ADAMTS13 autoantibody-specific anti-idiotypic antibodies is ongoing.
Disclosure: No significant relationships.

OC 11.4 Trauma-induced auto-heparinization: shedding of glycocalyx-derived heparan sulfate is not associated with endogenous anticoagulation

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Objectives: Heparan sulfate (HS) represents an integral component of the endothelial glycocalyx, a thin anticoagulant layer on the surface of the undisrupted vasculature. Hypoperfusion following major trauma results in endotheliopathy and the shedding of HS, which is suspected to promote the allosteric activation of plasmatic antithrombin. This endogenous mechanism of heparinization might represent a contributor to trauma-induced coagulopathy. We investigated whether the shedding of HS could exert heparin-like effects of anticoagulation.
Methods: ROTEM was performed on whole blood (WB) of trauma patients at admission, using a heparin-sensitive assay (INTEM /HEPTEM). To assess the course and magnitude of HS shedding following injury, rats were subjected to a well-characterized model of hemorrhage, trauma and resuscitation (HTR). INTEM /HEPTEM were performed at time points with peaking HS levels. Clinically relevant doses of HS were added to WB from healthy donors and INTEM was measured with heparin as a positive control.
Results: Severe injury was not associated with a prolongation of INTEM clotting time (CT) in trauma patients in the presence or absence of Heparinase (HEPTEM). In our rat model of HTR, shock and resuscitation resulted in the shedding of endothelial HS without a prolongation of INTEM CT or an effect of Heparinase. When compared with Heparin, clinically relevant concentrations of HS did not exert anticoagulant effects.
Conclusion: Severe injury results in the shedding of HS from the endothelium. Although structurally similar, shed HS does not appear to exert an anticoagulant effect, comparable to that of heparin. Our data do not substantiate the concept of clinical autoheparinization.
Disclosure: No significant relationships.

OC 12: Arterial thrombosis

OC 12.1 Evaluation of stroke risk in patients with end-stage renal disease and atrial fibrillation with the CHA2DS2-VASc score: The Vienna InVestiation of AtriaL fibrillation and thromboembolism in hemodialysis (VIVALDI)

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Objectives: Patients with atrial fibrillation (AF) have an increased risk of ischemic stroke and systemic embolism. In this investigation we questioned the performance of the CHA2DS2-VASc score for evaluation of the stroke risk in AF in the multi-morbid population of patients on hemodialysis (HD).
Methods: We identified patients with AF in a population-based prospective cohort study of patients on HD and followed them for the occurrence of a composite outcome comprising ischemic stroke, transient ischemic attack (TIA), and systemic embolism. We analyzed the risk of the composite outcome accounting for the competing risk of death and the time-dependent occurrence of new AF during follow-up.

Tab. 1 Distribution of risk factors for stroke in HD patients with AF included in the CHA2DS2-VASc score and further potential risk factors

Characteristic	Frequency in AF patients (N=206)
CHA2DS2-VASc score, median (25th to 75th percentile)	4 (3 – 5)
Congestive heart failure (%)	83 (40.3)
Hypertension (%)	189 (91.7)
Age	
65 – 74 years (%)	75 (36.4)
>75 years (%)	79 (38.3)
Diabetes (%)	88 (42.7)
History of stroke or TIA (%)	58 (28.2)
History of vascular disease (including coronary heart disease, peripheral artery disease) (%)	131 (63.6)
Female sex (%)	66 (32.0)
BMI, median (25th to 75th percentile)	25.6 (22.8 – 29.1)
Age, median (25th to 75th percentile)	72 (64 – 79)
Smokers (%)	97 (47.1)

Results: Of the total cohort of 625 patients, 165 patients had AF at baseline and 41 patients had new AF during follow-up of 515 days in median. ▶Table 1 describes the characteristics of the subset of patient with AF. Ten patients with AF had the composite outcome during follow-up. The association between risk of stroke and the CHA2DS2-VASc score was of borderline statistical significance (subdistribution hazard ratio [SHR] per 1 point increase 1.59, 95%CI 0.97–2.63, p=0.068). The CHA2DS2-VASc components age≥65 years (SHR 3.43, 95%CI 0.43–27.43, p=0.245), diabetes (SHR 3.26, 95%CI 0.87–12.24, p=0.080), history of stroke/TIA (SHR 2.60, 95%CI 0.76–8.95, p=0.130), and arterial vascular disease (SHR 5.15, 95%CI 0.64–40.84, p=0.121), were not individually associated with stroke.
Conclusion: The applicability of the CHA2DS2-VASc score may be limited for evaluating the risk of stroke in HD patients, because of the high frequency of comorbidities in HD patients.
Disclosure: No significant relationships.

OC 12.2 Platelet indices in heart failure, relation to different phenotypes and total mortality – results from the MyoVasc study

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Objectives: Platelet indices, mean platelet volume (MPV), a measure of platelet size, and platelet count are inexpensive and easy accessible laboratory parameters. Increased MPV has been correlated to increased risk of arterial and venous thrombosis and total and vascular mortality. This study aims to investigate the relation of both, platelet count and MPV, with different heart failure (HF) phenotypes and the link to total mortality.
Methods: In a highly standardized setting, platelet count and MPV have been investigated in 1979 subjects (HF, n=1818; Controls, n=161) enrolled in the MyoVasc study. Multivariable linear and cox regression analysis were used to investigate the associations between platelet indices and HF and total mortality, respectively.
Results: HF subjects showed positive association with MPV (beta estimate, β: 0.196 [0.097; 0.29], p=0.0001) and negative with platelet count (β:-6.83 [-13.4; –0.21], p=0.043). The left ventricular ejection fraction was strongly and negatively associated with MPV (p<0.0001) and positively with platelet count (p=0.00027) in an age and sex adjusted model. Increased MPV was related to HF with severe symptoms (NYHA class III+IV, β:0.215 [0.102; –0.33], p=0.0002), and no relevant relations were observed for platelet count. Cox-regression models demonstrated an increased risk of death in HF individuals with MPV > 85th percentile (HR=2.09, p=0.0050) or platelet count < 15th percentile (HR=2.19, p=0.0015), independent of age, sex, cardiovascular risk factors and cardiovascular diseases.
Conclusion: Lower platelet count and particularly increased MPV are linked with worse HF phenotype and to increased risk of death, independent of cardiovascular risk factors and cardiovascular comorbidities.
Disclosure: No significant relationships.

OC 12.3 Biodiesel Particulate Matter Negatively Affects the Atherosclerotic Plaque Phenotype in Mice

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Objectives: Long-term exposure to air pollutants increases cardiovascular morbidity and mortality. Particulate matter (PM) derived from diesel exhaust has been documented to be pro-atherogenic in animal studies. Biodiesels are widely introduced as new fuels with improved emission characteristics. However, biodiesel contains relatively more polycyclic aromatic carbohydrates (PAH) compared to diesel. Therefore, we hypothesize that exposure to biodiesel as compared to diesel exhaust results in increased atherosclerosis.
Methods: In a carotid cuff atherosclerosis model, the effects of exposure to exhaust PM of biodiesel vs. diesel PM vs. saline (control) on atherosclerosis were evaluated. Both common carotid arteries in LDLR$^{-/-}$ mice were cuffed at week 2 in the course of an 8-week high-fat diet regimen. All mice were intratracheally instilled with saline, PM biodiesel or biodiesel once-weekly for 5 times. Immunohistochemistry and primary human vascular smooth muscle cells (hVSMC) were used for evaluation.
Results: Exposure to both biodiesel and diesel didn't exacerbate atherosclerosis development, however, biodiesel affected plaque composition towards a more vulnerable phenotype as compared to diesel with decreased total collagen content and hVSMC (respectively –100%, p=0.09 and –339%, p<0.01 and tend to increase necrotic core volumes (37.5% vs 28.9 % p=0.2).

Exposure to biodiesel PM triggered loss of hVSMC in tunica-media, strongly correlating with apoptosis in the vessel wall (Pearson r=0.7, p<0.01). These findings were supported by dose-dependent apoptosis of hVSMCs upon 2-hour PAH treatment *in vitro*.
Conclusion: This study demonstrated that exposure to biodiesel exhaust PM modulates atherosclerotic plaque composition, resulting in an unstable plaque phenotype through enhanced pro-apoptotic mechanisms.
Disclosure: No significant relationships.

OC 12.4 Anemia is associated with decreased platelet inhibition by adenosine diphosphate P2Y12 receptor inhibitors after elective and acute percutaneous interventions with stent implantation

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Objectives: Anemic patients undergoing angioplasty and stenting are at an increased risk of ischemic events, which may in part be caused by an inadequate response to antiplatelet therapy with $P2Y_{12}$ inhibitors. In the current study, we therefore investigated the associations between anemia and on-treatment residual platelet reactivity in clopidogrel- (group1, n=306) and prasugrel-/ticagrelor-treated (group 2, n=109) patients undergoing elective and acute angioplasty with stent implantation, respectively.
Methods: Monocyte-platelet aggregate (MPA) formation was determined by flow cytometry in both groups. On-treatment residual platelet reactivity in response to adenosine diphosphate (ADP) was assessed by light transmission aggregometry (LTA) in both groups, and by the VerifyNow $P2Y_{12}$ assay and the Impact-R in group 1. P-selectin expression was measured by flow cytometry in group 2.
Results: In both groups, anemia was associated with significantly higher MPA formation in response to ADP (both p≤0.02). Moreover, by LTA maximal aggregation in response to ADP was significantly higher in patients with anemia in both groups (both p<0.05), and anemic patients in group 1 had a significantly higher on-treatment platelet reactivity to ADP by the VerifyNow $P2Y_{12}$ assay and the Impact-R than those without anemia (both p<0.001). In group 2, significantly higher platelet surface expression of P-selectin was seen in anemia after stimulation with ADP (p=0.02).
Conclusion: Anemia is associated with decreased platelet inhibition by ADP $P2Y_{12}$ receptor antagonists after elective and acute percutaneous interventions with stent implantation.
Disclosure: No significant relationships.

OC 12.5 Role of the Extracellular Matrix Protein Biglycan for Platelet Adhesion and Thrombus Formation

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Objectives: Biglycan (BGN) belongs to the family of the small leucine rich repeat proteoglycan (SLRP). Besides increasing the stability of the collagen network it is also responsible for tissue remodelling after myocardial infarction. The aim of this project is to determine the role of BGN as an ECM protein in platelet adhesion and thrombus formation.
Methods: In vitro and in vivo analysis of BGN deficient platelets and mice.
Results: Our ELISA and qPCR data provided strong evidence that platelets are not a source of biglycan (BGN). Additionally soluble BGN is not able to stimulate murine platelets. Ex vivo we detected increased platelet adhesion under static conditions and increased thrombus formation on a collagen-BGN matrix compared to collagen alone under flow conditions. Furthermore we observed reduced platelet adhesion at the injured carotid artery and prolonged tail bleeding times in bgn-/0 mice in vivo. Because of reduced platelet adhesion to immobilized recombinant BGN after inhibition of the major collagen receptor glycoprotein (GP)VI we suggest that GPVI might serve as a receptor for BGN. Phosphorylation studies confirmed this hypothesis showing phosphorylation of $PLC\gamma_2$ after stimulation of platelets with soluble BGN alone and together with collagen-related peptide (CRP). Moreover, phosphorylation of the Rac1/Cdc42 effector Pak was observed after stimulation of platelets with biglycan alone and biglycan and CRP.
Conclusion: This study reveals an important role of BGN for platelet adhesion and thrombus formation in hemostasis and thrombosis that might be mediated -at least in part- by GPVI.
Disclosure: No significant relationships.

Laboratory tests / Quality control

P 001 Preanalytical effects of pneumatic tube transport on routine measurement of platelet function tests

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Objectives: Pneumatic tube transport of blood samples reduces turnaround times and labour. However, the preanalytical effects on different platelet function tests are not fully known. The aim of this study was to evaluate the effect of pneumatic tube transport on routine platelet function tests, including PFA 100, platelet aggregation (light transmittance aggregometry, LTA), full blood method (Multiplate®), VASP and P-selektin expression.
Methods: Three venous blood samples from three healthy volunteers were obtained. The first sample was manuell transported, the second by pneumatic tube system and the third sample remained in the laboratory.
Results: The maximal transport distance was 590 m, duration of the transport 328 sec. in a mean velocity of 2.1 m/s (max. 5.5m/s) by stabile temperature (mean 23.5 grd, max 22.5 grd Celsius). No preanalytical effect of pneumatic tube transport could be seen for the LTA (with ADP, Collagen, TRAP-6, arachidonic acid) and the full blood method Multiplate® (ADP, TRAP). Also the measurements of P-Selektin and VASP-test by flow cytometry shown comparible results in all three analysis. The results in PFA 100 (Collagen ADP/Collagen epinephrine) were different in all three results of all three donors.
Conclusion: Pneumatic tube transport does not effect preanalytical errors in LTA, flow cytometry and full blood method (Multiplate) . The PFA 100 was impaired by the tube transport as well as by manuell transport. We recommend generally a different testing of all tube pneumatic systems, because of the different transport duration and used velocities. A comparible protocol should be developed for the quality management of the different pneumatic tube transport systems.
Disclosure: No significant relationships.

P 002 Assessment of aspirin and clopidogrel resistance by light transmittance aggregometry and PFA-100® in patients undergoing neuroendovascular procedures

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Objectives: Dual antiplatelet therapy with aspirin and clopidogrel reduces thromboembolic complications of cardio- and neurovascular procedures. Light transmittance aggregometry (LTA) is considered the gold standard of platelet function testing but is time-consuming and labor-intensive. The Platelet-Function-Analyzer (PFA-100®) is a rapid point-of-care test with uncertain sensitivity.
Methods: Patients were initiated on aspirin 100 mg and clopidogrel 75 mg 7–10 days before endovascular treatment of intracranial aneurysm or carotid stenosis. Non-responsiveness (resistance) to aspirin and clopidogrel was assessed by LTA using arachidonic acid and PFA-100® with the collagen/epinephrine cartridge and by LTA using adenosine diphosphate (ADP) and PFA-100® with the ADP/prostaglandin E1 (PGE1) cartridge, respectively.
Results: 141 patients (100 females; median age, 57 years) were analyzed. The prevalence of aspirin resistance was 2% (n=3) by LTA and 9% (n=13) by PFA-100®, while the prevalence of clopidogrel resistance was 33% (n=44) by LTA and 14% (n=20) by PFA-100®. There was no agreement between the two test systems for aspirin resistance (=0.00, p=0.701), and only a slight agreement for clopidogrel resistance (=0.36, p<0.001). In patients with clopidogrel resistance, as assessed by LTA, the clopidogrel dosage was increased to 150 mg per day before the intervention. Adverse outcomes (death, thromboembolism, or in-stent thrombosis) occurred in 9.9% of all patients independently of the initial LTA results.
Conclusion: While there is some agreement between LTA and PFA-100® in the assessment of clopidogrel resistance, there is no such agreement in the assessment of aspirin resistance. Both tests are thus not interchangeable in the setting of elective neuroendovascular interventions.
Disclosure: No significant relationships.

P 003 Long-term stability of thawed solvent/detergent-treated plasma OctaplasLGÒ

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Objectives: The Join United Kingdom Blood Transfusion and Tissue Transplantation Services' professional advisory committee has agreed to extend the shelf-life of thawed standard FFP from 24 hours to 5 days having FFP available to manage unexpected major hemorrhage without excessive wastage. The aim of our study was to assess the quality and haemostatic potency of solvent/detergent-treated plasma OctaplasLG® during 6 days of refrigerated storage.
Methods: Ten batches of OctaplasLG® manufactured from US plasma of different plasma sources and blood groups were investigated. Samples were collected immediately after thawing, and after 1, 3 and 6 days storage at +2–8°C. All samples were tested on thrombin generation (TGA), global coagulation parameters (aPTT), important coagulation factors (II, V, VII, VIII, IX, X, XI, ADAMTS13) and protease inhibitors (protein C and S, plasmin inhibitor), activation markers (VIIa, TAT, F1+2, D-dimer).
Results: All coagulation factors and protease inhibitors, with the exception of temperature sensitive parameters, were stable for 6 days during storage at +2–8°C. Factors V, VIII and protein S were stable for 24 hours but declined during longer storage at different ratios (i.e. by mean 10%, 30% and 35%). However, after 6 days, all activity levels in all batches remained between the approved specification limits. There was no activation of coagulation and fibrinolytic system observed in any of the batches investigated. TGA parameters remained in the normal ranges, indicating sufficient haemostatic potency after 6 days.
Conclusion: The stability studies support the usage of thawed OctaplasLG® units for up to 5 days of refrigerated storage.
Disclosure: All authors are Octapharma employees.

P 004 Buffy-coat-derived pooled platelet concentrates used for internal quality control of light transmission aggregometry: Five years of experience

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Objectives: So far standardized control material for internal quality control (IQC) of light transmission aggregometry (LTA) is commercially not available. Therefore co-workers' whole blood donations are used to perform necessary internal quality controls in routine laboratories leading to a need of up to 6 litres of whole blood per year. A robust and reliable alternative approach is to use buffy-coat-derived pooled platelet concentrates (PCs) for IQC material of LTA.

Methods: We implemented buffy-coat-derived pooled PCs from the local blood bank as IQC material for LTA. On each weekend one PC is prepared and aliquoted from the primary storage bag on a daily basis in four baby bags, which are delivered from Monday to Friday to the routine coagulation laboratory. LTA was performed on a four channel Chronolog 700 Aggregometer (Chronolog Corporation, Havertown, Pennsylvania, USA) (agonists used: collagen, adenosine diphosphate, arachidonic acid, thrombin receptor activator peptide-6, epinephrine, and ristocetine).
Results: The implementation of buffy-coat-derived pooled platelet concentrates improves the quality of the the daily platelet function analysis as required by international organizations for standardization. Primary results were published recently in the Journal of Thrombosis and Haemostasis (2017 Oct 13. doi: 10.1111/jth.13870.).
Conclusion: Buffy-coat-derived pooled PCs can be used as a reliable and robust IQC material for LTA measurements and are adventageous for the daily laboratory procedure and the staff safety and health.
Disclosure: No significant relationships.

P 005 Data mining of reference intervals for coagulation screening tests

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Objectives: Appropriate reference intervals are essential when evaluating coagulation test results. However, uncertainty exists regarding age- and sex-dependency of test results, and accurate interpretation of test results requires reference intervals specific to the examined population and analytical framework. Data mining of laboratory information systems is an emerging approach to reference interval determination and we evaluated its applicability to coagulation tests.
Methods: We analyzed measurements of activated partial thromboplastin time (aPTT), prothrombin time, thrombin time, international normalized ratio (INR), and fibrinogen performed during clinical care in the University Hospital Erlangen, Germany (1,778,738 samples from 116,754 patients, 45,577–509,859 samples per analyte). We identified the proportion of samples from healthy individuals using an established statistical approach (Reference Limit Estimator), in which the distribution of patient test results is approximated using a parametrical function, and used for the calculation of reference intervals (▶Figure 1).
Results: We established age- and sex specific reference intervals for aPTT, prothrombin time, thrombin time, INR, and fibrinogen. Exemplary results for aPTT are shown in ▶Table 1.
Conclusion: Data mining of laboratory test results enables the creation of reference intervals for coagulation tests that are specific to the examined population, age group, and analytical framework. This approach can complement conventional methods when establishing reference intervals and improve clinical decision-making based on coagulation tests.
Disclosure: No significant relationships.

P 006 Rapid centrifugation for the routine haemostasis laboratory

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Objectives: Implementation of full laboratory automation requires short and uniform centrifugation schemes. It is however unclear if this is applicable to the haemostasis laboratory. To assess the accuracy of measurements obtained with a rapid, high-speed centrifugation scheme in a comprehensive set of haemostasis parameters, and covering the full range of values obtained in clinical practice.
Methods: Two citrated plasma samples were obtained from consecutive patients with suspected abnormal haemostasis parameters and processed with two centrifugation schemes in parallel: 1500 g for 10 minutes and 3137 g for 7 minutes. The following tests were conducted: prothrombin time (n=125), INR (n=146), activated partial thromboplastin time (n=119), thrombin time (n=105), fibrinogen (n=125), D-dimers (n=34), antithrombin (n=31), anti-Xa activity (n=30), von Willebrand antigen (n=25), von Willebrand activity (n=27), and factors (f)II (n=69), V (n=64), VII (n=64), X (n=67), VIII (n=55), IX (n=37), XI (n=35), and XIII (n=20).

Tab. 1

Age (years)	Women (s)	Men (s)	Women & Men (s)
20–30	25.9–34.3	25.6–34.7	25.8–34.5
30–40	25.3–33.8	25.4–34.5	25.3–34.0
40–50	25.1–33.6	25.0–32.9	25.1–33.5
50–60	24.5–33.7	24.7–34.5	24.7–34.0
60–70	24.4–33.6	25.0–34.2	24.6–34.0
70–80	24.0–34.1	25.1–35.3	24.2–35.3
20–90 (combined)	24.7–34.4	25.1–34.7	24.9–34.5

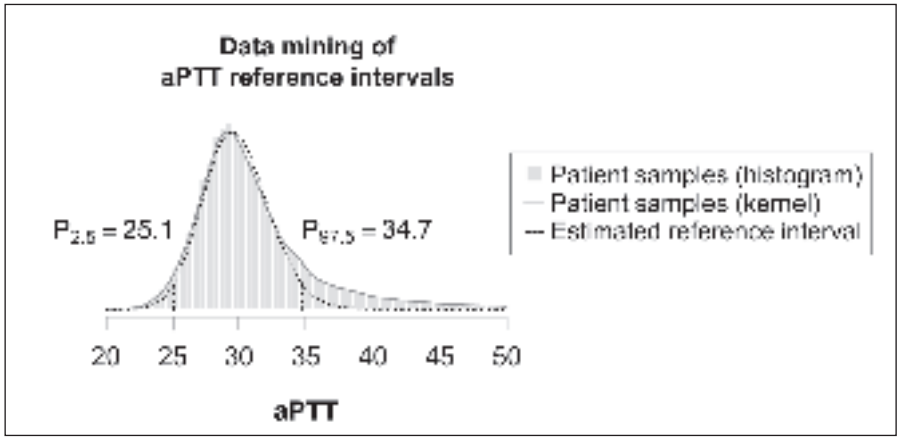

Fig. 1
Data mining of aPTT reference intervals

Results: Spearman's rank correlation coefficient was at least 0.95 for all tests but fV (0.92; 95% CI 0.86, 0.95), fIX (0.84; 0.70, 0.91) and fXI (0.79; 0.61, 0.89). A relevant bias of agreement was observed for fVIII (-17.5; 95%CI –22.3, –12.7). Higher or lower limits of agreement were above 15% in case of fV, fVII, fX, fVIII, fIX, fXI, and fXIII as well as von Willebrand activity.
Conclusion: Whereas a high concordance of haemostasis measurements obtained using a rapid, high-speed centrifugation scheme with an established low speed centrifugation scheme was observed for many routine parameters, some discrepancies were noted in case of fV, fVIII, fIX, and fXI.
Disclosure: No significant relationships.

Laboratory tests / Diagnostic methods

Poster

P 007 PANDA (Platelet Analyzed Number in Different Anticoagulants) is a biomarker for platelet dysfunction induced by leaky gut syndrome

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Objectives: Platelets are anucleate cells that play a crucial role in primary haemostasis. In addition, they also play an important role in inflammation and immunity by expressing Toll-like receptors (TLRs). In patients with leaky gut syndrome (LGS), a condition also referred to as "increased intestinal permeability". Endotoxin translocates into the gut microcirculation and binds to TLRs on platelet´s surfaces. Platelets can be also activated by interaction of heparin with GPIIIa, which is also present on platelet's plasma membrane. The activation threshold of endotoxin-bound platelets by heparin is lower than that of free platelets. The aims of this work were to investigate whether PANDA could be a biomarker for diagnostic of LGS as well as aspirin resistance in patients with gastrointestinal diseases.
Methods: Platelet count was determined in EDTA- and heparin-blood from 97 patients with LGS-associated gastrointestinal diseases and 10 healthy volunteers using commercial available blood collection systems (Sarstedt Inc.) in an automatized hematological counter. GraphPad Software was used for the statistical analysis. The results were considered significant if the p-value was less than 0.05.
Results: A highly significant difference in platelet count in heparin and EDTA blood was seen in patients with gastrointestinal diseases. In contrast, in healthy volunteers the platelet number in both blood samples was similar. PANDA also allowed the detection of aspirin resistance in these patients.
Conclusion: Our results indicate that PANDA assay is a low cost, fast, and reliable laboratory method for detecting LGS-associated inflammatory chronic diseases and platelet dysfunction as well as aspirin resistance.
Disclosure: No significant relationships.

P 008 Methodology of massive parallel sequencing on Ion Torrent platform – searching for rare genetic variants associated with thrombophilia

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Objectives: Methods of next generation sequencing (NGS) offer methodology for relatively fast sequencing of human genome. These methods are really useful for molecular diagnostics of human hereditary diseases. We are using massive parallel sequencing (MPS) in search for rare genetic variant in thrombophilia cases and this approach we are implementing to clinical practice. We designed NGS panel for genes (PROS1, PROC, SERPINC1 and PROCR) which encode proteins with important role in anticoagulation system. In these genes was recently found more than 800 mutations without mutational hots-pots.
Methods: NGS analysis was designed by Ampliseq Designer, performed by amplicon sequencing on Ion Torrent PGM platform. Our panel consists of 83 amplicons with 100% coverage of coding sequences and exon/intron border areas. For evaluation of founded variants we used Torrent Suite – Ion Reporter and IGV. Available databases of clinical variants are ClinVar and HGMD. Confirmation of potentially causative variants are performed by Sanger sequencing. For patient with negative MPS result we introduced CNV testing by MLPA (Multiplex ligation-dependent probe amplification).
Results: We tested 32 patients of our data set. They were selected by repeatedly low level of protein C, S or antithrombin in probands and their family members measured by functional tests. We found 16 probably pathogenic SNV: PROS1 (4), PROC (8), SERPINC1 (4) most of them were not yet published.
Conclusion: For our testing it is crucial very precise selection of probands and 100% coverage of amplicons. If this conditions are fulfilled, MPS is really fast, precise and economical advantageous tool for our problematics.
Disclosure: No significant relationships.

P 009 Evaluation of a new range of platelet agonists for the diagnosis of inherited or acquired platelet dysfunctions

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Objectives: Diagnosis of platelet dysfunctions (inherited or acquired) are performed by light transmission aggregometry (LTA) using specific agonists targeting the major receptors and signaling pathways (PAR-1; $P2Y_1$ & $P2Y_{12}$; a2A; GPVI; TxA_2). The aim of this study was to evaluate the performances of a new range of agonists (Arachidonic Acid, ADP, Collagen, Epinephrine and TRAP-6). Results obtained in healthy donors and patients with inherited or acquired platelet dysfunctions were compared to those obtained with reference agonists.
Methods: LTA (aggregometer TAV8, SD-Medical) was performed using citrated platelet-rich plasma (cPRP). Platelet aggregation was induced by different agonists (Agro-Bio a Stago brand) at different concentrations (low and high): ADP (2 and 10 µM), Arachidonic acid (1 mM), Collagen (2 and 10 µg/mL), Epinephrine (5 and 25 µM) and TRAP-6 (10 and 50 µM). Statistical analyzes were carried out using the MedCalc® software.
Results: Reference intervals (min, max) were defined on 70 healthy donors and ranged from 55% to 100% for all agonists regardless of concentration, except for ADP 2 µM. The performances of agonists were evaluated in the diagnosis of inherited platelet dysfunctions (d-storage pool disease, CalDAG-GEFI and Kindlin-3 deficiency), Glanzmann-like disease induced with anti-GPIIbIIIa antibodies (Abciximab) and drug-induced platelet disorders (Clopidogrel, Aspirin or dual therapies). Bland & Altman, correlation tests and ROC curves showed perfect matching between these new and reference reagents.
Conclusion: This new range of agonists has shown the same effectiveness as the existing reference in the diagnosis of acquired or inherited platelet dysfunctions.
Disclosure: No significant relationships.

P 010 Haematocrit correction of INR, Owren`s prothrombin time capillary citrated blood method

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Objectives: 2016 our laboratory performed 6383 Owren´s prothrombin time, capillary citrated blood method determinations. For each patient the current haematocrit-value was determined simultaneosly. For haematocrit-values < 0,40 and > 0,50 Owren´s PT and INR were automatically corrected.The plasma correction of the Owren´s-PT is obtained applying the formula: PT corrected [%] = PT [%] * 0,55 / (1 – haematocrit). INR corrected is calculated from the current PT calibration curve: INR corrected = (A / PT corrected [%] + B / Mean normal clotting time [s]) ^ ISI. The coefficients A and B emerge from the linear reciprocal regression of the PT calibration curve: PT [s] = 1 / PT [%] + B (Microsoft Excel).
Methods: STA-Hepato-Prest, Destiny Max (Stago Germany); Haematocrit-capillary HK 75 K (Kabe Labortechnik); Hämatokrit 210 (Andreas Hettich GmbH & Co.KG)
Results: For 1099 patients (17,2%) the PT and INR had to be corrected, for 628 patients (9,8%) the haematocrit-value was < 0,40 and 471 patients (7,4%) > 0,50. The extremum for haematocrit-values were found at 0,18 respectively 0,65. The need for haematocrit correction of INR is descriptively demonstrated with two patient examples.
Conclusion: Our laboratory was implementing the haematocrit correction of INR since 2013. The trigger was a patient for which the venous and capillary Owren´s PT was determined to adjust the Falithrom-dose and the results varied widely. The haematocrit-value was 0,60. The subsequent correction of INR explained the differences between the two analytical methods. Unfortunately, a planned study on 75 patients was rejected by the ethic committee in 2015.
Disclosure: No significant relationships.

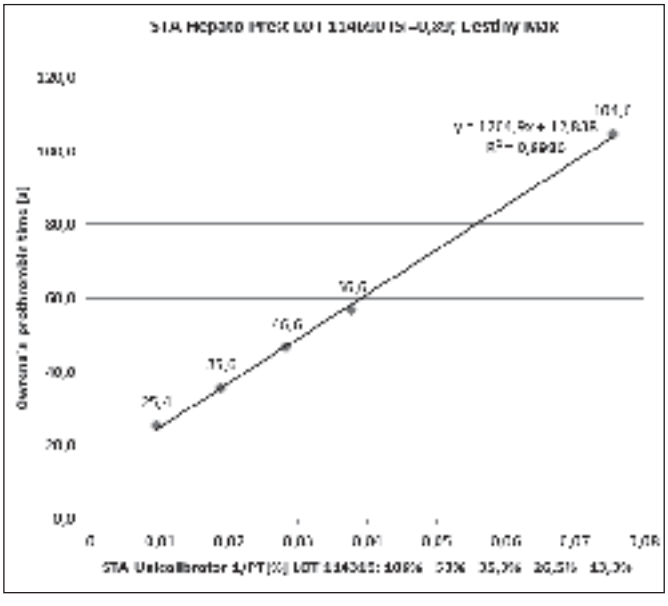

Fig. 1 STA-Hepato-Prest LOT 114690 ISI=0,89; Destiny Max

P 011 Evaluation of sample interferences on ADAMTS-13 activity measurement

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Objectives: Beside it's ability to directly inhibit plasma ADAMTS13 activity, unconjugated bilirubin interferes with certain fluorogenic assays with major impact on clinical diagnosis of TTP and treatment monitoring. Aim of this study was to evaluate the possible interference of unconjugated bilirubin and other potential interfering substances with TECHNOZYM® ADAMTS-13 activity ELISA.
Methods: 3 plasma samples with different ADAMTS-13 activity levels were spiked with increasing amounts of interfering substances and ADAMTS-13 activity was determined. A deviation of up to 15% from the un-spiked sample was regarded as acceptable.
Results: For hemolysis no interference was observed with samples containing up to 200 mg/dL haemoglobin. For lipemia no interference was observed with samples containing at least 500 mg/dL Intralipid. No interference was observed with samples containing up to 20 mg/dL unconjugated Bilirubin. A possible interference of rheumatoid factor was evaluated, plasma with known ADAMTS-13 activity was spiked with different samples of rheumatoid factor and compared to one sample receiving less than 20 U/mL which represented a normal sample. No interference was observed up to 40 U/mL. Within the spectrum of therapeutic intervention of TTP, CD20 antibodies, like rituximab, were described to be successful especially within refractory patients. No interference was observed up to a level of 200 µg/mL of CD20 blocking antibodies, which corresponds to the upper level of serum concentrations found after Rituximab administration.
Conclusion: In this study, we demonstrate that with TECHNOZYM® ADAMTS-13 activity ELSIA no significant interferences could be detected for all tested interfering substances, allowing an accurate clinical diagnosis of TTP and treatment monitoring.
Disclosure: All authors are employees of Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH

P 012 Utility of platelet function analyzer (PFA) in patients with suspected platelet function disorders

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Objectives: Platelet function analyzer (PFA) has been introduced as a screening tool in the perioperative and outpatient setting. However, the diagnostic performance regarding inherited platelet function disorders (PFD) is not fully established. We aimed to assess the diagnostic value of PFA for PFD in clinical practice.
Methods: Data of all consecutive patients referred between January 2012 and March 2017 with a suspected bleeding disorder to an outpatient unit of a university hospital were collected retrospectively. The diagnostic work-up was done according to current guidelines and platelet function was tested using light transmission aggregometry as well as flow cytometry.
Results: PFA (ADP, EPI) was conducted in 473 out of 555 patients referred (median age 41.7 years; 68.1% female). Possible PFD was diagnosed in 70 patients (12.6%), definite PFD in 48 patients (8.7%), von Willebrand disease in 43 patients (7.8%), other coagulation disorders in 39 patients (7.0%), and other disorders in 38 patients (6.9%). In patients with possible PFD, median PFA was 102 s (ADP; inter-quartile range [IQR] 89, 123) and 158 s (EPI; IQR 129, 219; ▶ Figure 1); in patients with definite PFD 107 s (ADP; IQR 92, 130) and 168 s (EPI; IQR 121, 201); and in patients without bleeding disorders 96 s (ADP; IQR 84, 110) and 139 (EPI; IRQ 117, 158).
Conclusion: PFA results were weekly associated with the presence of a PFD. Our results do not support the implementation of PFA for screening of PFD.
Disclosure: No significant relationships.

Poster

P 013 ClotPro – a new generation viscoelastic whole blood coagulation analyzer

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Objectives: Viscoelastic whole blood coagulation analysis provides a continuous, comprehensive assessment of the coagulation process, including thrombin generation, clot formation and fibrinolysis. The most widely used viscoelastic technique is thrombelastography / thrombelastometry presented by Hellmut Hartert in 1948, and further developed by our working group with the introduction of the ROTEM system in 1996.
Methods: With the ClotPro we are presenting a new viscoelastic testing system, which uses a modified detection technique (elastic motion thrombelastography), applying a cylindrical cup rotated alternately to the right and left by an elastic element and a stationary cylindrical pin in the original dimensions described for thrombelastography. The instrument provides 6 independent measuring channels and uses a proprietary "active tip" reagent format, which includes the reagents for each test in dry form in a pipette tip and therefore eliminates reagent handling by the user. Eight different assays have been developed using tissue factor activation (EX-test), contact phase activation (IN-test), fibrinolysis activation by TPA (TPA-test), fibrinolysis inhibition (AP-test), platelet blockade (FIB-test), heparin inhibition (HI-test), FXa activation (RVV-test) and direct prothrombin activation (ECA-test).
Results: Evaluations show an excellent agreement of viscoelastic assays performed with the use of the active tip reagent format vs. wet chemistry reagents, as well as elastic motion thrombelastography results compared to ROTEM analysis.
Conclusion: The ClotPro provides a fast and comprehensive evaluation of coagulation in whole blood, a simplified application, more testing channels for increased throughput and a larger variety of tests compared to previously introduced semiautomatic thrombelastography / thrombelastometry analysers.
Disclosure: Andreas Calatzis is a coowner of the manufacturer of the ClotPro analyzer.

P 014 Pre-analytical effects of pneumatic tube system transport with reduced speed on special coagulation tests

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Objectives: Pneumatic tube transport systems (PTS) for delivery of patient samples to a coagulation laboratory are often used to reduce turnaround time for vital analyses. PTS in our hospital has the ability to regulate the transport speed in the range of 2,5 – 6 $m.s^{-1}$.
Methods: We evaluated the effects of PTS transport for platelet function tests and special global coagulation tests. We used for validation group of 40 blood donors, which was be collected in duplicate. One sample was sent using PTS (normal speed 6 $m.s^{-1}$, reduced speed 3,9 $m.s^{-1}$) and the other was carried by personnel to the lab. We validated platelet aggregation on Apact 4004® analyzer using the inductors (ADP, Arachidonic acid and Ristocetin) and thrombin generation test as global coagulation test on Ceveron Alpha analyzer using the Technothrombin kit.
Results: Determination of TGT and aggregation showed diference more than 10% after transport of PTS with speed at 6 $m.s^{-1}$, compare to PTS with reduced speed at 3,9 m.s-1 with difference less than 3% in all samples of both aggregation and TGT tests.
Conclusion: We conclude that PTS (3,9 $m.s^{-1}$) transport not affect to platelet activity measured by LTA and also global coagulation test – TGT. The advantage of PTS transport is very rapid assessment laboratory testing. From the above validation study, it is clear that PTS should always be validated for specialized laboratory determinations and appropriately adapted to specific transport conditions. Supported by grant LF-2017–007 and MH CZ – DRO (FNOl, 00098892)
Disclosure: No significant relationships.

P 015 Perioperative monitoring of enzymatically active coagulation markers

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Objectives: The recent development of aptamer-based enzyme-capture assays for sensitive measurement of plasma levels of thrombin and activated protein C (APC) allows the assessment of the activity of the haemostatic system on the enzyme level. The present study was designed to investigate the perioperative levels of thrombin-markers and APC in the plasma of patients undergoing surgical intervention.
Methods: Thirty-five patients undergoing major surgery were included in the study. Blood samples were taken before, during and 24 h after the intervention and plasma analyzed for levels of thrombin and APC as well as conventional markers including prothrombin activation fragment F1.2 (F1.2), thrombin-antithrombin (TAT)-complexes, D-Dimer, and in vitro thrombin generation (TG) parameters. Low molecular weight heparin-based VTE prophylaxis was administered to all patients.
Results: Plasma levels of thrombin, TAT-complexes, and APC showed the largest changes with peak levels reached within the course of the surgeries. Plasma levels of thrombin and APC returned to baseline levels when measured 24 h after the surgery. In contrast, levels of D-Dimer were found to be highest in the post-surgery samples. No significant changes of TG parameters were observed. A clear threshold pattern was found between measured anti-FXa levels and levels of APC and F1.2.
Conclusion: The measurement of plasma levels of enzymatically active thrombin and APC allows for the direct measurement of the activity state of the blood coagulation system. Due to the observed combination of highly dynamic plasma levels and distinct response to anticoagulant therapy, especially circulating active APC, represents a promising real time coagulation marker.
Disclosure: No significant relationships.

P 016 First experience with educational case studies in bleeding disorders

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Objectives: The support of medical laboratories to enable physicians to diagnose patients with bleeding disorders and to monitor the effect of treatment is evident. Besides the delivery of accurate and reliable test results laboratories has also to initiate proper reflective testing on the basis of the results basic laboratory tests, like APTT, PT and fibrinogen. We therefore developed so-called case-studies in bleeding disorders to investigate whether proper reflective testing is performed and make the correct diagnosis is made on the patient sample provided.

Methods: Two case studies were performed using (A) a plasma of a mild haemophilia B patient with a factor IX level of approx. 0.10 – 0.15 IU/mL (number of participants: 136) and (B) a plasma of a type I Von Willebrand Disease patient (number of participants: 122). On the basis of a short case description laboratories were allowed to perform any test relevant to reveal to diagnosis.
Results: Case A: Ninety-two percent of the participants gave the correct diagnosis. The major reasons for the misdiagnosis were no APTT mixing test performed, an incorrect interpretation of the APTT mixing test results or insufficient reflective testing (e.g. only factor VIII was measured). Case B: Ninety-three percent of the participants gave the correct diagnosis. Here the major reason for the misdiagnosis was no performance of VWF assays.
Conclusion: Participation in these educational case studies is a valuable tool for medical laboratories and related clinicians to reveal shortcomings in reflective testing and interpretation of laboratory test results.
Disclosure: No significant relationships.

P 017 Evaluation of Diurnal Profile of Protein-S-Activity and Free-Protein-S on Patients on Rivaroxaban®-Therapy after Venous Thromboembolism (VTE) and Rivaroxaban®–Prophylaxis after Knee-Prothesis (KTP)

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Objectives: Protein-S-activity is a counter regulator of thrombosis. Low protein-S-levels are seen in patients with personal or family history of VTE. Substances such as Rivaroxaban® can interfere with the test, causing an overestimation. We evaluated the diurnal profile of protein-S-activity and free-protein-S on patients on Rivaroxaban®-therapy after VTE and Rivaroxaban®–prophylaxis after KTP.
Methods: Ten patients on 20mg-Rivaroxaban®-therapy (VTE) and ten patients on 10mg-Rivaroxaban-prophylaxis (KTP) were enrolled. Three blood-samples were taken, before, 3h and 6–8h after drug intake. Protein-S-activity was performed using STA®-STACLOT®, Stago-Diagnostics. Free-protein-S testing was performed using the Coamatic®-Protein-S-free, Haemochrom-Diagnostic. Both tests were performed on a BCS-XP®-Instrument, Siemens-Healthineers, Marburg-Germany. Statistical analysis was calculated using the MedCalc-Program v.15.11.4. In addition a Lab-database analysis for the last 10 years was done.
Results: Protein-S-activity increased 3h after Rivaroxaban®-20mg-intake by 28.5% and after 6–8h by 17.9% (both medians). Similarly, 3h after Rivaroxaban®-10mg-intake the protein-S-activity increased by 29.2% and after 6–8h by 21.3%. There was no statistically significant difference between these two groups (p=0.08). Rivaroxaban® did not interfere with the performance of free-protein-S. In the past 10 years a total of 4987 blood-samples were received for the analysis of protein-S-activity. Out of these, 240 blood-samples from 119 patients were shown to have protein-S-deficiency: 24% Type-I, 40% Type-II and 36% Type-III.
Conclusion: Intake of Rivaroxaban® 20 or 10mg results in an increased protein-S-activity but does not influence free-protein-S. For this reason and after our database analysis we suggest to measure both, the protein-S-activity as well as free-protein-S, to rule out protein-S-deficiency.
Disclosure: No significant relationships.

Predictive and diagnostic laboratory variables

P 018 The plasma concentration of activated protein C (APC) is increased in carriers of the factor V Leiden mutation (FVL)

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Objectives: Indirect markers of thrombin formation and subsequent generation of APC, including prothrombin activation fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT), APC-protein C inhibitor complexes (APC-PCI), and D-dimer have shown to be increased in FVL carriers. Aim of this study was to directly assess the effect of FVL on the plasma concentration of thrombin and APC.
Methods: Plasma levels of the active enzymes thrombin and APC were quantified using oligonucleotide-based enzyme capture assays (OECAs) in a population of 81 FVL-negative subjects (42 female) and 22 asymptomatic FVL carriers (15 female, eight homozygous). In addition, F1+2, TAT, and D-dimer were measured.
Results: With 1.39 (0.64–2.05) pmol/L in comparison to 0.41 (<0.39–0.68) pmol/L, median (interquartile range, IQR) plasma levels of APC were higher in the FVL-positive group than in the FVL-negative group ($p=10^{-4}$). In FVL-positive subjects, plasma levels of F1+2 and D-dimer were also higher than in FVL-negative subjects, with 0.19 (0.15–0.30) nmol/L versus 0.13 (0.09–0.18) nmol/L ($p=9\cdot10^{-4}$)and 0.39 (0.27–0.61) mg/L versus 0.21 (<0.17–0.29) mg/L ($p=10^{-4}$), respectively. Median thrombin levels were below the limit of detection (0.46 pmol/L) in both cohorts. TAT levels were 2.20 (<2.00–3.08) in the FVL-negative group and 2.46 (<2.00–3.25) ng/mL in the FVL-positive group. There was no statistically significant difference between FVL-positive and FVL-negative subjects regarding thrombin and TAT.
Conclusion: APC is a suitable marker of hypercoagulability in FVL carriers. Further studies are warranted to evaluate its predictive value in the assessment of thrombotic risk.
Disclosure: No significant relationships.

P 019 Need of Treating Residual Inflammatory Activity in Coronary Heart Disease: The Value of high sensitive CRP and LDL in a Real World Cohort

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Objectives: This study analyzes how many patients with coronary heart disease (CHD) on guideline conform therapy show an increased residual inflammatory as opposed to an increased residual lipid risk in order to define the need for an anti-inflammatory treatment in a real world setting.
Methods: High sensitive C-reactive protein (hsCRP) and low density lipoprotein (LDL) levels were determined in 700 all comer patients. Patients lacking CHD, such with chronic-inflammatory diseases, acute inflammation, and on immunosuppressive medication were excluded. Patients were divided in

following groups: elevated hsCRP (≥2mg/dl), normal hsCRP (<2mg/dl), off target LDL-cholesterol (≥70 mg/dl), on target LDL-cholesterol (<70 mg/dl).
Results: From 700 patients 221 fulfilled the inclusion criteria. HsCRP was increased in 45% of these patients. Patients with on target LDL levels showed lower hsCRP concentrations than those with off target values of LDL confirming a positive association between both (1,92 mg/dl vs. 3,15 mg/dl, p=0,0054). However, despite guideline-conform LDL-control 34% of patients with LDL-cholesterol <70mg/dl had elevated levels of hsCRP suggestive of residual inflammation. After logistic univariate regression LDL-cholesterol ≥70mg/dl (OR 2.15, p=0.014), heart failure (OR 3.07, p<0.001) and diabetes mellitus (OR 2.22, p=0.021) independently predicted elevated levels of hsCRP. Heart failure (OR 4.56, p<0,001) and diabetes together (OR 3.04, p=0.012) identified as co-predictors increased hsCRP following backward selection.
Conclusion: Our data suggest that a substantial part of patients with CHD shares a residual inflammatory risk defining a need for an anti-inflammatory therapy. Residual inflammation is particularly prevalent in patients with heart failure and diabetes.
Disclosure: No significant relationships.

P 020 Low molecular weight-heparin calibrated anti-Xa assays are safe predictors for the presence of rivaroxaban in emergency patients

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Objectives: Rapid assessment of the coagulation status in patients treated with rivaroxaban may be relevant in clinical settings such as, in stroke prior to lysis. Specific assays calibrated for rivaroxaban are not always available. As a surrogate, the use of low molecular weight-heparin (LMWH) calibrated anti-Xa assays has been suggested, but experimental evidence in literature is sparse.
Methods: Plasma samples from consecutive in- and out-patients with rivaroxaban intake were analyzed on the BCS XP coagulation analyser (Siemens, Marburg, Germany) by the use of a rivaroxaban-calibrated anti-Xa assay (CoaChrom, Maria Enzersdorf, Austria) and three different LMWH-calibrated anti-Xa assays: Biophen (CoaChrom), Innovance (Siemens), and Coamatic (Haemochrom, Essen, Germany). Data were analyzed with GraphPad Prism (La Jolla, CA, USA).
Results: Complete data were available for 78 patients. Rivaroxaban levels were between 30 and 100 ng/ml in 22, and between 101 and 550 ng/ml in 56 patients. Median concentration was 200 ng/ml. Pearson's correlation coefficient between rivaroxaban and LMWH assays was B=0.66, I=0.73 and C=0.84. All assays were informative (AUROC, 0.86–0.97). At a potentially safe plasma concentration of 100 ng rivaroxaban/ml or below, the three assays had 100% sensitivity and 81% specificity when the cut-offs were set at B=1.1, I=0.7, and C=0.42 U/ml
Conclusion: LMWH-calibrated anti-Xa assays are safe in predicting the presence of rivaroxaban in patient plasma with no apparent difference in specificity (approx. 80%) between them. The author recommends the use of LMWH-calibrated anti-Xa assays to guide further treatment in emergency patients after rivaroxaban intake whenever specific assays are not available.
Disclosure: No significant relationships.

Laboratory tests / Thrombin generation

P 021 Intra-assay reproducibility of thrombin generation in fresh and frozen-thawed platelet-poor plasma in the Calibrated Automated Thrombogram Assay

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Objectives: Applicability of thrombin generation tests (TGT) to clinical routine has been aspired since many years. Due to known challenges in standardisation comparability of the few published data addressing intra-assay variability is limited. The aim of our analysis was to investigate intra-assay variability of thrombin generation assessed in platelet-poor plasma (PPP).
Methods: In this prospective study thrombin generation was measured in twentyfold approach in 3,2% citrate PPP (n=54) obtained from male healthy blood donors aged 19–39 years. The tests were performed with fresh PPP using PPP-Reagent and repeated after storing the PPP frozen. For evaluation of intra-assay reproducibility coefficients of variation (CV) of LagTime, time to peak (ttPeak), Peak height of thrombin (Peak) and the area under curve (ETP) were calculated.
Results: All four parameters tested showed the same mean intra-assay CVs in fresh and stored PPP: LagTime 4%, ttPeak 3%, Peak 6% and ETP 5%. CVs of LagTime and ttPeak showed maxima of 8% or less in fresh and stored samples. Maximum CVs of Peak and ETP were outliers of 14% to 22%.
Conclusion: Our data showed high precision for LagTime and ttPeak and potential for high precision for Peak and ETP. We observed that Peak and ETP were highly sensitive to the performance of the operator in the semi-automated assay. It remains to be seen whether precision will be improved by the automated workflow of fully-automated TGTs.
Disclosure: No significant relationships.

P 022 Sensitive and Standardized TGA measurement in Haemophilia treatment with Ceveron® alpha TGA

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Objectives: To monitor treatment in haemophilic patients is crucial. It is well known that global assays or FVIII/FIX activity determination do not correlate with bleeding episodes. For patients treated with B domain deleted FVIII preparation (rFVIII) the factor activity determination is difficult. Thrombin generation assay (TGA) measures the entire thrombin generation process and a good correlation in treatment with anti-Inhibitor Coagulant Complex (*FEIBA*) or rFVIIa has been shown. Aim of this study was to show that TGA parameters, measured under standardized conditions, can be used with in haemophilic patient samples to monitor replacement therapy.
Methods: Haemophilia A patients plasma was used for spiking with rFVIII. FVIII/FIX activity was determined with one stage clotting assay. Samples were tested Ceveron®TGA RB, Ceveron®TGA RCL and Ceveron®TGA RCH assay kit on Ceveron® alpha TGA. A calibration curve was made once at the beginning of the study.
Results: Although FVIII/FIX activity was <1%, inter individual variation in Peak Thrombin values ranged from 12.3 to 40.4nM. In contrast to FVIII deficient sample where the Peak Thrombin showed a continues increase with increasing FVIII activity, the tested FIX deficient plasma the Peak Thrombin could be restored up to 50% with only 0.17 IU/mL. Peak Thrombin values correlate with the concentration of rFVIII, with 80 % restoration of normal plasma value.

Conclusion: When TGA is used under standardized conditions and with the appropriate trigger, it is a very sensitive tool to monitor factor replacement therapy and can be used to demonstrate correlation with individual bleeding risk during replacement therapy in clinical studies.
Disclosure: All authors are employees of Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH

P 023 Performance evaluation of automated thrombin generation measurement with Ceveron®TGA kits on Ceveron®alpha TGA

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Objectives: Fully automated thrombin generation on Ceveron®alpha TGA is a valuable tool for investigating blood coagulation activity, regarding elevated thrombin activity – thrombotic tendency – or depressed activity – bleeding tendency – when using standardized Ceveron®TGA kits. To enter routine use precise measurements between laboratories is mandatory. Aim of the study was to evaluate assay performance in regards of accuracy and precision between runs and between platforms.
Methods: To evaluate assay performance samples were measured with Ceveron®TGA RB, Ceveron®TGA RCL and Ceveron®TGA RCH assay kit, each on 3 different analyzer. Accuracy and precision were calculated for TGA parameters Peak thrombin, AUC and lag time.
Results: Calibration curves on each Ceveron®alpha TGA platform were made one at the beginning of the study using the standard analyzer settings for TGA calibration. The recovery of control were within 100% ± 10% of target value for all concentrations and for all 3 parameters, tLag, Peak and AUC, measured on the 3 platforms. Precision was very good with between-run and between-platform variations of <15% for tLag, Peak and AUC.
Conclusion: Our data demonstrate that using the standardized assay kits Ceveron®TGA RB, Ceveron®TGA RCL and Ceveron®TGA RCH in optimized settings on Ceveron®alpha TGA the determination of thrombin generation parameters can be performed with very good performance and excellent comparability between platforms.
Disclosure: All authors are employees of Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH

Laboratory tests / Issues related to new factor concentrates

P 024 Monitoring FIX prophylaxis and therapy with extended half-life factor IX concentrates in daily routine: approaching the challenge by applying Rox Factor IX assay.

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Objectives: The aim of the study was to evaluate the Rox Factor IX assay (Rossix AB, Mölndal, Sweden) applied on the automated STA-R Evolution System (Stago Deutschland GmbH, Düsseldorf) in comparison with the classic one-stage clotting assay of the system manufacturer for routine monitoring of patients suffering from haemophilia B as well as during prophylaxis or therapy with Idelvion®.
Methods: The Rox Factor IX assay is a chromogenic assay based on a method principle as shown in the ▶Figure 1. No FIX deficient plasma is used and like in vivo, thrombin is formed during the activation step. The amount of formed FXa is determined from hydrolysis of a FXa sensitive. chromogenic

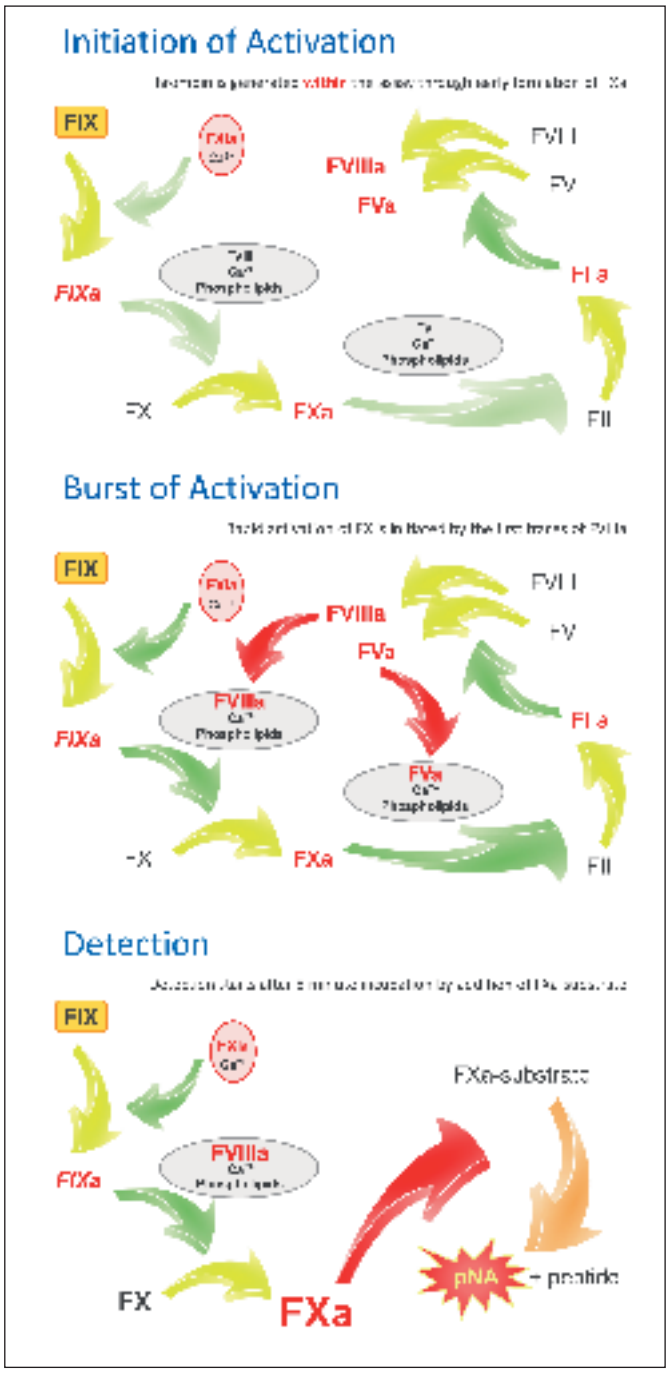

Fig. 1

substrate and is related to the sample FIX activity. The evaluation was performed on 6 plasma samples from haemophilia B-patients and 15 samples from patients undergoing Idelvion therapy.
Results: Using a dilution series of a FIX-standard (range 0.8 – 13%), the FIX-activity was found to be recovered according to the calculated values. While disclosing comparable results for the basic routine samples of haemophilia B-patients, the one-stage clotting assay revealed dramatically lower results (3,5–4 fold) compared to the chromogenic assay in samples containing the half-life extended FIX-concentrate Idelvion®.

Conclusion: The automated Rox Factor IX assay provides a robust and sensitive method to ensure an accurate monitoring of prophylaxis and therapy with the half-life extended factor IX-concentrate Idelvion®. The results support a general application of the Rox Factor IX assay in basic routine analyses as well as for post infusion monitoring.
Disclosure: No significant relationships.

Laboratory tests / Other related topics

P 025 Development of a specific and sensitive approach to measure serine protease inhibitors

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Objectives: Serine protease inhibitors (serpins) represent a family of proteins involved in maintaining homeostasis of import biological systems including coagulation. Representatives of this family of structurally related proteins are antithrombin (AT), C1-inhibitor (C1-inh) and a_1-antitrypsin (AAT). Their inhibitory activity is usually determined indirectly by measurement of residual protease activity of their main target proteases (thrombin, kallikrein and elastase), often using chromogenic substrates. Limitations of these well-established methods with regard to selectivity and sensitivity prompted us to develop an approach based on the direct measurement of serpin-protease complex formation.
Methods: For the measurement of C1-inh and AAT, purified human kallikrein and porcine elastase were coated to polystyrene microplates by passive adsorption. After incubation with the serpin-containing samples, the serpin-protease complexes formed were detected by peroxidase-conjugated anti-serpin antibodies. A polyclonal anti-AT antibody was used to capture AT, which then reacted under complex formation with biotinylated a-thrombin and streptavidin-peroxidase. Calibration curves were obtained using reference plasma pools or an AAT reference standard.
Results: Sensitive calibration curves were obtained for all three serpins ranging from 0.007 to 0.25 and 0.027 to 043 mU/mL for C1-inh and AT, respectively and from 6 to 192 ng/mL for AAT. The selectivity of the approach for the measurement of serpin activity was extensively demonstrated for AAT as heat-aggregated, oxidized and complexed AAT did not react in this complex formation assay.
Conclusion: The presented approach for serpin activity measurement surpasses current methodology in selectivity and sensitivity.
Disclosure: No significant relationships.

P 026 Development and performance characterization of a novel factor IX activity assay for the specific measurement of factor IX Padua in animal models

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Objectives: The naturally occurring single amino exchange (R338L) variant FIX Padua is used in a clinical gene therapy trial. Supportive nonclinical studies would benefit from a method that enables specific measurement of the transgene product. Therefore, a FIX Padua-specific Fab2 mini antibody was developed and applied to develop a FIX Padua-specific activity measurement. Presented data show the method's suitability for its use in the plasma of three mouse types, two of them expressing normal levels of wild type FIX.
Methods: A FIX Padua-specific Fab2 mini antibody, isolated by phage display, was applied to capture FIX Padua. Captured FIX Padua was then measured with a slightly adapted commercially available chromogenic FIX assay (Hyphen). The method's performance was shown by spike-recovery and precision analysis, carried out in citrated plasma from three mouse types (FIX ko, C57BL6 and NOD/SCID).
Results: Wild type FIX showed no response, even when measured at the minimum dilution of 1/10. Citrated mouse plasma samples spiked with 0.33 and 3.31 mU FIX Padua/mL showed mean recoveries within a 100 ± 7% range, thus clearly meeting the accuracy requirements of the EMA guideline for bioanalytical method validation. Intra-run precision, expressed as the relative standard deviation of six repeated measurements, did not exceed 9%, even at the low rFIX Padua concentration of 0.33 mU/mL. Inter-run precision, determined by repeated measurements (n=11) of an FIX Padua sample, was 7.0%.
Conclusion: These data confirmed the adequate performance of this new approach for the selective measurement of FIX Padua.
Disclosure: No significant relationships.

P 027 Multicenter Study of the High-volume Sysmex CS-5100 System Compared to the Sysmex CA-1500 System Using Reagents from Siemens Healthineers*

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Objectives: The objective of this study was to compare the performance of two automated coagulation analyzers, the high-volume Sysmex® CS-5100 System and the Sysmex CA-1500 System, using reagents from Siemens Healthineers. Instrument performance for factor V Leiden (FVL), factor VIII deficiency (FVIII), factor IX deficiency (IX) and Lupus Anticoagulant (LA1, LA2 & LAR) were compared.
Methods: Four clinical sites participated in method comparison (MC) studies by testing leftover samples. MC of the Sysmex CS-5100 System versus the Sysmex CA-1500 System was based on a total of (2430) results (sum of results over all parameters). Precision studies were performed according to appropriate guidelines at three clinical sites. Thirty-five samples (FVL: n = 5; FVIII, FIX, LA1, LA2 & LAR: n = 6) measured covered important medical decision points and the clinical reportable range. The complete dataset contained 8354 results. Additional performance data were determined for regulatory clearance.
Results: Data correlated well between the Sysmex CS-5100 System and the Sysmex CA-1500 System. The MC showing Passing-Bablok regression slopes between 0.94 and 1.05 and Pearson correlation coefficients ≥0.966 (depending on the application). Precision testing for the new device/reagent combinations showed low CV values. The median total precision CV was 3.0%, ranging from 0.9 to 7.3% (coefficient of variation; depending on application and sample).
Conclusion: The Sysmex CS-5100 System compares well to the CA-1500 and offers the benefits of improved functionality and ease of use in high-volume coagulation laboratories. *Application under FDA review. Product availability varies by country. ® SYSMEX is a trademark of SYSMEX CORPORATION
Disclosure: Martin Heisig is an employee of Siemens Healthineers, the commercial vendor of the described product.

P 028 Multicenter Study of the Mid-volume Sysmex CS-2500 System Compared to the Sysmex CA-1500 System Using Reagents from Siemens Healthineers*

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Objectives: The objective of this study was to compare the performance of two automated coagulation analyzers, the mid-volume Sysmex® CS-2500 System and the Sysmex CA-1500 System, using reagents from Siemens Healthineers. Instrument performance for factor V Leiden (FVL), factor VIII deficiency (FVIII), factor IX deficiency (IX) and Lupus Anticoagulant (LA1, LA2 & LAR) were compared.
Methods: Four clinical sites participated in method comparison (MC) studies by testing leftover samples. MC of the Sysmex CS-2500 System versus the Sysmex CA-1500 System was based on a total of (2500) results (sum of results over all parameters). Precision studies were performed according to appropriate guidelines at three clinical sites. Thirty-five samples (FVL: n = 5; FVIII, FIX, LA1, LA2 & LAR: n = 6) measured covered important medical decision points and the clinical reportable range. The complete dataset contained 8303 results. Additional performance data were determined for regulatory clearance.
Results: Data correlated well between the Sysmex CS-2500 System and the Sysmex CA-1500 System. The MC showing Passing-Bablok regression slopes between 0.92 and 1.04 and Pearson correlation coefficients ≥0.958 (depending on the application). Precision testing for the new device/reagent combinations showed low CV values. The median total precision CV was 3.0%, ranging from 0.7 to 10.5% (coefficient of variation; depending on application and sample).
Conclusion: The Sysmex CS-2500 System compares well to the CA-1500 and offers the benefits of improved functionality and ease of use in mid-volume coagulation laboratories. *Application under FDA review. Product availability varies by country. ® SYSMEX is a trademark of SYSMEX CORPORATION
Disclosure: Martin Heisig is an employee of Siemens Healthineers, the distributor of mentioned products.

P 029 Development of a fast and easy assay to determine DNase I activity in plasma samples

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Objectives: Upon activation, neutrophils release their chromatin and form neutrophil extracellular traps (NETs). NETs are highly pro-inflammatory and pro-thrombotic and have an important role in acute coronary syndrome. Deoxyribonuclease (DNase) I is a natural counter mechanism against NETs. Previous results exhibited a correlation between increased DNase I activity and smaller infarct size in patients. We aimed to establish a fast and easy method to quantify DNase I activity in human plasma.
Methods: A DNA substrate was provided that is degraded relative to DNase I content of the sample. Digestion was carried out in solution or when DNA was coated to a solid phase at 37 °C. Remaining DNA was inversely proportional to DNase I activity and measured using different dyes after varying time intervals. A DNase standard curve was included in each setup. Finally, DNA was coated to immunoplates and incubated with standards and plasma samples at 37 °C. Wells were emptied and washed before staining residual DNA with SYBR Green, and measured at an excitation and emission wavelength of 490 and 520 nm.
Results: The final assay included an optimized buffer system allowing comparable assay conditions between standards and plasma. Final plasma concentration in the assay had a strong influence on degradation kinetic as shown by assaying samples of different dilutions over time. The final protocol achieves high sensitivity and reproducibility.
Conclusion: We have established a reliable assay for quantification of DNase I in plasma samples allowing to further study the role of DNase I in cardiovascular disease.
Disclosure: No significant relationships.

P 030 Bioanalytical validation of a factor IX ELISA for the measurement of factor IX Padua expression in three different mouse models

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Objectives: The naturally occurring single amino exchange (R338L) variant factor IX (FIX) Padua is investigated in a clinical gene therapy trial since this gain-of-function mutation shows an up to 10 fold higher activity than FIX wild type. Nonclinical studies carried out in different mouse models (FIX ko, NOD/SCID and C57BL6 mice) required the measurement of FIX protein to determine FIX expression levels. Data obtained during the bioanalytical method validations are summarized.
Methods: Paired commercially available polyclonal sheep-anti human FIX antibodies (neat and peroxidase-labelled, not differentiating human FIX Padua from human wild type FIX) were applied. Bioanalytical method validation was carried out according to the EMA guideline on bioanalytical method validation using a recombinant FIX preparation. Accuracy, precision (intra-and inter-run), total error, linearity and robustness were addressed. Citrated mouse plasma pools were used as matrix.
Results: The FIX ELISA qualified for measuring recombinant FIX Padua in citrated mouse plasma (FIX ko, NOD/SCID and C57BL6) since all relevant assay performance characteristics met the criteria given in the EMA guideline on bioanalytical method validation. In particular, low total errors of 11.1% and 9.1% were determined for C57BL6 and NOD/SCID mouse plasma. The presence of endogenous FIX had no influence on the assay performance. For FIX-deficient mouse plasma a total error of 12.4% was found.
Conclusion: The FIX ELISA was demonstrated to allow accurate and precise measurement of human FIX Padua in citrated FIX ko, NOD/SCID and C57BL6 mouse plasma samples.
Disclosure: No significant relationships.

P 031 A Bispecific Antibody Lacks Measurability in Routine Coagulation Assays and Comparability to Factor VIII

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Objectives: Recently, clinical phase III results of the bispecific antibody emicizumab (ACE910) were reported (Oldenburg 2017). During development, ACE910's activity was tracked using thrombin generation assay (TGA), chromogenic assay, and activated partial thromboplastin time assay (aPTT). The most suitable method for efficacy-testing of non-factor therapy in a routine setting is not yet known.

Tab. 1 SIA (nM)

	SIA (nM)		
	60	200	600
Chromogenic assays			
(% FVIII)			
Biophen FVIII:C	2–3*	8–16*	20–50*
Activated partial thrombin time (APTT) assays			
standard clotting time evaluation (% FVIII)			
APTT-SP	111	> 200	> 200
All other assays	> 200	> 200	> 200
Activated partial thrombin time (APTT) assays			
clot waveform analysis (% FVIII)			
Dapttin	131	> 200	> 200
Actin FSL	44	102	134
Pathromtin SL	5	14	35
Triniclot	22	42	52
APTT-SP	7	14	26
Thrombin generation assay (Peak thrombin)			
(mean values from hemophilia A plasma pool) (% FVIII)			
TF	2	6	26
FXIa	2	5	10
Thrombin generation assay (ETP)			
(mean values from hemophilia A plasma pool) (% FVIII)			
TF	2	10	55
FXIa	6	16	> 100
Thrombin generation assay (Velocity index)			
(mean values from hemophilia A plasma pool) (% FVIII)			
TF	1	1	15
FXIa	2	4	9
Thrombin generation assay (Time to peak)			
(mean values from hemophilia A plasma pool) (% FVIII)			
TF	<1	<1	<1
FXIa	9	25	> 100
Thrombin generation assay (Lag time)			
(mean values from hemophilia A plasma pool) (% FVIII)			
TF	77	<1	<1
FXIa	> 100	> 100	> 100

Methods: A sequence identical analogue to ACE910 (SIA) was analyzed in 4 commercial chromogenic assay kits. Clotting times were measured by APTT using 5 different trigger reagents. In TGA, SIA was tested using extrinsic and intrinsic trigger conditions.
Results: SIA's lack of cross-reactivity to bovine factors rendered only Biophen FVIII:C test suitable for analysis. However, this assay was unsuitable for FVIII-equivalence assessment, since SIA maxed out and deviated from the FVIII reference curve. The aPTT test was highly sensitive to SIA. aPTT reagents yielded a FVIII-equivalence of 18–69% for 20nM SIA and >200% at ≥200nM SIA – patient's plasma concentrations observed in the phase III trial. SIA (600nM) only partially restored TG in hemophilia A patient plasma, resulting in FVIII-equivalence of 4–8% (intrinsic) and 16–36% (extrinsic) based on peak thrombin. Assessment of other TG parameters resulted in FVIII-equivalence of <1 to >100%.
Conclusion: Analysis of SIA and its equivalence to FVIII is challenged by its differing mechanism, rendering standard FVIII protocols unsuitable. Results are highly influenced by assay type, analytical conditions, and parameters used for calculation. Thus, new methods and likely a product specific standard are required to better predict the hemostatic effect of non-factor therapies.
Disclosure: All authors are employees of Shire.

Coagulation and fibrinolytic factors / Physiology

P 032 Pharmacokinetics and efficacy of fibrinogen concentrate in treating acute bleeding in adolescent patients with congenital fibrinogen deficiency

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Objectives: In patients with congenital fibrinogen deficiency, human fibrinogen concentrate (HFC) can correct the hemostatic defect and arrest bleeding. We investigated the pharmacokinetics (PK) and efficacy of Octafibrin, a highly purified, plasma-derived, lyophilized, double virus-inactivated HFC, in pediatric patients.

Tab. 1 Pharmacokinetic parameters of Octafibrin and Haemocomplettan P in pediatric patients with afibrinogenemia (n=5*)

	Octafibrin Mean (SD)	Haemocomplettan P Mean (SD)
AUC_{norm} (h·kg·g/L/mg)	1.57±0.44	1.29±0.67
Clearance (mL/h/kg)	0.68±0.18	0.91±0.33
C_{max} (g/L)	$1.53\pm^{0.32}$	1.15±0.29
Incremental *in vivo* recovery (mg/dL/(mg/kg))	1.95±0.41	1.52±0.21
Mean residence time (h)	100.7±20.1	94.8±18.0
$T_{½}$ (h)	72.8±16.5	64.8±11.9
T_{max} (h)	2.61±1.37	1.70±2.68

*One subject was excluded from the PK analysis due to a protocol deviation.
AUC_{norm}, area under the curve divided by dose per kilogram of body weight; C_{max}, maximum plasma concentration; SD, standard deviation; $T_{½}$, half-life; T_{max}, time taken to reach the maximum concentration.

Methods: Data from two multinational, prospective, open-label studies were summarized from adolescent patients aged 12–17 years with afibrinogenemia. Study FORMA-01 investigated comparative PK versus Haemocompletttan P, surrogate efficacy, and safety after infusion of 70 mg/kg Octafibrin. The surrogate efficacy endpoint was thromboelastometric maximum clot firmness (MCF) in plasma. FORMA-02 investigated hemostatic efficacy, MCF, and safety in the on-demand treatment of bleeding episodes (BE).
Results: In FORMA-01, 5 pediatric patients were included in the PK analysis (one was excluded due to a protocol deviation) and 6 patients in the analysis of MCF. PK data were similar to the comparator drug (▶Table 1). Mean MCF was 9.0±2.0 mm 1-hour post-infusion, from 0 mm at baseline. In FORMA-02, 2 patients received treatment with Octafibrin for BEs. In both patients, treatment efficacy was adjudicated on a 4-point objective scale as excellent. Mean MCF increased by 5.5±0.71 mm 1-hour post-infusion. There were no related serious adverse events (SAEs), thromboembolic events, allergic or severe hypersensitivity reactions, and no anti-fibrinogen antibodies.
Conclusion: In this analysis of data from two studies with HFC in adolescent afibrinogenemia patients, PK parameters of Octafibrin were comparable to those of the comparator. Following Octafibrin infusion, MCF increased significantly from baseline and hemostatic efficacy was rated as excellent. No related SAEs were observed.
Disclosure: B. Schwartz and C. Solomon: Employees of Octapharma. F. Peyvandi: Scientific advisor to Octapharma, Shire and Ablynx; consultant to Kedrion and LFB. All other authors have no conflicts of interest to declare.

P 033 Sensitivity of the aPTT, aPTT Lupus and aPTT Screen assays towards heparin and lupus anticoagulant

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Objectives: Activated partial thromboplastin time (aPTT) tests are used to monitor anticoagulation therapy and for the assessment of coagulopathies. To assist accurate diagnosis, a range of aPTT assays that give different responses to anticoagulants (heparin) and have varied lupus anticoagulant sensitivity, is required. We evaluated the sensitivity of three new aPTT assays (aPTT, aPTT Lupus and aPTT Screen) towards unfractionated heparin (UFH) and lupus anticoagulant. The aPTT assay has reduced lupus antibody sensitivity, whilst the aPTT Lupus assay has increased lupus antibody sensitivity.
Methods: For heparin sensitivity experiments, platelet-poor plasma samples from patients receiving UFH (n=117) were analysed. For lupus sensitivity experiments, lupus anticoagulant-positive commercially available plasma samples (n=96) were analysed. Therapeutic/reference ranges were determined (cobas t 711) or data from the package insert were used (Siemens BCS XP); prolongation of clotting times measured using aPTT, aPTT Lupus and aPTT Screen (cobas t 711) were then compared with the reference assay.
Results: Sensitivity to UFH (aPTT Screen > aPTT Lupus > aPTT) and lupus anticoagulant (aPTT Lupus > aPTT Screen > aPTT) varied between assays (▶Figure 1). Using aPTT Lupus or aPTT Screen, ≥80% of samples from UFH-treated patients within the therapeutic range (anti-Xa activity, 0.3–0.7 IU/mL) resulted in a measurable clotting time. For each assay, prolongation of clotting times in the presence of UFH and lupus anticoagulant were within 0.75–1.25 times that of the reference assay (▶Table 1).
Conclusion: aPTT, aPTT Lupus and aPTT Screen met the pre-specified acceptance criteria for sensitivity to UFH and lupus anticoagulant.
Disclosure: UG's organization received and receives research grants from Roche Diagnostics International AG, Rotkreuz, Switzerland

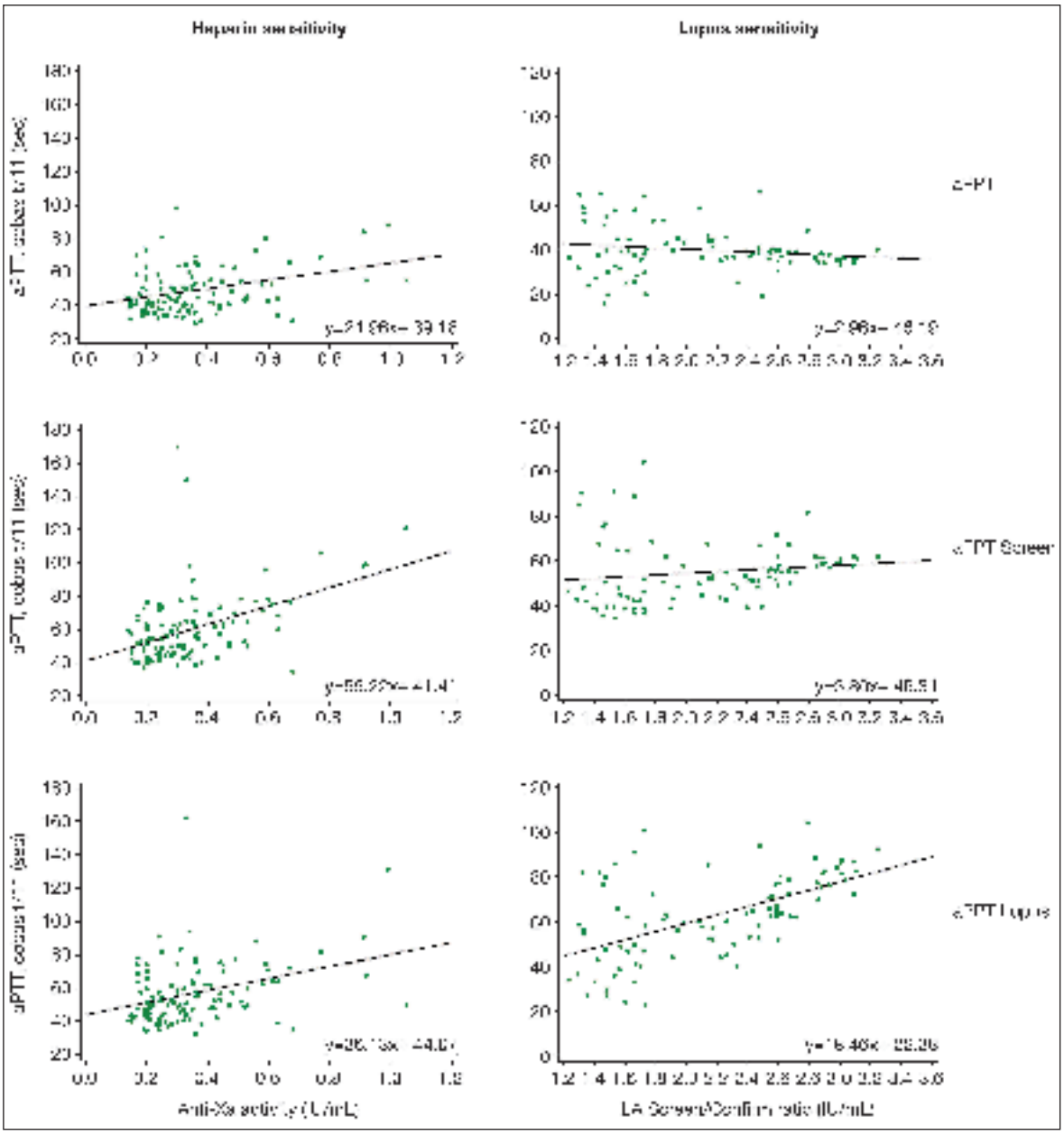

Fig. 1

Reagent	Reference reagent	Relative difference in clotting time ratio on cobas t 711 vs Siemens BCS XP analyser			
		Acceptance criteria	UFH	Acceptance criteria	Lupus anticoagulant
aPTT	Actin FS	0.75–1.25	0.97	0.75–1.25	1.00
aPTT Lupus	Actin FSL		0.95		1.04
aPTT Screen	Pathromtin SL		0.94		0.96

aPTT, activated partial thromboplastin time; UFH, unfractionated heparin

Tab. 1
aPTT, aPTT Lupus and aPTT Screen (cobas t 711 analyser) sensitivity to UFH and lupus anticoagulant, relative to reference reagents (Siemens BCS XP analyser)

P 034 The signaling lipid sphingosine-1-phosphate regulates gene and protein expression of both plasminogen activator inhibitor-1 and tissue factor in differentiated fat cells

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Objectives: The immunomodulatory signaling lipid sphingosine-1-phosphate (S1P) interlinks inflammation and coagulation as platelets generate and release large amounts of S1P upon activation. S1P regulates expression of the protease-activated receptors (PARs) to enhance cellular responses to thrombin and has been implicated in mechanisms of platelet activation. Since hyperlipidemia and obesity are typical risk factors for thrombosis, this study investigates possible effects of S1P on prothrombotic cascades in adipocytes *in vitro*.
Methods: Murine 3T3-L1 fibroblasts were differentiated with MDI (methylisobutylxanthine, dexamethasone, insulin) induction medium. Expression of adiponectin, PAR1–4, the S1P receptors S1PR1–5, plasminogen activator inhibitor-1 (PAI-1) and tissue factor (TF) were determined by RT-PCR; PAI-1 and TF protein by ELISA and Western blotting.
Results: MDI-induced differentiation resulted in characteristic phenotypical changes and a 600-fold increase in adiponectin expression. Expression of the PARs and S1PRs was reduced in differentiated adipocytes which fitted with cellular senescence. Incubation of adipocytes with S1P (0.3 to 10 µM) resulted in a significant upregulation of PAI-1 mRNA as well as protein expression and secretion to the culture medium. This was attenuated by pharmacological inhibition of S1PR2 and –3, but not of S1PR1. In comparison, TF mRNA was downregulated by S1P whereas protein levels were markedly increased. Moreover, Factor Xa (1 to 100 nM) which is known to stimulate S1P generation induced expression of both PA1–1 and TF protein in adipocytes.
Conclusion: S1P regulates expression of PAI-1 and TF in adipocytes *in vitro*. These mechanisms may modulate the pathogenesis of thrombosis in individuals at risk such as in obesity or metabolic syndrome.
Disclosure: No significant relationships.

P 035 Epigenetic determinants controlling the dynamics of F2 gene expression in the liver

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Objectives: Prothrombin (F2) is a multifunctional serine protease, which is primarily expressed in the liver. During blood coagulation activated thrombin cleaves fibrinogen to fibrin. Aberrations of Thrombin (F2a) regulation can lead to hemostatic imbalances, which can range from subtle subclinical to serious life-threatening coagulopathies, i.e. during septicemia. Recently it was shown that F2 expression is regulated by p38 MAPK, which plays an important role for the regulated (patho)physiology of F2 during septicemia and tumorigenesis. Hence, efficiently coordinated dynamics of gene expression, protein biosynthesis and secretion of F2 are critical for a proper balance of blood loss and thrombosis, and beyond.
Methods: Here, we were interested in deciphering regulatory factors that may account for (aberrant) extrahepatic F2 expression during embryogenesis and tumor formation. To that end we used a newly generated mouse model system ('D-Insight'), which was established to visualize gene expression and protein secretion of F2 in real-time and in vivo. Based on fluorescence and luminescence reporters this knock-in mouse permits us to monitor these processes in a living context by the use of non-invasive optical imaging. Additionally, we established a primary hepatic cell line obtained from this mouse model. This cell line allows us to elucidate F2 gene expression-, protein biosynthesis- and secretion dynamics in high resolution and in a high-through3put format with bioluminescence and fluorescence microscopy *ex vivo*.
Results: Applying this system, we obtained first insights that epigenetic regulation is a key mechanism controling F2 expression.
Conclusion: Epigenetic mechanisms control F2 expression. An update will be presented at the meeting.
Disclosure: No significant relationships.

P 036 Pregnancy Outcome in Mice with Protein S Complete Deficiency

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Objectives: Complete protein S (PS) deficiency is a rare fatal thrombophilia associating purpura fulminans (PF) and disseminated intravascular coagulation (DIC). We obtained a full rescue of *Pros1*$^{-/-}$ lethality by targeting FVIII, *F8*$^{-/-}$*Pros1*$^{-/-}$ mice showing neither PF or DIC. Because thrombophilias are associated with pregnancy loss, we investigated the effect of complete lack of PS on pregnancy outcome in mice.
Methods: Pregnancy monitoring in *F8*$^{-/-}$*Pros1*$^{-/-}$ and control mice by blood cell count, coagulation tests and histology.
Results: No litters were produced from *F8*$^{-/-}$*Pros1*$^{-/-}$ crosses. *F8*$^{-/-}$*Pros1*$^{-/-}$females were crossed with F8$^{-/-}$Pros1$^{+/-}$ males and sacrificed at different gestational stages. A high number of macerated embryos was found in *F8*$^{-/-}$*Pros1*$^{-/-}$ mice after E10: 57% (E12), 64% (E15) and 67% (E17). Most of alive embryos showed hemorrhages without PF and *F8*$^{-/-}$*Pros1*$^{-/-}$ embryos were absent after E12.5. The evaluation of DIC parameters in *F8*$^{-/-}$*Pros1*$^{-/-}$ and *F8*$^{-/-}$*Pros1*$^{+/+}$ pregnant females (E12–15, period of high embryos mortality) showed reduction of platelet counts (486±105 vs 806±134 G/L), fibrinogen level (1±0.1 vs 2±0.2 g/L) and increased TAT (33.2±5.1, 16.6±2.8 ng/L), respectively. LMWH or aspirin treated *F8*$^{-/-}$*Pros1*$^{-/-}$ pregnant mice had positive pregnancy outcome. Aspirin treated group showed smaller litter size compared to LMWH group (3±1 vs 6±1) with *F8*$^{-/-}$*Pros1*$^{-/-}$ genotype only rescued in LMWH group (n=1 over 7).
Conclusion: Targeting F8 did not prevent pregnancy loss in *F8*$^{-/-}$*Pros1*$^{-/-}$ mice. Few *F8*$^{-/-}$*Pros1*$^{+/-}$ embryos survived until E17. PS expression in the uteroplacental unit is indispensable for embryos development. Aspirin or better LMWH partially prevented pregnancy loss. Thromboprophylaxis might be beneficial for pregnancy development in case of very low PS.
Disclosure: No significant relationships.

P 037 Precision and method comparison data for three new coagulation assays measuring activated partial thromboplastin time (aPTT) on the new cobas t 711 and t 511 analysers

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Objectives: Assessment of aPTT is a good indicator of intrinsic coagulation pathway activity and is widely used to diagnose coagulopathies and monitor anticoagulant therapy. This multicentre study evaluated the precision of three coagulation assays (aPTT, aPTT Lupus and aPTT Screen) with different sensitivities to heparin, lupus anticoagulant and factor deficiencies, and compared their performance against commercially available assays/platforms.
Methods: Anonymised human 3.2% sodium citrate plasma samples were used (commercially sourced or residual clinical). For each assay, within-run precision was evaluated in a single run (three controls, five pool plasma samples, covering the relevant measuring ranges [21 replicates per sample]); reproducibility was evaluated over five days. Method comparisons were performed on the **cobas t** 711 versus reference reagents on the Siemens Sysmex CS or Stago STA-R Evolution systems (≥120 samples per assay, per site); Pearson's r correlation coefficients were estimated. Results were compared against prespecified acceptance criteria.
Results: Across all three assays, coefficients of variance (CV) for within-run precision and total reproducibility were within the acceptance range (▶Table 1). CVs for within-run precision were 0.2–1.5% **cobas t** 711) and 0.2–1.8% (**cobas t** 511); CVs for total reproducibility were 0.4–3.4% (**cobas t** 711) and 0.3–6.3% (**cobas t** 511). Method comparison experiments for all assays (**cobas t** 711) demonstrated excellent correlation versus their respective reference methods, with Pearson's correlation coefficients within the acceptance range (▶Table 1).
Conclusion: Each aPTT assay showed good within-run precision, reproducibility, and excellent correlation with commercially available assays/platforms, thereby demonstrating their suitability for use in core laboratories.
Disclosure: No significant relationships.

Hemophilia

P 038 c.5702T>C,p.Ile190Thr-a new mutation in the FVIII gene

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Objectives: Introduction: A 33-year-old patient presented after IUFT to control FVIIIc acitivity and VWD parameters. In her prefindings, a decreased FVIIIc and Von Willebrand-factor activity were seen. The first multimere analysis showed a VWD 1.
Methods: Anamnesis: In everyday life the patient suffers from menorrhagia, postbleeding after injury and easy bruising. During her first pregnancy bleedings occurred regularly. Family history was negative. No accompanying illnesses. Lab: Due to the remarkable finding that FVIIIc activity was lower than VWF- activities we did a repetition of the multimere analysis and a genetic testing.
Results: In the VWF gene no clinically relevant change could be proved despite the first multimere analysis. Sequence analysis of the F8 gene identified the previously undescribed variation c.5702T>C, p.Ile1901Thr (alternative nomenclature: p. Ile1882Thr) in exon 17. The affected amino acid position 1901 is highly conserved within 45 species and is located in the A3 domain. A software analysis with different in silico prediction tools classified this variation as likely pathogenic. Furthermore the related amino acid changes p.Ile1901Phe and p.Ile1901Met are described in the literature as causal for a mild haemophilia A (reference: Liu et al. (1998), Br J Haematol.103 (4):1051–60; Markoff et Al. (2009), Haemophilia. 15 (4):932–41; Rydz et al. (2013), In J Hematol. 88 (12):1030–4). In summary the detected variant p.Ile1901Thr can be classified as likely pathogenic (ACMG class 4).
Conclusion: The variant c.5702T>C, p.Ile1901Thr is probably a new pathological mutation predisposing for haemophilia A. Actually, second pregnancy course (actually 25th gestational week) is so far properly. No bleedings occured, no factor replacement therapy necessary.
Disclosure: No significant relationships.

Tab. 1 Within-run precision, reproducibility, and method comparison of the aPTT, aPTT Lupus and aPTT Screen assays versus reference methods.

Assay	Within-run precision* (range of % CV)		Total reproducibility† (range of % CV)		Method comparison (cobas t 711)				
	cobas t 711‡	cobas t 511§	cobas t 711‡	cobas t 511§	Reagent	Instrument	n	Acceptance criteria	Pearson's r
aPTT	0.2–1.5	0.2–1.8	0.4–2.9	0.4–3.8	Actin FS	Siemens Sysmex CS	594	≥0.85	0.980–0.986‡
					STA Cephascreen	Stago STA-R Evolution	175	NA	0.819¶
aPTT Lupus	0.3–1.2	0.2–1.4	0.8–3.1	0.3–2.2	Actin FSL	Siemens Sysmex CS	620	≥0.85	0.967–0.987‡
					STA Cephascreen	Stago STA-R Evolution	99	NA	0.943¶
					STA-LA	Stago STA-R Evolution	128	NA	0.9575¶
aPTT Screen	0.3–1.2	0.2–1.0	0.8–3.4	0.8–6.3	Pathromtin SL	Siemens Sysmex CS	579	≥0.85	0.964–0.985‡
					aPTT	Stago STA-R Evolution	153	NA	0.833¶

*acceptance criteria CV ≤4.0%; †acceptance criteria CV ≤25.0%; ‡range across four sites; §range across two sites; ¶performed at one site only
aPTT, activated partial thromboplastin time; CV, coefficient of variation; NA, not applicable

P 039 Prevention of bleeding in haemophilia A by prophylactic treatment with Octanate®, Wilate® or Nuwiq®: A prospective, multi-national, non-interventional study to evaluate routine practice prophylactic treatment schedules – NIS-Previq.

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Objectives: Since November 2014, Human-cl rhFVIII, Nuwiq®, a recombinant FVIII product derived from a human cell line, is available. A non-interventional study was further initiated with the primary objective to assess the influence of the weekly FVIII dose on annualized bleeding rates. A further objective is the description of prophylactic treatment schedules as applied in routine clinical practice. As of July 2017, also patients treated prophylactically with octanate® or wilate® may be enrolled.
Methods: All details of bleeds and factor VIII treatments are documented. This includes weekly distribution of injections, activity levels of patients and changes in schedules. Optional study elements comprise assessments of joint scores (HJHS), health related quality of life (SF-36) and a PK-assessment including dosing simulation for potential adaptation of therapy schedules.
Results: Data from 16 haemophilia A patients receiving continuous prophylaxis with Nuwiq have been documented so far. No inhibitor formation or other adverse drug reaction occurred. The SF-36 and HJHS are frequently documented elements. The majority of patients with injection on same weekdays schedules do not adapt dosing, e.g. a higher dose for a 3– over a 2-day interval. The general physical stress in most of the patients is "light". The time of day of injections varies considerably between patients and is more often than expected the evening.
Conclusion: The snapshot on the study data so far confirm the good tolerability and efficacy of Nuwiq. With progress of the study, further insight into the performance of real-world performance of prophylactic schedules is expected.
Disclosure: Employee of Octapharma

P 040 Practical utilization of Octapharma FVIII Concentrates in Previously Untreated and Minimally Treated Haemophilia A Patients Entering Routine Clinical Treatment with Nuwiq, Octanate or Wilate (Protect-NOW)

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Objectives: Introduction: Recent uncontrolled studies in previously untreated patients (PUPs) with haemophilia A have shown that the incidence of inhibitor development varies among FVIII concentrates. In the SIPPET[1] study, the cumulative incidence of high-titre inhibitors with hamster-cell derived recombinant FVIII (rFVIII) products was 28.4% vs 18.6% for plasma-derived FVIII (pdFVIII) products. However, these studies did not include all FVIII products available today. Octapharma's FVIII concentrates have also been tested in clinical trials in PUPs (high titre inhibitor rates pdFVIII/VWF: 5.9% to 10.7%, 50 EDs and human-cl rFVIII (published interim results[2]) 12.8%, 20 EDs); however, the numbers of PUPs treated in studies are limited.
Methods: Method: The purpose of the ongoing non-interventional Protect-NOW study is to evaluate product utilisation, effectiveness and safety, including inhibitor development information, in severe haemophilia A PUPs and MTPs, who have been treated with Octapharma's FVIII products in order to broaden the data base in this rare population.
Results: Protect-NOW is planned being initiated in at least 17 countries and 50 centres worldwide. One hundred and forty PUPs and MTPs of all ages and ethnicities are being studied for 100 EDs or a maximum of 3 years. No more than 4 previous EDs with other FVIII products are allowed for MTPs. No prior treatment with FVIII concentrates is permitted for PUPs.
Conclusion: In addition the study offers optional central laboratory substudies at the Institute of Experimental Haematology, Bonn, e.g. epitope mapping, non-neutralising inhibitor detection and gene analysis. First interim data are planned to be presented in 2019.
Disclosure: No significant relationships.

P 041 Do We Have the Correct Method to Assess Adherence in German Haemophilia Patients? – Evaluation of the VERITAS-PRO and VERITAS-PRN Questionnaires by German Haemophilia Treaters -

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Objectives: Non-adherence has a financial impact on healthcare systems and is influenced by social, economic, health-care-system related and condition-related aspects. Due to high treatment costs, the evaluation of adherence in people with haemophilia (PWH) is important. An evaluation by German PWH of the Veritas-PRO/Veritas-PRN, developed for the US environment, demonstrated that these adherence instruments are not entirely applicable for Germany.
Methods: Twenty-seven questionnaires were sent to 12 HTCs in Germany. Healthcare professionals (HCP) were asked to evaluate the questions of the US instruments concerning their relevance for German PWH on a 5-point-Likert-scale. Suggestions for additional questions were possible.
Results: So far, 19 HCPs sent back their evaluation (62.5% physicians, 25% nurses, and 2 study/haemostaseology assistants). 43.8% treated only adult PWH, 25% only paediatric PWH and 31.2% treated both. HCPs were treating PWH since 15.5±6.7 years (range 9–30). Based on the content validity ratio (CVR ≥0.6) five questions of the Veritas-PRO domains 'timing', 'planning' and 'communicating' (e.g., "I infuse the recommended number of times per week") and seven questions of the Veritas-PRN domains 'treating', 'dosing', 'planning', 'remembering' and 'communicating' (e.g., "I have enough factor and supplies at home to infuse when needed", "I call the treatment centre before medical interventions") were considered "very important" by HCPs for use in Germany. HCPs suggested to add a variety of questions concerning substitution diary, visits and communication with HTC, etc. In general, questions concerning novel therapies were missing.
Conclusion: It could be demonstrated that there is a need of an appropriate adherence instrument applicable for the German health-care-system.
Disclosure: No significant relationships.

P 042 Subcutaneous Administration of recombinant factor VIII by using von Willebrand Factor (vWF) Fragment: Pharmacokinetic assessment in a Hemophilia A mouse model

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Objectives: vWF functions as a chaperone, protecting factor VIII (FVIII) from proteolysis and from binding to phospholipid membranes. This protective capacity is mediated by the vWF domain D'D3. A novel low-molecular-weight vWF fragment containing the propeptide D'D3 domains (OCTA12) was generated, and the ability of OCTA12 to support FVIII absorption after subcutaneous (SQ) administration was analyzed.

Methods: The F8$^{-/-}$ hemophilia A mouse model was used to investigate the pharmacokinetics (PK) of recombinant human FVIII (simoctocog alfa, Octapharma) with or without OCTA12 after SQ or intravenous (IV) injection. Mice were treated with single doses of FVIII, either 200 IU/kg IV or 1000 IU/kg SQ. Plasma samples were collected 1 to 48 hours (h) after treatment. PK profiles were generated by analyzing FVIII:C chromogenic activity and FVIII:Ag.
Results: The peak FVIII:C activity was observed 1 h after IV administration with OCTA12 (133.4 ± 78.1 IU/dl), and decreased quickly thereafter with a half-life of 2.5 h. After SQ administration with OCTA12, FVIII:C activity increased slowly over the first 6 h (1 h = 10.9 ± 11.0 IU/dl; 3 h = 34.7 ± 21.0 IU/dl; 6 h = 49.6 ± 36.7 IU/dl) and was detectable for up to 32 h (6.1 ± 3.4 IU/dl). Estimated bioavailability of FVIII after SQ administration with OCTA12 was 18.4%.
Conclusion: In conclusion, results of these preliminary investigations show that the vWF fragment OCTA12 supports absorption of FVIII into vascular circulation after SQ administration. PK analyses after a single SQ administration showed promising bioavailability, clinically relevant peak levels, and prolonged FVIII:C activity.
Disclosure: N. Vollack, S. Werwitzke, and A. Tiede obtained research funding by Octapharma Biopharmaceuticals GmbH. B. Solecka and C. Kannicht are employed by Octapharma Biopharmaceuticals GmbH.

P 043 Differences of treatment of hemophilia patients treated in hemophilia centers and in other hospitals in Germany

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Objectives: Germany has well elaborated definitions of structural requirements for three levels of hemophilia treatment-centers: 23 Comprehensive Care Centers (CCC), 28 hemophilia treatments institutions (HBE), 38 regional centers (HB). The German health system gives hemophilia patients free choice of therapist. Here we research what share of patients prefers non-center-hospitals and whether treatment patterns differ.
Methods: Reports from German Statistical-Office and the hospital quality reports for 2010–2015 were analyzed for hemophilia cases and use of factorproducts. Statistical analysis was performed using Microsoft-Excel/Access version 2016.
Results: 2015 patients with diagnosis hemophilia-A were treated 53% in CCC, 15% HBE, 7% HB and 25% non-centers. Cases with FVIII use (units used) were 45% (55%) CCC, 19% (17%) HBE, 7% (6%) HB and 29% (22%) non-centers. Per case CCC used in their core departments 15,479IU FVIII; 10,815IU in other departments. HBE 10,420IU in core and 7,852IU others. Non-centers used 7,054IU FVIII. 28% cases with recombinant, 18% plasmatic FVIII in non-centers. The core adult CCC-departments used in average 17,163IU FVIII; 10,226IU HBE; 6,250IU HB and 5,462IU non-centers. Same pattern in pediatric, dental and ENT. In surgical and orthopedic departments the average FVIII-use was nearly equal, independent from the center level.
Conclusion: Germany has a sophisticated network of hemophilia treatment-centers. More than one quarter of patients with hemophilia are searching treatment in non-hemophilia centers, espacially surgery and orthopedics. Average use of factor products is here comparable in all levels of centers. Some aspects of the German network system may be questioned, patients partly ignore it and treatment patterns do not differ.
Disclosure: No significant relationships.

Tab. 1 Minimum EHL ratio required for a 2x/wk EHL rFVIII to achieve the same proportion of patients always >1 IU/dL as 3x/wk standard rFVIII

Dose regimen	Proportion of patients always above 1 IU/dL	Half-life extension ratio
Three times per week (benchmark); 30 IU/kg on Day 1 and 3, and 40 IU/kg on Day 5	56.6%	Not applicable
Twice weekly; 40 IU/kg on Days 1 and 3	56.6%	1.3
Twice weekly; 45 IU/kg on Days 1 and 3	56.6%	1.26

P 044 A model of the minimum half-life extension ratio needed to reduce the dosing frequency of extended half-life (EHL) recombinant FVIII (rFVIII) products

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Objectives: A key defining criterion of an EHL product is having an EHL ratio. However, a minimal clinically-meaningful half-life (HL) extension ratio has not yet been defined. We identify the HL extension ratio required to make a clinically meaningful (1 day) reduction in dosing interval while maintaining the same percentage of patients always above a target rFVIII concentration of 1 IU/dL.
Methods: The population PK model for standard rFVIII by Björkman et al. (2012) was used to estimate the percentage of patients always >1 IU/dL using a benchmark regimen for rFVIII dosed 3 times weekly. Then, dosing frequency was reduced to twice weekly and rFVIII HL extended until the percentage of patients always >1 IU/dL was equal to the benchmark regimen. The result is an estimate of the minimal clinically-important HL extension ratio required to meet the definition of an EHL rFVIII product.
Results: Benchmark doses for rFVIII of 100 IU/kg/week were tested to reflect common rFVIII utilization. This benchmark regimen resulted in 56.6% of patients always >1 IU/dL. Comparing the benchmark to doses of 80 and 90 IU/kg/week, the fold HL extension required to achieve 56.6% of patients >1 IU/dL was 1.30 and 1.26, respectively (▶Table 1).
Conclusion: rFVIII products should show a minimum HL extension ratio of 1.3 for a meaningful reduction in dosing while maintaining the same percentage of patients always >1 IU/dL FVIII.
Disclosure: Jason Booth is an employee of Shire.

P 045 An advanced reporting module within the smart medication™ platform simplifies mandatory reporting into the German Hemophilia Registry (DHR)

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Objectives: The German Hemophilia Registry (DHR) collects data from patients with bleeding disorders based on the German Transfusion Law (Transfusionsgesetz). The reporting process is characterized by time-consuming manual entry of bleeding information along with factor consumption into a form based interface at DHR. Alternatively, electronic diaries can utilize an automated electronic interface for direct communication with the registry, avoiding re-entering large amount of data manually again.

Methods: Within the smart medication™ platform an easy to use module was integrated to support processing and transferring diary data to the German Hemophilia Registry (DHR). This includes validation of factor consumption and bleeding information by doctors, secure data transfer to the registry as well as documentation of all reporting activities carried out by hemophilia centers.
Results: A pilot testing phase of the reporting module within smart medication™ confirmed that data handling and processing is greatly simplified for hemophilia centers using the smart medication™ platform. It could be shown that validation and transfer of bleeding information as well as factor consumption to the German Hemophilia Registry (DHR) already available in smart medication™ can be easily processed, saves significant time and lead to a maximum in data quality being reported to the registry.
Conclusion: Data processing within electronic diary smart medication™ for notification and reporting into the German Hemophilia Registry (DHR) is easy to use, avoid error-prone manual re-entry of large amount of data, saves time in the reporting process and at the same time ensures a maximum of data quality.
Disclosure: No significant relationships.

P 046 Analysis of the efficacy of rVIII-SingleChain in adult and adolescent patients with severe haemophilia A in Europe

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Objectives: rVIII-SingleChain is a novel B-domain truncated recombinant factor VIII (FVIII) comprising covalently bonded FVIII heavy and light chains, with a high binding affinity for von Willebrand factor. The safety, efficacy and pharmacokinetics of rVIII-SingleChain were assessed in the AFFINITY trial program. The aim of this analysis was to evaluate the efficacy of rVIII-SingleChain in the treatment of haemophilia A patients in Europe.
Methods: Previously treated haemophilia A patients 12–65 years with severe haemophilia A (FVIII <1%) received either on-demand or prophylactic infusions of rVIII-SingleChain. Prophylaxis dose and regimen were determined by the investigators, based on clinical bleeding phenotype and previous FVIII treatment, and could be adjusted at any time during the study. Bleeding events were rated by the investigator on a 4-point rating scale for hemostatic efficacy.
Results: Eighty-five patients from 11 European countries were included in the analysis, representing 49.1% of the total study population. Of these, 69 patients received prophylaxis and 16 on-demand treatment with rVIII-SingleChain. Prophylaxis with rVIII-SingleChain resulted in very low bleeding rates that were significantly lower than with on-demand treatment (median annualised bleeding rate (ABR): 0.00 vs. 28.08; p<0.0001; median annualised spontaneous bleeding rate (AsBR): 0.00 vs. 22.71; p<0.0001). In the European subset haemostatic efficacy was excellent/good in 92.5% (521/563) of bleeding events, similar to the overall study population (93.8% of 835 bleeds).
Conclusion: rVIII-SingleChain is efficacious in the prevention and treatment of bleeding events in haemophilia A patients in Europe, with a median ABR and AsBR of 0.00. These findings were consistent with the total study population.
Disclosure: CW: honoraria and/or consultancy fees from Baxalta/Shire, Bayer, Biotest, CSL Behring, LFB, Novo Nordisk, Pfizer and Sobi; RK: honoraria and research funding from Baxter, Bayer, Biogen Idec, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer

P 047 Non-neutralizing factor VIII antibodies as a marker for inhibitor development in a longitudinal cohort of haemophilia A patients

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Objectives: The development of neutralizing antibodies to FVIII (Nabs) is still the most severe complication in modern haemophilia A treatment. In clinical routine Nabs are determined by Bethesda Assay, which detects Nabs, but not non-neutralizing antibodies to FVIII (NNAs). The primary objective of this study was the characterization anti-FVIII drugs antibodies (ADA) development, ADA function and signature in correlation to treatment outcome in longitudinal samples of previously untreated patients.
Methods: Validated FVIII-ADA assays for detection of ADA, Nabs and IgG subclasses (IgG1, IgG2, IgG3 and IgG4) were used in combination with assays to identify the binding domains on FVIII (human-porcine FVIII constructs, FVIII heavy and light chain and individually expressed FVIII domains). A total of 21 previously untreated patients were analysed for >50 exposure days.
Results: In this retrospective, longitudinal analysis 10 patients had developed Nabs. For 6 with Nabs, NNAs were detected up to 150 days before Nabs. Two out of 11 Nab-negative patients showed ADAs in screening, but were not confirmed in competition tests. IgG subclass characterization showed a broad response in the beginning including IgG3, with a change to IgG1 and IgG4 only. Epitopes on FVIII were localised on FVIII heavy- and light-chain including domains A2, C1 and C2 with a change to A2 with Nab development.
Conclusion: For most patients, the development of NNAs preceded the detection of Nabs. None of the patients that did not develop Nabs showed confirmed ADAs. The relevance of ADA detection before Nabs development and possible clinical interventions needs to be assessed in clinical trials.
Disclosure: The research leading to these results has received support from Biotest AG and the Innovative Medicines Initiative Joint Undertaking under grant agreement n° [115303], resources of which are composed of financial contribution from the European Union's Sev.

P 048 Efficacy and safety of rVIII-SingleChain for surgical prophylaxis in haemophilia A

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Objectives: The novel recombinant Factor VIII, rVIII-SingleChain, is a B-domain truncated construct with a covalent bond between the heavy and light chains. The safety and efficacy of rVIII-SingleChain to control haemostasis in severe haemophilia A patients undergoing surgery was evaluated as part of the AFFINITY clinical development program.

Methods: rVIII-SingleChain was administered as either a bolus or continuous infusion during the perioperative period in patients undergoing procedures requiring general, spinal or regional anaesthesia. Haemostatic efficacy was rated by investigators; treatment success was defined as a haemostatic efficacy rating of either Excellent (haemostasis not clinically significant different from normal; blood loss ≤20% higher than predicted) or Good (haemostasis normal or mildly abnormal; estimated blood loss >20% but ≤30% than predicted). Studies in the AFFINITY program were approved by the relevant Ethics committee and national authorities and conducted according to GCP and the Declaration of Helsinki.
Results: A total of 28 adult, adolescent or paediatric patients underwent 35 surgical procedures during the AFFINITY program, with 5 of the procedures conducted in 4 paediatric patients. The most common surgical procedures performed were: circumcision (9), knee replacement (7) and dental extraction (3). rVIII-SingleChain was administered as a bolus in the majority (77%) of patients. Haemostatic efficacy was rated as Excellent (n=32, 91%) or Good (n=3, 9%) in all patients, and no serious or related adverse events were reported.
Conclusion: rVIII-SingleChain as either a bolus or continuous infusion provides safe and effective haemostatic control during a range of surgical procedures in adult, adolescent and paediatric patients.
Disclosure: IP: unrestricted grant, speaker and consultancy fees from CSL Behring; FK, CDK, JOng, SP: no conflicts to declare; JM: grant/research support from Baxalta, Bayer, Biogen, CSL Behring, Novo Nordisk and Roche and consultancy for Amgen, Biotest, Baxalta, Bay.

Poster

P 049 Estimation of Nuwiq® (human-cl rhFVIII) and Advate® Factor VIII activity using one-stage and chromogenic assays: results from an international comparative field study

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Objectives: One-stage (OS) and chromogenic (CHR) assays are commonly used to determine Factor VIII (FVIII) activity, but are subject to high variability, depending on the instrument and reagents used. Precise analysis of FVIII blood levels is essential for correct diagnosis, dosing and to ensure efficacy and safety of treatment. This international comparative field study evaluated the reliability of OS and CHR assays in measuring FVIII activity of two recombinant FVIII (rFVIII) products in routine laboratory practice.
Methods: FVIII-deficient plasma was spiked with Nuwiq® (B-domain deleted (BDD) rFVIII of human-cell line origin) and Advate® (full-length rFVIII of hamster-cell line origin) at 4 different concentrations (0.01, 0.05, 0.3 and 1.0 IU/dL) based on labelled potencies determined by chromogenic assay. Samples were prepared centrally, blinded and distributed to participating laboratories. Each laboratory performed FVIII activity testing using their routine protocols. Laboratories provided procedural information based on a questionnaire.
Results: Forty-four laboratories in 10 countries participated in the study. Most laboratories routinely performed both assays, OS and CHR. The results will be analysed for intra- and inter-laboratory variation, accuracy, and differences between the two rFVIII products. Furthermore, the results of the OS assay obtained with different APTT reagents and the results of the CHR assay obtained with different assay kits will be compared. Final study results are expected by the end of 2017.
Conclusion: This ongoing field study is investigating the performance of OS and CHR assays in measuring FVIII levels in human-cl rhFVIII (BDD) in comparison with one full-length rFVIII concentrate.
Disclosure: T.V., J.V., J.B. and L.B. are employees of Octapharma AG, Lachen, Switzerland.

P 050 Anticoagulation in patients with bleeding disorders: preliminary results of a multicentre cross-sectional study in Germany

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Objectives: Due to improved life expectancy of patients with bleeding disorders (PWBD), atrial fibrillation (AF), valve replacement and venous thromboembolism (VTE) become more common. Whether anticoagulation, which would be indicated in the normal population, is necessary and safe in PWBD is not clear.
Methods: Retrospective data collection from patient charts of PWBD (Haemophilia A (HA) and B (HB) all severities, von Willebrand disease (VWD) with activity < 30%), including type, severity, treatment of the bleeding disorder, co-morbidities and disease with indication for anticoagulation and its treatment.
Results: So far, 27 male PWBD (19 HA: 8 severe, 3 moderate, 8 mild; 5 HB: 1 severe, 4 moderate, 2 VWD, 1 VWD and HA), median age 71 years (range 29–90), have been included. Indications for anticoagulation were AF (n=19, median CHA_2DS_2VASc Score 3 (range, 1–6)), VTE (n=4, 3 after surgery with intensive factor replacement), 3 mechanical heart valves and 1 arterial thrombosis. 9 patients received anticoagulation, 5 for AF, 2 for mechanical heart valve replacement, and 2 for VTE. 4 PWBD received low dose Aspirin, 3 for AF, 1 for arterial thrombosis. In 2 patients with VWD and 1 Patient with mild haemophilia, prophylactic factor replacement was initiated with anticoagulation. Main reason for not initiating anticoagulation was bleeding risk in PWBD, second was expected lower event rate.
Conclusion: The majority of PWBD is not treated with long-term anticoagulation despite having a disease with indication for anticoagulation. To evaluate risk for thrombotic events and bleeding risk with anticoagulation in this specific population, a prospective study is needed.
Disclosure: This stady has been supported by an unrestricted grant of Bayer Healthcare

P 051 The Burden of Caring for a Child with Moderate or Severe Haemophilia – Results of the European BBC Study

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Objectives: Data on caregivers' burden in haemophilia are scarce. We aimed to assess caregivers' burden using the recently in the US developed 'HEMOphilia associated CAregiver Burden scale' (HEMOCAB™) in 7 European counties (France, Germany, Italy, Poland, Sweden, Turkey and UK).

Methods: Caregivers were asked to complete the HEMOCAB™ and socio-demographic data. In addition their child's clinical data were retrieved from medical records.
Results: So far, data from 88 caregivers (mean age 38.07±6.7) and their children (mean age 8.47±5.4 years; range 0.55–17.73) from 5 countries have been collected. Caregivers were caring for children with mainly haemophilia A (86.4%), severely affected (86.4%) and receiving prophylaxis (83%). Children had on average 3.58±4.9 total bleeds and 9.31±15.5 days lost in the past 12 months; 27.3% reported chronic pain. Most of the caregivers were female (85.2%), married (77.3%), living with a partner (83%), higher educated (61.3%), and working (64.7%). 20.7% had a long-term condition themselves; 64.8% reported that haemophilia affected the family life and had an economic impact on the family (34.5%). The HEMOCAB™ showed good psychometric characteristics in terms of internal consistency (Total Score α =.970). Caregivers reported highest burden in the HEMOCAB™ domains 'Perception of your Child' (M=43.94±25.3), 'Emotional Stress' (M=41.0±22.5) and 'Impact on You' (M=33.24±28.4).
Conclusion: Caregivers felt mainly impacted in the perception of their child and their emotional stress related to caring for a child with haemophilia. Thanks to effective prophylaxis and the decrease of bleeding frequency not only the development of joint damage can be reduced, but also caregiver's burden.
Disclosure: For the conduct of the BBC Study Haemnet received a grant from Shire.

P 052 Ensuring information security for the electronic patient diary smart medication™ by applying an Information Security Management System (ISMS) based on the international standards ISO/IEC 27001 and ISO/IEC 27799

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Objectives: Cybercrime and cyberattacks increasingly threaten information security, data privacy and data security of medical apps and medical app platforms. The series of ISO/IEC 27001 and its supplement ISO/IEC 27799 for health IT-systems are broad in scope and cover more than just privacy, confidentiality and technical and cybersecurity issues. They are applicable to organizations of all shapes and sizes, assess information risks and treat them according to their needs.
Methods: ISO/IEC 27001 and ISO/IEC 27799 provide general IT security controls as well as health specific controls together with implementation guidances. In total 14 areas, i.e. "Information security policies", "Access control" or "Cryptography" are addressed. The security controls and recommendations of the ISO standards have been analyzed and implemented to the smart medication™ platform to ensure a maximum of data security and privacy for patients and HCPs.
Results: It is shown how security controls and principles defined in the ISO/IEC 27001 and 27799 series are applied to the smart medication™ platform and how patients with hemophilia using this platform benefit from IT security measures. Furthermore, it is demonstrated how a PDCA (Plan-Do-Check-Act) iterative cycle ensures to keep up with constantly changing cyber threats.
Conclusion: The international standards for information security management based on ISO/IEC 27001 provide best practice recommendations on information security management. Platforms like smart medication™ for treatment of patients with hemophilia benefit greatly when best practices of these standards are applied.
Disclosure: No significant relationships.

P 053 High observed and steady-state trough FIX activity levels are maintained with rIX-FP prophylaxis in haemophilia B patients

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Objectives: Maintaining high factor IX activity (FIX:C) trough levels enables patients to transition from severe to mild disease states. The aim of this analysis was to summarize the observed and steady-state trough FIX:C levels in haemophilia B (FIX:C ≤2%) patients during prophylaxis with rIX-FP (IDELVION®).
Methods: Adults (≥12 years) received rIX-FP prophylaxis every 7– or 14-days and had trough FIX:C levels measured every 4 weeks before each infusion for up to ~92 weeks. Paediatric patients (<12 years) received prophylaxis every 7 days and had trough FIX:C levels measured at 4, 12, 24 and 36 weeks. Patients with ≥1 measurement obtained at observed trough were included in the analysis. Steady-state trough FIX:C levels included only FIX trough samples after 2 to 3 consecutive doses and excluded any trough where additional FIX product was administered.
Results: Mean steady-state trough FIX:C levels were 23% (n=41) in adults and 13% (n=23) in children for the 7-day regimen and 13% (n=18) in adults for the 14-day regimen. In adults, receiving 35–50 IU/kg rIX-FP every 7 days (n=32) or 50–75 IU/kg rIX-FP every 14 days (n=16) resulted in mean steady-state FIX:C levels of 21% and 13%, respectively. In paediatric patients receiving 35–50 IU/kg rIX-FP every 7 days (n=13), steady-state trough FIX:C levels were 14%. Steady-state trough was >5% across all doses and dose intervals in 96.2% of adult and 97.9% of paediatric patients.
Conclusion: rIX-FP maintains FIX:C activity levels with both 7– and 14-day prophylaxis regimens at levels consistent with a mild haemophilia B phenotype.
Disclosure: WH: Honoraria and travel support from Bayer, Baxalta/ Shire, CSL Behring, Grifols, LFB, NovoNordisk, Pfizer; JCG: honoraria for advisory boards for Baxalta/Shire, CSL Behring and Bayer, and support for Investigator Initiated Research from Baxalta/Shire an.

P 054 Cytokine profiles of children with Haemophilia A with and without FVIII inhibitors

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Objectives: In the last decade studies showed that some patients with Hemophilia A (HA) have a more pro-inflammatory immunological profile then healthy humans. Furthermore, patients with neutralizing antibodies (inhibitors) present a more regulatory immunological profile with higher production of IL-10.
Methods: In this study we investigated the difference in the cytokine levels of 10 children with HA (HA^-, age 1–7), 10 children with HA FVIII inhibitor (HA^+, age 1–6) and 12 healthy controls (age 2–8). In addition we observed longitudinally a toddler with HA who developed a FVIII inhibitor and started an immune tolerance induction. Frozen plasma samples were measured according to the human Cytometric Bead Array TH1, TH2, Th17 Enhanced Sensitivity Kit (BD).

Results: The concentration of proinflammatory Il-6 in HA children with inhibitor was significantly higher than in HA children without inhibitor or in healthy controls (median in pg/ml: 0,31 HA^-; 4,03 HA^{+}; 0,00 HC). The concentration of IL-10 did not differ significantly between these groups. In the longitudinal observation the patient started with higher levels of pro-inflammatory IL-6 and lower levels of regulatory IL-10. However, cytokine levels changed by time and were reversed after inhibitor development and start of ITI.
Conclusion: According to our study, children with FVIII inhibitor have higher levels of IL-6 but no significant difference of IL-10 compared to inhibitor-negative patients and healthy controls. For the first time we were able to do a longitudinal observation of the cytokine profile and visualize the switch from a proinflammatory to a more regulatory profile simultaneously with the start of ITI.
Disclosure: No significant relationships.

Poster

P 055 BAY 94–9027 Provides Effective Long-Term Prophylaxis: Interim Analysis of the PROTECT VIII Extension Study

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Objectives: BAY 94–9027 is a prolonged–half-life PEGylated recombinant factor VIII (FVIII) product. Safety and efficacy of BAY 94–9027 was studied in the PROTECT VIII study. This analysis reports interim data from the PROTECT VIII extension.
Methods: In the phase 2/3 PROTECT VIII study, 134 previously treated males aged 12–65 years with severe hemophilia A received BAY 94–9027 for 36 weeks on demand or as prophylaxis (twice-weekly [30–40 IU/kg], every-5-days (45–60 IU/kg), or every-7-days [60 IU/kg] regimens). Patients could continue in the extension with the same regimen or switch to a different regimen at any time.
Results: Results: 121/134 patients continued treatment in the extension. At start of extension, 24, 46, and 37 patients received twice-weekly, every-5-days, and every-7-days prophylaxis, respectively; 14 patients received on-demand treatment. At data cutoff (February 2017), patients (median age, 39 years) were observed for a median of 3.2 years (range, 45–1443 days) in the extension and ≤4.7 years including the main study. Twenty-four patients changed dosing frequency during the extension: 15 patients changed to higher frequency, 5 changed to lower frequency, and 4 patients changed twice (back to their original dosing frequency). Annualized bleeding rates varied by treatment regimen (▶Table 1). Cumulative doses were similar across all prophylaxis regimens. No patient developed FVIII inhibitors. Two patients discontinued from the study due to treatment-related serious adverse events.
Conclusion: Data from the PROTECT VIII extension show that BAY 94–9027 provides effective long-term prophylaxis across 3 individually tailored treatment regimens and is well tolerated up to 4.7 years on treatment.
Disclosure: Pabinger: Honoraria for lectures or advisory board meetings from Bayer, Biotest, CSL Behring, Pfizer, Novo Nordisk, SOBI, and Shire; unrestricted grants from CSL Behring and Novo Nordisk. Poulsen: Investigator on clinical trial for Bayer and Novo Nordisk.

P 056 FEIBA Global Outcome (FEIBA GO) study data read-out: Real world bleeding frequency in inhibitors patients on prophylaxis with APCC

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Objectives: "FEIBA GO" was designed to capture long-term outcomes on effectiveness, safety, and quality of life (QoL) in subjects with hemophilia and inhibitors treated with APCC in routine clinical practice. The primary objective is to describe the hemostatic effectiveness of APCC.
Methods: FEIBA GO is a prospective, non-interventional, observational, multicenter, cohort study in ~100 patients with hemophilia A or B and high-responding inhibitors treated with APCC prior to deciding to enroll in the study. Treatment regimens are at the discretion of the attending physicians

Tab. 1 Bleed Outcomes and FVIII Consumption by Treatment Regimen During Extension*

	Prophylaxis				Total Prophylaxis (n=107)	On Demand (n=14)
	2x/wk (n=23)	Every 5 Days (n=34)	Every 7 Days (n=26)	Variable Frequency† (n=24)		
Total ABR	1.9 (0.8; 3.6)	1.3 (0; 4.6)	0.7 (0; 1.6)	3.7 (1.4; 7.0)	1.5 (0.3; 4.6)	33.5 (20.3; 36.5)
Joint ABR	0.8 (0; 1.6)	1.0 (0; 3.1)	0.4 (0; 1.0)	2.7 (1.0; 4.7)	1.0 (0; 3.1)	20.5 (12.8; 33.4)
Spontaneous ABR	0.8 (0; 3.1)	0.7 (0; 2.9)	0.2 (0; 0.8)	2.4 (0.7; 5.4)	0.8 (0; 2.9)	20.7 (12.0; 28.6)
Patients with 0 joint bleeds, n (%)	9 (39.1)	11 (32.4)	10 (38.5)	3 (12.5)	33 (30.8)	0
Total dose, IU/kg/y	3917 (3247; 4244)	3414 (3230; 4093)	3114 (2901; 3239)	3912 (3344; 4059)	3417 (3153; 4012)	1394 (952; 1715)

ABR=annualized bleeding rate; FVIII=factor VIII.
Data are median (quartile 1; quartile 3) unless otherwise indicated.
*Data as of February 2017 cutoff (median time, 3.2 years).
†Patients who changed treatment regimen to higher frequency (n=15) or once weekly (n=5); 4 patients changed regimens twice (back to original dosing frequency).

Tab. 1

Patients on Prophylaxis Analyzed in the Data Read-out	N=28
Median Annualized Bleeding Rate (ABR)	**4.1 (0–50)**
Subjects with ABR "0"	10.5%
Subjects with ABR "≤2"	26.3%
Subjects with ABR "≤3"	36.8%
Median Annualized Joint Bleeding Rate (AJBR)	**2.3 (0–17)**
Subjects with AJBR "0"	31.6%
Subjects with AJBR "≤2"	47.4%
Subjects with AJBR "≤3"	68.4%

according to routine clinical practice, either prophylaxis or on-demand, including immune-tolerance induction. The observation period/subject is planned to be 4 years.

Results: A data read-out was carried out on May 18, 2017 on 41 subjects with severe hemophilia A and inhibitors from 14 hemophilia centers in 10 countries: median age 17 years (range: 3–71). At screening, 28 were on prophylaxis; among 38 patients with first FVIII inhibitor detection results available, median titer was 13.5BU (range: 1–2,410). Data were available for 19/41 (▶Table 1).

Conclusion: These preliminary findings demonstrate prevention of joint bleeding with FEIBA prophylaxis comparable to that reported in patients without inhibitors on replacement prophylaxis. This study will further augment the knowledge of long-term prophylaxis in the real-world clinical setting by assessing effectiveness, QoL and safety of FEIBA. It is anticipated that the FEIBA GO study will capture important outcomes data across and beyond the European Union on hemophilia A and B patients with high-responding inhibitors under a variety of prescribing regimens in routine clinical practice.

Disclosure: Employee of Shire

P 057 The First German Instrument for the Assessment of Subjective Physical Functioning in Children with Haemophilia – Psychometric Testing of the child-adapted HEP-Test-Q

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Objectives: For the appropriate assessment of subjective physical functioning in children with haemophilia standardized and validated instruments are necessary. Since such an instrument was not available in Germany we adapted the adult version of the HEP-Test-Q for children aged 6–17years and psychometrically tested this version in the context of different studies.

Methods: The adaptation of the HEP-Test-Q for children consisted of different phases: a) reformulation of items, b) pretesting (including feasibility testing, cognitive interview), c) psychometric testing.

Results: Items of the adult version were *reformulated* for better comprehensibility together with 5 children. *Pre-testing* in 34 children from the US, UK and Germany revealed a completion time of 8.2±4.1 minutes; most items were easy to understand, nine items had to be reformulated. *Psychometric testing* (EIS Study [n=67]; SO-FIT Study [n=127]) showed good psychometric characteristics in terms of reliability and validity. Cronbach's alpha ranged from α=.801 ('endurance') to α=.935 (Total Score). The child-adapted version of the HEP-Test-Q showed moderate correlations with subjective questionnaires PedHAL (r=.634, p<.0001) and Haemo-QoL SF (r=-.575, p<.0001) and low correlation with the objective HJHS (*r*=-.323). Known groups validity testing proved that the child-adapted HEP-Test-Q was able to discriminate between different clinical subgroups (OJS: p<.006, BMI: p<.015, target joints: p<.0001, type of prophylaxis: p<.038, pain: p<.0001).

Conclusion: The child-adapted HEP-Test-Q is the first German instrument for the assessment of subjective physical functioning. It is a short, well accepted, practical, and feasible self-report measure for children. Outcomes can be compared to adults since item concepts are identical to the adult version.

Disclosure:

P 058 Increasing von Willebrand Factor binding affinity for FVIII: Identifying amino acid substitutions in the D'D3 region

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Objectives: The frequent factor VIII (FVIII) infusions necessary for effective prophylactic treatment of severe haemophilia A represent a significant burden to patients. FVIII circulates in complex with von Willebrand Factor (VWF), and this association governs the clearance of FVIII. Improving the affinity of VWF for FVIII may therefore provide a strategy for increasing the half-life of FVIII, and so reduce the infusion frequency necessary for prophylaxis. Mutagenesis approaches have been utilised to identify residues in the D'D3 region of VWF that would increase the affinity of VWF for FVIII.

Methods: The N terminal region of VWF has been identified as a key region for FVIII binding. Over 300 directed individual and combination mutations at residues S764, L765, S766, P769 and S806 were generated as VWF D'D3 region fragments and expressed in HEK293 or CHOK1 cells, before screening by Surface Plasmon Resonance (SPR) to identify those with improved FVIII binding kinetics. A mammalian display library generated following random mutagenesis of D'D3 was also screened for enhanced FVIII binding variants.

Results: Substitutions at residues S764, S766, P769 and S806 were identified that resulted in an increased binding affinity to FVIII. Further VWF residues impacting binding to FVIII were identified using the mammalian display approach. In all cases, increased FVIII affinity was primarily due to off-rate changes.

Conclusion: Amino acid substitutions in the D'D3 region of VWF have been identified that increase the binding affinity for FVIII. These variants may assist with strategies to increase the half-life of FVIII for the treatment of haemophilia A.

Disclosure: AA, MH, KE, CP, MW, SD; employees at CSL Limited; TW: employee at CSL Behring.

Poster

P 059 Design of a Prospective, Non-interventional Study Evaluating Real-World Usage and Effectiveness of Elocta® (rFVIIIFc) and Alprolix® (rFIXFc) in the Prophylactic Treatment of Haemophilia A and B: The PREVENT study

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Objectives: The safety and efficacy of extended half-life (EHL) factor products Elocta® and Alprolix® have been established in phase 3 and extension studies that are ongoing. Here we describe the design of the ongoing PREVENT study (NCT03055611) with the objective to provide data on real-world usage and effectiveness of Elocta® and Alprolix® in Germany.
Methods: PREVENT prospectively collects data over a 24-month period from at least 100 patients with haemophilia A and B in approximately 30 German centres. Previously treated patients that have started prophylactic treatment with Elocta®/Alprolix® are eligible to join the study. The ratio of haemophilia A:B is not set, but the study aims to enrol a minimum 65 haemophilia A and a minimum 20 haemophilia B patients. Patient characteristics, including historical data, will be collected at baseline. Primary endpoints: annualised bleeding rate (overall, joint and target-joint), injection frequency and factor consumption. Secondary endpoints include rationale for initiating treatment with Elocta® and Alprolix®, treatment satisfaction, physical activity and quality of life. Results will be presented using descriptive statistics. When available, comparisons will be made against previous conventional FVIII and FIX treatment. Visits will be according to the haemophilia treatment centre's standard of care. Safety reporting will follow European Union regulation guidelines.
Results: The study is ongoing. Recruitment status of patients and centres will be presented.
Conclusion: PREVENT will provide insights into the rationale for initiating prophylactic treatment with EHL Elocta® and Alprolix® as well as the real-world usage and effectiveness in routine clinical practice in Germany.
Disclosure: Dr Tiede reports grants/personal fees for lectures and consultancy from Alnylam, Bayer, Biogen Idec, Biotest, Boehringer Ingelheim, CSL Behring, Leo Pharma, Novo Nordisk, Octapharma, Pfizer, Portola, Roche, Shire and Sobi. This research is funded by Sobi.

P 060 Prophylactic vs. on demand treatment in haemophilia patients – real life data according to electronic diary smart-medicationTM

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Objectives: Home treatment may be done on regular base (prophylaxis, P), in case of bleeding (on demand, OD) or as mixed regimens (temporary prophylaxis, M). Bleeding rates in real life were analyzed according to the three treatment groups.
Methods: 249 patients completed their electronic smart medication™ documentation in 2016 and were analyzed according to home treatment regimen and bleeding history.
Results: A majority (64%, n=160) was on prophylactic treatment. A minority (6%, n=15) used > 90% of factor VIII/IX for bleeds and < 10% for temporary prophylaxis (OD). Nearly a third (30%, n=74) of all patients used 10 – 90 % factor VIII/IX for on demand or temporary prophylaxis (M). Mean age was 25 (P), 42 (OD) and 34 (M). Annual bleeding rates were 2.4 (P), 12,9 (OD) and 11.4 (M). Total 129 joint bleeds in (P) (0,8/patient), 60 in (OD) (4/patient) and 370 in (M) (5/patient). Annual factor VIII/IX use (IU/kg BW) was 2852 IE/Kg (P), 536 IE(OD) and 2465 IE (M). The average factor VIII/IX dose per bleed (IU/kg BW/bleed) was 26 (P), 25 (OD) and 31 (M).
Conclusion: Real life data are in accordance to published data showing substantial less bleeding during prophylactic treatment in contrast to on demand treatment. Nearly one third of patients treat on mixed regimens with subsequently high bleeding rates, in spite of the highest dosing per bleed and may profit from more regular treatment.
Disclosure: No significant relationships.

P 061 Monocyte function is impaired in hemophilia

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Objectives: Monocytes are key regulators of inflammation and wound healing. Here we aimed to define monocyte function in hemophilia and their role in the imbalance between tissue regeneration and inflammation frequently observed in hereditary bleeding disorders.
Methods: Monocytes from hemophilia patients and healthy individuals were treated with M-CSF or GM-CSF and probed for morphological and molecular features of macrophage differentiation by phase contrast as well as fluorescence microscopy. Expression of CD163, Tie2 and CSF1R were measured on monocytes/macrophages by flow cytometry. Wound infiltration and phagocytosis were determined by probing macrophages for clot invasion and latex bead/red blood cell uptake.
Results: Morphological analysis of hemophilia macrophages revealed a defect in cell polarization and filopodia formation. This deficit was most pronounced in response to M-CSF, which promoted clot invasion and phagocytosis in donor but not in hemophilia macrophages. In addition, we detected a significant reduction of molecules supporting wound infiltration and blood removal such as CSF1R, Tie2 and CD163. This loss of regenerative macrophage functions, which is reflected by reduced expression of the M2 marker CD163, was mitigated after embedding hemophilia monocytes in clotted plasma from healthy blood donors.
Conclusion: Hemophilia monocytes exhibit a defect in macrophage maturation resulting in a significant impairment in regenerative macrophage functions that can be partially restored by normalizing clotting.
Disclosure: No significant relationships.

P 062 rIX-FP prophylaxis regimens result in high compliance in adult and paediatric haemophilia B patients in clinical studies

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Objectives: In clinical studies, rIX FP (IDELVION®) prophylaxis regimens with 7-, 10 and 14 day dosing intervals resulted in median annualized spontaneous bleeding rates of 0.00. The aim of this analysis was to evaluate compliance to rIX-FP prophylaxis regimens in haemophilia B (FIX ≤2%) patients in two clinical studies (PROLONG-9FP).
Methods: In the adult study (≥12 years), 23 patients received on-demand treatment for 6 months followed by 7-day prophylaxis for 6 months, and 40 patients received 7-day prophylaxis for 6 months and then, if eligible, switched to a 10– or 14-day dosing interval for 6 months. In the paediatric study, 27 patients <12 years of age received 7-day prophylaxis. Dose, dosing frequency and rIX-FP consumption was recorded in an e-diary and verified by the number of used vials returned at study visits. Compliance to prophylaxis regimens and prescribed dose (adults only) was assessed.
Results: Compliance to prophylaxis (receiving ≥80% of the expected number of infusions) was high in adult (94.9%) and paediatric patients (97.9%). In adults, mean compliance rates were similar between regimens; 94.7% (n=40) and 95.1% (n=23) for 7-day prophylaxis, 90.7% and 97.2% for 10– and 14-day prophylaxis, respectively. The mean compliance rates were similar between 1–5 (97.3%) and 6–11 year olds (98.3%). In adults, 85.7% of patients received within 10% of their prescribed dose ≥80% of the time.
Conclusion: In clinical studies with rIX-FP, haemophilia B patients demonstrated a high degree of compliance to prophylaxis with a range of dosing intervals, enabling them to achieve low bleeding rates.
Disclosure: SH: received honoraria for specking Bayer Healthcare GmbH, Baxalta Innovations GmbH, Biotest AG, CSL Behring GmbH, Novartis Pharma GmbH, Novo Nordisk GmbH, Octapharma GmbH, Pfizer and research grants from ayer Healthcare GmbH, Baxalta Innovations GmbH, Bi.

P 063 LongLife-9FP: a prospective, non-interventional study to observe the effectiveness of recombinant factor IX albumin fusion protein (rIX-FP) in patients with haemophilia B in real life

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Objectives: rIX-FP is a recombinant long-acting factor IX albumin fusion protein with a considerably improved pharmacokinetic profile compared to standard factor IX products. In clinical trials rIX-FP demonstrated in pretreated haemophilia B patients: i) long-lasting efficacy for 7,10 or 14 days in prophylaxis regimen, ii) instant and reliable efficacy in on demand and iii) sustained efficacy and safety in patients undergoing surgery. Besides clinical data however, there is growing interest in real world use of rIX-FP. LongLife-9FP is a non-interventional study which aims to complement results of previous rIX-FP clinical trials with data from a larger haemophilia B population in real world clinical practice.
Methods: Haemophilia B patients of any age and severity receiving rIX-FP as prophylaxis or on demand therapy are eligible for enrolment. About 150 patients are planned to be documented in 30 German haemophilia treatment centres and their routine treatment with rIX-FP will be observed for 3 years or until 100 exposure days have been reached. Patients are routinely seen at centres every 3 to 12 months. At these visits, occurrence of bleeding, adverse drug reactions, quality of life using the Hemo/a-QoL-questionnaire, patient satisfaction, as well as factor consumption and bleeding-related absenteeism are to be documented, if available.
Results: A methodological description of this non-interventional study will be presented and a status update will be given.
Conclusion: This non-interventional study collects data from the everyday use of rIX-FP in real world clinical practice and is intended to confirm the very good efficacy and safety of rIX-F as found in clinical trials.
Disclosure: JO: research support: Baxter, Bayer, Biotest, CSL Behring, Grifols, NovoNordisk, Octapharma, Pfizer, consultancy fees: Baxter, Bayer, Biogen Idec, Biotest, Chugai, CSL Behring, Grifols, NovoNordisk, Octapharma, Pfizer, Roche, SOBI; MG: employee at CSL Be.

P 064 Bleeding vs. Factor VIII/IX consumption – comparison of annual real-life results between 2014 and 2016 according to electronic diary smart-medicationTM

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Objectives: Bleeding frequency and factor VIII/IX consumption (FC) differ widely among patients with severe haemophilia. Year to year patterns among individual patients and centers may, however, be similar.
Methods: Annual FC and Joint bleeds (JB) were compared among 246 patients with haemophilia A/B from 9 Haemophilia centers between 2014 and 2016 according to electronic documentation smart medication™.
Results: The average FC (IU/kg BW) was 2,442 (±2,038) in 2014, 2,701(±1.837) in 2015 and 2,575 (±1,877) in 2016. The average number of JB was 2.1 (±3.9), 2.5 (±4.9) and 2.27 (± 7.3), respectively. Four groups, comparing above or below average FC and JB, were compared between 2014, 2015 and 2016: The majority (45%/40%/44%) had 2 or less JB with less than average FC, followed by a group(31%/35%/32%) who also had 2 or less JB but above average FC. A minor group (14%/11%/15%) had more than 2 JB and more than average FC and was similar in size to a group (10%/14%/8%) who had more than 2 JB but less than average FC.
Conclusion: Between 2014 and 2016 a majority (76%/73%/76%) of patients documented 2 or less JB per year as a result of optimal home treatment. Patients with high bleeding frequency in spite of above average FC revealed a small (14%/11%/15%) but important group requiring intensified attention. The results were consistent comparing three consecutive years. The electronic diary smart medicationTM is suitable to focus on groups of patient which may require more or less factor treatment or need otherwise intensified treatment.
Disclosure: No significant relationships.

Von Willebrand disease

P 065 Recombinant Von Willebrand Factor (rVWF) Administration: Dosing Considerations and Rapid Stabilization of Endogenous Plasma FVIII Levels in Patients with Severe Von Willebrand Disease

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Objectives: We analyzed prospective clinical trial data to better inform dosing decisions related to rVWF treatment and the pharmacodynamic effect of administering rVWF on endogenous FVIII:C.

Methods: Two phase 3 clinical trials were included in this post-hoc analysis: an on-demand study (NCT01410227) and an elective surgery study (NCT02283268). All patients had severe VWD. Patients who received rVWF alone at doses of 50 or 80 IU/kg VWF:RCo were included in the analysis of mean endogenous FVIII:C over time.

Results: Analysis included 25 patients treated with rVWF 50 IU/kg VWF:RCo and 15 treated with 80 IU/kg VWF:RCo. rVWF alone resulted in rapid and sustained increases in endogenous FVIII:C, regardless of the VWD type (▶Figure 1). Among 36 patients with a preinfusion FVIII:C level of <40 IU/dL, the mean (range) time to reach 40 IU/dL FVIII:C was 5.1 h (0.8–11.5) in all patients and 5.6 h (1.6–11.5) in patients with type 3 VWD. Patients with type 1, 2A, and 2B achieved the 40-IU/dL level faster (1.5, 4.2, and 0.8 h, respectively).

Conclusion: Treatment with rVWF alone results in rapid stabilization of endogenous FVIII:C, which was more rapid in patients with higher baseline FVIII:C levels. FVIII:C increases at a mean rate of 7.7 IU/dL per hour (range: 1.0–17.2). Therefore, a rise in FVIII:C to hemostatically effective levels (≥40 IU/dL) was reached in the majority of patients within 6 h, and sooner in patients with higher FVIII:C levels at baseline.

Disclosure: Joan C. Gill has the following disclosures: consultancy (Shire, Bayer, CSL Behring), research funding (Shire, CSL Behring), membership on an entity's board of directors, speakers bureau, or advisory committee (Shire, Bayer, CSL Behring).

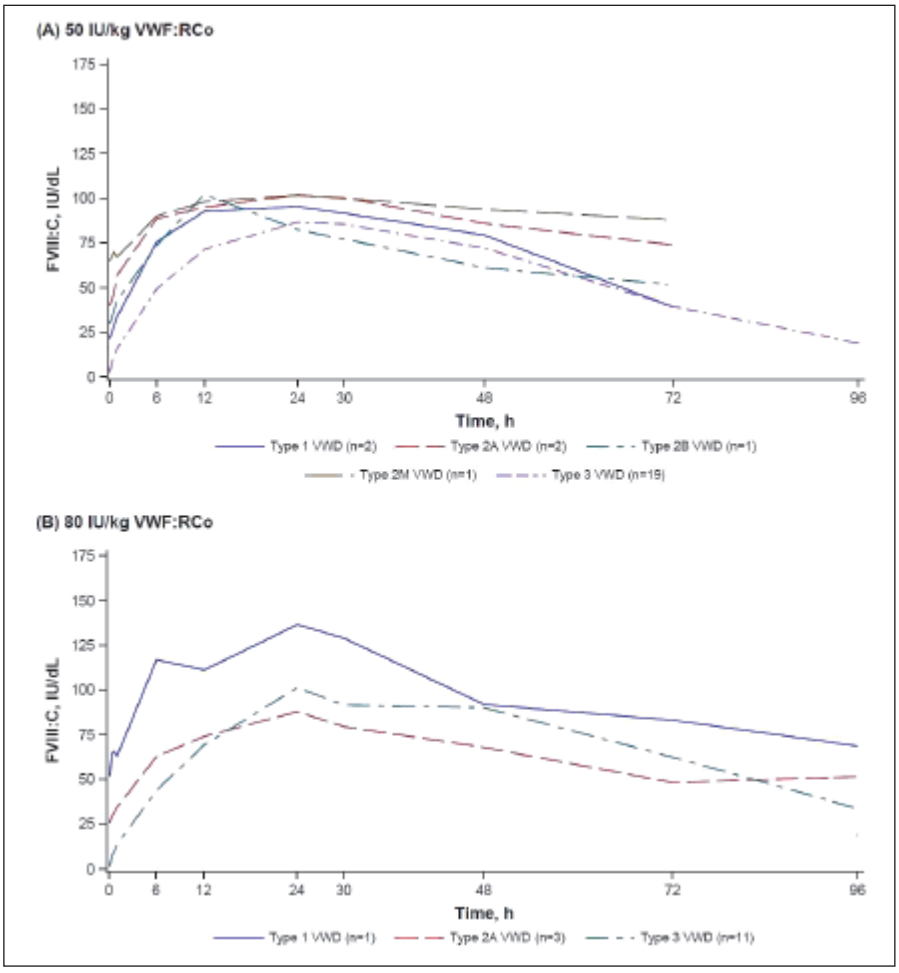

Fig. 1
Mean FVIII stabilization for patients with severe VWD who received rVWF alone. **A** 50IU/kg VWF:RCo **B** 80 IU/kg VWF:RCo

P 066 Platelet function analyzer measurement of closure time as a biomarker for activity of high and ultralarge multimers of recombinant von Willebrand factor (rVWF)

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Objectives: Recombinant von Willebrand factor (rVWF), licensed in the US under the brand name VONVENDI™, has the multimeric distribution of freshly secreted VWF with ultralarge (UL) and high molecular weight (HMW) multimers from endothelial cells and megakaryocytes since it has never been in contact with ADAMTS13 or any other proteolytic enzyme. The platelet function analyzer-200 (PFA-200) is highly sensitive in detecting UL-HMW VWF multimers. We hypothesized that (a) von Willebrand disease (VWD) patients´whole blood samples, spiked with rVWF shows a normalization in PFA closure time (PFA-CT) and (b) that a dose-response relationship could be demonstrated.
Methods: Twelve patients diagnosed with VWD were selected. A therapeutic dose of rVWF product (1 IU/ml) was spiked in VWD patients´ whole blood samples and PFA-CTs were measured. Further, PFA-CTs under incremental doses of rVWF (0.1, 0.2 and 0.5 IU/ml) were investigated.
Results: The PFA-CTs were normalized in VWD patients´ whole blood samples spiked with rVWF. Also, incremental doses of rVWF resulted in a progressive and dose-dependent correction of PFA-CT.
Conclusion: Spiking experiments of rVWF in VWD plasma indicate that the PFA-200 is a useful tool to detect rVWF activity. As the PFA-CT correction is dose dependent, rVWF might be reliably monitored with PFA-200 as a point-of-care analytical method during replacement therapy.
Disclosure: travel support (LFB, Novo Nordisk).

P 067 A homozygous large deletion of transcription factor TEAD4 is implicated in severe VWF deficiency

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Objectives: Genetic diagnostic tests including DNA sequencing and multiplex ligation-dependent probe amplification failed to reveal a causative mutation in VWF gene (VWF) of an index patient (IP) with type 3 von Willebrand disease. In this study, we aimed to elucidate disease genetics etiology through in-depth study of the patient-derived blood outgrowth endothelial cells (BOECs) as well as further genomic rearrangements exploration.
Methods: Von Willebrand factor (VWF) antigen levels in lysate and medium of BOECs isolated from the IP and healthy individuals were measured. Confocal immunofluorescent analysis of the BOECs was performed. The VWF-RNA transcript analysis and quantitative real time RT_PCR was done. Furthermore, array comparative genomic hybridization (aCGH) technique was applied to detect putative genome variations. The VWF promoter sequence was put into the transcription factor predictor TRANSFAC platform.
Results: The mean of total biosynthesis of VWF (sum of intracellular and secreted VWF) in patient-derived BOECs was considerably reduced compared with that of healthy donors (~6% vs. ~96%). Confocal immunofluorescent images showed that in spite of substantial reduction in intracellular VWF staining of patient-derived BOECs, small VWF storage bodies are still formed. RNA analysis assured undamaged biosynthesis of VWF transcript. However, the amount of VWF RNA was quantitatively reduced. The aCGH analysis revealed a homozygous large deletion in TEAD4 gene whose protein product was predicted to bind to VWF promoter at + 217 bp upstream from transcription start site.
Conclusion: Our results suggested that deleted TEAD4 is implicated in substantial reduction in VWF transcription and subsequently strong deficiency in VWF manufacture.
Disclosure: No significant relationships.

P 068 Quality of Life in women with von Willebrand Disease and menorrhagia depending on the Bleeding Score

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Objectives: Patients with VWD often present with menstrual bleeding events which may influence quality of life.
Methods: We systematically investigated the extent to which the bleeding score, laboratory parameters and menorrhagia correlates to the quality of life of affected patients with VWD. Patients were examined using a bleeding score questionnaire according to ISTH BAT, SF-36 and a menorrhagia questionnaire.
Results: This study enclosed 72 women (median 35.5 years, 20 – 49 years), of whom 52 were affected by type 1 VWD, 17 women type 2 VWD, and 3 women type 3 VWD. Menorrhagia was present in 81.9% of the patients. The hemoglobin level (median 13.1g/dl, 9.2g/dl – 15.7g/dl) and the ferritin level (median of 44ng/ml, 2ng/ml – 154ng/ml) did not show abnormalities. The bleeding score was increased with a median of 12 (3 – 34). There was a strong correlation between menorrhagia and ISTH BAT Bleeding (p=0.0063 [<35 years]; p=0.0162 [>35 years]). The Bleeding Score (p=0.0388 [<35 years]; p=0.0496 [>35 years]) and manifestation of menorrhagia (p=0.03 [type 1 VWD]; p=0.015 [vWF:Act <50%]) correlated with the physical quality of life. By multiple regression analysis the von Willebrand activity is the most crucial parameter concerning the Bleeding Score (p=0.075). Essential for the physical quality of life were the extent of menorrhagia, the Bleeding Score and the von Willebrand activity.
Conclusion: VWF activity seems to be an essential parameter for the prediction of the quality of life. Menorrhagia can be adequately estimated by a short questionnaire after de Wee et al. (2011).
Disclosure: The study has been sponsored by an unrestricted grant by CSL Behring.

P 069 Tolerability of a plasma-derived von Willebrand factor (VWF) concentrate (WILFACTIN/WILLFACT): more than 15 years of clinical development in patients with inherited von Willebrand disease

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Objectives: The von Willebrand disease (VWD) treatment objective is to correct the VWF deficiency and the secondary deficiency of factor VIII (FVIII). Because VWD patients have intact endogenous FVIII production, a purified plasma VWF concentrate almost devoid of FVIII has been specifically developed for VWD treatment when desmopressin is contraindicated or ineffective.The tolerability of this concentrate was assessed throughout 15 years of clinical development.
Methods: This analysis evaluates drug-related adverse events (AEs) reported during seven completed clinical studies conducted from January 1999 to August 2014 in patients treated with LFB's VWF concentrate.

Results: Cumulated data indicated that more than 49 million IU of VWF:RCo were administered in 226 patients on 16,640 treatment-days in all therapeutic situations (on-demand treatment and control of bleeding episodes, routine prophylaxis and perioperative management of bleeding). A total of 30 drug-related AEs were reported in 15 patients (6 females, 9 males) following 24 administrations. None were serious. Four AEs were device-related reactions (inflammation). Regarding the reactions of special interest for this class of product, no neutralising antibodies, thromboembolic events or virus transmissions were reported. There were 26 AEs with symptoms indicating potential allergic reactions in 11 patients (4.9%) following 0.11% of injections (20/17664 injections). Most symptoms resolved within 15 minutes, were isolated and did not reoccur following subsequent injections. All the 30 drug-related AEs were mild to moderate in intensity and resolved without sequelae.

Conclusion: This assessment based on significant exposure during clinical studies indicates that WILFACTIN®/WILLFACT® was well tolerated in all clinical situations with few mild/moderate, reversible adverse reactions.

Disclosure: All authors are employed or subcontracted by LFB.

Poster

Rare bleeding disorders

P 070 Characterization of patients with a mild to moderate bleeding phenotype: results of the Vienna Bleeding Biobank (VIBB)

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Objectives: Mild bleeding disorders (MBDs) find expression in various clinical phenotypes. Only an exact diagnosis allows targeted treatment. We established the Vienna Bleeding Biobank to characterize and thoroughly investigate Austrian patients with MBDs.

Methods: To quantify the severity of bleeding, a standardized bleeding score (BS) was used (Rodeghiero et al., JTH, 2005). Routine coagulation tests were performed, and von Willebrand factor (VWF), single clotting factors, and platelet function, were determined.

Results: Five hundred twenty-eight patients (81.3% female) were included. A diagnosis of an established bleeding disorder was made in 156 patients (29.5%) (▶Table 1). A large part of the patient population (372 patients, 70.3%) showed normal test results and were categorized as patients with bleeding of unknown cause (BUC). The median BS [Interquartile range (IQR)] was 5 [3–7] in patients with and those without a definite or assumed diagnosis of a bleeding disorder. There was also no difference in parameters like age (median 37 [28–51] and 41 [29–54]) and BMI (median 23.3 [21.6–26.1] and 23.3 [21.0–26.5]). A diagnosis of a bleeding disorder was more frequently made in men than in women (49.5% versus 24.9%). Male sex (OR 2.95, 95%CI:1.88–4.63; p<0.001) and blood group 0 (OR 1.72, 95%CI:1.18–2.51; p=0.005) were independently associated with the diagnosis of a bleeding disorder.

Conclusion: Over 70% of patients had BUC. The severity of bleeding, however, was similar between patients with a diagnosis and those without. Men were more frequently diagnosed with a bleeding disorder than women. Further mechanisms responsible for MBDs still have to be identified.

Disclosure: No significant relationships.

Tab. 1 Diagnoses of patients with mild bleeding disorders

Diagnosis	n	%
Bleeding of unknown cause	372	70,3
Low VWF (VWF 30–50%)	32	6,1
Definite VWD (VWF < 30%)	11	2,1
Possible PFD*	55	10,4
Definite PFD†	33	6,3
FVIII deficiency (FVIII ≤ 50%)	11	2,1
FIX deficiency (FIX ≤ 50%)	5	0,9
mild FXI deficiency (FXI ≤ 60%)	3	0,6
FXIII deficiency (FXIII ≤ 10%)	1	0,2
Possible PFD+ FXI deficiency	2	0,4
Possible PFD + Low VWF	1	0,2
Hypo-/Dysfibrinogenemia‡	2	0,4
Total	528	100

* Abnormal platelet function tests at one time point (or only 1 assessment available) †Repeatedly abnormal platelet function tests ‡One patient with confirmed, one patient with suspected dysfibrinogenemia; VWF, von Willebrand Factor; PFD, platelet function disorder; FVIII, Factor VIII; FIX, Factor IX; FXI, Factor XI; FXIII, Factor XIII

P 071 Efficacy, safety and pharmacokinetic studies of a triply secured fibrinogen concentrate (FibCLOT) in children with congenital fibrinogen deficiency

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Objectives: A triply secured fibrinogen concentrate (FibCLOT®) has been shown to be effective and well tolerated in afibrinogenaemic adults. Treatment in children was investigated in this study.

Methods: An international, open-label study investigated the pharmacokinetic, efficacy and safety of FibCLOT® for the treatment of bleeding episodes and prevention of excessive bleeding during surgery in afibrinogenaemic children. Recovery-guided dosing was performed to obtain optimal plasma fibrinogen concentrations.

Results: Sixteen patients with afibrinogenaemia (1–12 years) including 8 children ≤6 years were enrolled. Incremental Recovery (IR) was lower in children (n=12) with a geometric mean at 19.1 compared to 23.3 (g/L)/(g/kg) in adults/adolescents. Eleven children were treated on-demand with a median infusion dose of 0.090 g/kg [range 0.028–0.132] for 17 major and 0.070 g/kg [range 0.039–0.088] for 15 minor bleeding episodes. Physicians' global assessment of response to FibCLOT treatment was excellent or good for 97% of episodes. Ten children underwent 11 surgical procedures. Six circumcisions including 1 orchidopexy were in ≤6 years group and other procedures, all dental extractions were in >6 years group. Haemostasis was rated as excel-

lent in all procedures. Most of the surgical or bleeding events (91%, 39/43) were managed with a single dose of FibCLOT. Median fibrinogen level after first FibCLOT administration was 1.37 g/L for the control of bleeding and 0.97 g/L before surgery. No serious adverse reactions were observed.
Conclusion: These results provide clinical evidence for the efficacy and safety of FibCLOT® in paediatric afibrinogenaemic patients ≤12 years. IR was lower compared to adults and adolescents.
Disclosure: A Dahmane, S Pujol, C Henriet, W Stevens and Françoise Bridey are employed by LFB. C Djambas Khayat , M El Khorassani, S Aytac and C Rothschild acted as principal investigators for the study received honoraria for conducting the research. E fuseau is sub

P 072 Utility of the ISTH bleeding assessment tool in patients with suspected platelet function disorders

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Objectives: Bleeding assessment tools (BAT) have been widely implemented in the work-up of patients with suspected bleeding disorders. However, the utility regarding inherited platelet function disorders (PFD) is still elusive. We aimed to assess the diagnostic value of the ISTH BAT in clinical practice retrospectively.
Methods: Clinical characteristics and laboratory data of all consecutive patients referred between 01/2012 and 03/2017 to an outpatient unit of a university hospital with a suspected bleeding disorder were collected. The diagnostic work-up was done according to current guidelines and platelet function was tested using LTA as well as flow cytometry.
Results: 555 patients were assessed, 68.1% were female, median age was 41.7 years (inter-quartile range [IQR] 28.0, 60.7). PFD was diagnosed in 48 patients (8.7%), possible PFD in 70 patients (12.6%), von Willebrand disease or low von Willebrand factor associated with blood group 0 in 43 patients (7.8%), other coagulation disorders in 39 patients (7.0%) and other disorders in 38 patients (6.9%). Median scoring of the ISTH BAT was 2 in patients without bleeding disorder (IQR 1, 4), 4 in patients with possible PFD (3, 7), and 7 in patients with PFD (5, 9; p<0.0001; Mann-Whitney U test). Area under the ROC curve was 0.80 (95% CI 0.74, 0.86). At a threshold of 4, sensitivity was 87.5% (95% CI 74.8, 95.3) and specificity 55.8% (95% CI 51.4, 60.2).
Conclusion: Presence of a PFD was associated with relevantly higher BAT scorings compared to patients without an identifiable disorder. Our data suggest that BAT might be a useful screening tool for PFD.
Disclosure: No significant relationships.

P 073 A study on selected mutations and severity of clinical presentation in patients with factor V deficiency in Iran

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Objectives: Factor V deficiency (FVD) is a rare bleeding disorder (RBD) mostly present in regions with high rate of consanguinity. FVD after FXIII deficiency is the next more prevalent RBD in Sistan and Baluchistan in south east of Iran. The aim of this study is to evaluate clinical manifestations and severity of bleeding diathesis in patients with FVD. We also assessed the prevalence of two previously described mutations of F5 gene (IVS8+6T >C and IVS19+3A→T) in Iranian population.
Methods: This descriptive study was conducted on 23 patients with FVD in Sistan and Baluchistan province. In this study FVD was diagnosed by clinical findings and routine laboratory tests. Bleeding diatheses were classified into three grades depending on the severity of symptoms grade I, II and III. The Severity of bleeding episodes in our patients was compared with other RBDs. We also evaluated our patients for two above mentioned FV gene mutations.
Results: Based on residual plasma FV activity 6 (26%), 16 (69.5%) and 1(4.5%) patients had mild, moderate and severe factor deficiency, respectively. Twenty seven percent of patients had grade III life threatening bleeding episodes .grade II and grade I bleeding diathesis were observed in 52.5% and 17.5% of patients, respectively. Both *FV gene mutations* had been negative in all 23 patients.
Conclusion: We concluded that FVD is the second most common type of RBDs in this province and grade II bleeding episodes were the major bleeding presentation.
Disclosure: No significant relationships.

P 074 GGCX mutations identified in VKCFD1 patients show different γ-carboxylation pattern for different vitamin K dependent proteins

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Objectives: Vitamin K Dependent Coagulation Factor Deficiency type 1 (VKCFD1) is a rare hereditary bleeding disorder caused by mutations in γ-glutamyl carboxylase (GGCX) which is often characterized by additional non-bleeding phenotypes including skin hyperlaxity or facial hypoplasia. GGCX γ-carboxylates vitamin K dependent (VKD) proteins. The aim of this study is to characterize the effect of GGCX mutations on VKD proteins to evaluate effective dose of vitamin K (K) needed for treatment.
Methods: A GGCX knockout HEK293T cell line was generated by CRISPR/Cas9 technology. The cDNAs of GGCX together with F2, Growth Arrest Specific –6 (Gas6) or Osteocalcin (BGLAP) were cloned into a bicistronic vector. Cells were transfected with GGCX wild-type and mutants and treated with different K concentrations to determine γ-carboxylation by ELISA.
Results: Elevated K concentration increases γ-carboxylation of F2, BGLAP, and Gas6 for S284P and W493S, whereas S300F shows no recovery. Certain mutants show differential effect on γ-carboxylation for different VKD proteins as H404P and T591K can restore γ-carboxylation for F2 but not for BGLAP and Gas6, which is vice versa for R485P.
Conclusion: Our data suggests that patients harboring S284P will show reversible coagulation (and non-coagulation) phenotypes where therapy with K will lead to normal coagulation. Residual clotting factor activities can be achieved for H404P, W493S, T591K. Patients with mutation S300F will never reach physiological coagulation and γ-carboxylation of other VKD proteins indicating that vitamin K binding or catalytic activity is abolished. Non-coagulation phenotypes as observed for H404P and T591K can be potentially explained by reduced γ-carboxylation of BGLAP or Gas6.
Disclosure: No significant relationships.

Poster

P 075 Functional diversity of structural disulfide bonds in Coagulation Factor XIII B subunit

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Objectives: Coagulation Factor XIIIB (FXIIIB) is the protective/regulatory partner of the catalytic Factor XIIIA (FXIIIA) subunit. It circulates in plasma in both free ($FXIIIB_2$) and bound forms (zymogenic $FXIIIA_2B_2$). A monomeric FXIIIB subunit is composed of 10 repititive sushi domains, each held together by a pair of disulfide bonds at its N- and C-terminals. Three out of eight missense mutations detected in the *F13B* gene from homozygous/heterozygous FXIII deficient individuals occur on these Cysteines and result in secretory, folding and protein-protein interaction defects. We aim to ablate individually all 20 disulfide bonds of FXIIIB subunit and to express them heterologously to study impact on protein structure and function.
Methods: Mammalian expression vectors carrying *F13B* cDNA were mutated individually at 20 Cysteines to Alanine. The 20 mutated variants and wild type were transiently transfected to *HEK293T* cells and the transfection products collected. Immunostaining, BN-PAGE, ELISA, mutant purification and Size-exclusion-chromatography (SEC) was performed from transfection products to analyze the specific functional effect of each mutation. Effect on FXIIIA activation was analyzed by spiking FXIIIAa-generation assay with purified mutants.
Results: Twelve out of 20 mutants could be expressed and purified. Co-Immunostaining/ELISA reveals differential intracellular retention. BN-PAGE/SEC reveals differential oligomeric states of mutant FXIIIB. Generation assay shows differential impact of mutations on FXIIIA activation.
Conclusion: Disrupting specific sushi domain disulfide bond influences specific structural-functional aspect of FXIIIB protein. Clearly, although the nature of all FXIIIB disulfide bonds is uniformly structural, they do show functional diversity with respect to the whole protein.
Disclosure: No significant relationships.

P 076 FXI deficiency: to clot or not to clot?

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Objectives: In FXI deficiency the risk of bleeding is not predictable since it does not correlate with FXI levels. It is known that lower fibrin network density and stability are associated with bleeding phenotype in FXI deficient patients. Thrombodynamics assay (TD) is a new global haemostasis test that provides information on thrombin generation (TG), clot formation and fibrinolysis. Our aim was to correlate TD parameters with the clinical phenotype of FXI deficient patients.
Methods: TD assay is performed in platelet free plasma upon initial activation with tissue factor (TF) immobilized on a plastic surface. Different parameters describing TG, clot formation, and fibrinolysis are calculated. Thus far, we analyzed plasma from two patients with FXI deficiency, exhibiting different phenotypes: a non-bleeder (patient 1, FXI 40%), and a thrombophilic (patient 2, FXI 5%). Healthy donor plasmas (n=22) were used as control.
Results: In patient 1 the initiation phase of coagulation was normal, the amplification phase was slightly decreased, and clot formation was normal. In patient 2 the initiation phase was normal, the amplification phase was enhanced and clot formation was increased with the appearance of spontaneous clots, despite a FXI at 5% and no detectable laboratory thrombophilia.
Conclusion: Based on our preliminary results TD analysis seems to be a useful tool to describe FXI clinical phenotype. We are currently extending our investigations to FXI deficient patients with bleeding phenotypes.
Disclosure: No significant relationships.

Treatment of bleedings disorders

P 077 Comparison of the kinetic and haemostatic properties of a new specific plasmin inhibitor and known antifibrinolytic agents

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Objectives: Antifibrinolytics – ε-aminocaproic acid (ACA) and tranexamic acid (TXA) are used to stop the bleeding in surgery, trauma, and other medical practice. **Aims.** Comparison of kinetic and hemostatic properties of ACA, TXA and a new specific inhibitor of plasmin Ac-Ala-Phe-Lys-Pip (AFK).
Methods: Inhibition of plasmin, urokinase (Uk) and tissue plasminogen activator (tPA) activities by the antifibrinolytics was studied using their specific substrates. Haemostatic effect of AFK, ACA, TXA and saline solution was compared in a model of liver parenchymal bleeding of anesthetized rats (4 groups of 6 animals).
Results: AFK much more inhibited the plasmin activity (K_i 0.25±0.02 mM), than ACA (K_i 58±3 mM) and TXA (K_i 23±2 mM). AFK inhibited Uk activity (K_i 0.206±0.007 mM), but not tPA. In contrast to the ACA and TXA, AFK did not inhibit the plasminogen activation by Uk or tPA. Time before bleeding arrest was 217±25, 221±41, 216±25 and 303±24 s for TXA, ACA, AFK and saline solution, respectively. The blood weight loss was 1.81±0.3, 1.93±0.28, 1.91±0.11 and 2.17±0.46 g for TXA, ACA, AFK and saline solution, respectively. Comparative toxicity of TXA, ACA and AFK (LD_{50}) in mice and rats was 1.3, 3.3 and 6.5 g/kg, respectively.
Conclusion: ACA and TXA inhibit mainly the plasminogen activation and, partially, plasmin, while haemostatic action of AFK is associated with the direct blocking of active sites of plasmin and Uk. The toxicity of AFK is lower in 2–5 times, and its hemostatic efficacy is comparable to the efficiency of clinically used ACA and TXA.
Disclosure: No significant relationships.

P 078 Biochemical characterization, stability, and pathogen safety of a new fibrinogen concentrate (Fibryga®)

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Objectives: Fibryga®, a new lyophilized, plasma derived fibrinogen concentrate for treatment of congenital fibrinogen deficiency, was investigated.
Methods: Functional characterization of the final product was performed by activity measurement, cross-linking studies and thromboelastometry. Purity and integrity of the fibrinogen molecule was demonstrated by size exclusion chromatography and identification and quantitation of co-purified plasma

proteins and activation makers. Pathogen safety of Fibryga® was proven by downscaling studies for the two dedicated pathogen inactivation/removal steps, i.e. solvent detergent treatment and nanofiltration using filters with a pore size of 20 nm.
Results: the final product is stable for at least 36 months at room temperature fast reconstitution within less than five minutes when using the Octajet device high specific activity and nearly 1:1 ratio in fibrinogen activity to antigen only traces of accompanying proteins, high molecular weight proteins and activation markers were detected almost complete γ-γ chain formation was shown by crosslinking experiments *in vitro* efficacious clot formation was proven using the thromboelastometric tests FIBTEM, EXTEM and NATEM in whole blood (ex-vivo) effective global pathogen reduction factors were ≥ 9.04 $\log_{10}$ (HIV-1), ≥ 13.15 $\log_{10}$ (PRV), ≥ 10.77 $\log_{10}$ (BVDV), ≥ 5.21 $\log_{10}$ (HAV), 4.53 $\log_{10}$ (PPV) and [3] 4.39 $\log_{10}$ (PrP^{Sc} HAS 263K).
Conclusion: Fibryga® meets the requirements for a state-of-the-art fibrinogen concentrate, by displaying a satisfactory activity profile combined with a favorable pathogen safety profile and stability.
Disclosure: All authors are employees of Octapharma

P 079 Assessing the use of fibrinogen-binding Affimer proteins to modify clot formation and prolong lysis

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Objectives: Affimer proteins consist of two randomised nine amino acid peptides attached to a scaffold. We hypothesise that fibrinogen Affimer binder can be used to modify clot formation and lysis and their use as an antifibrinolytic therapy may provide a novel approach to target bleeding disorders.
Methods: Turbidimetric assays were used to assess the effect of Affimer binder F9b on fibrin clot formation and lysis. The use of the combination of two binders F9b and E6b was also tested. Confocal laser scanning microscopy was used to determine the impact of F9b on the fibrin clot structure. SDS PAGE was used to confirm the purity of the Affimer protein used.
Results: In human plasma, Affimer binder F9b increased the maximum OD from 0.197±0.006 AU (mean±SD) to 0.415±0.05 AU ($p<0.008$) and decreased the lag time of plasma clot formation from 388±30 seconds to 170±19 seconds ($p<0.001$) at a molar ratio of 5:1 (binder:fibrinogen). Moreover, prolongation of clot lysis was found in the presence of F9b. Similar results were observed in purified fibrinogen systems and mouse plasma, which were enhanced further when both E6b and F9b were present. Confocal microscopy revealed dramatic changes to the clot structure with dense bundles of fibrin and large pores caused by F9b.
Conclusion: Fibrinogen-binding Affimer protein F9b alters clot structure and prolongs fibrinolysis. Certain Affimer binders, when used in combination may cause an additive effect on clot formation and lysis. Specific Affimer binders may be used as tools for the modulation of clot structure and lysis.
Disclosure: No significant relationships.

Bleeding disorders, coagulation and fibrinolytic factors / Other related topics

P 080 Genetic Analysis of PMM2 (Phosphomannomutase-2) in patients with suspected Anithrombin defects

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Objectives: *PMM2* mutations are the main causes of congenital disorders of glycosylation type I (CDG-I) which leads to defects in the N-glycosylation pathway. CDG-I is an autosomal recessive disorder with heterogeneous symptoms. In some cases patients show a deficiency of anticoagulation and procoagulation factors and bleeding or thrombotic events respectively. Our aim was to analyse molecular defects in *PMM2* in patients with suspected Antithrombin defects, without mutations in the *SERPINC1* gene itself.
Methods: In 135 patients PMM2 gene was analysed by Sanger Sequencing, after previous exclusion of *SERPINC1* mutations. The identified variants were classified into previously described CDG-I causing mutations with known impact on PMM2 function and unknown mutations.
Results: In 23 of 135 patients mutations *PMM2* mutations were detected. Altogether, 1 unknown and 7 already described heterozygous variants of *PMM2* were identified. The unknown genetic modification is a potential regulatory variant in the 5' untranslated region of the *PMM2* gene. The previously described variants consist of 1 Nonsense mutation and 6 Missense mutations. Missense mutations were mapped onto 3-D-structure of Phosphomannomutase-2 and showed an impact on the protein.
Conclusion: *PMM2* mutations seem to be a cause of Antithrombin deficiency due to the influence on the glycosylation state of the protein and furthermore a risk factor for thrombotic events. The mechanisms and extent of the effect on Antithrombin glycosylation as well as the subsequent influence on the function of Antithrombin has to be further elucidated.
Disclosure: No significant relationships.

Pediatric and neonatal thrombosis and hemostasis

P 081 Management of moderate factor XI deficiency in dental extraction: A case report

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Objectives: Factor XI deficiency is a rare autosomal recessive bleeding disorder which can cause severe bleeding in patients requiring surgery. Bleeding symptoms and their intensity can widely vary and do not always correlate with factor levels. This can complicate management of surgery in patients with factor XI deficiency. Data guiding the management of factor XI deficiency in patients undergoing dental extraction is rare.
Methods: We report on a 17 year old girl with moderate factor XI deficiency (5%). Monthly menstruation is well controlled with tranexamic acid. Contemporary she was admitted for dental extraction of 4 teeth. One hour before tooth extraction she was substituted with 1000IE Hemoleven® (LFB Pharma) (18IE/kg). 24 hours later factor Xl level was 21%. In the further course no more substitution was necessary. Additionally she took tranexamic acid 3 x 1000 mg for 7 days after the operation.
Results: Using this therapy there was neither a bleeding nor a thrombosis and the patient could be discharged from hospital after 2 days.

Conclusion: Factor XI concentrate (Hemoleven®) is safe in the context of dental extraction. The substitution depends on the initial factor level. Proposed factor XI peak level is 30–40%, with 1 IE/kg Hemoleven increasing the factor level up to 2%. In our patient 18 IE/kg was enough. Regular monitoring of factor XI levels is important, especially before a second substitution because of its high thrombogenicity. In our patient with moderate factor XI deficiency we were able to maintain a normal perioperative haemostasis with only one substitution of factor XI.
Disclosure: No significant relationships.

P 082 Different clinical manifestation of mild hypofibrinogenemia

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Objectives: Patients with hypofibrinogenemia and fibrinogen levels of > 0.5g/l are reportedly asymptomatic or have a mild bleeding phenotype. Menorrhagia, intramuscular hemorrhage and gastrointestinal bleeding are most common.
Methods: Here, we report 3 families with mild hypofibrinogenemia and very different clinical manifestations.
Results: Family 1: The mother and 2 sons have fibrinogen levels around 0.8–1.0 g/l. Both boys are suffering from congenital heart disease (CHD). The mother and the older son suffer from bleedings. The mother has in addition a possible von Willebrand Syndrome with von Willebrand parameters in the lower limit. The older son has in addition an intermittent platelet dysfunction. He had various bleedings. The younger brother has up to now no bleedings. Family 2: The 17 years old boy has a fibrinogen between 0.9 – 1.2 g/dl and a platelet dysfunction. He had a bleeding after adenoidectomy. Bleeding occurred after surgery for a sinus pilonidalis and poor wound healing was observed. The older brother and the father had epistaxis, the mother easy bruising. They have fibrinogen in the normal range. Family 3: All 3 children have fibrinogen around 1.3 and 1.5g/dl. The parents were not evaluated. Two girls of the family showed, in addition, von Willebrand parameters in the lower limit. One girl is suffering from poor wound healing, even of minor wounds. The other girl and a brother have non problems.
Conclusion: The bleeding symptoms of hypofibrinogenemia were very heterogeneous even in the same family. Additional risk factors seem to be crucial. When anticoagulation is required unexpected severe bleeding can occur.
Disclosure: No significant relationships.

P 083 Sepsis-like Viral Infection and Cerebral Microembolism by Human Parechovirus Typ 3 in a Newborn

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Objectives: We report on a newborn who after uncomplicated birth and postnatal appearance on day 8 post natum was admitted to hospital with fever and sepsis-like illness.
Methods: Procalcitonin was elevated, but physical examination, chest X-ray, abdominal ultrasound, echocardiography and spinal tap at first revealed no focus of infection. After further laboratory examinations with D-dimers elevated up to 6535 µg/l, prothrombin time reduced to 43 %, PTT prolonged to 87 sec and coagulation factor IX reduced to 12 %, cranial MRI was performed in search for intracranial hemorrhage or sinus venous thrombosis, but instead showed ischemic areas in the white matter.
Results: By use of multiplex-PCR human parechovirus type 3 could be detected in the patient´s CSF as well as in the feces of the parents. FFP was applicated and anticoagulation with unfractionated heparin was started and continued for 3 months, resulting in an excellent clinical outcome.
Conclusion: We postulate para-infectious vasculitis leading to cerebral microembolism as the most probable cause of ischemia in this patient. We recommend to look for human parechovirus type 3 in children with sepsis-like illness and signs of disseminated intravascular coagulation, even if CSF cell count is normal.
Disclosure: No significant relationships.

Platelets

P 084 Plasma ADAMTS13 Activity in Chronic Thromboembolic Pulmonary Hypertension

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Objectives: Deficiency of ADAMTS13 activity leads to von Willebrand factor giant multimers with high affinity for platelets and high thrombotic risk. Because elevated levels of vWF are associated with thrombosis, we hypothesized that ADAMTS13 activity is involved in major vessel thrombosis of pulmonary hypertension. Therefore, we determined ADAMTS13 activity in non-thromboembolic pulmonary arterial hypertension (PAH), and in chronic thromboembolic pulmonary hypertension (CTEPH).
Methods: ADAMTS13 activity was measured in a kinetic assay using the fluorescence resonance energy transfer substrate VWF 73, and ADAMTS13 concentration was measured in an enzyme-linked immunosorbent assay. Plasma samples of 89 patients (mean age 55±14 years) were obtained at time of diagnosis.
Results: 45 patients (51%) were diagnosed with CTEPH, 36 patients (40%) with PAH, and 8 (9%) patients with pulmonary hypertension (PH) due to lung disease and/or hypoxia. ADAMTS13 activity and concentration showed no significant difference between patients with CTEPH (0.97±0.26U/mL; 0.59±0.17µg/mL), PAH (0.98±0.29U/mL; 0.58±0.21µg/mL) and PH (0.80±0.22U/mL; 0.54±0.17µg/mL). However, a significant reduction of ADAMTS13 activity and concentration was found in patients with PAH associated with CTD (0.77±0.27U/mL; 0.46±0.22µg/mL) compared to patients with idiopathic PAH (1.06±0.27U/mL, P≤0.01; 0.62±0.19µg/mL, P≤0.05). Gel-based analysis of the size of vWF multimers was in accordance with these findings.
Conclusion: No significant difference of ADAMTS13 activity and concentration was observed in plasma levels of patients with CTEPH compared to PAH or PH. However significantly reduced ADAMTS13 activity and concentration were found in patients with PAH associated with CTD, and PH compared to patients with idiopathic PAH.
Disclosure: No significant relationships.

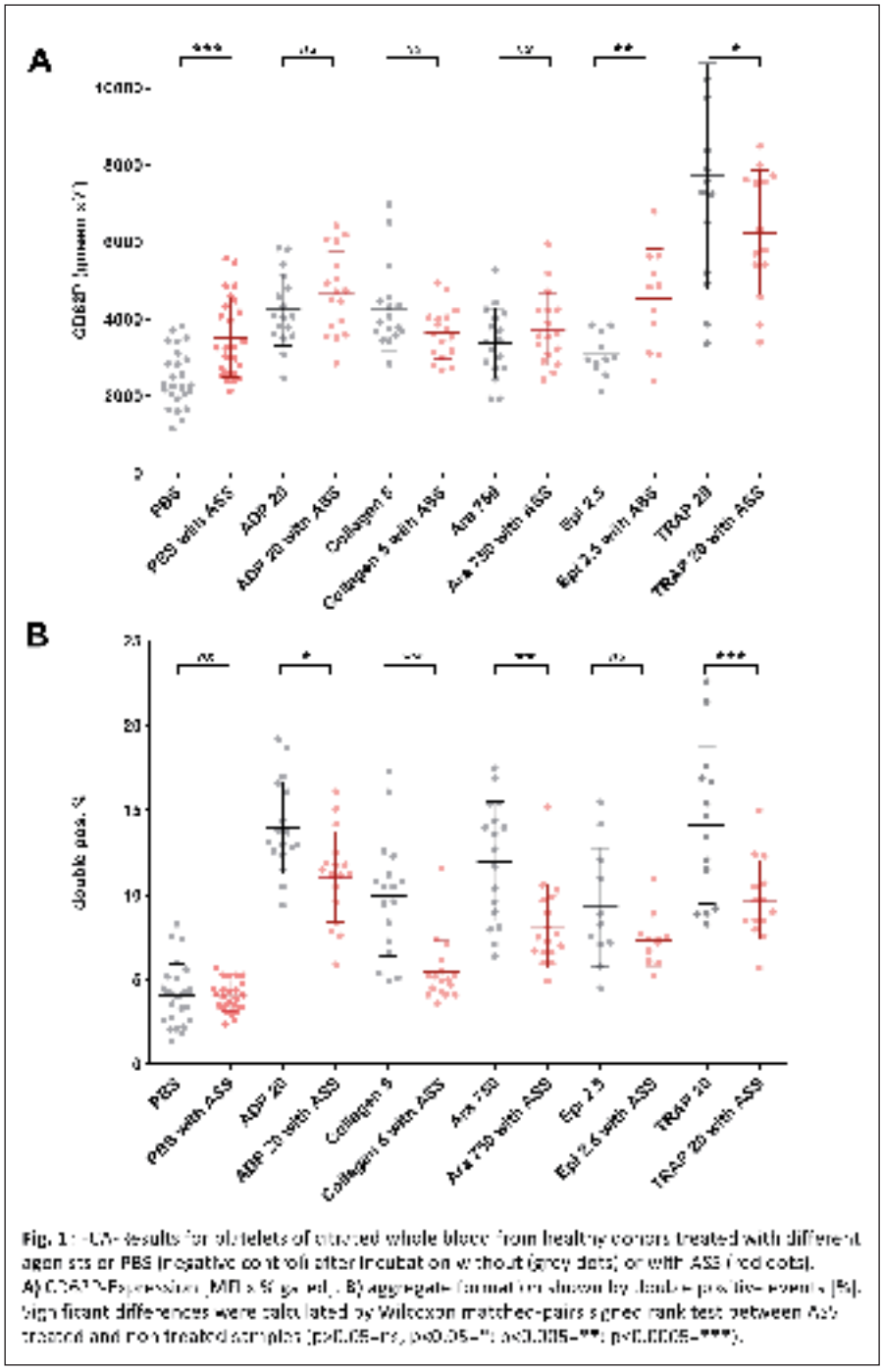

Fig. 1
FCA-Results for platelets of citrated whole blood form healthy donors treated with different agonists or PBS (negative control) after incubation without (grey dots) or with ASS (red dots). A) CD62P-Expression [MFI x % gated]. B) aggregate formation shown by double positive events [%]. Significant differences were calculated by Wilcoxon matched-pairs signed rank test between ASS treated and non treated samples. (p>0.05=ns; p<0.05=*; p<0.005=**; p<0.0005=***).

Fig. 1 FCA-Results for platelets of citrated whole blood from healthy donors treated with different agonists or PBS (negative control) after incubation without (grey dots) or with ASS (red dots). A) CD62P-Expression [MFI x % gated]. B) aggregate formation shown by double positive events [%]. Significant differences were calculated by Wilcoxon matched-pairs signed rank test between ASS treated and non treated samples (p>0.05=ns; p<0.05=*; p<0.005=**; p<0.0005=***).

P 085 Evaluation of a flow-cytometer based platelet aggregation assay to test platelet inhibition by acetylsalicylic acid in small volume blood samples

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Objectives: Platelet aggregation assays require high volumes of platelet rich plasma or whole blood. Sometimes only small sample volumes are available for platelet function testing. A low volume flow cytometer-based aggregation assay was introduced by De Cuyper et al. (2013). We evaluated this assay with blood from healthy donors treated with or without acetylsalicylic acid (ASS) for its differences in aggregation and P-selectin (CD62P) expression.

Methods: 200µL citrated whole blood (pre-incubated with or without 50mg/mL ASS for 10min) was aliquoted into two 100µL tubes. Platelets were stained with CD31-FITC or -PE, respectively. Samples were washed twice, resuspended in 100µL hirudinized buffer (PBS with Ca/Mg, containing 20% normal plasma) and mixed. CD62P-AF647 was added and 20µL of each sample were incubated with ADP (20µM), TRAP (20µM), arachidonic acid

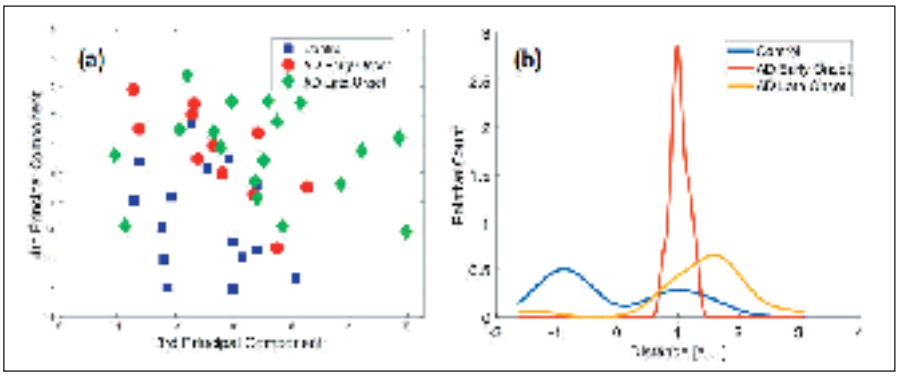

Fig. 1

(750µM), epinephrine (2.5µM), collagen (5µg/mL) or buffer. Platelet activation was stopped using 0.5% paraformaldehyde after 3min. FITC- and PE-double positive events (=aggregate formation) and CD62P-expression were analyzed by flow cytometry.

Results: Comparing platelets treated with and without ASS, CD62P-expression was not decreased using ADP, collagen, arachidonic acid or epinephrine (▶Figure 1) but only using TRAP. Contrary, the aggregate formation was significantly decreased using ADP, collagen, arachidonic acid and TRAP but not with epinephrine. The negative control revealed an activation of CD62P when adding ASS but no aggregate formation.

Conclusion: While CD62P-expression is not affected by ASS, this aggregation method might be helpful to detect ASS intake from patients when only small blood volumes are available.

Disclosure: No significant relationships.

P 086 Multivariate Analysis of Platelet Shape, Blood-, Aggregation- and Flow Cytometry-Markers Reveals Difference Between Controls, Early and Late Onset Alzheimers Disease

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Objectives: The current study investigates the possibility to differentiate early and late onset Alzheimers Diseases (AD) patients from each other and from a healthy control population by employing a combined multivariate analysis of platelet shape data, patient blood count as well as aggregation- and flow cytometry markers.

Methods: A total of 47 markers for geometrical shape change [1], platelet aggregation, flow cytometry and standard blood count were obtained in age-matched groups of 15 healthy volunteers, 11 early onset and 19 late onset AD patients. Principal components (PC) were determined from those markers and components without a significant contrast between each of the three groups were removed. Employing the remaining PCs, the signed Euclidean distance (dE) of each individual datapoint (control, early/late onset AD) from the centre-of-gravity of the control group was determined and plotted for each of the three groups.

Results: ▶Fig. 1a shows the scatter between the 3rd and 4th PC. The distribution of dE is shown in ▶Fig. 1b. A clear difference between the groups is observed, with a small overlap between controls and late onset AD patients. The early onset patients are somewhere "in between" the two other groups.

Conclusion: A difference between controls, early/late onset AD patients was observed using a multivariate analysis approach of platelet data and clinical blood count. [1] Kraus, et al. Platelets (11); 2013.

Disclosure: No significant relationships.

P 087 Natriuretic peptides weaken platelet aggregation in patients with ST-segment elevation myocardial infarction

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Objectives: Cyclic guanosine-3',5'-monophosphate (cGMP) is a key second messenger for both nitric oxide and natriuretic peptides (NP) cardiovascular effects. Particulate guanylyl cyclase (pGC) is a second guanylyl cyclase enzyme to control cGMP synthesis, which is known to be activated by NP. There is certain gap in evidence supporting NP role in regulating platelet function. The role of NP-pGC-cGMP in platelets under pharmacological suppression is controversial.

Methods: We studied on-treatment platelet aggregation and secretion induced by ADP (10 mcM) and collagen (2 mcg/mL) in 80 male patients with ST segment elevation myocardial infarction (STEMI), on admission to the ICU and on the 7th day. Antiplatelet therapy consisted of aspirin and ticagrelor in standard regimen. Platelet function was also assessed with a brain natriuretic peptide (BNP) preincubation in STEMI patients and practically healthy volunteers (PHV).

Results: In patients with BNP > 100 pg/mL ADP induced aggregation was significantly lower than in those with BNP < 100 pg/mL ($p = 0.019$). ADP-induced aggregation in patients with BNP > 100 pg/L was also significantly slower ($p = 0.013$). Pharmacodynamic profiles of prescribed antiplatelet therapy differs in patients with high and low BNP levels. Preincubation with a BNP solution resulted in a significant decrease in aggregation parameters in PHV and STEMI patients ($p < 0.05$). No changes in dense granules secretion was detected in patients with high and low BNP levels, and after BNP preincubation.

Conclusion: Received results support the suggestion that natriuretic peptides play a noticeable role in the regulation of platelet function including modification of antiplatelet agents' pharmacodynamics.

Disclosure: No significant relationships.

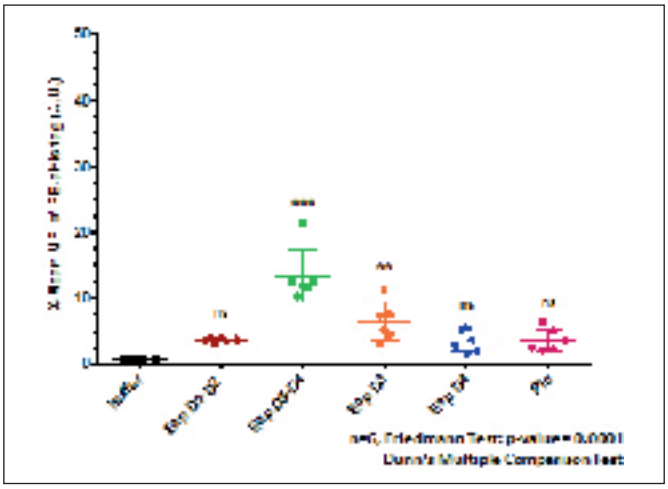

Fig. 1 Binding of Eap domains to platelets (MFI)

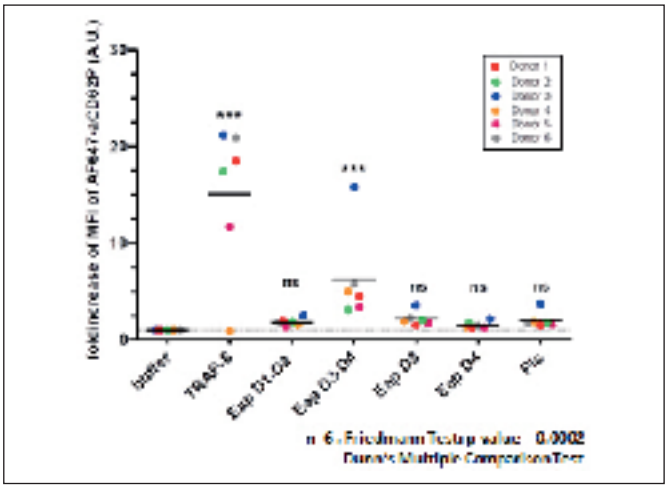

Fig. 2 CD62P expression (fold increase)

P 088 Serum sphingosine-1-phosphate is decreased in subjects taking aspirin: Association analyses with blood cell-related parameters in the Study of Health in Pomerania cohort

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Objectives: The platelet-derived sphingosine-1-phosphate (S1P) is a bioactive signaling lipid involved in numerous biological processes such as inflammatory and immunomodulatory responses as well as vascular homeostasis. The secretion of S1P from platelets is dependent on the formation of thromboxane and subsequent activation of the thromboxane receptor. S1P does not only play a role as mediator but also as a biomarker for cardiovascular pathologies as altered concentrations are associated with coronary artery and peripheral vascular diseases. Recently, we determined a reference range for serum S1P (0.534–1.242 µmol/L) in individuals without cardiometabolic disorders. The present study aims to identify associations between individual S1P concentrations and blood cell-related parameters and the impact of platelet aggregation inhibitors, e.g. aspirin, on serum S1P levels.
Methods: We determined serum S1P concentrations in 4194 participants of the Study of Health in Pomerania (SHIP)-TREND cohort using liquid chromatography-tandem mass spectrometry and performed association analyses, e.g. analyses of variance and linear regression models, with respective blood cell-related parameters and medication data.
Results: S1P concentrations were significantly associated with white and red blood cell counts, hematocrit as well as platelet counts ($p<0.01$ for all). Individuals treated with platelet aggregation inhibitors, i.e. aspirin and clopidogrel, showed decreased S1P concentrations (0.807 vs. 0.753 vs. 0.730 µmol/L, $p<0.01$).
Conclusion: The main source for S1P in blood is reflected by the strongest association found for platelet counts. Decreased S1P levels due to aspirin intake imply systemic effects of reduced S1P secretion from platelets. Modulating circulating S1P may be a potential therapeutic strategy in individuals at cardiovascular risk.
Disclosure: No significant relationships.

P 089 Extracellular Adherence Protein (Eap) Tandem Repeat Domain EapD3D4 from Staphylococcus aureus binds to Human Platelets and Triggers Platelet Activation

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Objectives: Staphylococcus aureus and its secreted products can interact directly with platelets or indirecty through recruitment of serum components acting as molecular bridges. The staphylococcal secreted extracellular adherence protein (Eap), with four tandem repeat domains (D1 through D4) is a member of the secretable expanded repertoire adhesive molecules (SERAM), and is known to activate platelets. Here we show that only the EapD3D4 domain binds to platelets and triggers platelet activation.
Methods: His_6-tagged $EapD_1D_2$, D_3D_4, D_3 and D_4 domains were heterologously expressed in *E. coli.* Binding of Eap domains to washed platelets was detected by anti- His_6 antibody and platelet activation was quatintified by measuring CD62P expression by flow cytometry and confocal fluorescence microscopy. In addition, a novel microbead array based "bacteriomimetic" microfluidic platform was developed to investigate functional interactions between individual Eap domains and platelets under flow conditions.
Results: Binding of $EapD_3D_4$ to washed platelets was significantly higher [▶Fig 1: X-mean of MFI 13.38 ± 4.07 mean±SD, n=6 donors and $P<0.001$] in comparison to $EapD_1D_2$, D_3, D_4 and buffer controls. CD62P expression increased in the presence of $EapD_3D_4$ [▶Fig 2: Fold increase 6.24 ± 4.8 mean±SD, n=6 donors and $P<0.0001$] but not in the presence of the other domains. Fluorescence confocal microscopy revealed $EapD_3D_4$ binding to the platelet surface and subsequent formation of platelet aggregates [▶Fig 3]. On "bacteriomimetic"microbead arrays, platelets showed specific interaction and adhesion only with those immobilized with $EapD_3D_4$ [▶Fig 4].
Conclusion: The domain D_3D_4 from S.aureus Eap protein mediates Eap binding to platelets and triggers platelet activation and aggregation.
Disclosure: No significant relationships.

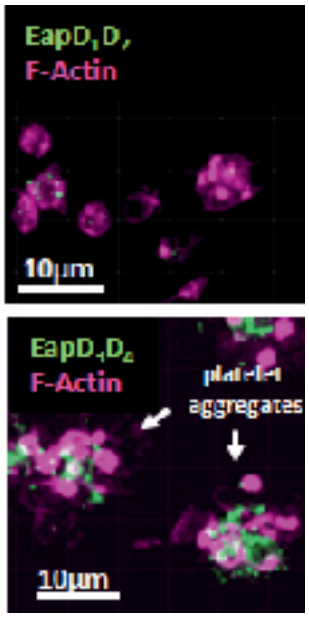

Fig. 3
$EapD_3D_4$ binds to platelets

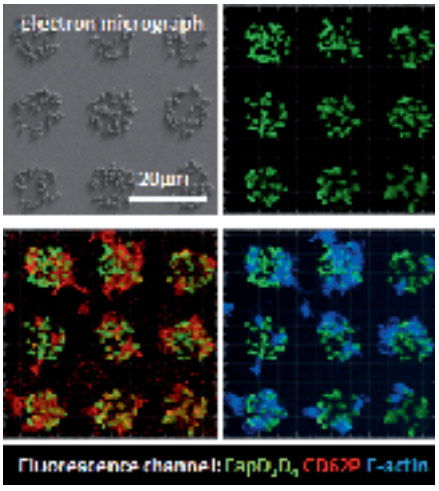

Fig. 4
Platelets bind to "bacteriomimetic" microbead arrays with $EapD_3D_4$

P 090 Anti-PF4/heparin antibodies bind to PF4 on Gram-positive Bacteria

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Objectives: Heparin induced thrombocytopenia (HIT) is likely a misdirected bacterial host defence mechanism. The chemokine platelet factor 4 (PF4, CXCL4) binds charge-dependently to polyanions, such as heparin, which leads to a conformational change of PF4 and expression of antigenic neo-epitopes. HIT antibodies bind to these neoepitopes. PF4 expresses the same epitopes after binding to Lipid A on Gram negative E.coli, as we have shown recently. We now systematically investigate binding of PF4 to Gram-positive bacteria.
Methods: We tested binding of PF4 to living bacteria using a protein-A deficient strain of *Staphylococcus aureus* (SA113Δspa) and a capsule-free pneumococcus (*Streptococcus pneumoniae* D39Δcps). To quantify conformational changes of bound PF4, we used the PF4/H specific monoclonal antibodies KKO and 5B9, and purified IgG from HIT-patients, which was preadsorbed with *S.aureus* or *pneumococcus* to deplete bacteria-specific-IgG. Binding of antibodies was quantified by flow cytometry, using fluorescent secondary antibodies.
Results: Binding of PF4 is dose-depended and shows saturation at 10µg/mL for *S. aureus* and 20µg/mL for *S. pneumoniae*, respectively. PF4 changes its conformation after binding to both, Gram-positive bacterial species. This was demonstrated by binding of the PF4/H complex specific monoclonal antibodies KKO and 5B9, as well as of IgG antibodies from HIT patients.
Conclusion: We provide further evidence that PF4 binds to Gram-positive bacteria, hereby expressing a neoepitope, recognized by PF4/H specific antibodies. This further supports the role of PF4 as part of a broad reacting bacterial host defence system.
Disclosure: No significant relationships.

P 091 Clopidogrel in Critically Ill Patients

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Objectives: Only limited data are available on treatment of critically ill patients with clopidogrel. The aim of this trial was to investigate the effects and the drug concentrations of the CYP450 activated pro-drug clopidogrel and the half-life of the similarly metabolized pantoprazole in critically ill patients.
Methods: Critically ill patients with prior clopidogrel (75mg) (n=43) or pantoprazole (40mg) (n=16) therapy were included. Platelet inhibition was assessed by ADP-induced whole blood aggregometry and measurement of vasodilator-stimulated phosphoprotein phosphorylation (VASP-P). Plasma concentrations of clopidogrel, its active metabolite and of pantoprazole were quantified by tandem mass spectrometry.
Results: ADP-induced aggregometry in whole blood classified 74% (95% confidence intervals (CI) 59–87%) of ICU patients as poor responders (n=43), and 65% (95% CI 49–79%) responded poorly according to the VASP-P assay. Whereas the plasma levels of clopidogrel active metabolite normally exceed the inactive prodrug ~30-fold, parent drug levels even exceeded those of the metabolite 2-fold in critically ill patients. The half-life of pantoprazole was several-fold longer in ICU patients compared to reference populations.
Conclusion: The majority of critically ill patients respond poorly to clopidogrel and may therefore be at increased risk to experience cardiovascular events. The inverse ratio of prodrug/active metabolite indicates insufficient metabolization of clopidogrel, which is independently confirmed by the ~5-fold increase in half-life of pantoprazole. Thus, high-risk patients in the ICU may benefit from treatment with alternative platelet inhibitors.
Disclosure: No significant relationships.

P 092 Impact of glycoprotein VI signalling in platelet mediated amyloid β fibril formation

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Objectives: Platelets display all enzymatic activities necessary to produce amyloidβ (Aβ) peptides and therefore play a role in Aβ plaque formation in Alzheimer's disease (AD). Additionally, studies depicted increased platelet activation in AD patients and AD mice. Recently we were able to show that platelets contribute to Aβ aggregation through integrin $\alpha_{IIb}\beta_3$ induced outside-in signalling. Experiments were performed to further investigate potentially related receptors in the plaque formation process. In particular the interaction between the collagen receptor GPVI and Aβ40 was investigated.
Methods: Cell culture experiments and Western Blot to analyse Aβ and platelet interaction.
Results: GPVI inhibiting studies in platelet cell culture using a GPVI antibody indicated reduced Aβ aggregation. Additionally performed analysis of the downstream GPVI signalling pathway by determining LAT phosphorylation showed an increased phosphorylation rate after prolonged stimulation with soluble Aβ. Inhibition of integrin $\alpha_{IIb}\beta_3$ leads to reduced phosphorylation of LAT after Aβ stimulation and therefore gives strong evidence towards a secondary loop of activation. This is supported by immunoprecipitation studies showing no interaction of GPVI and Aβ. The formation of fibrin upon platelet mediated Aβ aggregation suggests that fibrin might play a role in GPVI signaling during platelet-mediated Aβ fibril formation.
Conclusion: The experiments provided first evidence that no direct binding of Aβ to GPVI occurs but indicate a secondary loop of GPVI activation via Integrin $\alpha_{IIb}\beta_3$ binding. Further investigation of ligands involved in this process is currently in progress.
Disclosure: No significant relationships.

Platelet dysfunction

P 093 Hemorrhagic diathesis caused combined mild thrombocytopenia and platelet dysfunction due to heterozygous mutations in β1 tubulin and thromboxane A2 receptor

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Objectives: Introduction: Chronic hemorrhagic diathesis in patients showing normal levels of plasmatic clotting factors strongly suggests for congenital platelet disorders (CPD). We report on a pediatric patient (male, 3 years) with clinical signs of bleeding. The diagnostic work up included morphologic, hemostaseologic and molecular genetic investigation of the patient and his family.
Methods: The index patient (D1), a sibling (D2), his mother (D3) and father (D4) were included for laboratory investigation. Light transmission aggregometry was performed using standard concentrations of ADP, collagen, AA, epinephrin and U46619. Blood smears were processed for immunostaining of several platelet proteins. The *TUBB1* and *TBXA2R* genes were sequenced from genomic DNA. PCR methods with sequence-specific primers (PCR-SSP) were developed for genotyping the *TUBB1*c.920G>A* and *TBXA2R*c.908T>C* variants.
Results: Platelet counts in D1, D2 and D4 indicated mild thrombocytopenia with approximately 100 Gpt/L. In D1 and D2 immunofluorescence microscopy indicated defects in ß1-tubulin. The *TUBB1* gene was heterozygous for the c.920G>A variant (rs6070697; p.R307H) in D1 and D4. Platelet aggregation on AA and U46619 stimulation was significantly diminished D1 and D3. The *TBXA2R* gene revealed a novel heterozygous mutation c.908T>C (p.L303P) in D1 and D3. Screening for the *c.908C* allele of 11,426 healthy blood donors was negative.
Conclusion: In the present case bleeding is caused by thrombocytopenia inherited from the father and platelet dysfunction inherited from the mother. However, it remains unclear whether the common *TUBB1*c.920G>A* variant is associated with low platelet counts. Platelet dysfunction presumably is caused by the novel *TBXA2R*c.908T>C* mutation.
Disclosure: No significant relationships.

P 094 Functional defect of integrin αIIbβ3 without reduced antigen expression

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Objectives: Glanzmann thrombasthenia (GT) is an inherited bleeding disorder caused by changes in the platelet receptor integrin αIIbβ3 (GPIIb/IIIa). Changes in the responsible genes, ITGA2B and ITGB3, can lead to an absence (GT type I) or a major reduction in the expression (below 20%, GT type II) of the receptor on the platelet surface. In rare cases variations of the receptor display a dysfunctional protein without major impairment of the receptor abundance (GT variant).
Methods: In this study we present the case of a patient with a bleeding disorder due to functional defect of αIIbβ3. The patient presented with severe hematomas (face, ear, thorax) without previous trauma. Apart from severe bruising no other hemorrhages could be detected. The family history is negative for severe bleeding adverse events following trauma or surgery apart from easy bruising. Both platelet count and plasmatic coagulation did not display any significant abnormality, the patient does not take any regular medication.
Results: When analyzing platelet function using light transmission aggregometry, the patients' platelets showed an absent activation with both collagen and epinephrine. Interestingly, antigen expression measured by flow cytometry was only minimally decreased for GPIIb and unaltered for GPIIIa as well as other platelet surface receptors (GPIb, GPIX, GPIV, GPVI).
Conclusion: As the patient did not show any other remarkable phenotype and the family history was negative for severe bleeding following trauma or surgery, no medication was started but due to the young age of the index patient a close follow up in the outpatient clinic was initiated.
Disclosure: No significant relationships.

Poster

P 095 High platelet activity in hypothermic patients with acute ST-elevation myocardial infarction

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Objectives: Myocardial infarction (MI) is one of the leading causes of death and morbidity worldwide. Therapeutic hypothermia (TH) is used in patients with cardiac arrest and infants suffering from birth asphyxia. Cardioprotective mechanisms of TH in MI are in discussion. Effects of TH on platelets and hemostasis in-vivo are still to be clarified, especially in MI patients.
Methods: The STATIM (Strategic **TA**rget Temperature management **I**n **M**yocardial Infarction) study investigated the impact of TH during STEMI, aiming at a temperature below 35°C during reperfusion. All patients received prasugrel and acetylsalicylic acid. 120 patients underwent a 1:1 randomization and biological samples were immediately processed for laboratory multiplate®, ELISA and flow cytometry analysis.
Results: Platelet multiplate® analysis revealed increased responsiveness (TRAP-test) of platelets from cooled patients. Plasma levels of ADP, an important *in-vivo* platelet stimulus, were found to be significantly elevated in patients receiving TH. We observed significant longer bleeding times in TH patients, which were accompanied by more clinical bleedings, whereas platelet count and fibrinogen were not affected by TH. In patients treated with TH, mean platelet volume was identified as an independent predictor of bleeding events.
Conclusion: Our *in-vivo* study of MI patients revealed that TH leads to high platelet responsiveness and clinical bleeding events, which can be predicted by the mean platelet volume. Correlation with outcome data will identify the clinical importance of these findings regarding ischemia and cardiac function.
Disclosure: No significant relationships.

P 096 Impaired platelet function caused by Vibrio cholera outer membrane vesicles

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This abstract has been withdrawn

Inherited thrombocytopenias

P 097 Delayed diagnosis of MYH-9 related disorder in a man with azoospermia and desire for fertility

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Objectives: MHY-9 related disorders (MYH-9 RD) belong to the group of autosomal- inherited giant platelet disorders. They are characterized by macrothrombocytopenia and inclusion bodies in leukocytes or as syndromic forms combining these features with hearing loss and/or nephropathy and/or pre- senile cataracts. MYH-9 gene codes for the cytoplasmic contractile protein non-muscular myosin heavy chain IIA. Bleeding tendency is usually mild to moderate.
Methods: We report about a 46 year old male with more than 20 years history of chronic immune thrombocytopenia which was non- responsive to corticosteroids. Despite thrombocytopenia multiple surgeries were performed without any treatment and without increased bleeding. In our outpatient clinic the patient was presented before testicular biopsy due to non-obstructive azoospermia and desire for fertility. Anamnesis revealed presenile cataract surgery in the age of 36 years and beginning high tone hearing loss.
Results: Laboratory testing demonstrated platelet count of 31×10^9/L but no free or bound platelet autoantibodies by standard glycoprotein-specific assay. Von Willebrand disease type IIB and Bernard Soulier syndrome were excluded. Mean platelet volume was increased to 16.8 fL. Diagnosis of MYH-9 RD was based on identification of the granulocyte inclusion bodies using blood smears and confirmed by genotyping. Genetic testing of the MYH-9 gene revealed c.5717C>T; p.Thr1906Met (het) and a heterozygous duplication of intron/exon crossing to exon 37. Testicular biopsy was successfully done without any treatment, sperms were obtained and in vitro fertilisation is planned despite of hereditary disease.
Conclusion: Patients with MYH-9 RD may be misdiagnosed as having immune thrombocytopenia.
Disclosure: No significant relationships.

P 098 Immunofluorescence analysis on a blood smear – validation of stability of blood smears during storage

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Objectives: Inherited platelet disorders can pose diagnostic challenges. Many inherited platelet disorders are associated with morphological defects of platelet receptors, granule proteins and cytoskeletal proteins. A specialized testing to evaluate these inherited platelet disorders is the immunofluorescence microscopy. However, the preanalytic requirements for immunofluorescence microscopy are not well known.
Methods: Anticoagulated blood (EDTA, Citrate, Hirudin) from 3 different donors with two technical replicates and blood smears of 3.5µl blood were prepared within 4h after drawing. Air dried blood smears were stained on day 0, 1, 3 and 7 after preparation, using different antibodies against the GP IIbIIIa and GP IbIX and granula markers (P-Selectin, vWF, Thrombospondin, CD63, Lamp1, Lamp2). We also visualized cytoskeleton structures like NMMIIa, alpha and ß1 tubulin and filamin A. Intensity and distribution of markers were evaluated by two independent investigators.
Results: Stability of morphological markers with EDTA-anticoagulated blood is superior. The receptor markers GPIIbIIIa and GPIbIX are stable during storage of air dried blood smears with a slight reduction in intensity after 3 days. We also see a reduction in intensity in all granule markers. Tubulin stabilizes during storage and got better up to day one, NMMIIA remain stable during storage. Filamin A has a diffuse distribution at day 0 and showed a ring structure on day 7 in all samples.
Conclusion: Preanalytic requirements of immunofluorescence microscopy are suitable for patients without direct access to a specialized laboratory and air dried blood smears from EDTA-blood are stable for shipment for several days.
Disclosure: No significant relationships.

Acquired thrombocytopenias

P 099 Binding Characteristics of Anti-Platelet Factor 4/Polyanion Antibodies on Different Substrates

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This abstract has been withdrawn

P 100 Correlation between the activation state of Integrin alpha IIb beta 3 and changes in protein structure

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Objectives: Integrin alpha IIb beta 3 is a heterodimeric transmembrane platelet receptor which plays an essential role in hemostasis and is involved in the autoimmune disease Immune thrombocytopenia (ITP). The capacity of the immune system to induce an immune response are likely related to different conformations of αIIbβ3. We investigate in a membrane environment whether there is a correlation between the activation state of αIIbβ3 by manganese ions (Mn2+) and changes in protein secondary structure.
Methods: Integrin αIIbβ3 was incorporated into 1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol (DMPG)/ 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) liposomes. The protein was then activated by Mn2+ and the activation state of αIIbβ3 was verified by conformation specific antibody (PAC-1) binding using enzyme-linked immunosorbent assay (ELISA). and related interaction analysis with PAC-1 and other ligands were performed by surface plasmon resonance (SPR) and quartz crystal microbalance (QCM).Changes in αIIbβ3 structure induced by Mn2+ were investigated by circular dichroism (CD).
Results: The successful reconstitution of αIIbβ3 into liposomes was proved by SDS-PAGE and flow cytometry. We showed by ELISA using PAC-1 that a concentration of 1mM Mn2+ activates αIIbβ3. The binding of PAC-1 to active protein was further confirmed by SPR and QCM analyses. First CD experiments indicate changes in protein structure upon Mn2+-induced activation of αIIbβ3 incorporated into liposomes. This suggests a correlation of activation state and changes in protein secondary structure.
Conclusion: Using a combination of biophysical methods and ELISA, we showed that αIIbβ3 reconstituted into liposomes is activated by Mn2+ and the activation state is related to changes in the secondary structure of the protein.
Disclosure: No significant relationships.

P 101 Effect of platelet size on the platelet reactivity in the HIPA-test

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Objectives: Heparin-induced thrombocytopenia (HIT) is a prothrombotic adverse drug reaction caused by antibodies which activate platelets by cross-linking Fc gamma receptors lla (FcγRlla) after binding platelet factor 4 (PF4)/ heparin complexes. HIT-antibodies occur more frequently than clinical HIT with thrombocytopenia and thromboses. We hypothesize that besides antibody-formation other platelet related risk factors are involved. Our objective was to investigate the influence of platelet size on the platelet reactivity in response to HIT-antibodies.
Methods: Large and small platelet fractions were separated from volunteers by differential centrifugation. The quantity of FcγRlla was determined using flow cytometry. Platelet activation was measured with two sera from HIT patients (Serum 1+2: anti-heparin/PF4 ELISA positive, heparin-induced platelet activation (HIPA) test positive) and the PF4/heparin specific 5B9 antibody in the HIPA-test.
Results: Large and small platelet fractions had different mean platelet volumes (median large vs. small): 11.2fl vs. 6.8fl, p<0.001. Large platelets contained more FcγRlla receptors than smaller platelets (MFI large vs. small): 10.9 vs. 8.8; p= 0.0234, which was proportional to the size difference. Large platelets aggregated significantly faster in the HIPA-test compared to small platelets (median time large vs. small): 9min vs 20min, p=0.0313 (Serum1); 10min vs 17min, p=0.0156 (Serum2) and 20min vs 26min, p=0.0313 using the 5B9 antibody.
Conclusion: Platelet size has an influence on platelet reactivity in response to HIT antibodies. Large platelets respond faster and contained more Fc-gamma-receptors, suggesting a potential role of platelet size in the pathogenesis of HIT.
Disclosure: No significant relationships.

Platelets / Physiology

P 102 Differential platelet phenotype and function of PON2– and NOX2-deficient mice

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Objectives: Paraoxonase-2 (PON2) exhibits anti-oxidative properties and counter-acts inflammation and atherosclerosis. The NADPH oxidase-2 (NOX2) represents a major pro-oxidative enzyme. Using PON2– and NOX2-deficient mice we investigated the effect of PON2 and NOX2 on platelet ROS production, phenotype and activation.
Methods: Platelet count and MPV were analyzed by a cell counter. Surface receptors, intracellular ROS and platelet activation were assessed by flow cytometry in diluted platelet-rich plasma in the presence of different Ca^{2+}-concentrations.
Results: PON2-deficient mice had decreased platelet count but increased MPV associated with elevated ROS levels *ex vivo* whereas NOX2-deficient mice had unaltered platelet count and MPV associated with decreased basal ROS levels compared to wildtypes. PON2-deficient platelets showed increased GPIba-surface expression and vWF-binding as well as increased P-selectin surface expression and annexin-V binding with additional Ca^{2+}ex vivo and after convulxin-treatment. In contrast, agonist-induced αIIbβ3 integrin activation and P-selectin surface expression were decreased without additional Ca^{2+}. NOX2-deficient platelets presented diminished GPIba-surface expression and vWF-binding, but unaltered P-selectin surface expression and annexin-V binding *ex vivo*. However, αIIbβ3 integrin activation, P-selectin surface expression and annexin-V binding of NOX2-deficient platelets were increased in response to convulxin independent of the extracellular Ca^{2+}-concentration.
Conclusion: Our data indicate that PON2 and NOX2 regulate platelet ROS generation and platelet activation reversely. Hypo-or hyperfunction of PON2-deficient platelets is dependent on extracellular Ca^{2+}-concentration.
Disclosure: No significant relationships.

Poster

P 103 Platelet CXCR7 Acts an Anti-Thrombotic Therapeutic Target in Regulating Thrombo-ischaemic Pathophysiologies

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Objectives: Elevated platelet surface expression of the chemokine CXCL12 and its receptors CXCR4-CXCR7 in acute coronary syndrome (ACS) influences functional recovery following myocardial infarction (MI), and prognosis. CXCR7 engagement by chemokines/cytokines exerts a prosurvival and anti-thrombotic effect. This study explores the anti-thrombotic potential of a specific pharmacological CXCR7 agonist and its therapeutic implications.
Methods: Flow chamber assay, T-TAS, **S**canning**I**on**C**onductance**M**icroscopy, thrombinoscopy, thromboelastography, aggregometry, flowcytometry,**M**ass**S**pectrometry
Results: Platelet-megakaryocyte lineage specific *Pf4-Cre+CXCR7$^{flox/flox}$* CXCR7 deficient mice exhibited a pro-thrombotic disposition but normal platelet count and bleeding time. CXCR7-agonist counteracted activation-induced *ex vivo* platelet degranulation, integrin activation (flowcytometry), aggregation, thrombus formation, (ex vivo flow chamber assay, T-TAS), in blood from healthy subjects and ACS patients alike. CXCR7-agonist regulated spreading and morphology dynamics of platelets on collagen and fibrinogen. CXCR7-agonist controlled activation-induced phosphorylation of PLC-γ, Src, PKC, PI3K, Akt, p38MAPK, intracellular calcium mobilization, but induced cGMP-cAMP levels over time and PKG-PKA dependent VASP phosphorylation to regulate platelet activation. CXCR7-agonist decreased activation-induced externalization of phosphatidylserine on platelets, checked platelet dependent (PRP) but not plasma dependent (PPP) thrombin generation (thrombinoscopy), also did not influence clot formation (thromboelastography). *In vivo*, CXCR7-agonist administration counteracted platelet activation, thrombus formation (ligation and $FeCl_3$-induced) without affecting bleeding time or coagulation profile (APTT, PT). *Pf4-Cre^{+}CXCR7$^{flox/flox}$* mice showed increased area-at-risk, reduced ejection fraction, infiltration of inflammatory cells to ischaemic myocardium following MI (LAD-ligation). CXCR7-agonist administration prior to MI controlled platelet activation, reduced area-at-risk, and improved ejection fraction deciphered 1 day post MI.
Conclusion: CXCR7-agonist as an anti-thrombotic strategy could counteract platelet hyper-reactivity and subsequent thrombo-ischaemic complications without compromising physiological haemostasis.
Disclosure: No significant relationships.

P 104 Hypoxia impairs agonist-induced integrin αIIbβ3 activation and platelet aggregation

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Objectives: Ischemia, caused by vascular thrombosis, leads to hypoxia in the affected tissues. Although human physiology, to a certain extent, can adapt to hypoxic conditions, the impact of low oxygen levels on platelet function is largely unresolved. Therefore, we explored how low oxygen levels influence $\alpha_{IIb}\beta_3$ integrin-dependent platelet function.
Methods: Isolated human platelets, washed or in platelet-rich plasma (PRP), were kept for 30 minutes in a COY hypoxic chamber at 1% or 8% oxygen, with or without re-oxygenation. After platelet stimulation with TRAP-6 or convulxin, we analyzed platelet aggregation of normoxia and hypoxia-exposed washed platelets, PRP and in diluted PRP (14% v/v). In diluted PRP, we tested the activation of $\alpha_{IIb}\beta_3$, following stimulation with several agonists (ADP, TRAP-6, convulxin) in hypoxia or normoxia. Furthermore, we investigated the static adhesion of the platelets on collagen, fibrinogen and vitronectin. In addition, we studied the platelet granule secretion as well as the time course of $\alpha_{IIb}\beta_3$ activation under normoxic and hypoxic conditions.
Results: Hypoxia impaired the activation of the fibrinogen receptor integrin $\alpha_{IIb}\beta_3$ and the agonist-induced aggregation of washed human platelets, but had no effect on granule secretion. The presence of plasma protected the platelet fibrinogen receptor $\alpha_{IIb}\beta_3$ from hypoxia-dependent functional impairment. Defective αIIb function was further demonstrated by decreased static platelet adhesion on fibrinogen coatings. This hypoxia-dependent effect on the $\alpha_{IIb}\beta_3$ integrin function was fast and reversible.
Conclusion: Hypoxia affects $\alpha_{IIb}\beta_3$ integrin function of washed platelets in a fast and reversible fashion
Disclosure: No significant relationships.

P 105 ACD vs. sodium-citrate as an anticoagulant for platelet rich plasma (PRP) preparation influences the extent of platelet shape change during spreading – quantitative morphometric data from standardized robotic darkfield microscopy.

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Objectives: Platelet shape change and spreading is regarded a process particularly sensitive to a multitude of preanalytical factors such as for example the type of anticoagulant used. We initiated a prospective and blinded study to quantitatively evaluate the impact of different preanalytical conditions on platelet morphology.
Methods: A standardized protocol was established for blood withdrawal, PRP and specimen preparation, microscopic examination and morphometric analysis [1,2] . Blood from seven healthy volunteers was drawn into standard vacutainer tubes containing acid citrate dextrose (ACD-A) or 0,105 M tri-sodium citrate (CIT). PRP and slides were prepared and automated micro-

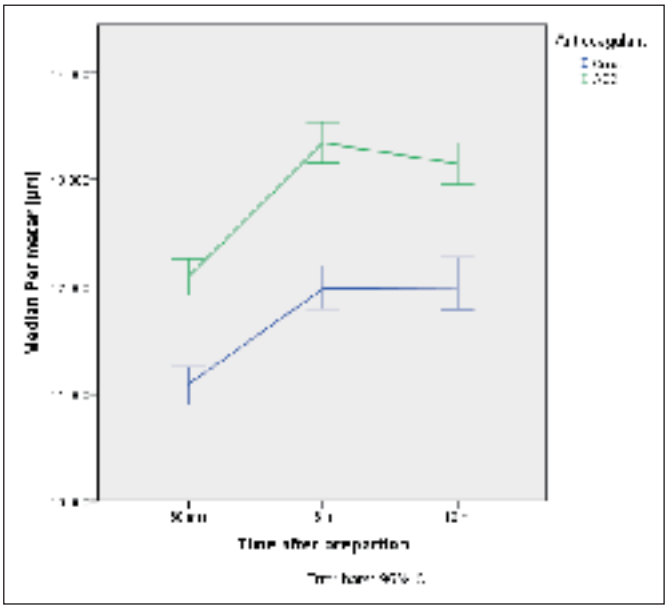

Fig. 1 In standardized darkfield microscopy, the median perimeter (error bars: 95% confidence interval) of platelet outlines differs significantly between PRP from ACD- vs. sodium citrate-anticoagulated blood.

scopic analysis was performed 30 minutes, 6 and 12 hours after preparation. Morphometric parameters such as area, perimeter, circularity and fractal dimension were determined using the GROUP-IT-software [1].

Results: 19462 platelet outlines were segmented for ACD and 17320 for CIT. As ▶Fig. 1 shows, the morphometric change between baseline, 6 and 12 hours was comparable for both anticoagulants. However, the median values (95%CI) of all measured parameters were significantly different for ACD vs. CIT at each time point (e.g. at 6 hours: Perimeter=13.35 (13.16–13.54) vs. 11.98 (11.80–12.20) µm, p<0.001, Circularity=0.68 (0.67–0.69) vs. 0.71 (0.70–0.72), p<0.001)

Conclusion: Significantly more platelet outlines with significantly stronger morphological signs of activation can be segmented from ACD-as compared to CIT-anticoagulated PRP. [1] Kraus, et al., Platelets. 11, 2013 [2] Kraus, et al., FGNAMB. 1, 2015 ▶Fig. 1.: In standardized darkfield microscopy, the median perimeter (error bars: 95% confidence interval) of platelet outlines differs significantly between PRP from ACD- vs. sodium citrate-anticoagulated blood.

Disclosure: No significant relationships.

P 106 Novel network of protein phosphatase 2A (PP2A) with α-endosulfine (ENSA) in human platelets

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Objectives: Protein kinases, protein phosphatases and their substrates are major components of the human genome and essential for the regulation of pathways in all cells including platelets. While protein kinases have been at the forefront of biomedical research, protein phosphatases only recently received similar attention. In *Drosophila* and *Xenopus*, cell cycle/mitosis is tightly controlled by Greatwall kinase (Gwl), its substrate α-endosulfine (ENSA) and the protein phosphatase 2A (PP2A).Gwl phosphorylates ENSA at S67 and converts it to a potent PP2A inhibitor. The aim of this study was to investigate the presence and regulation of the Gwl/ENSA/PP2A network in human platelets.

Methods: Platelet proteomic studies were done as described (Burkhart J. Blood, 2012; Beck F. Blood, 2017). Human ENSA (wildtype and phosphomutants), cloned and expressed in HEK293 cells and *E.coli* BL21, was studied with intact human platelets and platelet extracts. Phosphatase activity of PP2A was assessed with a novel PP2A selective chromogenic assay.

Results: ENSA, PP2A but not MASTL (human Gwl) were detected in human platelets. Despite the absence of MASTL, strong ENSA-S67 protein kinase activity was detected in intact human platelets and extracts when PP2A was inhibited by okadaic acid (see ▶Figure 1). Other Gwl/MASTL-related kinases present in platelets are now probed for ENSA-S67 phosphorylation. Phosphorylation of ENSA at S109 by both cAMP/PKA and cGMP/PKG, attenuated ENSA-S67 phosphorylation. Inhibition of PP2A was analyzed by okadaic acid and recombinant ENSA.

Conclusion: ENSA, its protein kinases and its target PP2A are central regulatory nodes in human platelets.

Disclosure: No significant relationships.

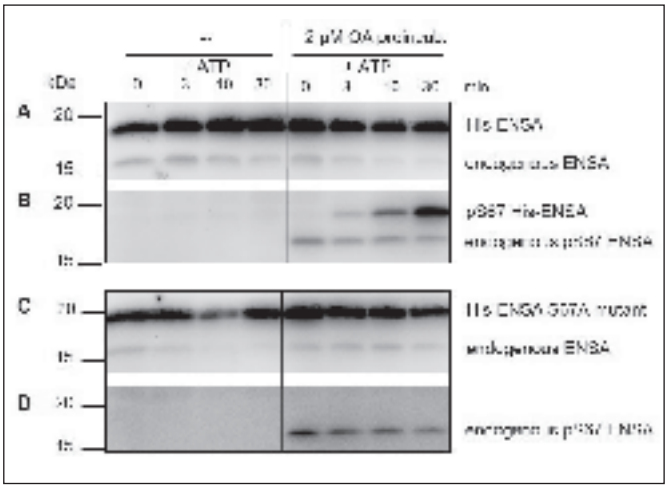

Fig. 1 Demonstration of specific ENSA S67 protein-kinase activity in soluble extracts of human platelets. Washed human platelets were pre-treated without (-) or with 2 µM okadaic acid (OA) for 15 minutes at 30°C. Then, platelets were lysed by an EDTA/EGTA containing NP40 buffer at 0°C and recombinant His-tagged ENSA protein (wildtype and the S67A mutant as control) was added to the soluble platelet extracts. The phosphorylation reaction of endogenous protein kinase(s) was started by the addition of $MgCl_2$/ATP (0 min) and incubated at 30°C. Aliquots were removed from the reaction mixture before ATP addition (0 min) and after 3, 10 and 30 minutes of incubation. Western blots were probed for the presence of endogenous (~17 kDa) and recombinant His-tagged (~ 19 kDa) ENSA with a general antibody against human ENSA (A and C; cell signalling) and for the presence of phosphorylated endogenous and His-tagged pS67-ENSA with a phosphospecific antibody (B and D; cell signalling).

P 107 Real time deformability cytometry provides unique information on platelet quality

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Objectives: Platelet concentrates (PC) are stored at room temperature (RT). Storage at 4°C is an attractive option, especially as standard tests for quality testing show similar results of platelet metabolism, aggregation, and activation. We introduce a new method, real-time deformability cytometry (RT-DC), which in contrast, clearly differentiates platelet quality after storage at RT vs 4 °C.

Methods: Apheresis PC of 8 healthy donors were stored either at RT or at 4°C under agitation. Samples were taken at day 1, 4, 7, and 10 after donation. RT-DC is a novel real time analysis allowing measurement of platelet mechanical properties. Platelets are flowed through a microfluidic channel and deformed without contact by shear stress and pressure gradients. In parallel all samples underwent fluorescence microscopy with f-actin and α-tubulin staining, and platelets adhesion/aggregation on collagen surfaces were assessed.

Results: RT-DC showed a clear differentiation between RT and 4°C stored platelets. Due to cold storage deformation was reduced to 0.034±0.004 in contrast to 0.133±0.032 for RT platelets, p=0.0007. Surface area decreased during cold storage (24.8±1.5µm^2 for cold and 29.0±2.2µm^2 for RT stored platelets, day 4; p=0.0047). Results from fluorescence microscopy confirm the observations. Cold stored platelets show a deformed tubulin ring already at day 1. Most importantly, platelets from cold stored PCs formed microaggregates on collagen starting from day 4.

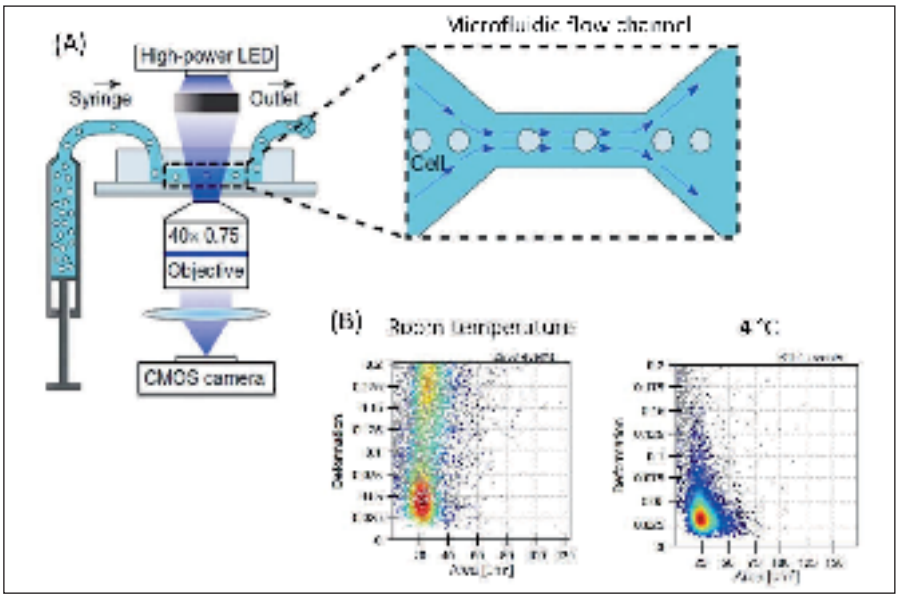

Fig. 1
A) Scheme of RT-DC with microfluidic channel, **B**) Deformation scatterplots of APC platelets stored at room temperature or 4 °C at day 1

Conclusion: RT-DC is the only method showing differences between platelets stored at 22°C or 4°C. Assessing mechanical properties of platelets is highly relevant for transfusion medicine. Platelets stored at 4°C > 3 days bear the risk for thromboembolism.
Disclosure: No significant relationships.

P 108 Continuous flow cytometric monitoring of intracellular calcium and sodium ions upon platelet activation

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Objectives: The interaction of platelet (PLT) agonists with their respective receptors triggers intracellular signaling events. Cytosolic ion fluxes play an important role to mediate and sustain PLT activation and procoagulant activity. Here we propose a continuous flow cytometric monitoring of intracellular free calcium and sodium ion fluxes upon PLT activation and procoagulant activity development.
Methods: PLTs were preloaded with calcium or sodium ion indicators, Fluo-3 AM and Asante NaTRIUM Green-2 AM, respectively. After measurement of a stable baseline, PLTs were activated with thromboxane analogue U46619, ADP, thrombin, TRAP6 (PAR-1 agonist), AYPGKF (PAR-4 agonist), or convulxin (collagen receptor GPVI agonist). Then, ion indicator fluorescence was continuously acquired over time, up to 10 minutes, on an Accuri C6 flow cytometer.
Results: We obtained continuous monitoring of cytosolic kinetics for calcium and sodium fluxes upon PLT activation with single agonists or combinations thereof. Weak ion mobilizations were observed with U46619 and ADP. TRAP6 and AYPGKF demonstrated an important calcium increase but modest sodium fluxes. Strong cytosolic increases were observed for both ions after activation with convulxin or thrombin. Simultaneous stimulation with thrombin and convulxin generating procoagulant COAT PLTs potentiated ion kinetic responses. Annexin-V co-staining revealed a dichotomous cytosolic ion mobilization leading to procoagulant activity.
Conclusion: The present work highlights the use of continuous intracellular ion monitoring by flow cytometry to demonstrate characteristic calcium and sodium mobilization patterns following PLT activation with various agonists. Moreover, this technique will sharpen the ability to analyze PLT signaling and may be useful to investigate PLT pathophysiology.
Disclosure: No significant relationships.

P 109 Large platelets expose a procoagulant phenotype which is driven by epinephrine receptor signaling

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Objectives: Platelets consist of heterogeneous populations of smaller and larger cells. We developed a method to separate large and small platelets from the same blood sample in sufficient quantities to allow comparative studies. Our first objective was to characterize the protein composition of large and small platelets and to assess their response to standard agonists.
Methods: Large and small platelet fractions were separated by differential centrifugation (n>5). Platelet receptors ($P2Y_{12}$, GPIb, GPVI, alpha-2A) were quantified by flow cytometry. The cytosolic proteome was analysed by LC-ESI-MS/MS. Platelet aggregation was performed with collagen (5µg/ml), ADP (20µM) and epinephrine (10µM) using light transmission aggregometry. Phosphatidylserine (PS) exposure was determined by Annexin V binding after stimulation with TRAP, collagen and epinephrine.
Results: Large platelets showed a higher epinephrine receptor density (MFI 19.35 ±9.4 x; p=9.93±5.3; p=0.0051) and more GTP binding proteins relevant for epinephrine receptor signaling compared to small platelets. Consistently, platelet aggregation in response to epinephrine was more pronounced in large compared to small platelets (max. aggregation 70% ±24 vs 40% ±32; p=0.0313), whereas it did not differ after stimulation with collagen and ADP. Addition of epinephrine to collagen and TRAP resulted in significantly more procoagulant PS-positive large platelets compared to small platelets (4.2% ±0.9 vs 2.1% ±1; p=0.0313).
Conclusion: Large platelets express more epinephrine receptors and downstream signaling proteins compared to small platelets and display a stronger response to epinephrine. Epinephrine preferentially stimulates phosphatidylserine expression of large platelets. This indicates a procoagulant phenotype of large platelets under steady state conditions.
Disclosure: No significant relationships.

DIC, sepsis and other inflammatory conditions

P 110 Differential regulation of LPS-induced monocyte tissue factor (TF) by protein disulfide isomerase (PDI) inhibitors

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Objectives: Extracellular PDI is an abundant oxidoreductase with pleiotropic effects in vascular biology. Although inhibition of PDI is currently being investigated as a therapeutic approach to prevent thrombosis, the effect of PDI inhibitors on monocyte TF expression has not yet been systematically investigated. Quercetin-3-rutinoside (rutin) reversibly binds to the substrate recognition pocket of PDI, thus inducing a conformational change of the enzyme. Cell-permeable PACMA-31 irreversibly blocks the catalytic cysteines within the active motif of PDI responsible for its oxidoreductive activities.
Methods: Citrate-anticoagulated whole blood or isolated peripheral mononuclear cells (PMCs) were incubated with lipopolysaccharide (LPS) or buffer control for 5 hours at 37°C in the presence or absence of rutin or PACMA-31. Monocyte TF antigen and procoagulant activity (PCA) were assessed by flow cytometry and clotting assay, respectively. Additionally, TF-specific PCA of microvesicles (MVs) was analyzed by Xa generation assay.
Results: Rutin prevented LPS induction of monocyte TF expression and release of procoagulant MVs in whole blood and with isolated PMCs, albeit the inhibitory effect was less pronounced in the latter model system. Surprisingly, PACMA-31 exerted opposing effects under these conditions. Whereas PACMA-31 largely abolished LPS-induced expression of cellular TF, PCA and release of procoagulant MVs in PMCs, PACMA-31-mediated PDI inhibition in whole blood resulted in a significant increase in monocyte TF antigen and MV-associated Xa generation.
Conclusion: These experiments emphasize the crucial role of PDI in contributing to TF-dependent coagulation in endotoxinemia, but also indicate additional functions of PDI in regulating cellular TF expression dependent on the extracellular environment.
Disclosure: No significant relationships.

P 111 Monitoring the hemostatic balance in a murine model of hepatotoxin-induced advanced chronic liver disease: effects of protein S and combined Gas6-protein S deficiencies.

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Objectives: The hemostatic balance in advanced chronic liver disease (ACLD) is highly sensitive to changes and contribute to fibrosis progression/complications. No tests are available to monitor globally hemostasis in ACLD.Here, thrombin generation assay (TGA) was used to assess hemostasis in a murine ACLD model using wild-type and mice carrying partial protein S (Pros1) and Gas6 complete deficiency.
Methods: $Pros1^{+/+}$, $Pros1^{+/-}$, $Gas6^{-/-}Pros1^{+/+}$, $Gas6^{-/-}Pros1^{+/-}$ mice received 200mg/kg thioacetamide i.p. 3x/week/12weeks.
Results: 20–30% of mice died. The survivors developed fibrosis with a wide range of severity: severe fibrosis (METAVIR F4) in 11% $Pros1^{+/+}$(n=4) and 31% $Pros1^{+/-}$ (n=4) mice, advanced stage (F3) in 50% $Pros1^{+/+}$(n=12) and 54% $Pros1^{+/-}$ (n=7), moderate fibrosis (F2) in 39% $Pros1^{+/+}$(n=10) and 15% $Pros1^{+/-}$ (n=3). $Gas6^{-/-}Pros1^{+/+}$ mice developed less severe fibrosis: F3 in 37% (n=3) and F2 63% (n=5). This trend was reversed in $Gas6^{-/-}Pros1^{+/-}$ mice: 54% (n=6) at F3 and 46% (n=5) at F2. PT, Fb, FV, FVIII and platelets were unchanged. TAT was increased in $Pros1^{+/+}$ and $Pros1^{+/-}$ mice while levels in $Gas6^{-/-}Pros1^{+/+}$ mice were comparable to the untreated group ($Pros1^{+/+}$=50.2±34.8, $Pros1^{+/-}$=47.7±57.9 vs $Gas6^{-/-}Pros1^{+/+}$= 10.8±5.5, $Gas6^{-/-}Pros1^{+/-}$=13.9±17.9). Preliminary TGA data showed no APC resistance (nAPCsr: $Pros1^{+/+}$=1.0±0.3, $Pros1^{+/-}$=1.3±0.7, $Gas6^{-/-}Pros1^{+/+}$= 1.3±0.3 and $Gas6^{-/-}Pros1^{+/-}$=1.1) but an acquired TM resistance in $Pros1^{+/+}$ and $Pros1^{+/-}$ (nTMsr: $Pros1^{+/+}$=2.1±0.4, $Pros1^{+/-}$=1.9±0.5, $Gas6^{-/-}Pros1^{+/+}$= 1.2±0.4 and $Gas6^{-/-}Pros1^{+/-}$=1.1). A positive correlation between nTMsr and the METAVIR score for $Pros1^{+/+}$ mice (r=0.46, n=6) was found.
Conclusion: These data suggest that TM resistance might be discriminatory for the murine ACLD severity. Further TGA analysis on treated and control mice are needed.
Disclosure: No significant relationships.

P 112 Extracellular RNA boosters the inflammatory function of damaged (DAMP)- or pathogen-associated patterns (PAMP) in macrophages

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Objectives: Self-extracellular RNA (eRNA) has been characterized as universal alarm signal upon tissue damage as well as cofactor in inflammation and intrinsic coagulation. Here, it was studied whether eRNA can sensitize macrophages in response to damaged- or pathogen-associated patterns (DAMP, PAMP), such as toll-like receptor (TLR)-agonists.
Methods: Mouse macrophages were differentiated from bone marrow-derived stem cells by colony stimulating factor and characterized by CD68-, F4/80-, and CD11b-staining using FACS analysis. Highly purified RNA was harvested from NIH 3T3 cells and used as eRNA. Cytokines were quantified by ELISA.
Results: When exposed to isolated agonists of TLR2 (Pam2CSK4) and TLR4 (LPS) at concentrations between 10 pg/ml – 1 µg/ml, macrophages responded with a liberation of TNF-a in a concentration-dependent manner. eRNA enhanced Pam-induced cytokine liberation synergistically by causing a considerable left-shift of the dose-response curve of the TLR2-agonists Pam2CSK4 (Pam) or FSL1. The synergistic response of eRNA on TLR4-agonists was much weaker, and not detectable with TLR3– or TLR7-agonists. Synergistic responses of eRNA and Pam were abolished by RNase1, by antibodies against TLR2, or by an inhibitor of the NFkB-signaling pathway. Since inhibition of the p38 MAPK or ERK pathway also reduced the combined eRNA/Pam response, several pathways may feed the Pam/eRNA-TLR2 signaling route.
Conclusion: The damage signal eRNA sensitizes macrophages towards DAMPs/PAMPs in a synergistic manner via TLR2-NFkB-signaling mechanisms. This supports the concept that eRNA sensitizes our body towards external activators of inflammation and innate immunity and may thereby serve as a threshold factor for life-threatening diseases like sepsis.
Disclosure: No significant relationships.

Poster

P 113 Refractoriness to exogenous TFPI in pediatric patients with inflammatory bowel disease.

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Objectives: Inflammatory Bowel Diseases (IBD) are characterized by chronic inflammation of the digestive tract which is associated with a heightened risk to develop thrombosis. In an inflammatory state polyphosphate released from platelets or bacteria can induce a prothrombotic shift in the coagulation system. Polyphosphate can inhibit TFPI action, which makes it a unique endogenous TFPI inhibitor. We hypothesized that polyphosphate might be involved in the pathophysiology of IBD, and tested whether IBD patients exhibit a resistance to exogenous TFPI.
Methods: Pediatric patients with active Crohn's disease (N=5) or ulcerative colitis (N=5) were compared with age-matched healthy controls (N=10). Thrombin generation was performed using Calibrated Automated Thrombography using 1 pM tissue factor with or without addition of recombinant TFPI (150 ng/ml).
Results: Without addition of recombinant TFPI IBD patients exhibited a shorter lagtime than controls (IBD: 2.59±0.52 min; Controls: 3.67±0.57 min, P<0.001) In healthy controls, addition of recombinant TFPI resulted in substantial lagtime prolongation (5.32±0.45 min; P<0.001). Plasma from patients with Crohn's disease exhibited slightly prolonged lagtime (0.84±0.23 min; P<0.001). No significant changes in lagtime were observed when TFPI was added to plasma from patients with ulcerative colitis.
Conclusion: Plasma of pediatric IBD patients exhibits refractoriness to exogenous TFPI activity, which is potentially mediated by the TFPI inhibiting action of polyphosphate. This refractoriness may extend to endogenous TFPI, which is in line with shorter lagtimes in IBD patients compared to controls, and may contribute to the heightened risk to develop thrombosis.
Disclosure: This work was supported by funds from Biotest, Novo Nordisk, Baxalta, and CSL Behring.

Perioperative hemostasis

P 114 Protocol for a phase 3, non-inferiority, randomized comparison of a new fibrinogen concentrate vs. cryoprecipitate for treating acquired hypofibrinogenemia in bleeding cardiac surgical patients: the FIBRES study

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Objectives: Coagulopathic bleeding is a serious complication of cardiac surgery involving cardiopulmonary bypass (CPB), often requiring massive transfusion with allogeneic blood products (ABPs). Acquired hypofibrinogenemia (plasma fibrinogen <1.5–2.0g/L) is a primary causative factor. Randomized trials evaluating fibrinogen supplementation are needed in this setting.
Methods: FIBrinogen REplenishment in Surgery (FIBRES) is a multi-center, randomized (1:1), active-control, single-blinded trial in adult cardiac surgical patients experiencing clinically significant bleeding due to acquired hypofibrinogenemia (NCT03037424). The primary objective is to determine if efficacy of fibrinogen concentrate (Octafibrin/Fibryga, Octapharma) is non-inferior to cryoprecipitate. Patients will receive 4g fibrinogen concentrate or 10 units cryoprecipitate (dose-equivalent to 4g) whenever fibrinogen supplementation is ordered within 24 hours after CPB; all randomized patients will receive fibrinogen supplementation as clinically indicated. The primary outcome is total ABPs administered within 24 hours. Secondary outcomes include: major bleeding; fibrinogen levels; adverse events (AEs) and serious AEs within 28 days. Need for patient consent is waived, and the study will include 1,200 patients; assuming 20% non-inferiority margin and ~10% drop-out after randomization, ≥550 patients/group provides >90% power. Interim analysis will include 600 patients. The pragmatic design and algorithm align with standard practice, aiding adherence and clinical relevance. ▶Figure 1. Study Design.

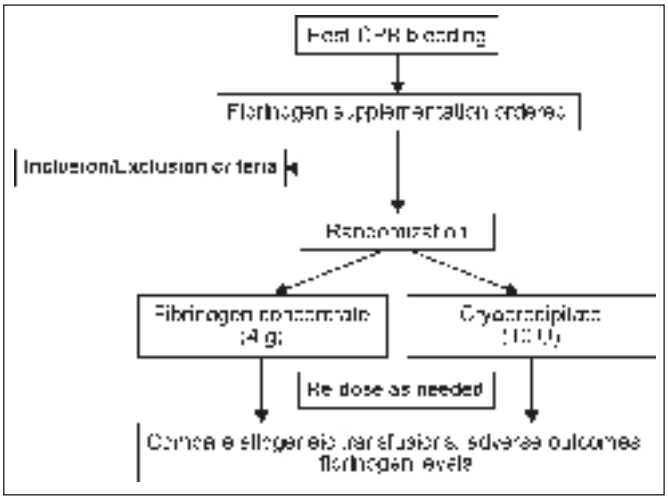

Fig. 1

Results: Completion late 2018; results early 2019.
Conclusion: This is the largest randomized comparison of fibrinogen concentrate and cryoprecipitate in this setting. Non-inferiority of the new, highly purified fibrinogen concentrate would support its use in acquired hypofibrinogenemia. The results are likely to improve care for cardiac surgical patients experiencing significant bleeding, an under-studied, high-risk population.
Disclosure: Research support and honoraria from Octapharma.

Transfusion medicine

P 115 Quality of fresh frozen plasma after thawing with a new radio wave device

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Objectives: Uncontrolled haemorrhage caused by serious injuries after trauma results in rapid need of blood products. The aim of this study was to analyse the quality of clotting factors after thawing with a new radio wave device UFT 100 and after storage at 4°C for 48 hours.
Methods: Samples of 30 different FFP units were drawn aseptically and investigated on various clotting factors and protein proteases (Fibrinogen, Antihrombin, FII, FV, FVII, FVIII, FIX, FX, FXI, FXIII, vWF, protein S, protein C) before freezing, at the time of product thaw and after storage at 4°C for 48 hours.
Results: Activities of factor V, VII and VIII remained stable immediately after thawing compared to baseline, but declined after storage at 4°C for 48 hours. At the time of product thaw and after storage, slight variations in other clotting factor activity levels and protein proteases could be observed, but none of them reached statistical significance.
Conclusion: All FFP units contained adequate coagulation factor activities to maintain haemostatic activity at the time of product thaw. The storage at 4°C for 48 hours causes an anticipated decrease in Factor VIII-activity, but retains normal coagulation factor levels of many other plasma proteins. Therefore we conclude that the UFT 100 has no significant influence on the activity of clotting factors and plasma proteases in FFP units.
Disclosure: No significant relationships.

Cancer

P 116 Androgen deprivation differentially affects peripheral blood leukocyte population in young and old mice

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Objectives: Androgen deprivation therapy (ADT) is still the standard of care for advanced prostate cancer patients. ADT does, however, cause cardiovascular side effects, including myocardial infarction and stroke. These adverse events could be a direct cause of ADT as it also induces immunological alterations. Especially monocytes are of interest as they control systemic coagulation and inflammation. In mice, there are two major monocyte subsets, Ly-6c^{high} and Ly-6c^{low}, which are considered pro-inflammatory and patrolling, respectively. As prostate cancer is a disease of the elderly but most experiments are performed with young mice we set up a study to compare the ADT effects on peripheral blood leukocyte populations between young and old mice.
Methods: We castrated young (10-weeks-old) and old (10-month-old) male mice and analyzed leukocyte populations over several weeks using flow cytometry. Monocytes were defined as CD45+ CD11b+ B220– CD3– cells and further divided into Ly-6c^{high}, Ly-6c^{medium} and Ly-6c^{low} cells.
Results: Under basal conditions, young and old mice showed similar levels of peripheral blood leukocytes. Upon castration, old but not young mice showed a significant increase in total monocytes, starting at day 6 after castration. This effect was associated with an increase of Ly-6c^{high} monocytes. In young mice, monocyte numbers and monocyte subsets were similar in sham operated and castrated mice.
Conclusion: Upon castration monocytes are differently affected in young and old mice. Old mice might be a better tool to analyze the adverse effects of androgen depletion therapy observed in prostate cancer patients.
Disclosure: No significant relationships.

P 117 Circulating tumor cells and thromboembolic events in patients with glioblastoma multiforme

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Objectives: Patients with glioblastoma multiforme (GBM) are at increased risk for arterial and venous thromboembolic (TE) events. Risk factors include surgery, the use of corticosteroids, radiation and chemotherapy, but also prothrombotic characteristics of the tumor itself such as expression of tissue factor, VEGF or podoplanin. Although distant metastases are extremely rare in this tumor entity, circulating tumor cells (CTCs) have been detected in a significant proportion of GBM patients, potentially linking local tumor growth characteristics to systemic hypercoagulability.
Methods: We performed a post-hoc analysis of a previous study, in which GBM patients had been systematically investigated for CTCs [1]. Information on TE events was retrieved from electronic patient charts. 1. Muller, C., et al., *Hematogenous dissemination of glioblastoma multiforme*. Sci Transl Med, 2014. **6**(247): p. 247ra101.
Results: In total, 133 patients (median age, 62 years; IQR, 53–70 years) were analyzed. During follow-up, TE events were documented in 14 patients (11%), including 8 venous and 6 arterial events. CTCs were detected in 26 patients (20%). Of the patients with detectable CTCs, 3 (12%) experienced a TE event compared to 11 (10%) of the patients without detectable CTCs. Thus, there was no difference in the frequency of TE events between patients with and those without detectable CTCs (P=0.85).
Conclusion: Although our study confirms a high risk of vascular thrombosis in GBM patients, it does not point to an obvious association between TE events and CTCs.
Disclosure: No significant relationships.

P 118 Prevalence and Risk of Arterial and Venous Thromboembolism in Patients with and without Cancer: Analysis of a Nation-Wide Database

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Objectives: An interrelation between cancer and both venous and arterial thromboembolism (VTE/ATE) has been suggested. We have compared the frequency of a history or concurrent ATE diagnosis in cancer- and non-cancer patients, and analyzed the association of ATE and VTE with cancer in a nation-wide cross-sectional study.
Methods: ICD-10 diagnosis codes of all publically-insured Austrian adults (18–89 years) were extracted from the Austrian Association of Social Security Providers dataset covering the years 2006–2007 (n=6.806,932). Patients with a history of cancer or active cancer were defined as having at least one ICD-10 "C" diagnosis code, and patients with ATE and/or VTE as having at least one of I21, I24, I63, I64, I74, I26, I80, I82 code. Random-effects (RE) meta-analysis models were applied for analyzing prevalence and relative risk.
Results: In patients with cancer, a history or concurrent diagnosis of ATE was more frequent than VTE. In detail, among 157,781 subjects with cancer, 56,763 had an ATE diagnosis (RE-prevalence=28%) and 22,388 a VTE diagnosis (RE-prevalence=19%). In contrast, among 6.649,151 subjects without cancer, only 51,580 had an ATE diagnosis (RE-prevalence=1%) and 18,870 a VTE diagnosis (RE-prevalence<1%). This corresponded to a RE-risk ratio of 65.1 (95%CI: 34.5–123.0, p<0.001) for ATE (▶Figure 1) and 69.7 (95%CI: 25.3–192.3, p<0.001) for VTE.
Conclusion: The prevalence of both VTE and ATE is significantly higher in patients with a diagnosis of cancer compared to those without cancer. Our population-level meta-data indicates a strong association between cancer, ATE and VTE, and supports the concept of shared risk factors and pathobiology between these diseases.
Disclosure: No significant relationships.

P 119 Fibrin supports growth and invasion of glioma stem cells

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Objectives: Glioblastoma is a highly aggressive brain tumor characterized by diffuse growth and resistance to therapy. Angiogenesis in glioblastomas is poorly organized and, therefore, tends to be associated with tumor cell necrosis, hemorrhage and thrombosis. This, in turn, leads to the formation of a fibrin-rich matrix, which could provide important adhesive cues for glioblastoma growth and proliferation.
Methods: Glioblastoma cell lines and primary tumor cells from patients with glioblastoma were embedded in a 3-dimensional matrix of clotted plasma, fibrin or Matrigel™ and scored for invadopodia formation as well as proliferation using phase contrast microscopy. Tumor stem cells were generated by culturing glioblastoma cells in the presence of Neurocult™ media.

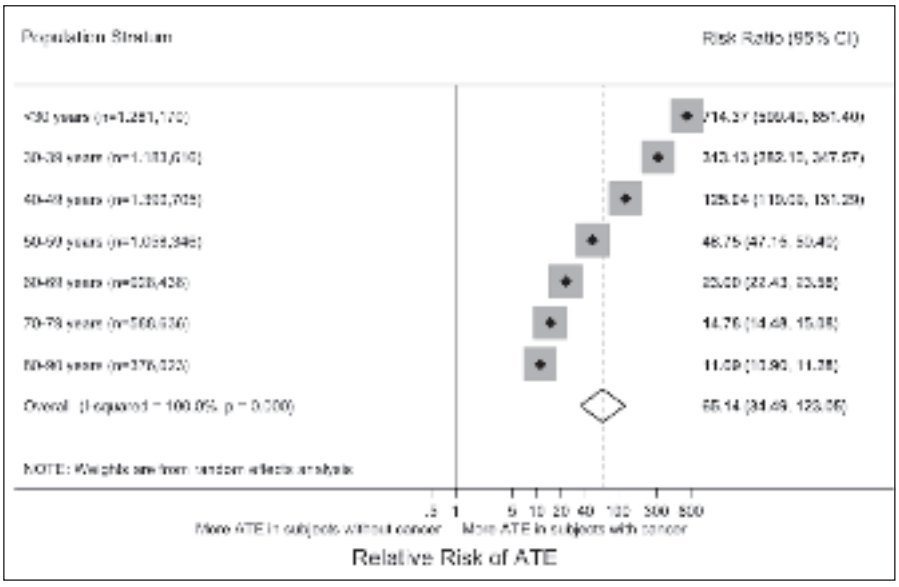

Fig. 1
Relative Risk of ATE

Tab. 1 Results: For positive control lot 2K52B00 BUs

2K52B00	Technochrom FVIII:C	Siron LS	DAPTTIN TC	Pathromtin SL	STA CK-Prest	SythASil
Mean BU	26.8	27.4	19.4	31.7	27.0	32.0
SD	1.83	1.95	1.42	2.96	5.05	2.55
CV %	0.68	0.71	0.73	9.33	8.71	7.97

Tab. 2 Results for positive control lot 2K71B00

2K71B00	Technochrom FVIII:C	Siron LS	DAPTTIN TC
Mean BU	16.4	16.7	14.5
SD	1.79	2.74	2.84
CV %	1.09	1.64	1.96

Results: By probing glioblastoma cells in 3D culture, we identified strong clot invasion and proliferation in cell lines as well as in primary tumor cells freshly isolated from patients with glioblastoma. Clot invasion and proliferation was mediated by integrin β3 and fibronectin, which were highly expressed in clot invasive glioblastoma. Non-invasive cell lines or low grade primary gliomas, on the other hand, expressed only moderate amounts of these adhesion proteins. Clot-invasive glioblastoma cells grew significantly better in fibrin than in matrigel and this growth advantage was accompanied by significant upregulation of the stem cell marker nestin.
Conclusion: Our data show that clotted plasma, which is present in the fibrin-rich edema of the tumor extracellular matrix, strongly promotes glioblastoma infiltration and colonization. Moreover, they suggest that integrin β3-mediated adhesion in fibrin promotes the self-renewal of tumor-initiating cells.
Disclosure: No significant relationships.

Acquired hemophilia

P 120 Performance of the FVIII INH kit using different clotting methods for FVIII determination

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Objectives: The positive control contained in the FVIII INH reagent kit is a pool of native plasma obtained from donors with high FVIII inhibitor titer. In the last batches of FVIII inhibitor plasma, the determined BUs showed a dependency on the method used for FVIII determination. Aim of the study was to investigate the performance of the assay using different clotting methods for FVIII determination.
Methods: The modified Bethesda assay uses buffered normal plasma in a 1:1 mixture with patient plasma and imidazole buffer for control mixture. To compensate for protein dilution, imidazole buffer was also supplemented with BSA. After incubation, the residual FVIII activity was determined with Technochrom FVIII:C assay and FVIII clotting methods, using different aPTT reagents.
Results: For positive sample BUs are ▶Table 1:
As the donors are treated with rFVIII preparations, the developed antibodies may interfere with phospholipid binding of FVIII. This is reflected in different BUs, using aPTT reagents with different phospholipid composition/concentration.
Conclusion: The FVIII INH kit can be used with different FVIII clotting methods with good performance.
Disclosure: All authors are employees of Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH

Women issues in thrombosis and hemostasis

P 121 Folic acid supplementation reduces plasma homocysteine in postmenopausal women

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This abstract has been withdrawn

P 122 Peripartum management in women receiving anticoagulant treatment

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Objectives: Hereditary risk factors or a history of venous thromboembolism (VTE) exacerbate the already increased risk for VTE in pregnancy. Although anticoagulant treatment during pregnancy is well established, less is known about its impact on obstetric and anesthesiological peripartum management.
Methods: The medical records of 150 women who delivered at the University Hospital Bonn and received peripartum anticoagulant medication with low molecular weight heparin (LMWH) were evaluated and matched at a 1:2 ratio with women who did not receive heparin. Matching criteria were age, date and gestational age at delivery. Indications for LMWH were VTE in the current pregnancy (18%), history of VTE (53%), and asymptomatic thrombophilia (29%).
Results: In the LMWH group the odds ratio for caesarean section without neuraxial anesthesia was statistically significant elevated (OR=2.27, 95% CI=1.04–4.97, p=0.035). In addition the evaluated items included the mode of delivery and mode of anesthesia, blood loss during birth, pre- and postpartal hemoglobin levels, and Apgar score of the newborn infant, but no significant differences between both groups were observed. The recommended interval for cessation of LMWH before caesarean section or neuraxial anesthesia was not respected in four cases. In another five cases, in which LMWH was not paused in time, a vaginal delivery without neuraxial anesthesia was performed.
Conclusion: Our data demonstrate that bleeding complications at birth does not occur frequently in women receiving anticoagulant treatment with LMWH. A higher proportion of women with anticoagulant treatment in pregnancy might be eligible for neuraxial anesthesia.
Disclosure: No significant relationships.

P 123 Von Willebrand disease and unintentional childlessness: results from a large screening study

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Objectives: Women with von Willebrand disease (VWD) have not been shown to have lower rates of fertility than women without VWD. However, conflicting data has been published on the risk of pregnancy loss in female VWD patients. Female VWD patients may be at increased risk of unintentional childlessness for a variety of complications including conception problems, miscarriage, and fetal loss. A screening study was introduced with the aim to prove this association and to establish its magnitude.
Methods: Women presenting to a regional fertility center for unintentional childlessness were offered initial laboratory screening for VWD. A total of 1413 patients aged between 16 and 48 years agreed to participate in the study and were screened for von Willebrand factor antigen (vWF:Ag) and ristocetin cofactor activity (vWF:RCo) at the time of their initial consultation.
Results: Thirty nine patients (2.8%) demonstrated vWF:Ag levels below 50% (range 12–48%) and are considered likely VWD patients. In addition, 96 patients (6.8%) were considered possible VWD patients (vWF:Ag levels 50–59%).
Conclusion: In a large screening study aiming to establish an association between VWD and unintentional childlessness, cases of likely and possible VWD amount to 9.6%. Considering VWF levels less than 50% as indicative of VWD, 2.8% of the presenting women are likely VWD patients. An additional 6.8% of the women are preliminary defined as possible VWD patients for the known fluctuating nature of the VWF complex and require follow-up investigations. Pregnancy in women with established VWD will be closely monitored for bleeding-related complications and therapeutic measures will be defined individually.
Disclosure: No significant relationships.

P 124 Management of deliveries in carriers for haemophila A and low FVIII levels with recombinant FVIIIFc fusion protein

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Objectives: Introduction: We report the outcome of deliveries in three carriers of hemophilia A who had previously experienced post-partum haemorrhage. The women were treated with efmoroctocog alfa (Elocta®, Sobi) two hours before delivery. We speculated that this Fc fusion-factor VIII product could cross the placenta into fetal blood by binding to FcRNn receptors and thus reduce the risk of bleeding in the neonate.
Methods: Samples and Methods: We assayed FVIII activity in three carrier females at three time points: before pregnancy; at week 38 of gestation; and 4 hours after delivery. Each woman received 2000 IU efmoroctocog alfa 2 hours before delivery. A cord blood sample was drawn from the neonates (one with severe hemophilia A, one wild type and twins with mild hemophilia) immediately after birth and a venous blood sample was also drawn one day after birth.
Results: Results: In the carriers we observed an increase of the FVIII activity of 2%, 89% and 90%. In the newborn with severe hemophilia A and also in the other newborns, we did not see any effect after infusion of the mother. No thrombotic complications were observed.
Conclusion: Discussion/Conclusion: The infusion of a recombinant FVIII linked to the Fc-region does not effect the FVIII-level of the newborns. It seems, that also these kind of FVIII concentrate does not pass the placenta in a measureable amount.
Disclosure: No significant relationships.

P 125 Referral for thrombophilia testing in an obstetric patient population: a tertiary centre experience

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Objectives: Thrombophilias increase the risk of pregnancy-related morbidity. Current guidelines recommend testing women when they present with recurrent miscarriage (3 or more consecutive pregnancy losses before 24 weeks) or with a late miscarriage (loss between 12–24 weeks). Our aim was to evaluate our testing for thrombophilia in pregnancy-related events.
Methods: Data was collected retrospectively on 151 thrombophilia screens requested for obstetric reasons from July 2016 to July 2017 at Waikato Hospital. Cost analysis was done using NZ $300 (US $223) for a full screen and NZ $30 (US $22) for protein S alone.

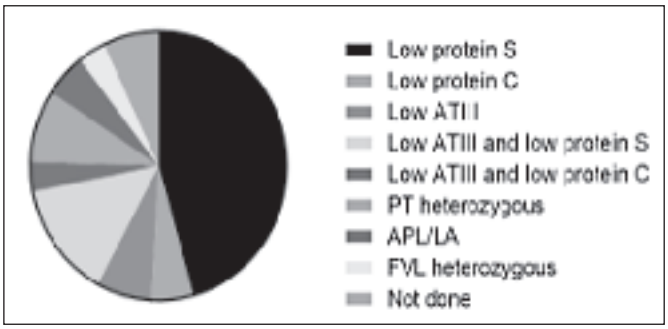

Fig. 1 Breakdown of positive test results (57 positive results from 151 tests).

Tab. 1 Cost of thrombophilia screens and protein S tests.

	Cost in NZ dollars	Cost in US dollars
Full thrombophilia screen	$28,000	$20,992
Protein S (inc repeat testing for pregnancy-related low levels)	$879	$648

Results: Mean patient age was 31 years (range 18–48). The commonest reason for testing was recurrent miscarriage (49/151). 83% (126/151) of tests requested were for a full thrombophilia screen. There were 57 positive tests (38%). The commonest abnormal result was low protein S levels (26/57) (▶Figure 1). Of these, 4 tests were repeated with 1 confirmed protein S deficiency. Cost of negative thrombophilia screens was estimated at NZ $28,000 (US $20,992); while protein S testing (including repeat tests) was estimated at NZ $879 (US $648). Results that would change management (e.g. APL antibodies) were few (6/151).
Conclusion: The majority of obstetric thrombophilia testing was negative. Most positive results (low protein S) were attributable to pregnancy. There was a potential change in management for only 6 patients. Our results are in line with the literature which shows little evidence supporting thrombophilia screens in pregnancy-related morbidity. We aim to educate our colleagues on the utility and cost of thrombophilia testing in pregnancy.
Disclosure: No significant relationships.

Animal models

P 126 Protein tyrosine phosphatase non receptor type 22 (PTPN22) function impacts neutrophil extracellular trap formation

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Objectives: Neutrophils undergo NETosis via peptidylarginine deiminase 4 (PAD 4) activation and release extracellular traps (NETs) into the extracellular space to combat pathogens. NETs also have a significant role in thrombotic disease. Coronary NET burden correlates positively with infarct size in ST-elevation myocardial infarction (STEMI) patients. It was reported that a missense mutation (R620W) in the protein tyrosine phosphatase non receptor type 22 (PTPN22) results in abrogated PAD4 inhibition and consecutively leads to enhanced NETosis. Deoxyribonuclease (DNase) is a natural counter mechanism against NETs. Therefore, we analyzed the effect of PTPN22 deficiency on NET formation in a murine model and studied the R620W single nucleotide polymorphism (SNP) in coronary artery disease (CAD) patients with regard to outcomes.
Methods: Blood was drawn from PTPN22 knockout (KO) mice, NETosis was induced by ionomycin and compared to wildtype (WT) mice (each n=10). NETotic neutrophils were measured by flow cytometry. DNase activity in murine plasma samples was measured by an in-house built activity assay. Furthermore, we tested the R620W SNP in 711 CAD patients who suffered from ST elevation myocardial infarction using allelic discrimination polymerase chain reaction (PCR).
Results: PTPN22 KO mice displayed significantly reduced NETosis compared to WT. Interestingly, PTPN22 mice had a significantly increased plasmatic DNase activity, which correlated with reduced NETosis. CAD patients carrying the R620W showed no altered mortality compared to controls.
Conclusion: In contrast to present literature, we found decreased NETosis in PTPN22 KO mice. In this ongoing project, we will further evaluate NETosis and DNase in connection to PTPN22.
Disclosure: No significant relationships.

P 127 Demonstration of both hypo- and hypercoagulability by the thrombin generation assay during experimental sepsis

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Objectives: The activation of blood coagulation has been demonstrated in most cases of sepsis, however previous studies in humans could not detect hypercoagulability with global hemostasis assays. We hypothesized that in a sepsis model, thrombin generation assay can be useful do identify different hemostatic alterations if multiple measuring conditions are utilized.
Methods: To elicit a fulminant sepsis, live Escherichia coli were inoculated to pigs and prothrombin time, activated partial thromboplastin time, thrombin time and fibrinogen were measured by coagulometry. Platelet counts, platelet aggregates and platelet phosphatidyl serine (PS) expression were studied and the PS-inducing ability of septic and control plasmas was investigated by flow cytometry using human red blood cells. Thrombin generation was carried out by the Ascent Fluoroscan reader and results were evaluated by the Thrombinoscope software.
Results: Clotting assays showed a large variability but no systematic changes during the 4-hour observation period. Platelet count significantly decreased and the number of platelet aggregates increased already by two hours compared to baseline values. Although the increase in platelet PS expression was non-significant in the septic group, the septic plasma elicited PS expression on normal human red blood cells. Thrombin generation became significantly faster, but the quantity of formed thrombin demonstrated both hypo- and hypercoagulability depending on the setting of the assay.
Conclusion: Enhanced thrombin generation measured without activators and the PS-inducing capacity of septic plasma are signs of hemostatic activation during fulminant sepsis while the decreased amount of generated thrombin upon tissue factor and phospholipid induced activation demonstrates consumption coagulopathy.
Disclosure: No significant relationships.

Vascular wall biology and disorders / Endothelial cells

P 128 Expression patterns of F8-binding-TFs in different endothelial cells

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Objectives: F8 deficiency causes hemophilia A. Main source of F8 production/secretion is the liver sinusoidal endothelial cells (HHSECs), whereas other ECs also express and secrete F8 but to a less extent; this variability in expression could be caused by epigenetic regulation and/or by differential expression of transcription factors (TFs) binding to the promoter region.
Methods: Toward explaining the molecular reasons for differences in expression of F8 between different ECs, we used TRANSFAC-database to identify potential TFs binding to the F8 promoter and determine their expression levels in adult and fetal HHSEC and in human umbilical vein endothelial cells (HUVECs) using microarrays. Additionally we analyzed methylation of the F8 promoter region using Illumina-EPIC methylation arrays
Results: F8 expression is highest in adult HHSEC, than fetal HHSEC than HUVEC. We found 2 CpGs in the 1,5 kb upstream of F8-TSS, whose methylation patterns distinguish HUVECs from both fetal and adult HHSEC but with no significant separation power between adult and fetal HHSEC. Twenty eight TFs matrices were found to bind to the 1,5 kb region upstream and 200 bp region downstream of F8-TSS. These matrices represent unique 75 TFs, after background filtering, in our dataset. We than identified differential expressed TFs that are specifically over expressed in F8 expressing cells. We found that 1 TF were common between adult and fetal HHSEC against HUVEC
Conclusion: We detected TFs predicted to bind the F8 promoter that are specifically expressed in adult HHSEC. These TFs could be linked to expression and thus secretion potential of F8 by HHSEC.
Disclosure: No significant relationships.

Angiogenesis and tissue repair

P 129 Endothelial activation of NF-kB leads to impaired embryonic angiogenesis

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Objectives: The process of the formation of blood vessel can generally be subdivided into vasculogenesis and angiogenesis, which are both fundamental in embryonic development and organogenesis. Nuclear factor-kB (NF-kB) are important mediators for inflammatory and immune responses and regulate are variety of processes including proliferation, apoptosis and development. Previous work has shown that NF-kB signaling is required for adequate angiogenesis and it might therefore be an interesting target for anti-angiogenic cancer therapies. It is known that NF-kB targets a variety of factors, which can affect angiogenesis, including angiogenic factors as, for example, VEGF and the VEGF-receptor but also angiostatic factors as VEGI and thrombospondin 1.
Methods: To further evaluate the role of NF-kB during angiogenesis we generated mice, which activate NF-kB specifically in endothelial cells. To that end, we crossed mice, which express the well-characterized Tie2–Cre allele to mice harbouring a constitutive active IKK2^{ca} allele after a flox-stop cassette.
Results: Endothelial expression of IKK2^{ca} resulted in severe defects in the embryonic vascular system, as evident by reduced yolk sac angiogenesis and hemorrhagic lesions. Mice positive for Tie2 and homozygous for the IKK2^{ca} allele die at embryonic day (E) 12.5 – 13.5 dpc. In the liver, these mice show severe apoptosis and reduced proliferation. This is most likely due to reduced blood vessel formation, which leads to impaired blood supply and apoptosis of hepatocytes.
Conclusion: Constitutive endothelial activation of NF-kB inhibits angiogenesis during mouse embryonic development. Impaired angiogenesis does particularly affect the development of the liver.
Disclosure: No significant relationships.

Biorheology

P 130 Prothrombotic role of hemorheologic disturbances

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This abstract has been withdrawn

Extracellular microvesicles

P 131 New method for determination tissue factor positive microparticles as coagulation system activator

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Objectives: In this part of the study, where we determined the causes of endothelial dysfunction at cardiac surgery patients indicating for aortocoronary bypass with extracorporeal circulation , we focused on the role of tissue factor (TF) in the activation of these pathophysiological processes. Microparticles are too small to be visualised using conventional forward and side light scatter, thus we used carboxyfluorescein diacetate succinimidyl ester staining for their identification, CD142 Briliant Violet antibody for detection of tissue factor producing subpopulation and BD Trucount tubes for absolute counting. We demonstrated that CFSE can be successfully used to label vesicles with closed intact membrane. Multiparametric flow cytometry seems to be valuable tool for detection, identification and enumeration of microparticles for both biomedical reasearch and clinical application.
Methods: We used the BD FACS Canto II flow cytometer with instrument setting optimized for very small objects just above the noise level (threshold operator OR with parameters FITC 1500 and BV 2200) for microparticle analysis in plasma samples. CD142 Briliant Violet (BD) antibody for detection of tissue factor producing subpopulation and BD Trucount tubes for absolute counting. Thrombin generation was measured using a Ceveron Alpha analyzer with fluorescent detection method.
Results: We verified the model on patients undergoing cardiac surgery with the use of extracorporeal circulation, where is a high risk of endothelial dammage.

Conclusion: We demonstrated overexpression of TF on microparticles at cardiac surgery patients and a significant increasing of thrombin generation in plasma. Supported by grant LF-2017–007 and MH CZ – DRO (FNOl, 00098892)
Disclosure: No significant relationships.

Vascular wall biology and disorders / Other related topics

P 132 Expression and release of ribonuclease inhibitor by vascular cell types

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Objectives: Background: Extracellular ribonucleases (RNases) of the RNaseA family are produced and secreted by different tumor and vascular cell types and express ribonucleolytic and other activities, related to tissue protection (RNase1) and angiogenesis (RNase5). Upon uptake of such RNases, all cell types are protected against unwanted RNA-degradation and oxidative stress by providing a cytosolic 50-kDa ribonuclease inhibitor (RI), which binds RNases with femto-molar affinity to render them inactive. No information exists on the cellular release and extracellular functions of RI. Aims: To investigate the mechanism and the regulation of RI release from tumor and vascular cells.
Methods: Methods: Following treatment of endothelial (Ea.hy926) and tumor cells (HT1080) with different agents, the expression and release of RI, RNase1 and RNase5 were examined on mRNA and protein level using quantitative PCR, Western blot analysis and RNase activity tests.
Results: Results: RI was found to be secreted in variable quantities into the cell supernatants of various blood cell types as well as tumor and endothelial cells under normoxic conditions. Hypoxia for 24 h enhanced the release of RI protein, whereby RI mRNA expression was only slightly increased. In contrast, mRNA expression of RNase5 increased up to 50-fold, comparable to the elevation of VEGF mRNA. RI and RNase5 (but not RNase1), liberated from cells, were associated with the microvesicle fraction as was demonstrated by Western blot analysis.
Conclusion: Conclusion: Extracellular RI, particularly released under stress conditions from vascular and tumor cells, may influence vascular homeostasis in various ways that are being currently explored.
Disclosure: No significant relationships.

P 133 Angiotensin-II enhances neutrophil extracellular trap formation in an AT1R and NADPH oxidase-dependent manner

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Objectives: Arterial hypertension is a major risk factor for coronary artery disease (CAD). By formation of neutrophil extracellular traps (NETs), neutrophils release their nuclear content into the extracellular space, fighting pathogens. NETs have been implicated in CAD. In preliminary studies of CAD patients, we observed a positive correlation between blood pressure and NETosis *ex vivo*, implicating blood pressure modulating NETosis. Angiotensin-II (Ang-II) mediates blood pressure via its potent vasoconstrictive properties, but also exerts pro-inflammatory functions via the angiotensin type 1 receptor (AT1R). AT1R is expressed on neutrophils. We thus hypothesized that Ang-II might influence NETosis.
Methods: We stimulated isolated neutrophils with ionomycin ex vivo. NETosis was measured using Sytox® Green, a dye that exclusively stains extracellular DNA, a hallmark feature of NETs. The detergent Triton served as positive control. To assess the role of the Ang-II pathway, we pre-incubated neutrophils with Ang-II, AT1R antagonist losartan or NADPH oxidase inhibitor diphenyleneiodonium (DPI).
Results: We observed a dose-dependent NET release by ionomycin. Irrespective of ionomycin concentration, pre-treatment with Ang-II significantly enhanced NETosis to 80–90% of positive control. Losartan abolished this effect, suggesting an AT1R-dependent pathway. NADPH oxidase is crucial for NETosis due to release of reactive oxygen species. DPI abolished the effect of Ang-II on NETosis.
Conclusion: Our results implicate that via Ang-II, arterial hypertension aids neutrophils to undergo NETosis by increasing intracellular ROS production, which makes neutrophils more susceptible to pro-NETotic stimuli. This provides new insight in how effective blood pressure lowering might lead to more favorable outcomes in CAD.
Disclosure: No significant relationships.

P 134 White blood cell count to mean platelet volume: A novel marker for critical limb ischemia in peripheral arterial occlusive disease Patients

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Objectives: White blood cell count to mean platelet volume (WMR) was published recently as a possible prognostic marker in coronary artery disease patients with st-elevation myocardial infarction. As platelets play a key role in atherosclerosis and atherothrombosis we investigated WMR and its association with critical limb ischemia (CLI) in peripheral arterial occlusive disease (PAOD) patients.
Methods: In our cross sectional study we included 2124 PAOD patients treated at our institution. To analyze potential predictors for CLI univariate logistic regression was performed. Significant variables in univariate analyses were selected for multivariate logistic regression. Variables in the final model were selected with a backward stepwise procedure. ROC analyses were performed to identify the best cut off value for WMR to predict CLI. Therefore the Youden´s index was calculated.
Results: Multivariate regression analysis revealed diabetes ($p<0.001$, OR 2.4, 95% CI 1.9–2.9), CRP ($p<0.001$, OR 1.01, 95% CI 1.007–1.014), age ($p<0.001$, OR 1.05, 95% CI 1.04–1.06), and WMR ($p<0.001$, OR 2.3, 95% CI 1.5–3.5) as significant predictors for CLI. ROC analysis showed ≥0.83 as the best cut off value for WMR to predict CLI.
Conclusion: High WMR is significantly associated with a high risk for CLI in PAOD patients. WMR is an easily determinable, broadly available and cheap marker, which could be used to highlight patients at high risk for CLI.
Disclosure: No significant relationships.

P 135 Platelet BMP4 is Critical for Leukocyte-Endothelium Interactions during Vascular Inflammation

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Objectives: Bone morphogenic proteins (BMPs) are members of the TGFß superfamily. BMPs have distinct functions during hemostasis and play a central role in various pathologic conditions, including cardiovascular diseases. Recent findings demonstrate that BMPs are also expressed in platelets. How-

ever, their function is poorly understood. Here, we investigate the role of platelet BMP4 during vascular inflammation.
Methods: BMP4 floxed mice were crossed with PF4 Cre mice to generate platelet-specific deletion of BMP4 ($BMP4^{Plt-/-}$). Intravital microscopy of mesenteric veins was performed to evaluate leukocyte adhesion upon stimulation with TNFα. Expression of adhesion molecules and chemokines were analyzed by RT-PCR and Western Blot. P-selectin and plateletleukocyte aggregates were evaluated using flow cytometry.
Results: Platelet morphology and function did not differ between $BMP4^{Plt-/-}$ and control mice. Stimulation with TNFα resulted in increased rolling and adherence of leukocytes to the vessel wall which was reduced in $BMP4^{Plt-/-}$ mice (175 ± 25 versus 50 ± 7 rolling cells and 16 ± 3 versus 7 ± 2 adherent cells, respectively). Expression of adhesion molecules and the chemokines RANTES and PF-4 were reduced in $BMP4^{Plt-/-}$-mice. Deletion of platelet BMP4 was associated with less expression of P-selection and reduced formation of plateletleukocytes aggregates upon activation.
Conclusion: We demonstrate for the first time that platelet BMP4 is involved during vascular inflammation. Deletion of platelet BMP4 prevented expression of adhesion molecules and chemokines, and reduced platelet-leukocyte complex formation. This data suggest a potential implication for platelet BMP in inflammatory diseases, including atherosclerosis and restenosis.
Disclosure: No significant relationships.

P 136 Effect of plasma, human albumin, Ringer's lactate and coagulation factor concentrates on acute endotheliopathy in a rodent model of hemorrhagic shock

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Objectives: Recent studies suggest that transfusion of fresh frozen plasma (FFP) to treat hemorrhagic shock (HS) ameliorates the endotheliopathy after trauma. The specific role of plasma derived coagulation factors to treat endothelial injury remains poorly understood. We investigated the effects of FFP, Ringer's lactate (RL), human albumin (HA) and hemostatic therapy using RL or HA in combination with either fibrinogen concentrate (FC) or prothrombin complex concentrate (PCC), on endothelial cell damage (VEGFR1), glycocalyx shedding (syndecan-1, heparan sulfate) and sympatho-adrenal activation (adrenaline) in a rat model of HS.
Methods: Rats were subjected to a model of HS and endothelial injury. At the end of shock (EOS) animals were randomly divided into 7 groups: Ringer's lactate (RL), human albumin (HA), FFP, RL + FC, RL+PCC, HA+FC and HA+PCC.
Results: Hemorrhagic shock led to pronounced sympatho-adrenal activation and increased shedding of both syndecan-1 and heparan sulfate. Levels of heparan sulfate were restored to baseline (BL) following plasma based resuscitation, but remained high among all other groups. None of the therapeutic regimes yielded significantly lower concentrations of syndecan-1 and sVEGFR1, while a tendency towards higher levels of syndecan-1 was observed in the HA-FC group.
Conclusion: In the current study, we found that plasma concentrations of heparan sulfate were restored to BL values following FFP-based resuscitation. We were not able to detect a significant effect of purified coagulation factor concentrates on circulating markers of glycocalyx shedding. There was a tendency towards higher values for syndecan-1 following treatment with HA+FC, suggestive for an aggravation of reperfusion injury following HS.
Disclosure: No significant relationships.

P 137 Thrombin selectively induces transcription of macrophage genes involved in inflammation

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Objectives: Besides its role in blood coagulation, thrombin is known to act as a cell signaling molecule via activation of protease-activated receptors (PARs), thereby connecting coagulation with immune/inflammatory responses. Yet, no detailed data exist on the gene expression profiles of thrombin-stimulated macrophages. Here, the contribution of PAR1 in thrombin-induced gene expression as well as the signaling pathways involved in this process were investigated.
Methods: Bone marrow-derived macrophages (BMDM) were generated from bone marrow stem cells from C57BL/6J mice and were stimulated with different concentrations of thrombin at 37°C. To investigate the contribution of PAR1, macrophages were treated with PAR1-activating peptide (PAR1-AP) or an inactive control peptide, as well as with the PAR1 antagonist SCH79797. For inhibition studies, signaling pathway inhibitors PD98059, SB203580 or BAY11–7082 were included. Gene expression was analyzed by RT-PCR using specific primers.
Results: Several proinflammatory genes (including iNOS, IL1β, TNFα, MIP1α, MIP2, IP10, CXCL1) were differentially expressed in macrophages in response to thrombin. The PAR1-AP mimicked the effect of thrombin, while its inactive control peptide had no significant influence on cell stimulation. Moreover, SCH79797 inhibited the thrombin-induced gene expression in macrophages. RT-PCR data also showed the necessity of NFkB activation for thrombin-induced gene expression.
Conclusion: Our results provide new experimental evidence for the existent link between coagulation and innate immunity, whereby PAR1 and NFkB play important roles in transmitting thrombin-mediated gene expression responses in macrophages.
Disclosure: No significant relationships.

P 138 Deficiency in Platelet BMP4 Reduces Neointima Hyperplasia after Wire Injury

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Objectives: Bone morphogenic proteins (BMPs) are members of the TGFβ superfamily. BMPs play a pivotal role during organogenesis, hemostasis, and in various pathologic conditions, including cardiovascular diseases. Recent findings demonstrate that BMPs are also expressed in platelets. However, their function is poorly understood. We investigated the role of platelet BMP4 on vascular remodeling.
Methods: Transgenic mice with platelet-specific deletion of BMP4 were generated ($BMP4^{Plt-/-}$) and crossed with $LDLr^{-/-}$ mice ($BMP4^{Plt-/-}/LDLr^{-/-}$). At 8 weeks of age, $BMP4^{Plt-/-}/LDLr^{-/-}$ mice and control littermates received a 2-week diet containing 15.8% *wt/wt* fat and 1.25% cholesterol. Carotid wire injury was performed at the age of 10 weeks. Re-endothelialisation and neointima hyperplasia were evaluated.
Results: Loss of platelet BMP4 prevented neointima formation after carotid wire injury ($4.2x10^4$±$0.9x10^4$ um^2 versus $14.9x10^4$±$2.2x10^4$ um^2 in $BMP4^{Plt-/-}/LDLr^{-/-}$ and control mice, respectively). Monocyte infiltration and circulating leukocyte-platelet complexes were reduced in $BMP4^{Plt-/-}$ mice. Expression of ICAM and VCAM was decreased. Furthermore, platelet activation by thrombin was reduced in $BMP4^{Plt-/-}$ mice, resulting in diminished P-Selectin and JONA expression. Release of the chemokines RANTES and PF4 was reduced in $BMP4^{Plt-/-}/LDLr^{-/-}$ mice. Interestingly, endothelial regeneration after injury was decelerated in $BMP4^{Plt-/-}$ mice. This is further demonstrated in-vitro, where platelet BMP4 promoted endothelial cell proliferation and migration.

Conclusion: Platelet BMP4 deficiency ameliorated intima hyperplasia after wire injury. This is partly mediated by inhibition of platelet activation, reduced platelet-leukocyte interaction, and less inflammation. Our findings suggest that BMP4 is a promising target for the treatment of vascular restenosis.
Disclosure: No significant relationships.

Oral anticoagulants

P 139 DOACS in APS patients presenting only venous thromboembolism. A cohort study and a systematic review of the literature.

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Objectives: The recommended treatment for secondary thromboprophylaxis in Antiphospholipid Syndrome (APS) is vitamin K antagonist (VKAs). However, VKAs monitoring is challenging. Direct oral anticoagulants (DOACs) could be useful in this situation. The aim of this study was to determine factors associated with thrombosis (TH) recurrence, rate of thrombosis recurrence and bleeding (BLEED) in APS patients under DOACs for venous thromboembolism (VTE).
Methods: A descriptive analysis was performed on patients presenting APS under DOACs for VTE: in a cohort pooling patients from the Hopital Pasteur Nice and cases described in the literature.
Results: 139 patients with APS and VTE under DOACs were identified: 98 women (n=70%), 56 patients with a secondary APS and 30 patients triple positive, 93% of all patients were under rivaroxaban. Over a mean follow-up of 15 months, 14 TH recurrences occurred (5 patients with arterial TH). Factors associated with TH recurrence were male gender (OR 3,7, IC95% 1,2–11,5, p=0,03), antibody triple positivity (OR 4,4, IC95% 1,4–13,9, p=0,01), aCL positivity (OR 6,6, IC95% 1,4–30,8, p=0,01) and anti-β2GP1 positivity (OR 5,2, IC95% 1,4–19,7, p=0,01). The incident thrombotic recurrence risk was 7,8% person-years and the arterial TH recurrence rate is 2,8% person-year. The incident risk of BLEED was of 12% person-years: 22 patients with minor BLEED, no patients experienced major BLEED.
Conclusion: DOACs may be interesting in this subset of APS patients who experienced only VTE. Controlled randomized trial which compare clinical events in APS patients under VKAs and DOACs are needed to confirm those results.
Disclosure: No significant relationships.

P 140 Successful Replacement of an Inadequate Phenprocoumon Anticoagulation by Rivaroxaban in an 80-Year-Old Factor VII-Deficient Patient with Severe Peripheral and Coronary Artery Disease

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Objectives: The management of patients with factor VII (FVII) deficiency requiring oral anticoagulation is challenging, as coumarin derivatives inhibit the synthesis of FVII. In a male patient with peripheral artery disease grade 3 of the lower limbs femoro-crural arterial bypasses recurrently became thrombotically occluded despite apparently sufficient anticoagulation with clopidogrel, high-dose enoxaparin and phenprocoumon.
Methods: Haemostasis was monitored by INR, prothrombin time (PT Quick %), factors II (FII), FVII and FX activity, D-dimer, anti-factor Xa activity (AXa) for enoxaparin and rivaroxaban (modified AXa assay). Molecular genetic analysis was performed by direct sequencing.
Results: During the treatment course after his last emergency presentation, despite an increase of INR to high therapeutic range (3.6–3.9) and low FVII levels (6–14%) (FII 42–49%, FX 19–25%) within 55 to 83 days, gradually increasing D-dimer levels to 1.4–1.7 mg/l indicated an inadequate anticoagulation. When phenprocoumon was then replaced by rivaroxaban 2.5 mg b.i.d., D-dimers declined to normal range (0.75 mg/l) within 35 days. So far under rivaroxaban therapy no new thrombosis in the bypasses have evolved. Molecular genetic analysis of the factor VII gene (F7) revealed the heterozygous mutation p.(Gln48Leu) and two functional polymorphisms: 10bp insertion in promotor and p.(Arg413Gln). The latter predisposes a 20–25% decline of FVII activity per allele. These polymorphisms together with the mutation explained the 70–75% loss of activity.
Conclusion: Anticoagulation of patients with FVII deficiency with phenprocoumon may be functionally inadequate despite sufficiently high INR levels. Rivaroxaban in contrast is able to modulate the clotting activation to a sufficient anticoagulant level already at low doses.
Disclosure: Bayer Healthcare: Sponsoring of Participations to Scientific Meetings

P 141 Activated factor X-based testing of antithrombin activity is not superior over a thrombin-based method in a real-world patient population using different oral anticoagulant drugs

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Objectives: Antithrombin (AT) activity tests, that are used for diagnosing hereditary AT deficiency, are either based on inhibition of thrombin (FIIa) or activated factor X (FXa). FXa-based assays are presumed to be more sensitive to certain AT deficiency causing mutations than FIIa-based assays.

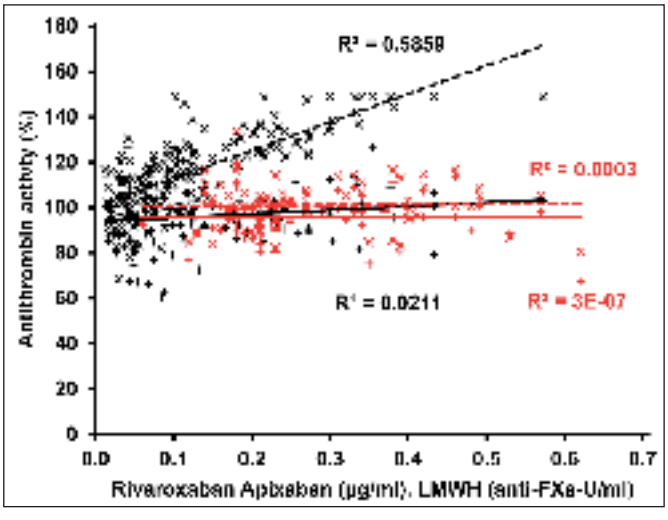

Fig. 1 Influence of anticoagulant drug levels on antithrombin activity (AT) results. Correlation of AT determined by assays based on inhibition of thrombin (+, solid lines) or FXa (x, intersected lines) with plasma levels FXa inhibitors (rivaroxaban, apixaban, black symbols) and low molecular weight heparin (LMWH, red symbols).

Methods: In order to assess the performance of these two methods in a real-world scenario, 745 consecutively collected samples from patients referred to our institute for thrombophilia testing were analysed with both assays.
Results: In samples from patients not receiving direct-acting oral anticoagulants or heparins (n=485) both methods showed good agreement (r=0.874, Bland-Altman limits of agreement 6.57%, –15.76%). While similar results were obtained in patients receiving low molecular weight heparin (LMWH, n=76, r=0.891, 4.09%, –14.35%), the agreement was lower in patients receiving rivaroxaban (n=86, r=0.570, 5.97%, –49.43%) and apixaban (n=72, r=0.735, 3.77%, –42.45%). Measurement of anti-FXa activity revealed that results of the FXa-based assay increased with plasma levels of FXa-inhibitors but not LMWH in a dose-dependent manner, while the FIIa-based test was unaffected (▶Figure 1). 2 out of 11 hereditary AT deficient samples from patients not receiving FXa-inhibitors showed pathologically reduced AT levels only in the FXa-based test.
Conclusion: These data suggest that FXa-based AT testing can be preferred over FIIa-based methods only in the absence of FXa-inhibitors as these anticoagulant drugs falsely increase AT activity levels. FIIa-based AT testing is feasible in these patients, if borderline results are further clarified, e.g. by measurement of antigen levels or genetic analysis.
Disclosure: No significant relationships.

P 142 Recurrent ischemic events in a patient with secondary APS and Libman Sacks endocarditis on apixaban

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Objectives: Currently, evidence for use of DOACs in patients with anti-phospholipid syndrome (APS) is rare. We report on a patient with APS and Libman-Sacks endocarditis.
Methods: We describe the management of anticoagulation in a 47-year old female patient with systemic lupus erythematodes, secondary APS, hereditary factor VII-deficiency and Libman-Sacks endocarditis.
Results: The patient was on long-term prophylaxis with ASS but suffered a stroke in 2014. Anticoagulation was changed to phenprocoumon which was left due to impossible guiding by INR. The following drug, rivaroxaban, was not tolerated. Then, apixaban was started at 2x2.5mg. On this therapy, the patient suffered a new stroke in August 2015 and the dose was increased to 2x5mg. In February 2016, Libman-Sacks endocarditis of the aortic and mitral valve was suggested by transoesophageal echocardiographic findings. Therefore, belimumab was added to hydroxychloroquin and azathioprine. However, a NSTEMI occurred in September 2016 and coronary angiography was accompanied by new minor cerebral infarctions. At this stage, apixaban was changed to phenprocoumon again. The dose was guided by factor II activity with a target of 20–30% but not by INR which achieved values of 3.5–6. No further embolisms or bleeding complications developed under this therapy in combination with the extended immunosuppression. Complete regression of the valvular deposits of the assumed Libman-Sacks endocarditis was observed during the next 7 months. The patient is stable now for more than one year.
Conclusion: This case indicates that apixaban seems to be insufficient for prevention of embolic events in patients with APS and, in specific, with endocarditis.
Disclosure: No significant relationships.

Antiplatelet agents

P 143 The anticoagulant action of ethylpyruvate

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Objectives: Ethyl pyruvate (EP) is known for its anti-inflammatory and anti-oxidative properties. Recent studies suggest that EP also exerts anticoagulant action by reducing mRNA expression of tissue factor, the most important trigger of the coagulation cascade. It was the aim of our study to examine the anticoagulant action of EP in whole blood samples.
Methods: Haemostatic profiling was performed using a platelet function analyzer (PFA) 200, impedance aggregometry, a Cone and Platelet analyzer (CPA), calibrated automated thrombogram, and thrombelastometry (TEM).
Results: EP exerted significant anticoagulant action in whole blood samples. Closure times were dose-dependently prolonged (PFA 200), amplitudes dose-dependently decreased (impedance aggregometry), and coagulation times were dose-dependently prolonged (thrombelastometry) in the presence of increasing amounts of EP (0 up to 1000 µg/mL).
Conclusion: EP is a potent anticoagulant drug. Chronic administration of EP might be beneficial to impede the development of atherosclerotic lesions and thrombotic events.
Disclosure: No significant relationships.

Thrombolytic agents

P 144 Single-center experience with thrombolysis in high- and intermediate-risk pulmonary embolism

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Objectives: Pulmonary embolism (PE) is a potentially life-threatening acute cardiovascular syndrome. While thrombolysis is the guideline-recommended standard of care treatment for high-risk patients, it may be considered in a subset of intermediate-risk patients.
Methods: We followed 87 patients with pulmonary embolism either at high- or intermediate risk (2013–2017). Patients at intermediate risk were treated with alteplase, either full- (100 mg) or low-dose (0.6mg/kg, 50mg max) at the treating physician's discretion when these patients were considered at especially high risk or had already signs of hemodynamic decompensation.
Results: Fourtyone (47%) of the 87 patients were at intermediate risk. Sixteen of these patients (39%) received thrombolysis. 7 were treated at full dose and 9 at low dose. Thirtyfour of the patients at high risk (73.9%) received full-dose thrombolysis. Survival in the intermediate risk group was 97.5% (40 patients), compared to 63% (29 patients) in the high risk group. In the intermediate risk group, relevant bleedings occurred in 3 patients (7.3%) while in the high risk group 21 patients (45.7%) had relevant bleedings.

Conclusion: 30-day survival of patients at intermediate risk PE was 97.5%, after approximately 40% of these patients had received thrombolysis. The risk of relevant bleedings in patients at intermediate risk receiving thrombolysis was relatively low. According to current guideline recommendations, the choice for thrombolysis in intermediate-risk PE patients needs to take into account each individual patient's risk for bleeding and PE-related death. In our study, patients with a low bleeding risk and at younger age appeared to benefit from thrombolysis and low-dose alteplase was safe.
Disclosure: No significant relationships.

P 145 Stability in human plasma and thrombolytic activity of prourokinase-dendrimer conjugates in vitro

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Objectives: Prourokinase (proUK) is an effective plasminogen activator, which widely used as thrombolytic agent. The disadvantage of proUK is rapid clearance from the bloodstream. Dendrimers containing peripheral functional groups are a new class of polymers as drug carriers. **Aim.** Increase in proUK stability in human plasma by its modification with nanosized poly(amidoamine) dendrimer of 2.5 generation.
Methods: Peripheric COOH-groups of the dendrimer was activated with methiodide EDC and N-hydroxysulfosuccinimide (pH 2.0). ProUK was conjugated with the purified activated dendrimer in ratios of protein:polymer 1:1, 1:5, 1:10 and 1:20 (M:M) at pH 8.5. The following properties proUK and proUK-dendrimer conjugates were studied in vitro: stability of fibrinolytic activity in human plasma using fibrin-plate method; plasminogen activator activity using conjugated method and thrombolytic activity by lysis rate of human plasma clots.
Results: Increase in modification degree of proUK (from 1: 1 to 1:20) with dendrimer slowed slightly the conversion of proUK-dendrimer conjugates by plasmin into UK-dendrimer conjugates but the latter have retained the high amidase activity (92–85 %). Plasminogen-activator and thrombolytic activities of proUK-dendrimer conjugates and free pro-UK were comparable. Clot lysis in 4 h induced by all proUK-dendrimer conjugates was 90–95% compared to free proUK. $\tau_{1/2}$ of fibrinolytic activity stability in plasma *in vitro* was 48 min for proUk and from 60 to 130 for proUk-dendrimer conjugates (1:1 to 1:20).
Conclusion: The proUK- poly(amidoamine) dendimer G2.5 conjugates were significantly more stable in human plasma, and have retained the high levels of plasminogen-activator and thrombolytic activities as compared to free proUK.
Disclosure: No significant relationships.

Monitoring antithrombotic therapy

P 146 Evaluation of assay performance monitoring edoxaban plasma concentration with Technoview® Edoxaban and Technochrom® anti-Xa

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Objectives: The aim of this study was to evaluate the performance of measurement of edoxaban with a system composed of an anti-Xa assay using lyophilized calibrators and controls, as well as correlation of patients sample results with those measured with Mass Spectrometry Liquid Chromatography (LC-MS).

Methods: The one stage chromogenic assay for the determination of Xa inhibitor activity in human citrated plasma, Technochrom® anti-Xa, is used for edoxaban measurement on Ceveron® alpha. Lyophilzed calibrators and controls with assigned edoxaban values are used for assay calibration and to calculate assay performance parameters. Anti-Xa assay results of patient samples are compared to those obtained with LC-MS reference method to evaluate assay correlation.
Results: Calibration curves in low range 0–150ng/mL and in high range 0–500ng/mL are made using two adjusted analyzer settings. All calibration curves have a linearity of R^2=1.0±0.1. LLoQ is determined at 25ng/mL. The recovery of control is within 100% ± 10% of target value for all concentrations and precision is very good with intra-assay and inter-assay variations of <15%. For method comparison the correlation of spiked samples as well as patient samples shows a Passing and Bablock regression with a Slope of 1.0 +/- 0.1, an Intercept <15ng/mL and r >0.95.
Conclusion: Our data demonstrate that using the lyophilized calibrator set Technoview® Edoxaban for calibration of Technochrom® anti-Xa assay in optimized settings on Ceveron alpha the determination of edoxaban plasma concentrations in patient samples can be performed with very good performance, patient sample results correlating with the LC-MS results.
Disclosure: All authors are employees of Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH

Platelet dysfunction

P 147 TRPM7 kinase controls calcium responses in arterial thrombosis and stroke in mice

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Objectives: Transient receptor potential melastatin-like 7 channel (TRPM7) is a ubiquitously expressed bi-functional protein comprising a TRP channel segment linked to a cytosolic a-type serine/threonine protein kinase domain. TRPM7 forms a constitutively active Mg^{2+} and Ca^{2+} permeable channel, which regulates diverse cellular processes in both healthy and diseased conditions but the physiological role of TRPM7 kinase remains largely unknown.
Methods: We show that point mutation in TRPM7 kinase domain deleting the kinase activity in mice (*Trpm7*$^{R/R}$) causes a marked signaling defect in platelets.
Results: *Trpm7*$^{R/R}$ platelets showed an impaired phosphatidylinositol-4,5-bisphosphate (PIP_2) metabolism and consequently reduced Ca^{2+} mobilization in response to stimulation of the (hem)ITAM receptor GPVI and CLEC-2, and also thrombin receptors. These activation defects were partially due to the abnormal phosphorylation and activation of PLC gamma 2 and PLC beta 3 isoforms. Spleen tyrosine kinase Syk phosphorylation was also defective downstream of GPVI and CLEC-2 receptors. To bypass the platelet-receptor activation defects, thapsigargin, a non-competitive inhibitor of SERCA, was used to deplete the Ca^{2+} store and directly activate STIM1 mediated store-operated Ca^{2+} entry (SOCE) through Orai1. In *Trpm7*$^{R/R}$ platelets SOCE was strongly reduced. All these signaling defects translated into

impaired platelet aggregate formation under flow and protection of the animals from arterial thrombosis and ischemic stroke in vivo.
Conclusion: Our results identify TRPM7 kinase as a key modulator of PLC mediated Ca^{2+} store depletion and SOCE in platelets. The protection of *Trpm7*$^{R/R}$ mice from acute ischemic disease without developing intracranial hemorrhage indicates that TRPM7 kinase might be a promising antithrombotic target.
Disclosure: No significant relationships.

Pathophysiology of thrombosis / Arterial

P 148 Fibrocytes accumulate at the culprit lesion site in STEMI and are functionally impaired by neutrophil extracellular traps

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Objectives: Inflammation is important in the pathogenesis of ST-elevation myocardial infarction (STEMI). Neutrophil extracellular traps (NETs) are enriched at the culprit lesion site (CLS) of patients. Fibrocytes, mesenchymal progenitor cells with both leukocyte and fibroblast properties, accumulate in cardiac tissue of a murine ischemia/reperfusion model and contribute to tissue repair and Collagen-I deposition. In advanced atherosclerotic plaques, expression of bone morphogenic protein receptor II (BMPRII) is lost. We studied fibrocyte frequencies and their BMPRII expression at the CLS of STEMI patients.
Methods: We drew blood samples from the CLS and femoral site during primary percutaneous coronary intervention from STEMI patients (n=50, male=78%, mean age=61±13y). Fibrocytes were characterized using flow cytometry. Double-stranded (ds)DNA, a surrogate marker of NETosis, was measured in plasma using PicoGreen®. To assess the influence of NETs on Collagen-I and BMPRII expression, fibrocytes were stimulated *in vitro* with isolated NETs.
Results: Fibrocytes were increased two-fold at the CLS compared to femoral blood. No differences were found in BMPRII expression between CLS and femoral blood. dsDNA was highly increased at the CLS and negatively correlated with both Collagen-I and BMPRII expression of fibrocytes. *In vitro* treatment of fibrocytes with NETs induced a decrease of Collagen-I and BMPRII. DNase 1, which degrades NETs, abolished this effect.
Conclusion: We report the accumulation of fibrocytes at the CLS and STEMI. Furthermore, our data suggest a functional link between NETs and fibrocytes, leading to Collagen-I and BMPRII downregulation. NETs might thereby impair reparative functions of fibrocytes after STEMI.
Disclosure: No significant relationships.

P 149 Neutrophil extracellular trap formation is impaired in patients with Howell Jolly body-positive splenectomy

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Objectives: Neutrophils are able to release their nuclear content into the extracellular space by formation of neutrophil extracellular traps (NETs). NETs have a role in host defense, but are also implicated in thrombotic and autoimmune diseases. Splenectomy is a risk factor for the development of bacterial infections of chronic thromboembolic pulmonary hypertension (CTEPH). In splenectomized patients, dysfunction of neutrophils has been reported with regards to bactericidal function and reactive oxygen species

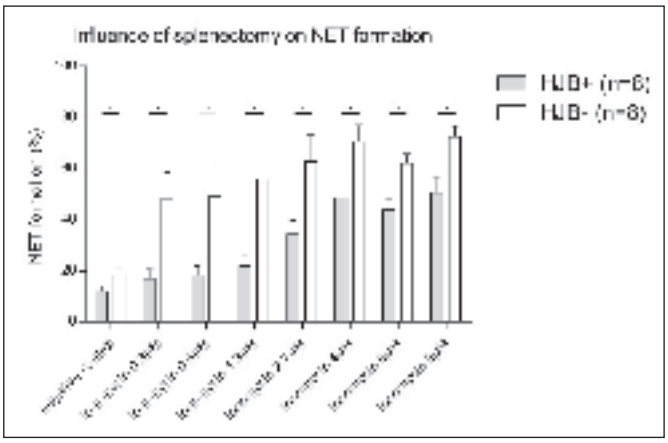

Fig. 1 Influence of splenectomy on NET formation

formation. NETosis in splenectomy has not been investigated. We hypothesized that NETosis was altered in splenectomized patients.
Methods: We drew venous blood from patients with a history of splenectomy (n=16, 50% female, mean age 57±12 years). Howell Jolly bodies (HJB), nuclear remnants in erythrocytes considered to be indicative of severe splenic dysfunction, were detected by microscopy. *Ex vivo* NET formation of isolated neutrophils upon stimulation with ionomycin was measured using Sytox® Green, a dye exclusively staining extracellular DNA released in the course of NETosis.
Results: Five (31%) patients had relative neutropenia; eight (50%) patients were HJB+. In HJB+ patients, NET formation *ex vivo* was significantly decreased compared to HJB- patients (▶Figure 1). Interestingly, spontaneous NET formation in the absence of ionomycin was also decreased.
Conclusion: Our findings indicate impaired NETosis, an important effector mechanism of neutrophils, in in HJB-positive splenectomy patients. This could be one factor explaining increased susceptibility to bacterial infection.
Disclosure: No significant relationships.

P 150 Clinical characteristics of cerebral infarction patients with essential thrombocythemia

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Objectives: Essential thmocythemia (ET) is known to be associated with increase vascular event, but the clinical characteristic regarding stroke associated with ET has not been reported.
Methods: Between January 2013 and December 2016, acute ischemic stroke patients with ET who were admitted to two university stroke centers in Seoul, Korea were included. We retrospectively reviewed their clinical, laboratory and imaging data. Stroke subtype was determined based on TOAST criteria, and ET associated stroke was defined when no other potential etiology existed. For outcome analysis good outcome was defined as modified Rankin scale (mRS) between 0 to 2 at discharge and grave outcome as mRS 5 or 6.
Results: A total of 29 patients were included, and their mean age was 66±16 years (10 female patients). Stroke subtype included 12 ET associated stroke, 10 large artery atherosclerosis, 5 small vessel occlusion, and two cardioembolism cases. Embolic infarction pattern including multiple vascular territory was more prevalent in ET associated stroke (3 vs 8 patients, Fisher exact test, p=0.018). For 12 ET associated stroke patients, antithrombotic prescription included dual antiplatelet agent combination in 7 patients, warfarin with or without antiplatelet in 3 patients, aspirin in one patient, and no antithrombotic in one patient due to hemorrhagic transformation. Overall, symptomatic hemorrhage occurred in two patients, and five patients experienced grave outcome. Old age, low hemoglobin level, female sex, and cardioembolic stroke subtype were associated with grave outcome.

Conclusion: Stroke etiology among ET patients is diverse, and age, sex and hemoglobin level are suggested as potential poor prognostic factors.
Disclosure: No significant relationships.

P 151 Toll-like receptor-4 augments thrombin generation and supports platelet deposition to the ligation injured carotid artery

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Objectives: Introduction The activation of Toll-like receptors (TLRs) of platelets and the endothelium by endogeneous and exogeneous stimuli promotes thrombosis. Activation of vascular TLRs results in the activation of distinct prothrombotic pathways that support thrombus growth. Here, we investigate the role of TLR4 on platelet deposition following carotid artery ligation injury.
Methods: Methods We applied fluorescence intravital microscopy to investigate platelet deposition following carotid artery ligation injury in TLR4-/- and WT mice. TLR4 was inhibited with CLI-095 to study platelet deposition in vivo and ex vivo thrombin formation by the calibrated automated thrombin generation (CAT) assay in platelet-rich plasma (PRP). In vivo, platelet deposition to the injured carotid artery following inhibition of thrombin with Argatroban was analysed.
Results: Results TLR4-/- mice showed a marked reduction of platelet deposition to the injury site 10 minutes after carotid artery ligation. Involvement of TLR4 signaling was corroborated by pharmacologic inhibition of TLR4 in WT mice. CAT analyses showed that activation of platelet TLR4 signaling contributes to thrombin generation. To explore if the TLR4-dependent effects on coagulation could contribute to the platelet deposition defect, we blocked thrombin. In vivo treatment with Argatroban resulted in reduced platelet deposition in WT mice.
Conclusion: Conclusion Our results indicate a role for steady-state TLR4 signaling in tissue factor-triggered thrombin generation. In vivo, impaired TLR4 signaling resulted in reduced platelet deposition to the carotid artery ligation injury site, which was dependent on thrombin activity.
Disclosure: No significant relationships.

P 152 The Q222R deoxyribonuclease 1 single nucleotide polymorphism is associated with mortality in patients after ST-elevation myocardial infarction

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Objectives: Neutrophils can release their chromatin forming neutrophil extracellular traps (NETs). NETs are implicated in autoimmune and thrombotic diseases, including ST-elevation myocardial infarction (STEMI). Deoxyribonuclease (DNase) 1 degrades NETs. DNase 1 Q222R single nucleotide polymorphism (SNP), impairing DNase 1 function, was associated with increased incidence of MI. In STEMI, dysfunctional DNase 1 activity was correlated with increased NET burden and infarct size. We hypothesized that DNase 1 is crucial to counteract NET formation in coronary artery disease (CAD) and that dysfunction of the enzyme might thereby induce chronic NET burden with influence on long-term outcome.
Methods: We enrolled CAD patients with a history of STEMI between 2006 and 2016 (n=711). Genotyping using allelic discrimination qPCR was performed to identify DNase 1 Q222R SNP (rs1053874). By multivariable Cox regression, we assessed the influence of DNase 1 SNP on all-cause and cardiovascular mortality, adjusting for established cardiovascular risk factors.
Results: Homozygous mutation of the DNase 1 SNP was present in 64 (9.0%) patients; 304 (42.8%) and 343 (48.2%) were heterozygous and homozygous for the wild-type allele, respectively. Median survival was 60.0 [interquartile range 30.3; 91.5] months. A total of 133 (18.7%) patients deceased; 78 (11.0%) died of cardiovascular causes. Homozygous mutation of DNase 1 was independently associated with all-cause mortality (hazard ratio 2.05, 95% CI 1.22–3.46, p=0.006) and cardiovascular mortality (hazard ratio 2.02, 95% CI 1.02–4.01, p=0.046).
Conclusion: We report a negative influence of the Q222R DNase 1 SNP on survival after STEMI. Our findings argue for a deleterious role of NETs not only in CAD.
Disclosure: No significant relationships.

Pathophysiology of thrombosis / Venous

P 153 Plasma levels of activated protein C (APC) significantly increase in carriers of the factor V Leiden (FVL) mutation in response to low dose administration of recombinant activated factor VII (rFVIIa)

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Objectives: Aim of this study was to demonstrate directly and in vivo the influence of the FVL mutation on plasma levels of thrombin and APC after extrinsic activation of coagulation.
Methods: 15 µg/kg rFVIIa were injected intravenously in 12 FVL-negative subjects as wells as 12 heterozygous and 3 homozygous asymptomatic FVL carriers. During a follow-up period of eight hours, plasma levels of thrombin and APC were measured using oligonucleotide-based enzyme capture assays (OECAs), in addition to prothrombin activation fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT), and D-dimer.
Results: Administration of rFVIIa was well tolerated, and its pharmacokinetics showed an expected course in all subjects. F1+2 (mean±standard deviation) increased from 0.14±0.06 to 0.18±0.07 nmol/L (p=0.007) in FVL-negative subjects and from 0.21±0.16 to 0.27±0.17 nmol/L (p=0.001) in heterozygous FVL carriers, indicating mild activation of thrombin. APC increased from 0.88±0.51 to 3.77±03.12 pmol/L (p=0.009) in the FVL-negative group. Heterozygous and homozygous FVL carriers showed a greater increase from 1.68±0.82 to 10.58±4.70 pmol/L ($p=10^{-5}$) and from 1.66±0.78 to 17.11±6.36 pmol/L, respectively. Throughout the follow-up period, APC levels were significantly higher in heterozygous FVL carriers than in FVL-negative controls ($p=0.047–9{\cdot}10^{-4}$). They remained significantly elevated over several hours in all groups whereas plasma levels of thrombin, TAT, and D-dimer did not change significantly (▶Figure 1).
Conclusion: The presented approach allows assessment of the functionality of the anticoagulant APC pathway in vivo. Plasma levels of APC are significantly increased in FVL carriers compared to normal individuals after extrinsic activation of coagulation.
Disclosure: No significant relationships.

P 154 Another view on factor X in coagulation.

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Objectives: Factor X in coagulation is mainly considered for its activation of prothrombin in clot formation. Evaluation of factor Xa inhibitors in global clotting tests is however not fully understood.

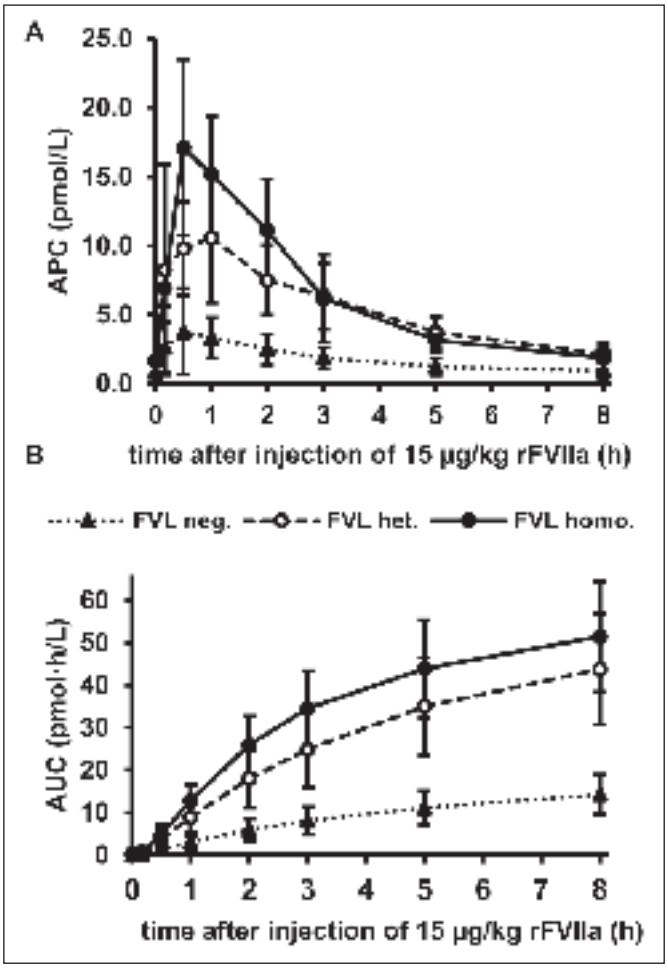

Fig. 1 APC generation induced by rFVIIa. (**A**) Plasma levels of APC in FVL-negative subjects (FVL neg.; n=12), heterozygous (FVL het., n=12), and homozygous (FVL homo., n=3) FVL carriers. Data are presented as mean standard deviation. (**B**) Area under the APC generation curve.

Methods: We evaluated the effects of factor Xa inhibition in a new phenotype of clot growth from a surface with immobilised tissue factor (Thrombodynamics), using plasma substrate supplemented with high lipids (4 µM)
Results: The pathway TF-VIIa/X-V/II/fibrin was studied in factor IX deficient plasma. Inhibition of growth by factor Xa inhibitors was very minor for rivaroxaban, apixaban, fragmin, bemiparin, clexane and fondaparinux (tested in the therapeutic plasma range). It is concluded that factor Xa inhibitors do not inhibit clotting in this clot growth phenotype. Testing the same inhibitors in normal plasma showed a dose dependent inhibition of clot growth to a plateau. The residual clot growth at the plateau was quantitatively similar to the growth in factor IX deficient plasma. It is concluded that the factor Xa inhibitors inhibit the participation of the Josso loop (factor IX-VIII). The only target for factor Xa in this process is the activation of factor VIII.
Conclusion: We concluded that the factor Xa inhibitors inhibited the formation of factor VIIIa. It is proposed to consider factor Xa inhibitors as inhibitors of extra factor Xa formation via the Josso loop. Apparently the inhibition of factor Xa in clotting directly on prothrombin/fibrin is not served yet by the tested inhibitors.
Disclosure: C. Kluft and J. Begieneman are employees of Good Biomarker Sciences. N. Dashkevich is employee of Hemacore

P 155 Coagulation factors under influence of multiple sclerosis IgG

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This abstract has been withdrawn

P 156 Prevalence of common thrombophilic disorders in patients with ocular thrombosis

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Objectives: Ocular thrombosis (OT) is a common retinal vascular disease that may result in significant visual loss. The influence of thrombophilia in these cases is still controversial. Prevalence of thrombophilic disorders as in other non-ocular thrombosis patients is not yet well studied. We here report upon thrombophilia screening in 167 patients with OT.
Methods: 141 patients with ocular venous thrombosis and 23 with ocular arterial thrombosis and 3 patients with both arterial and venous thrombosis were examined. Four to six weeks after episodes, patients have been sent to carry out thrombophilia screening.
Results: – Thrombophilia screening: antiphospholipid antibodies were detected in 31, lipoprotein (a) elevation in 25, factor VIII:c elevation in 5, antithrombin deficiency in 4, prothrombin (G20210A) polymorphism in 8, factor V Leiden mutation in 16 patients. – Treatment: 106 patients received acetylsalicylic acid (ASA), 2 ADP antagonists, 2 low molecular weight heparin, 25 vitamin K antagonists, 1 direct oral anticoagulant (antifactor Xa) and 31 patients had no anticoagulant treatment. Thromboembolic history was positive in 65 patients (38 %).
Conclusion: These results demonstrate that common risk factors for venous thromboembolism like factor V Leiden mutation, prothrombin polymorphism, antithrombin deficiency and factor VIII:c elevation are less frequent in patients with OT. In contrast, antiphospholipid antibodies and lipoprotein (a) elevation seem to play a major role in OT patients. Positive history of deep venous thrombosis and pulmonary embolism in 38 % of these patients has an important impact on the prognosis and on the choice of therapeutic approach.
Disclosure: No significant relationships.

P 157 Identification of microRNAs as biomarkers for predicting the risk of venous thromboembolism

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Objectives: Currently available risk assessment models for venous thromboembolism (VTE) are not predictive enough to precisely assess the risk of a first or a recurrent VTE. To improve risk prediction of (recurrent) VTE, more appropriate biomarkers are needed. Therefore, our purpose was to identify microRNAs (miRs) which could clinically useful biomarkers of VTE.
Methods: To generate a relevant and convincing miR database, plasma from patients with a high thrombotic risk (male, history of two unprovoked VTE, mean age 59) and age-matched healthy men were subjected to miR expression analysis. The Affymetrix miRNA Microarray includes 2.578 human miR samples, which cover the majority of all thus far known human miRs. Potential VTE-associated miRs were further verified by qRT-PCR in independent patient and control samples.

Tab. 1 Significantly upregulated miRs between VTE patients and controls

miR	Fold
hsa-miR-6816–5p	3.13
hsa-miR-3185	2.62
hsa-miR-8069	2.60
hsa-miR-3196	2.59
hsa-miR-8072	2.43
hsa-miR-6125	2.40
hsa-miR-1237–5p	2.32
hsa-miR-3940–5p	2.25
hsa-miR-4516	2.23
hsa-miR-1228–5p	2.19
hsa-miR-6821–5p	2.18
hsa-miR-1908–5p	2.14
hsa-miR-4763–3p	2.08
hsa-miR-4734	2.04
hsa-miR-4745–5p	2.02
hsa-miR-4651	2.02
hsa-miR-2861	2.00

Sorted according to their regulation factor (cut off ≥2.0). Underlined: validated miR regulation confirmed.

Results: Microarray and bioinformatic analysis (SAM-analysis) reflect major changes between seven patients and seven controls. Thirty-nine miRs showed significantly higher expression levels in patients compared to controls, whereas 120 miRs exhibited no differences. Among those upregulated 39 miRs, 17 were modulated twofold higher (▶Table 1). No miRs were expressed solely in VTE patients or controls. Four miRs (hsa-miR-3185, hsa-miR-3196, hsa-miR-6816, hsa-miR-8072) were validated by qRT-PCR in 20 patients and matched controls and upregulation was consistent in more than 16 samples.
Conclusion: We identified four miRs as candidates for the development of minimally invasive biomarkers for predicting VTE. These VTE-related miRs also provide a basis to further investigate into the molecular mechanism underlying the pathogenesis of VTE.
Disclosure: No significant relationships.

Cancer

P 158 Prevalence and clinical significance of the JAK2 V617F mutation in a selected cohort of thrombophilia patients

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Objectives: The Janus kinase 2 (*JAK2*) V617F somatic mutation plays a central role in the pathogenesis of Philadelphia-negative myeloproliferative neoplasms (MPNs). The mutation, however, can also be found in a significant proportion of patients without overt signs of MPN, particularly in those with atypically located or recurrent thromboembolism. Still, it remains uncertain which patients should be tested for the $JAK2^{V617F}$ mutation.
Methods: Of all adult patients referred to the University Medical Center Hamburg-Eppendorf for the diagnostic work-up of thrombophilia between 2012 and 2016, we identified subjects in whom additional testing for the $JAK2^{V617F}$ mutation was performed. Medical records were retrospectively analyzed regarding demographic, clinical and laboratory parameters in order to define distinct criteria for $JAK2^{V617F}$ testing.
Results: Of 2,038 patients, 253 (12.4%) were evaluated for the presence of the $JAK2^{V617F}$ mutation within the scope of thrombophilia testing. Of these, 37 patients (14.6%) were $JAK2^{V617F}$ -positive with 20 of them (54%) meeting the diagnostic criteria for MPN during follow-up. While there was no significant difference in the rate of splanchnic vein thrombosis or total recurrent thromboembolic events, $JAK2^{V617F}$ -positive patients had more frequently experienced recurrent venous thromboembolism (VTE) while on oral anticoagulant therapy than $JAK2^{V617F}$ -negative patients ($P<0.01$). In a logistic regression analysis, advanced age ($P<0.01$) and increased hemoglobin ($P<0.05$) and platelet counts ($P<0.001$) were significantly associated with the presence of the $JAK2^{V617F}$ mutation.
Conclusion: Patients with thromboembolic events and abnormal blood counts should be tested for the $JAK2^{V617F}$ mutation. Furthermore, $JAK2^{V617F}$ testing seems reasonable in patients with recurrent VTE despite oral anticoagulant therapy.
Disclosure: No significant relationships.

Hemophilia

P 159 A novel von Willebrand factor fragment increases bioavailability of recombinant human FVIII (simoctocog alfa) in hemophilia A dogs and FVIII/VWF double knockout mice

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Objectives: Subcutaneous (SC) drug delivery for FVIII therapy in hemophilia A (HA) is hindered by poor FVIII bioavailability. von Willebrand factor (VWF) protects FVIII from proteolytic degradation and could increase FVIII bioavailability. We analyzed the effect of engineered VWF fragments on FVIII binding affinity, half-life and bioavailability.
Methods: Recombinant human FVIII (rhFVIII, simoctocog alfa) complexed with a modified VWF fragment (OCTA12), VWF D'D3 domains, or full-length plasma-derived VWF (flVWF, Wilate®) was administered via intravenous (IV) route to FVIII/VWF double knockout (DKO) mice to assess stabilization of FVIII in circulation. FVIII activity after IV and SC administration of the rhFVIII/OCTA12 complex was subsequently evaluated in a HA dog model according to the treatment scheme in ▶Table 1.

Tab. 1 HA dog treatment scheme

	First injection	Second injection (after 96 hours)
Dog #2	rhFVIII/OCTA12; 100 IU FVIII/kg; IV	rhFVIII /OCTA12; 200 IU/kg; SC
Dog #3	rhFVIII; 200 IU FVIII/kg; IV	rhFVIII /OCTA12; 200 IU/kg; SC
Dog #4	rhFVIII /OCTA12; 200 IU FVIII/kg; SC	rhFVIII /OCTA12; 100 IU/kg; IV

Results: FVIII activity levels in DKO mice were significantly higher when rhFVIII was administered with any VWF variant in comparison to rhFVIII alone (peak activity levels (% mean±SEM, n = 15): rhFVIII 78.9±6.1; rhFVIII/D'D3 351.2±22.3; rhFVIII/OCTA12 295.9±17.4; rhFVIII/flVWF 404.4±20.5). Co-administration with flVWF, D'D3 or OCTA12 resulted in a 4.5-, 6.4– or 14.7-fold prolongation of the rhFVIII half-life respectively. In HA dogs, peak FVIII activity levels were 215% (rhFVIII) and 134% (rhFVIII/OCTA12) after IV administration, and 24.6% after SC administration (rhFVIII/OCTA12). Bioavailability of FVIII after rhFVIII/OCTA12 administration by IV and SC was 90% and 37.7% respectively, relative to IV administration of rhFVIII (twice the dose) alone.
Conclusion: The novel engineered VWF fragment OCTA12 leads to high FVIII bioavailability after SC administration in HA dogs, and IV administration in HA dogs and FVIII/VWF DKO mice.
Disclosure: B.S. and C.K. are employees of Octapharma Biopharmaceuticals GmbH, Berlin, Germany. D.L. has acted as a speaker and paid consultant and participated in studies for Octapharma.

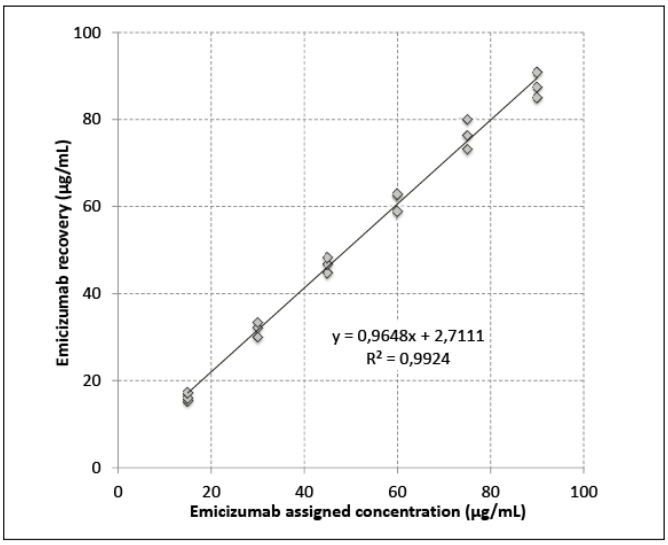

Fig. 1 Linearity evaluation

Laboratory tests / Diagnostic methods

P 160 Quantification of emicizumab based on a modified one-stage FVIII assay

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Objectives: Emicizumab bridges FX and activated FIX to restore the function of activated FVIII. Once-weekly emicizumab prophylaxis was shown to significantly lower bleed rates compared with no prophylaxis in persons with hemophilia A with inhibitors (OIdenburg, et al. *NEJM 2017*). A method of quantifying emicizumab concentration based on the one-stage FVIII assay is presented.
Methods: A modified procedure was developed using a 1:8 citrated plasma sample pre-dilution by the coagulation analyzer, followed by the standard one-stage FVIII test. A dedicated emicizumab calibrator (100 µg/mL) and controls (25 and 75 µg/mL) were developed (r^2 Diagnostics, South Bend, IN, USA). Analyses were performed on BCS XP, using Actin FSL reagent and FVIII-deficient plasma (Siemens, Marburg, Germany).

Results: Linearity over the calibration range of 10 –100 µg/mL is shown in the ▶Figure 1. Calculations of total precision of samples with 75, 25 and 12.5 µg/mL emicizumab were 5.1%, 5.6% and 9.6%, respectively. In 20 samples from patients treated with emicizumab, there was a statistically significant correlation between emicizumab levels assessed using the presented method versus an ELISA method (R^2 = 0.98). Adding recombinant FVIII (Advate, Shire, 0.74 U/mL) to samples with emicizumab led to an increase in reported emicizumab concentration by approximately 18 µg/mL using the modified one-stage method. No interference was observed when recombinant activated FVII (rFVIIa, Novo Nordisk) or activated prothrombin complex concentrate (aPCC, Shire) was spiked into emicizumab-containing samples.
Conclusion: A modified one-stage FVIII method, using a dedicated calibrator and controls for emicizumab, allows quantification of emicizumab concentration over the clinically-relevant dose range.
Disclosure: Andreas Calatzis is a consultant to Genentech, Inc. David C. Chen and Joanne I. Adamkewicz are employees of Genentech, Inc. Walter Calhoon and Michael Morris are employees of r2 Diagnostics. Mags McInerney is a consultant to r2 Diagnostics.