64th Annual Meeting

Society of Thrombosis and Haemostasis Research

Novel concepts for a lifetime challenge

Bremen, Germany | 18–21 February 2020

Book of Abstracts

WELCOME ADDRESS

Dear colleagues and friends,

our congress motto “Novel concepts for a lifetime challenge” points out that many disorders in the field of thrombosis and haemostasis affect patients throughout their entire life. Thus, basic science and patient care should involve both, an interdisciplinary approach at a patient’s given age and a close collaboration of physicians and scientists specialised on different phases of the patient’s life, such as neonatologists, paediatricians, general practitioners and geriatricians. Understanding the natural history of bleeding and thrombotic disorders from a clinical and scientific perspective is key to providing our patients with the best possible treatment in a rapidly changing landscape of novel diagnostic tests and therapeutic agents.

We feel that the Town Musicians of Bremen, as depicted in the annual meeting’s logo, nicely illustrate the urgent need for trustful teamwork in basic science and clinical practice. Only when we build on each other’s knowledge and experience, we can come up with “Novel concepts for a lifetime challenge”.

We have received many outstanding contributions on a broad spectrum of basic and clinical research topics and hope that you enjoy reading the annual meeting's abstracts as much as we did!

Florian Langer
Congress president

Thomas Renné
Congress president

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ORALS

SOA01 State of the art & oral communications: Cancer & coagulation

SOA01-2-AB

Biomarkers of haemostasis predict response to chemotherapy in patients with advanced lung cancer

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Objectives: The haemostatic system has been implicated in tumour promotion and metastasis formation in lung cancer. Furthermore, selected haemostatic biomarkers have been reported to predict risk of lung cancer development and progression. The aim of this analysis was to investigate the role of haemostatic biomarkers for prediction of therapy response in patients with advanced lung cancer initiating chemotherapy.

Methods: We analysed the association of pretherapeutic levels of preselected haemostatic biomarkers (D-dimer, sP-selectin, fibrinogen, prothrombin fragment F1+2 (F1+2)) with response to chemotherapy in patients with advanced lung cancer (n=277; metastatic: n=196, inoperable: n=81) within the framework of the Vienna Cancer and Thrombosis study, a prospective observational cohort study. Median age of the cohort was 61 years (interquartile range (IQR): 56-67) and 37% were female. 231 patients had non-small-cell lung cancer (adenocarcinoma: n=165, squamous-cell carcinoma: 45) and 46 patients had small-cell lung cancer. The most frequent chemotherapy combination was platinum-vinca alkaloid (n=74) followed by platinum-gemcitabine (n=52) and platinum-antifolate (n=52). Median number of chemotherapy cycles during 1st line of chemotherapy was 4 (IQR: 2-5).

Outcome measures for this analyses as surrogate markers for therapy response were (1) mortality and overall survival (OS), (2) progression free survival (PFS) and (3) disease control rate (DCR). Biomarkers were analysed on a continuous scale per doubling of levels and as dichotomized variable for survival times (OS and PFS).

Results: Mortality was significantly increased in multivariable analysis (adjusting for stage, grade and histology) in patients with elevating levels of D-dimer (hazard ratio (HR) for death per doubling of levels: 1.70 [95%CI: 1.41-2.04]) and F1+2 (HR: 1.35 [1.09-1.67]). Overall survival was significantly shorter in patients with levels above the 75th percentile of distribution compared to those below in D-dimer (median OS: 7.6 vs 15.6 months), F1+2 (9.9 vs 14.5 months), sP-selectin (9.6 vs 13.7 months) and fibrinogen (8.5 vs 14.1 months). Risk of disease progression per time was significantly higher for increasing levels of D-dimer (HR for disease progression per doubling of levels: 1.41 [1.20-1.67[) and F1+2 (HR: 1.29 [1.06-1.58]) with significantly shorter PFS for patients with high (≥75th percentile) vs low levels of D-dimer (median PFS: 4.3 vs 6.6 months) and F1+2 (5.3 vs 5.5 months). Probability of disease control at restaging during 1st line of chemotherapy was significantly lower in patients with higher levels of D-dimer (OR for DCR per doubling of levels: 0.60 [0.41-0.87]), F1+2 (OR: 0.63 [0.41-0.96]) and sP-selectin (0.56 [0.32-0.98]).

Conclusion: Biomarkers of haemostatic activation such as D-dimer and F1+2 predict response measures to chemotherapy in patients with advanced lung cancer.

Biomarker	Mortality	Median overall survival (OS)		Disease progression	Median progression free survival (PFS)		Disease control rate (DCR)
	adjusted HR for death per doubling of levels*	Biomarker <Q3	Biomarker ≥Q3	adjusted HR for disease progression per doubling of levels*	Biomarker <Q3	Biomarker ≥Q3	adjusted OR for disease control per doubling of levels*
D-dimer	1.70 [1.41-2.04] p<0.001	15.6 [12.8-18.5] p<0.001	7.6 [5.6-9.6]	1.41 [1.20-1.67] p<0.001	6.6 [5.8-7.5] p<0.001	4.3 [3.5-5.1]	0.60 [0.41-0.87] p=0.008
Prothrombin fragment F1+2	1.35 [1.09-1.67] p=0.005	14.5 [11.7-17.2] p=0.009	9.9 [8.6-11.1]	1.29 [1.06-1.58] p=0.012	5.5 [4.2-6.7] p=0.018	5.3 [4.0-6.7]	0.63 [0.41-0.96] p=0.030
sP-selectin	1.34 [0.98-1.85] p=0.068	13.7 [11.6-15.7] p=0.018	9.6 [8.3-10.8]	1.18 [0.89-1.57] p=0.247	5.7 [4.5-6.8] p=0.484	4.8 [2.9-6.6]	0.56 [0.32-0.98] p=0.043
Fibrinogen	1.28 [0.88-1.87] p=0.199	14.1 [11.9-16.3] p=0.006	8.5 [6.7-10.2]	1.15 [0.82-1.59] p=0.419	6.2 [5.3-7.2] p=0.073	3.9 [2.7-5.0]	0.72 [0.38-1.36] p=0.313

*adjusted for stage, grade and histology; mortality: risk of death per time; overall survival: time to death from any cause; progression free survival: time to radiological progression of disease or death from any cause; disease control rate: composit outcome of radiological remission or stable disease at restaging; Abbreviations: HR: hazard ratio, Q3: third quartile of biomarker distribution; OR: odds ratio;

[Response measures to 1st line chemotherapy in patients with advanced lung cancer according to pretherapeutic levels of haemostatic biomarkers]

SOA01-3-AB

Transforming growth factor β released by platelets primes macrophage activation and extracellular trap formation, thereby influencing breast cancer growth in mice

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Background: Leukocytes release their chromatin fibers, referred to neutrophil extracellular traps (NETs), which are composed by DNA, histones and granule content. NET formation facilitates tumor cell aggressiveness in the context of systemic inflammation. Earlier, it has been shown that platelets could promote NET formation during bacterial infections. Recent studies indicated that activated platelets may also induce monocyte/macrophage-dependent extracellular trap (MET) formation, a process termed as METosis, thereby exacerbating organ injury and inflammation. However, the role of activated platelets in METosis during tumor growth has not been investigated.

Aims: We examined the role of platelets in the formation of METs during breast cancer growth.

Methods: Breast tumors were induced by orthotopic injection of two different breast cancer cell lines called AT-3 and E0771. Tumor growth was analysed in wild-type (WT) mice and megakaryocyte/platelet specific TGFß-knockout mice (*TGFß$^{fl/fl\text{-}PF4\text{-}Cre}$*) which were treated with (i) DNAse I, (ii) macrophage-depleting chlodronate liposomes, (iii) platelet- and (iv) neutrophil-depleting antibodies. Infiltration of immune cells into the growing tumors was followed by immunofluorescence confocal microscopy and flow cytometry.

Results: We show that macrophage depletion decreased tumor volume in WT mice to a similar extent as DNAse I treatment. In contrast, depletion of neutrophils did not alter tumor growth in WT mice, indicating that neither neutrophils, nor NETs are critical for breast cancer growth. Moreover, we

found that platelet depletion or TGFß deficiency in platelets inhibited METosis. Depletion of macrophages and MET formation attenuated tumor growth and this process was strongly dependent on TGFß released by activated platelets. Platelet-derived TGFß enhances tumor growth by priming macrophage activation and METosis.
Conclusion: Our findings reveal that inhibition of TGFß released by activated platelets may represent a new therapeutic strategy to decrease breast cancer growth by interfering with pro-tumorigenic macrophage function.

SOA01-4-AB

Fibrin as a target for glioblastoma detection and treatment

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Glioblastoma (GBM) is a highly aggressive brain tumor characterized by diffuse growth and resistance to therapy. GBM growth is associated with necrosis, hemorrhage and thrombosis. This leads to the formation of a fibrin-rich extracellular matrix, which could provide important adhesive cues for GBM growth and proliferation.
Fibrin formation, invasion and proliferation was tested in tumors from patients with astrocytoma. GBM stem cells were generated using neural stem cell media. Tumor xenografts were established by injecting GBM cells under the skin and into the brain of immune compromised mice.
Compared to normal brain tissue, which is essentially fibrin-free, immunohistochemistry revealed a marked upregulation of clot formation in the interstitial spaces of patients with astrocytoma 3 and GBM. Astrocytoma 2 expressed 3-3.5 fold less fibrin than was found in tissues from patients with high-grade astrocytoma. Primary GBM cells, that were freshly isolated from patients after tumor surgery proliferated strongly after embedding in a three dimensional (3D) matrix of clotted plasma *ex vivo* whereas tumor cells from astrocytoma 2 and 3 infiltrated clot but were unable to proliferate. GBM proliferation in 3D depended on fibrin, which mediated upregulation of the stem cell marker nestin, whereas culturing glioblastoma cells in a 3D matrix of matrigel™ failed to promote nestin expression as well as GBM proliferation. Moreover, formation of GBM xenografts in mice *in vivo* depended strictly on the presence of plasma that was mixed into the tumor cell suspension prior to subcutaneous injection.
To determine the interaction of GBM cells with fibrin on a molecular basis, we transfected GBM cells with siRNA against integrin β3, which abolished invadopodia formation and caused a sustained growth inhibition. GBM growth in 3D fibrin also depended on the formation of a fibronectin matrix as knockdown of fibronectin led to a growth arrest. Freshly isolated tumor cells from patients with GBM colonized most efficiently in 3D fibrin *in vitro* as well as in mice *in vivo* when they express fibronectin in combination with integrin β3.
Mice with orthotopic GBM xenografts were injected intravenously with the fluorescein-coupled dekapeptide CGLKIQKNEC. Using a fluorescence endoscope *in situ*, we detected strong green fluorescence over the right parietal lobe, where tumor growth had been established by MRI beforehand. We confirmed tumor binding of the peptide in brain tissue by fluorescence microscopy *ex vivo*, which demonstrated specific green fluorescence in the tumor xenograft while adjacent normal brain tissue and tissues from distant organs only exhibited background fluorescence.
Our data demonstrate a specific upregulation of fibrin in GBM, which promotes proliferation of GBM stem cells via integrin β3 and fibronectin. Moreover, we present a strategy to identify fibrin in the tumor extracellular matrix as a possible means to identify astrocytoma progression *in vivo*.

SOA01-5-AB

Chronically elevated Interleukin-6 levels lead to a disturbance in the coagulation system in mice

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Introduction: Pro-inflammatory cytokines play an important role as activators of the hemostatic system as well as in the downregulation of physiological antithrombotic mechanisms. Interleukin-6 (IL-6) has been described to influence platelet production and activation. IL-6 was associated with accelerated clotting and intravascular coagulation in tissue factor (TF)-driven murine thrombosis models. The precise role of myeloid cell derived IL-6 on thrombosis formation and the hemostatic system, however, is still unknown.
Methods and Results: To investigate the role of IL-6, we generated a new mouse model with Cre-recombinase driven constitutive overexpression of IL-6 in lysozyme M-positive myelomonocytic cells (LysM-IL-$6^{OE/+}$, Control mice: IL-$6^{OE/+}$). LysM-IL-$6^{OE/+}$ mice did not develop deep venous thrombosis (DVT) 3 days post inferior vena cava ligation compared to controls that displayed normal thrombus formation. Nevertheless, platelets were more reactive in LysM-IL-$6^{OE/+}$ mice compared to control mice. Platelet number was not altered in LysM-IL-$6^{OE/+}$ mice and there were no differences in D-Dimer levels. However, we found unstoppable post operative bleedings in LysM-IL-$6^{OE/+}$. They showed a prolonged aPTT (LysM-IL-$6^{OE/+}$: 104 s, IL-$6^{ind/+}$: 29 s) and a significantly increased INR (LysM-IL-$6^{OE/+}$: 0.72, IL-$6^{OE/+}$: 0.65) as well as moderately reduced levels of coagulation factors V and IX. An increase of von Willebrand factor in LysM-IL-$6^{OE/+}$ mice as well as elevated levels of antithrombin compared to control mice. Besides, we detected increased levels of fibrinogen in LysM-IL-$6^{OE/+}$ mice compared to control mice (LysM-IL-$6^{OE/+}$: 536 mg/dl, IL-$6^{ind/+}$: 211 mg/dl). Most importantly, hepatic levels of proteinase inhibitor α2 macroglobulin (α2M) mRNA and protein were highly increased in LysM-IL-$6^{OE/+}$ mice compared to controls. We found significantly elevated erythrocyte sedimentation in line with agglutinating erythrocytes, which seemed to be at least partly mediated by IL-6 and α2M. Platelet erythrocyte interaction seems to be essential in this severe bleeding phenotype.
Conclusions: Chronic myeloid overexpression of IL-6 results in a severe bleeding phenotype.

This work was supported by the DFG KA4035/1-1 and by the German Ministry for Education and Research (BMBF 01EO1503)

SOA02 State of the art & oral communications: Regulation of coagulation

SOA02-2-AB

In vivo thrombin generation and subsequent formation of activated protein C (APC) down-regulates plasminogen activator inhibitor-1 (PAI-1)

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Scientific research question: It has been suggested that APC contributes to a hyperfibrinolytic state in various acquired coagulation disorders including coagulopathies induced by sepsis or trauma. However, *in vivo* evidence for the proposed underlying mechanism of proteolytic degradation of PAI-1 by APC remains inconclusive. Recently, we have shown increased APC generation in response to *in vivo* thrombin formation in carriers of the thrombophilic mutations factor V Leiden (FVL) and prothrombin (FII) 20210G>A. In this approach of stimulated hemostasis activity pattern evaluation (SHAPE), low-dose administration of recombinant activated factor VII (rFVIIa) was utilized to trigger *in vivo* thrombin formation. Aim of the present study was to investigate the resulting effects on the fibrinolytic system.
Methodology: The study population consisted of 30 FVL and 28 FII 20210G>A mutation carriers (thereof 13 with a history of thrombosis each), and 18 healthy controls. Blood samples were collected immediately before and during a period of 8 hours following injection of 15 µg/kg rFVIIa. Plasma levels of APC were quantified using an oligonucleotide-based enzyme capture assay (OECA). Other monitored parameters included prothrombin activation fragment 1+2 (F1+2), PAI-1, tissue-type plasminogen (t-PA), PAI-1, α2-antiplasmin, plasmin-α2-antiplasmin complexes (PAP), soluble fibrin monomers, d-dimer, and thrombin-activatable fibrinolysis inhibitor (TAFI) antigen and activity levels.
Findings: Compared with the controls, FVL carriers showed higher median levels of APC at baseline (0.87 vs. 1.39 pmol/L, *P*=0.003). PAI-1 levels were higher in FLV carriers (30.1 ng/mL, *P*=0.002) and in FII 20210G>A carriers (28.3 ng/mL, *P*=0.026) than in the controls (15.5 ng/mL). The other baseline parameters did not differ significantly. In all three cohorts a comparable increase of F1+2 was

observed after administration of rFVIIa, whereas the APC increase was greater in FVL carriers (by 6.40 pmol/L, $P< 10^{-4}$) and in FII 20210G>A carriers (by 4.76 pmol/L, P=0.002) than in healthy controls (by 2.53 pmol/L). Concurrently, median PAI-1 levels decreased more in FVL carriers (by 19.8 ng/mL, P=0.024) and in FII 20210GA carriers (by 20.1 ng/mL, P=0.042) than in healthy controls (by 9.2 pmol/L) (Figure 1). Plasma levels of TAFI antigen, PAP, and d-dimer increased within their respective reference ranges in thrombophilic mutation carriers and controls, but the extent of these changes did not differ between the three cohorts. t-PA and the other parameters did not show significant changes after rFVIIa administration.
Conclusion: Increased APC formation rates in FVL carriers were associated with a greater decline of PAI-1 levels in the absence of interfering changes in t-PA levels. These data provide further *in vivo* evidence that APC down-regulates PAI-1. Overall, the SHAPE approach utilized here does not induce a significant profibrinolytic response, even in patients with thrombophilic mutations.

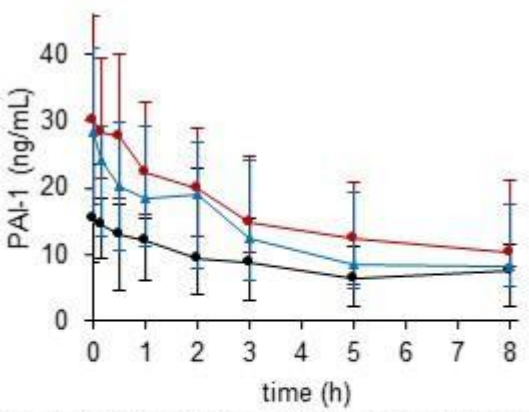

Fig. 1. In-vivo plasma levels of plasminogen activator inhibitor-1 (PAI-1). After t=0 recombinant activated factor VII (15 µg/kg) was administered to carriers of the factor V Leiden mutation (red symbols, n=30), prothrombin 20210G>A mutation (blue symbols, n=28) and to healthy controls (black symbols, n=18). Data are shown as median and interquartile range.

SOA02-3-AB

VWf containing therapeutic concentrates induce FXII activation, leading to kallikrein activation and bradykinin formation in vitro

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Scientific research question: Alegre et al. described 1986 the activation of the kinin prekallikrein system after infusion of FVIII concentrates in haemophilia patients. These concentrates contained quite high amounts of misfolded proteins and quite large amounts of vWf. As the structural stability of vWf is highly influenced by shear stress, we asked ourselves whether current FVIII and vWF concentrates. might either directly and/or indirectly activate FXII.
Methodology: Three pdFVIII products (Octanate®, Immunate®, Haemoctin®) and four rFVIII products (Kovaltry®, Kogenate®, NovoEight® and Advate®), two pd vWF products (Haemate®, and Wilate®) and rVWf (Vonvendi®) were studied. NanoSight tracking analysis (NTA) was used for visualization of protein aggregates. FXII-Prekallikrein activating activity was measured in the presence of various concentrations of FVIII or vWF products using the chromogenic substrate S-2302. S-2302 cleavage by autoactivation without FXII was used as negative control. Platelet activation releasate was used as positive control. For measurement of bradykinin formation by kallikrein, single chain high molecular weight kininogen (HMWK) was added to the reaction mixture without chromogenic kallikrein substrate and incubated for 10 min. Unreacted HMWK was removed after 30 minutes and bradykinin was quantified by a competitive ELISA. VWF binding to FXII was studied by Elisa.
Results: The tested pdFVIII products contained significantly more protein nanoparticles than all tested recombinant FVIII products and all contained vWf. Immunate, and Octanate induced a significant activation of FXII/kallikrein pathway, while this effect was not observed with rFVIII. All vWf concentrates, pdvWf as well as rvWf, activated the FXII/kallikrein pathway. Bradykinin formation induced by rFVIII did not differ from the negative control. Immunate and all vWf concentrates induced a significant bradykinin formation. Recombinant vWf as well as vWf from plasma derived concentrates bound to FXII. “Mistreating”

of vWf containing concentrates by inducing shear had a strong influence on FXII activating activity.
Conclusions: As the FXII/kallikrein pathway is proinflammatory and as its product bradykinin activates signalling pathways resulting in increased vasodilation, vascular permeability, edema formation and chemotaxis of neutrophils as well as inducing pain, our findings might be of clinical interest. Further research aims to analyse the role of vWF FXII interaction in more detail.
Part of the work was supported by a grant of Bayer Vital GmbH, 51368 Leverkusen, Germany

SOA02-4-AB

Contact and complement system: Comparison of direct and C1 inhibitor-mediated inhibition of FXIIa, FXIa and C1s by glycosaminoglycans and other sulfated glycans

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Scientific research question: Numerous diseases and clinical situations are associated with activation of contact and complement system, which consequently leads to stimulation of inflammation as well as thrombosis. This is based on the linkage of these systems by several components like factor XII (FXII), factor XI (FXI) and the regulator C1 inhibitor (C1-INH). Therefore, inhibition of these factors or improvement of their regulation represent promising therapeutic strategies. Due to the known potentiating effect of heparins on C1-INH, the questions arise, whether endothelial heparan sulfate (HS) is involved in the physiological regulation and whether sulfated glycans (SG) may represent an option to enhance the activity of C1-INH. The aim of this study was to compare the effects of gylcosaminoglycans (GAG) and other sulfated glycans on the activity of FXIIa, FXIa, and the complement factor C1s in absence and presence of C1-INH. Based on the evaluation of structure-activity relationships, suitable lead structures of sulfated glycans should be identified.
Methodology: The concentration-dependent (0.0125-12.5 µg/ml) direct and C1-INH-mediated effects of SG on FXIIa, FXIa, and C1s were measured by chromogenic substrate assays. The up to > 40 structurally defined SG tested in this study included (1) heparins and fondaparinux, (2) genuine and chemically modified glycosaminoglycans (GAGs), (3) semisynthetic β 1,3 glucan sulfates(1,3-GS), and (4) genuine and degraded fucoidans.
Findings: Depending on the enzyme, the SG differed in their direct effects, i.e. no effect on FXIa activity, slight to moderate inhibition of FXIa activity, and either inhibition or increase of FXIIa activity. In absence of SG, C1-INH inhibited the three enzymes in the order FXIIa > C1s >> FXIa. Whereas SG did not modify the C1-INH-mediated FXIIa activity, they potentiated the inhibition of C1s and even more pronounced that of FXIa. Both their direct inhibitory activity (FXIIa and FXIa) and their C1-INH potentiation (FXIa, C1s) were not only dependent on their degree of sulfation and molecular weight, but also on their glycan structure. The low-sulfated HS showed to improve C1-INH, especially against FXIa, whereby it was some less active than unfractionated heparin. Among the drugable SG, the 1,3-GS PhyS4 turned out as most potent compound resulting in almost complete inhibition of all three enzymes due to both direct inhibition and C1-INH potentiation.
Conclusion: The study revealed that SG structure-dependently inhibit FXIIa, FXIa, and C1s by C1-INH potentiation and / or direct inhibition. The results warrant further investigations on the physiological role of GAG in the network of contact and complement system as well as on well-designed SG as potential therapeutic inhibitors.

SOA02-5-AB

Mechanism and therapy of fat embolism

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Objective: Fat embolism is a common and severe complication in patients after long bone fractures or during orthopaedic procedures and is associated with high mortality. Released procoagulant bone marrow components that enter the circulation can cause fat embolism. The pathomechanism of fat embolism has remained enigmatic and its therapy is mostly symptomatic.
Methodology and Findings: Natural bone marrow-derived lipids and the synthetic triglyceride triolein that constitutes a major bone marrow fat component, initiate contact activation of factor XII (FXII) in a dose-dependent manner. Together with chromogenic analysis, real time thrombin formation and clotting assays revealed that natural and synthetic lipids induce FXII-driven coagulation. Pharmacologic targeting of activated FXII largely interfered with procoagulant lipid activities in human plasma. Intravenous injections of triolein or lipids derived from bone marrow led to lethal pulmonary embolism in wild-type mice. In contrast, mice with genetic deficiency in FXII or its substrate of the intrinsic coagulation pathway, factor XI, were protected from fat-initiated

pulmonary embolism. Histological sections of lung tissue from lipid-challenged wild-type mice revealed diffuse fibrin deposition in occluded blood vessels. However, no vascular thrombosis was detectable in lung sections of factor XI- and XII-deficient mice after lipid injection.
Conclusions: Here, we show that bone marrow-derived lipids initiate contact activation, trigger the FXII-driven intrinsic coagulation pathway and induce lethal pulmonary embolism in mice. Interference with activated FXII may offer a novel strategy for prophylactic or therapeutic treatment of fat embolism.

SOA03 State of the art & oral communications: Thrombo-inflammation

SOA03-2-AB

Identification and functional characterization of a thrombus-resolving regulatory T cell population

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Background: $CD4^+Foxp3^+$ regulatory T (T_{reg}) cells have a major direct and non-redundant role in tissue repair and maintenance in addition to their role in suppression of immune responses. Whether and how T_{reg} cells participate in thrombus resolution has not been investigated.
Aims: Based on the observation that T_{reg} cells accumulate in venous thrombi, we aimed to clarify their functional role in venous clot resolution.
Methods: Experimental approaches included the selective manipulation of T_{reg} cell numbers during thrombus resolution, comprehensive characterization of the histological and cellular changes induced by them, generation of T_{reg} cell expression profiles and the identification of their clot-resolving function at the molecular level.
Result: We describe a specialized population of resident 'clot-busting' T_{reg} cells that that accumulates in venous blood clots, and directs thrombolysis by regulating monocyte recruitment and differentiation. Clot T_{reg} cells express a repair T_{reg} cell profile and produce the matricellular protein SPARC in response to inflammatory mediators. T_{reg} cell deficiency in SPARC delays clot resolution, while preserving suppression of autoimmunity.
Conclusion: Our study reveals a crucial role of specialized T_{reg} cells in ordered blood clot resolution. Increasing T_{reg} cell activity appears to be an attractive therapeutic approach for improving thrombus resolution and restoring organ function in chronic thromboinflammatory diseases. T_{reg} cell-modulating reagents, that are already clinically tested, may be used for this purpose.

SOA03-3-AB

Procoagulant extracellular vesicles impair trophoblast function by a thrombo-inflammatory pathway in preeclampsia

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Scientific research question: Procoagulant extracellular vesicles (EV) and platelet activation have been associated with pregnancy complications. We have recently identified that EV cause preeclampsia by platelet mediated placental inflammasome activation. Whether this thrombo-inflammatory pathway alters trophoblast differentiation and function remains unknown. We therefore aim to identify whether the EV induced thrombo-inflammatory pathway modulates trophoblast proliferation, differentiation and invasion in PE.
Methodology: We injected C57BL/6 mice with procoagulant EVs to study the role of *EVs in-vivo*. Blood pressure, kidney histology (PAS staining and EM), proteinuria, sFlt-1 were assessed to evaluate PE. We exposed human and mouse trophoblast cells to EV and platelets to study their role *in-vitro*. Trophoblast proliferation and cell death was studied using Ki-67 immunostaining, BrdU incorporation and TUNEL staining. RT-PCR for marker genes (PL-II, Tpbpa, Gcm1) was done to study trophoblast differentiation. Matrigel based tube formation assays and MMP expression were used to assess trophoblast invasion. Translational relevance was studied in human PE placenta.
Findings: EV injection into pregnant mice results in PE and accumulation of activated platelets in the placenta. EV treatment enhanced cell death and

impaired trophoblast differentiation and proliferation both *in-vitro* and *in-vivo*. EV treatment resulted in impaired trophoblast tube formation and reduced MMP expression indicating impaired trophoblast invasion. Platelet depletion and genetic (NFE2-/-, Gαq-/-) or pharmaceutical (Apyrase, Aspirin) platelet inhibition abolished these effects. EV induced inflammasome activation in trophoblast cells, and NLRP3 or Casp-1 deficiency or IL-1-receptor antagonist (Anakinra) abolished the effects of EV. Inflammasome activation, platelet activation and cell death were positively correlated in human PE placenta.
Conclusion: These results demonstrate that EV mediated platelet activation impairs trophoblast function by reducing trophoblast proliferation, differentiation and invasion by platelet mediated inflammasome activation. These results support the pathophysiological relevance of enhanced maternal platelet activation at the feto-maternal interface. Monitoring platelets activation and/or EVs in maternal blood may provide insights into placental failure.

SOA03-4-AB

Inflammatory release of platelet serotonin is dispensable for the regulation of endothelial adhesion molecules

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Scientific research question: Platelets store peripheral serotonin in high concentrations and release it upon their activation. Serotonin was shown to modulate the immune response in various mouse models of inflammation including myocardial ischemia/reperfusion injury, metabolic syndrome, rheumatoid arthritis, endotoxic shock or sterile peritonitis. We and others reported that platelet-derived serotonin enhances the recruitment of leukocytes to sites of acute inflammation and tissue damage, thereby worsening disease outcome. Consequently, serotonin action appears to be a promising therapeutic target. For successful therapeutic application the exact mechanisms of serotonin action in immune cell recruitment must be clarified. We investigated whether serotonin alters the function of endothelial cells in leukocyte recruitment and extravasation during sterile and unsterile inflammation.
Methodology: We applied two experimental models: acute high dose intraperitoneal (i.p.) lipopolysaccharide (LPS) injection and mesenteric ischemia/reperfusion injury in wild type (WT) C57BL/6 and tryptophan hydroxylase 1 deficient (Tph1-/-) mice that do not synthesise peripheral serotonin. Leukocyte recruitment and activation were analysed via intravital microscopy and flow cytometry. The endothelial phenotype was investigated by histological and flow cytometric analysis of endothelial adhesion molecules involved in leukocyte recruitment. The mouse studies were complemented by in vitro analyses in human umbilical vein endothelial cells (HUVEC).
Findings: Upon i.p. LPS challenge, significantly fewer neutrophils transmigrated into the peritoneal cavity (as analysed in lavage fluid) in Tph1-/- mice compared to WT. This was in part due to diminished CD11b expression on circulating neutrophils in Tph1-/-. Flow cytometry analysis of endothelial cells of abdominal blood vessels showed an LPS-dependent increase in E-selectin, P-selectin, vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) expression. No difference was observed between WT and Tph1-/- mice. These results were confirmed by fluorescence immunohistochemistry of mesenteric veins. In the sterile inflammation model of mesenteric ischemia/reperfusion the number of rolling leukocytes was significantly lower in Tph-/- compared to WT in the beginning of the reperfusion phase. In line with the LPS model, endothelial adhesion molecules E-selectin, P-selectin, ICAM-1 and VCAM-1 were not expressed differently in Tph1-/- than in WT. In support of the in vivo data, serotonin failed to induce endothelial adhesion molecules in HUVEC in vitro.
Conlusion: The observed serotonin-dependent alterations in leukocyte-endothelial interaction and leukocyte transmigration were independent from a direct effect of serotonin on endothelial adhesion molecule expression.

SOA03-5-AB

Thrombin receptor PAR4 drives inflammatory signaling in the heart

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Scientific research question: Thrombin antagonists reduce cardiac remodeling and dysfunction in diabetic mice, in part by suppressing fibrin-driven inflammatory signaling. The role of cellular thrombin receptors in this regard is not known. We

recently reported upregulation of protease-activated receptor 4 (PAR4) in cardiac fibroblasts isolated from diabetic mice, and here tested the hypothesis that PAR4 mediates thrombin-driven inflammatory signaling in the diabetic heart.
Methodology: Whole tissue lysates were prepared from left ventricles (LV) of mice with and without diet-induced type-2 diabetes, and from right atrial appendages (RAA) from patients with and without type 2 diabetes undergoing heart surgery. Human cardiac fibroblasts (CF) were cultured in low (LG, 5.5. mmol/L) or high glucose (HG, 25 mmol/L) to simulate normo- and hyperglycemia, respectively. Target gene and protein expression were assessed by realtime PCR and Western blot respectively, cytokine release was determined by ELISA.
Findings: Diabetic mouse LV showed increased expression of pro-caspase-1 and pro-interleukin (IL)-1β, indicating transcriptional priming of the NLRP3 inflammasome, as well as augmented auto-cleavage to active caspase-1, indicating NLRP3 inflammasome activation. Accordingly, IL-1β maturation and formation of the pyroptotic pore protein N-terminal gasdermin D, prerequisite for IL-1β secretion, were also higher in diabetic vs. control mouse LV. Abundance of PAR4, but not of PAR1, increased in diabetic mouse LV and correlated positively with active caspase-1. Genetic deletion of PAR4 abrogated the proteolytic activation of caspase-1, IL-1β and gasdermin D in LV of mice with diet-induced diabetes, indicating causal involvement of PAR4 in diabetes-driven inflammation. RAA from patients with type 2 diabetes also showed higher levels of PAR4 than non-diabetic RAA, together with an increased abundance of active caspase-1, mature IL-1β and cleaved (pore-forming) gasdermin D. Finally, human CF maintained in HG conditions expressed more PAR4 than LG cells and showed higher PAR4-dependent IL-1β transcription and secretion in response to thrombin, confirming a functional link between PAR4 and inflammatory signaling through IL-1β.
Conclusion: The PAR4 thrombin receptor is a relevant driver of caspase-1-dependent IL-1β production in the diabetic heart, with CF likely to contribute substantially to PAR4-mediated inflammatory signaling. We provide a mechanistic explanation for the chronic low-grade thromboinflammation that characterizes diabetic cardiomyopathy and for the cardioprotective effects of thrombin inhibition in this setting. The strong bleeding side-effects of the direct thrombin inhibitors might limit their use to patients with a clear indication for anticoagulation, but the emerging PAR4-selective antagonists may provide a feasible alternative approach to blunt cardiac inflammation in patients with diabetes.

SOA04 State of the art & oral communications: DOACs

SOA04-2-AB

Higher case-fatality rate after stroke compared to major bleeding in end-stage renal disease patients on hemodialysis

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Background: The assessment of risk for stroke and major bleeding in hemodialysis (HD) patients is difficult due to the high incidence rates of both events in this patient population, but important when considering antithrombotic treatment. We investigated 30-day case-fatality rates in HD patients after stroke and major bleeding events to gauge the immediate impact on mortality.
Methods: In a prospective cohort study of HD patients in Vienna, Austria, we recorded the occurrence of a composite outcome of stroke, transient ischemic attack (TIA) and systemic embolism as well as the outcome major bleeding according to the ISTH definition. All outcomes were verified by an independent adjudication committee.
Results: In the cohort of 625 HD patients (397 men [63.3%], median age 66 years [25th to 75th percentile 55 - 75 years] with a median observation time of 870 days (25th to 75th percentiles: 391 - 1234 days), 40 composite outcomes of strokes, TIA, and systemic embolism (6.4%) occurred with an event-rate of 29.0 per 1000 patient-years. All-cause death occurred in 256 patients (41.0%) with a rate of 181.9 per 1000 patient-years. The 30-day case-fatality after stroke/TIA/systemic embolism was 22.5%. Major bleeding occurred in 89 patients (14.2%) including 14 intracranial hemorrhagic events, with an event-rate of 67.8 per 1000 patient-years. The 30-day-case-fatality after major bleeding was 19.1% and was especially high after intracranial hemorrhage (28.5%). There were 165 patients with atrial fibrillation (AF) in the cohort at study baseline (26.4%) and 73 patients developed AF during the observation time (15.9% of previously non-AF patients). In patients with AF the

30-day case-fatality rate after stroke was 28.0% and was 21.4% after major bleeding.
Conclusion: Although major bleeding event occurred more than twice as often as stroke events in HD patients, the 30-day case-fatality rates were higher after stroke. The difference in 30-day case-fatality after stroke and major bleeding is greater in AF patients on HD.

SOA04-3-AB

Performance characteristics of DOAC Dipstick test for direct oral factor Xa or thrombin inhibitors in urine from a German multicentre study and analysis of inter-centre agreement

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In emergency situations, direct oral anticoagulants (DOACs) may need to be detected quickly, but this is not always feasible; thus, confirmation of the presence or absence of DOACs remains a major challenge. All DOACs are excreted into urine.
In this prospective, open-label, controlled, non-randomized, multicentre study performed in Germany, subjects treated with an oral direct factor Xa inhibitor (DXI; apixaban, edoxaban, and rivaroxaban) or the oral thrombin inhibitor (DTI) dabigatran were included. The primary endpoint of the study was to analyse the true positive and true negative rate of the factor Xa inhibitor and thrombin inhibitor DOAC Dipstick test compared with the results obtained by liquid chromatography tandem mass spectrometry (LC-MS/MS) from urine samples of subjects. Specifically, the inter-centre variability was analysed because visual identification of the test pad colours could not be quantitatively analysed.
Nine hundred and fourteen subjects were included at 18 German centres, of which 880 subjects were evaluable for the analysis (451 in the DXI group (apixaban: n=170, edoxaban: n=131, rivaroxaban: n=150) and 429 in the DTI group). Demographic data were comparable between both groups. In the DXI group, specificity, accuracy, and positive predictive value were significant at 95% ($p < 0.002$) and the sensitivity and negative predictive value were significantly non-inferior at a proportion of 95% including the 0.5% margin ($p < 0.04$). In the DTI group, these parameters were significant at a proportion of 97.5% ($p < 0.001$). The agreement (kappa value) between results of the factor Xa and thrombin inhibitor pads and of LC-MS/MS was 0.945 and 0.987, respectively. The intention-to-analyse evaluation confirmed the results of the per-protocol evaluation. The visual evaluation of the factor Xa inhibitor and thrombin inhibitor pads were not significantly different between centres.
The present study shows that DOAC Dipstick test sensitively and specifically determines the presence and absence of direct oral factor Xa and thrombin inhibitors in urine samples compared to the gold standard LC-MS/MS method. The high agreement between centres analysing DOAC Dipstick test encourages further multinational evaluation in emergency medicine and other patient groups.

SOA04-4-AB

Venous thromboembolism therapy with apixaban in daily-care patients: Results from the *DRESDEN NOAC REGISTRY* (NCT01588119)

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Objectives: The effectiveness and safety of acute venous thromboembolism (VTE) treatment with apixaban, demonstrated in phase-III trials, needs to be evaluated in daily care patients.
Methods: The *DRESDEN NOAC REGISTRY* is a prospective regional registry in which patients with oral anticoagulation undergo prospective follow-up (FU). So far, more than 4500 patients have been enrolled, including 469 VTE patients with apixaban treatment. For this analysis, only patients with acute VTE who started apixaban within 14 days after diagnosis of VTE and were enrolled within these 14 days were evaluated. All reported outcome events were centrally adjudicated based

on source documentation and standard event definitions.
Results: Until May 31st, 2019, 351 patients received apixaban for acute VTE treatment (51.6% male, 78.6% DVT; 21.4% PE±DVT, mean age 63 years, mean time between VTE diagnosis and initiation of apixaban 1.0±2.7 days). At baseline, apixaban doses consisted of 10 mg BID in 91.5%, 5 mg BID in 6.6% and 2.5 mg BID in 2.0% of patients. Reasons for not using 2x10 mg apixaban BID were pre-treatment with therapeutic parenteral anticoagulants for ≥7 d in 19 cases, comorbidities (e.g. bleeding history, renal impairment) in 4 cases and unknown in 8 cases.
During active apixaban therapy (mean FU 20.7±6.7 months; mean apixaban exposure 13±9.5 months), 3/351 patients (0.9%) experienced a recurrent VTE (0.8/ 100 pt. years). During treatment, 122/351 (34.8%) patients reported bleeding complications, including 5 cases of ISTH major bleeding (1.4%; 1.3/100 pt. years).

	on treatment (last intake ≤ 3d)	off treatment (interruption and permanently discontinuation)
recurrent VTE		
n %	3 in 3 pts. 3/351 (0.9%)	17 in 15 pts. 15/351 (4.3%)
- recurrence as PE	2	7
- recurrence as DVT	1	8
- unusual site of VTE	0	2
ISTH major bleeding		
n %	5 in 5 pts. 5/351 (1.4%)	11 in 10 pts. 10/351 (2.8%)
- intracranial	3	3
- genitourinary	1	1
- gastrointestinal	0	4
- intraocular	0	1
- other	1	2

[Recurrent VTE and major bleeding in patients during apixaban intake and after discontinuation]

11 patients died during FU (1.8/100 pt.years). Most common causes of death were terminal malignant disease (n=4), followed by sepsis/infection (n=2), age related death (n=2), fatal cardiovascular event (n=1), fatal bleeding (n=1) and other reasons (n=1).
At 6 months respective 12 months, (FU completed in 349and 341 pts.), 66.5% and 52.8% of patients were still taking apixaban. The remaining patients had a scheduled end of treatment (24.4% and 30.8%) or were switched to other anticoagulants (3.7% and 3.8%). Therefore, the rates of unplanned complete discontinuation at 6 or 12 months were 3.7% and 6.5%, respectively.
After apixaban interruption for more than 3 days or permanent discontinuation, 15 patients experienced a recurrent VTE (7 PE±DVT, 8 DVT) with a mean time between last intake of apixaban and VTE recurrence of 4.8±3.8 months (range 14-417d).
Conclusions: This is one of the first real-world reports on apixaban in VTE treatment. Our results indicate high effectiveness and safety of apixaban in this setting. Initial dosing was according to label in over 90% of patients and, at 6 and 12 months, persistence to apixaban therapy was excellent with low rates of unplanned discontinuation. Fatal VTE and fatal bleeding are rare events during apixaban therapy. However, a relevant number of patients developed recurrent VET within the first 6 months after apixaban discontinuation.

SOA04-5-AB

Unified calibration curves for the measurement of direct anti-Xa-inhibitors

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Objectives: Anti-Xa assays are the standard of care for the estimation of plasma levels of anti-Factor Xa (FXa) inhibitors. Laboratories have to establish a

calibration curve for each of the anti-FXa-inhibitors, which is expensive and cumbersome to handle. The aim of this study was to show that the DOAC plasma levels can be estimated independently from the anti-Xa-assay and the specific calibrator used in the test.
Methods: A total of 240 blood samples (80 each with apixaban, edoxaban and rivaroxaban) from patients at different time points after DOAC intake were included into the analysis. Plasma levels were analyzed using all six anti-Xa-assays currently licensed in Germany and were calibrated against each available anti-Xa calibrator. A combined calibration curve was established for each anti-FXa-inhibitor by merging all calibration curves for that anti-FXa-inhibitor ("like vs. like"). The determined drug levels were compared to drug levels estimated using calibration curves of a different drug (e.g. apixaban vs. rivaroxaban).
Results: The mean difference in the slope between the combined calibration curve and the specific calibration curve of the same drug was 1.03 ± 0.09 for apixaban, 1.03 ± 0.06 for edoxaban and 0.99 ± 0.10 for rivaroxaban. In apixaban containing plasma samples calibrated with the combined edoxaban calibration curve, the mean difference in the slope compared to the specific calibration curves was 1.13 ± 0.18 for apixaban and 0.81 ± 0.12 for rivaroxaban, while it was 0.97 ± 0.13 and 0.75 ± 0.08 for edoxaban containing plasma samples calibrated with apixaban and rivaroxaban and 1.29 ± 0.16 and 1.51 ± 0.35 for rivaroxaban containing plasma samples calibrated with apixaban and edoxaban, respectively.
Conclusions: A unified calibration curve for all anti-FXa-inhibitors can be established. The variation of the drug levels when edoxaban is calibrated with apixaban and vice versa is about 10%. A correction factor of 0.8 and 0.75 should be used for the estimation of rivaroxaban plasma levels with edoxaban and apixaban calibration curves. Conversely, apixaban levels or edoxaban levels need to be corrected by 1.3 or 1.5, respectively, if rivaroxaban is used as calibrator.

SOA05 State of the art & oral communications: VWS/ECMO

SOA05-2-AB

Acquired von-Willebrand-syndrome in ECMO patients: a hospital-based 3-year cohort study

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Scientific research question and methods: While von-Willebrand-syndrome (VWS) is the most common inherited bleeding disorder acquired (a) VWS is a rare condition, which is increasingly being diagnosed associated with complex medical conditions. In line with literature data the yearly incidence of clinical relevant aVWS derived from a two-hospital based reporting system "Orbis" was recently calculated as 8% in a pilot study. Since approximately 100% of patients undergoing ECMO develop laboratory aVWS during their hospital stay in the majority of cases without clinical bleeding, in a second cohort we additionally evaluated consecutively hospitalized patients admitted to the cardiac-cardio surgery department (CCS) to collect more relevant data with respect to adverse outcome, i.e. *i)* development of clinically relevant aVWS and/or *ii)* death during a 1-year follow-up period.
Findings: Within a 3-year period 338 white patients aged 18-88 years (median: 59.7 male; 61.7 female) were enrolled (male 64.5%). The yearly incidence of patients with aVWS with clinical relevant bleeding symptoms in this high risk cohort was 23% (78/338) with a death rate of 74% within a median time of 9 days (1-229) following ECMO start. Of note, compared with non-bleeding patients with aVWS 15 of 78 subjects and bleeding symptoms received aspirin or clopidogrel, vitamin-K-antagonists, heparins, argatroban and/or anti-factor-Xa inhibitors. As treatment for bleeding episodes blood product administration (platelet transfusions, erythrocyte substitution, FFP) was performed concomittant with PPSB, rFVIIa, Fibrinogen or factor XIII concentrates. The 78 subjects with aVWS received different sources of von-Willebrand-factor concentrates, corresponding to a median (min-max) amount of 6.000 IU per patient affected (1.000-157.000 IU). Logistic-regression analysis adjusted for age at ECMO start

and gender revealed that *i)* the presence of blood group 0 versus non-0 (Odds ratio(OR)/95% CI: 1.76/1.005-3.11; p=0.04) and the overall need for blood product unit administration per unit (OR/95%CI: 1.1/1.1.01-1.02; p< 0.001) were independenly associated with the development of clinically relevant aVWS. *ii)* Whereas in the entire ECMO cohort older age (increase per year) at ECMO start (OR/95%CI: 1.01/1.0-1.03; p=0.050) and the amount of blood product units necessitated were related with death (OR/95%CI: 1.01/1.002-1.014; p=0.01) during the follow-up period, death was not associated with the development of clinically relevant aVWS.
In conclusion, in the present cohort study derived from consecutively patients admitted to the CCS we found a clinical relevant bleeding incidence of 23% in subjects with aVWS. The latter, however, was not associtated with death following the ECMO procedure.

SOA05-3-AB

Modulating apoptosis in platelet: a promising approach for cold storage of apheresis platelet concentrates

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Scientific research question: Transfusion of platelet concentrates is routinely used to treat or prevent bleeding. Bacterial infection caused by storage at room temperature (RT) still remains a major problem of this established therapeutic approach. Recently, we showed that cold-stored apheresis platelet concentrates (APCs) are associated with better PLT functionality, like adhesion and aggregation ability, but also with shorter PLT half-life in vivo (Haematologica 2018, PMID: 30115655). Cold-induced apoptosis was identified as a potential mechanism of the accelerated PLT clearance. In this study, we investigated the effect of apoptosis inhibitors during cold storage of APCs on platelet function and survival.
Methodology: APCs were collected and stored at RT and 4°C in the presence or in the absence of a caspase-9 inhibitor. PLT apoptosis was assessed measuring the phosphatidylserine exposure and the mitochondrial membrane potential (MMP) using flow cytometry. The protein expression was quantified by western blot. The PLT aggregation ability was analyzed using an aggregation assay.
Findings: The expression of the apoptotic marker phosphatidylserine was enhanced in cold-stored APCs (C-APCs) compared to RT ones (RT-APCs) (% apoptotic PLTs mean±standard error means [SEM]: 13±1% vs. 5±1%, C- vs. RT-APCs, respectively, p=0.018). In order to investigate if the apoptosis specifically involved the intrinsic pathway, the MMP was analyzed using TMRE as a marker of alive cells. Interestingly, a significant decrease in MMP was observed after cold storage in comparison to RT indicating the activation of the intrinsic pathway (mean fluorescence intensity [MFI] of TMRE±SEM: 6.13±1.89 vs. 18.53±3.64, C- vs. RT-APCs, respectively, p=0.038). In addition, a decrease of the procaspase-9 protein level was found after cold storage using western blot. When PLTs were stored in the presence of a caspase-9 inhibitor a significant rescue of the cold-stored cells viability was observed (MFI of TMRE±SEM: 1.95 ± 0.23 vs. 4.39 ± 0.49, without vs. with caspase-9 inhibitor, respectively, p=0.0453). More importantly, PLT aggregation ability was not affected by the presence of caspase-9 inhibitor and comparable with PLTs stored in the absence of the inhibitor (% maximal aggregation in response to thrombin receptor activator peptide mean±SEM: 76±6% vs. 67±16%, without vs. with caspase-9 inhibitor, respectively, p=0.638).
Conclusion: Our results show that the decrease of cold-stored PLT viability is mediated by the activation of the intrinsic apoptosis pathway. Most importantly, our data suggest that cold-lesions can be prevented by a specific caspase-9 inhibitor without affecting PLT aggregation capability. Consequently, cold storage, associated with a better PLT functionality, may become an efficient strategy for APC storage in combination with apoptosis inhibitors.

SOA05-4-AB

Activation of platelet Syk is regulated by a novel crosstalk between protein kinase C and Syk-serine phosphorylation

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Scientific research question: The spleen tyrosine kinase Syk plays an essential role in platelet proximal signaling mediated by ITAM-dependent receptor activation. Recently, we published that

the crosstalk between platelet activation mechanisms mediated by GPIbα and inhibitory pathways mediated by cAMP/PKA and cGMP/PKG resulted in a significant inhibition of the overall platelet aggregation, but surprisingly, induced prolonged Syk activation and only partial inhibition of InsP3 production and Ca^{2+} release. Syk was differentially regulated by cAMP/cGMP elevating agents since Y525/526 [located in the kinase domain] and Y352 [in the regulatory interdomain B], showed a hyperphosphorylation profile, whereas the Syk S297 site [also the regulatory interdomain B] was significantly downregulated. Because the role of Syk S297 phosphorylation in human platelets is not known so far, we investigated Syk S297 phosphorylation in relation to Syk activation and function in human platelets.
Methodology: Echicetin beads (EB) and convulxin (cvx) were used as specific GPIbα and GPVI agonists, respectively. Platelet aggregation was determined by light transmission aggregometry. Phosphorylated proteins were analyzed by immunoblotting. Intracellular messengers inositol monophosphate (InsP1) as marker for InsP3, and Ca^{2+}i were quantified by ELISA IPOne assay and flow cytometry, respectively.
Findings: Syk S297 was significantly upregulated in a transient manner similar to Syk Y525/526 and Y352 phosphorylation upon ITAM-mediated platelet signaling induced by EB or cvx, and Syk S297 phosphorylation was dependent on SFK and Syk activation. Pre-incubation of platelets with the cAMP/cGMP elevating agents iloprost/riociguat resulted in a significant downregulation of Syk S297 phosphorylation but Y-hyperphosphorylation, which was associated with prolonged Syk activation. Interestingly, these effects were also seen when PKC was inhibited by the global-PKC inhibitor GFX. These hyperphosphorylation profiles of Syk tyrosine sites led to a significant increase of InsP3 production and Ca^{2+} release in GPIbα and GPVI-activated platelets. Accordingly, PKC inhibition affected only partially the overall platelet aggregation induced by EB or cvx. PKC activation by the phorbol ester PDBu induced S297 upregulation but not Y-phosphorylation of Syk in intact platelets as well in platelet lysates, which was similar to ADP-stimulated platelets.
Conclusion: Our data demonstrate that PKC activated by ITAM/Syk pathways stimulates Syk S297 phosphorylation and that this is a possible feedback regulation after Syk activation via GPIbα and GPVI. Thus, the S297 site within the crucial interdomain B, an important switch for Syk activation, might represent a potential diagnostic and/or therapeutic target in human platelets.

SOA05-5-AB

Prospective validation of a rapid and accurate diagnostic algorithm for heparin-induced thrombocytopenia

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Objectives: Heparin-induced thrombocytopenia (HIT) carries a high mortality and morbidity if left untreated, while switching unnecessarily to alternative anticoagulants leads to bleeding complications and increased costs. Clinical suspicion of HIT therefore requires rapid laboratory assessment to guide clinical management. We aimed to develop and prospectively validate a diagnostic algorithm able to accurately confirm or exclude HIT with a short laboratory turn-around-time (TAT).
Methods: Based on our previous derivation studies (05.2014 - 12.2016, n = 526), we developed an in-house diagnostic algorithm that relies on the Bayesian combination of pre-test clinical probability (4Ts-score) and quantitative results of sequential rapid immunoassays (IAs) for anti-PF4/heparin antibodies [AcuStar HIT-IgG (IgG-specific CLIA) and PaGIA-H/PF4 (PaGIA)] in order to predict the outcome of the functional gold-standard assay (HIPA). We prospectively validated this algorithm (01.2017 - 07.2019, n = 590).
Results: 100% negative (NPV) and positive (PPV) predictive cut-off values for a positive HIPA were set at IgG-specific CLIA values of < 0.13 U/ml and >3.0 U/ml, respectively. For PaGIA, the cut-off titers were < 2 and >8, respectively. Likelihood ratios were determined for intermediate results. During the prospective validation study, IgG-specific CLIA was employed as a single IA in 480/590 (81.4%) of cases (TAT 30 min); PaGIA was performed as a second line IA in 110/590 (18.6%) of initially unsolved cases (additional TAT 30 min). Our Bayesian diagnostic approach could predict HIT in 45/590 (7.6%) and excluded it in 519/590 (88.0%) patients, leaving only 18/590 (3.0%) cases unresolved. Additionally, we identified 8/590 (1.4%) positive predictions not confirmed by HIPA. In at least seven of these eight patients, laboratory work-up and clinical evolution were very suggestive of HIT.

Conclusions: The combination of the estimated clinical probability of HIT and the sequential application of two rapid IAs for anti-PF4/heparin antibodies enables a rapid and accurate diagnostic work-up within 60 minutes for more than 95% of patients with suspected HIT.

SOA06 State of the art & oral communications: Haemostasis in the placenta

SOA06-2-AB

Activation potential and neutrophil extracellular trap formation of neutrophils in patients with antiphospholipid syndrome

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Background and scientific research question: Antiphospholipid syndrome (APS) is an autoimmune disorder characterised by the presence of antiphospholipid antibodies as well as the occurrence of arterial and/or venous thromboembolism (TE) and/or pregnancy complications. The underlying mechanisms leading to these complications are still not fully understood. Especially in the last decade, new functions of neutrophils and their diversity was an important focus research in this field. High density neutrophils (HDN) are found in health and disease, whereas low density neutrophils (LDN) are increased in pathologic conditions. LDNs were first found after gradient centrifugation in patients with cancer and infection, and are more prone to undergo neutrophil extracellular trap (NET) formation. Neutrophils and especially NETs are of specific interest in diseases with an increased risk of thrombosis.

It was the aim of this study to investigate differences in neutrophil subpopulations, their potential of activation and potential of NET formation between patients with APS and healthy controls, to gain mechanistic insights into the role of neutrophils in APS.

Methodology: HDN and LDN from 20 patients with APS and 20 age- and sex-matched healthy donors were isolated by density gradient centrifugation and activated with various stimuli (IO, ionomycin; PMA, phorbol 12-myristate 13-acetate). Flow cytometry was applied to analyse the increase of CD11b expression after 30 min (defined by % of CD11b high cells, according to immediately fixed samples set to 1%) and NET formation, as indicated by H3Cit-positive cells after 3 h of activation.

Findings: Patients with APS have increased levels of LDNs in their PBMC layer (peripheral blood mononuclear cell) ($p < 0.01$, figure A). After 30 min stimulation, neutrophils from patients with APS showed increased levels of CD11b high neutrophils, especially in HDNs ($p < 0.01$, figure B). Neutrophils from patients did not only show enhanced ability for activation, but had also increased NET formation capacity, even more pronounced in LDNs from patients with APS (Figure C). Even in the absence of activation, baseline LDNs (imfix) from patients with APS had increased levels of H3Cit-positive cells ($p < 0.01$, figure C).

Conclusion: In conclusion, neutrophils from patients with APS have altered neutrophil subpopulations, a higher potential for activation after short term stimulation and enhanced NET formation after long term activation compared to healthy controls. Our results support the hypothesis that HDNs and LDNs from patient with APS are already pre-activated as they show increased H3Cit positive cells at baseline. This increased NET production and potential for activation might be the result of their primed state due to the disease. In line, the altered neutrophil activation state may contribute to increased risk of thrombosis in patients with APS.

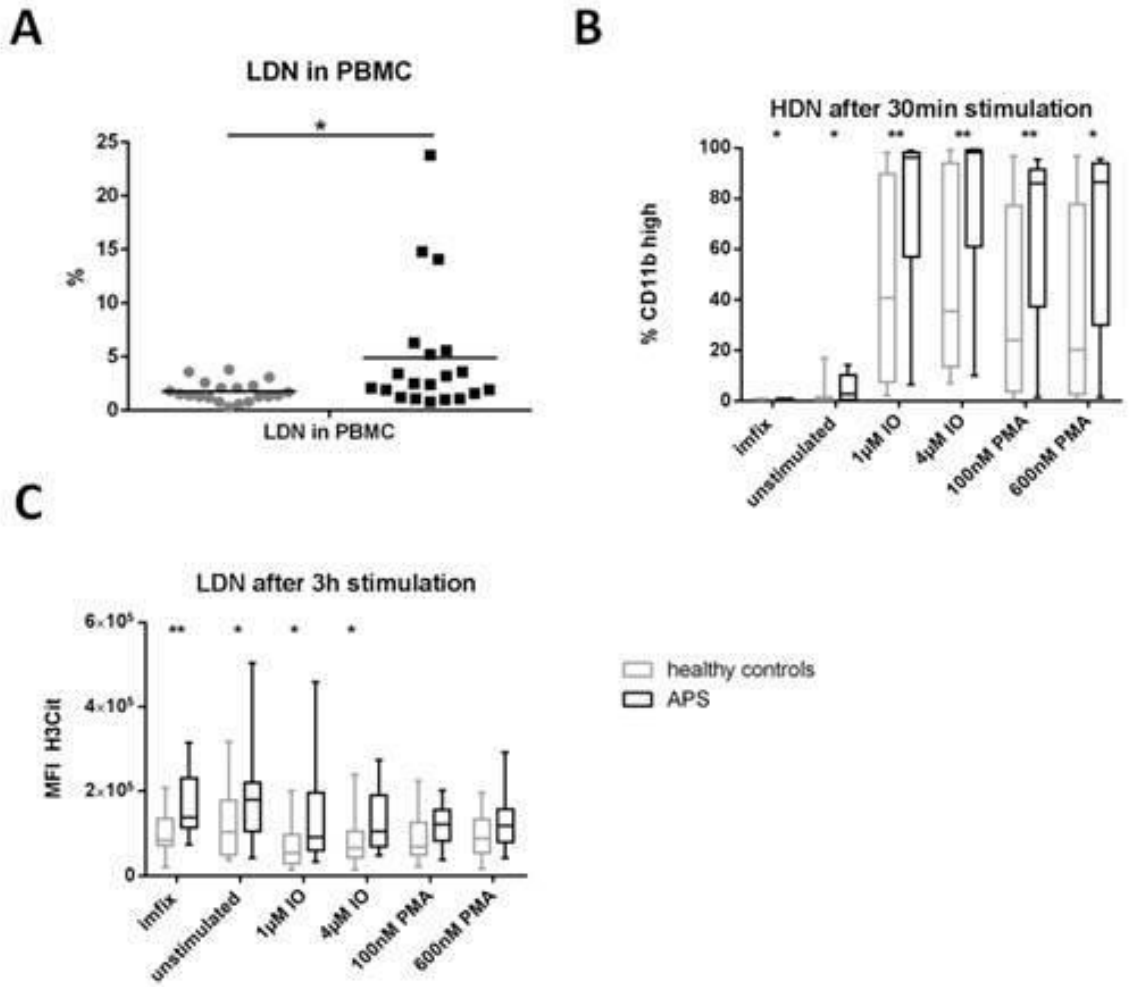

Figure legend: Neutrophil subset count, potential of activation and ability of NET formation. (A) Percent of low density neutrophils (LDNs) in PBMCs (peripheral blood mononuclear cells) in healthy controls (grey) and patients with APS (black). (B) Percentage of CD11b high HDNs (high density neutrophils) in healthy controls and patient with APS (defined by % of CD11b high cells, according to immediately fixed samples set to 1%). (C) Mean fluorescence intensity (MFI) of the NET marker H3Cit (citrullinated histone H3) after 3h stimulation of LDNs. IO... ionomycin, PMA... phorbol 12-myristate 13-acetate

SOA06-3-AB

Kynurenine, an endothelial derived factor that modulates platelet function

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Background: Platelets are small, discoid anucleated blood elements derived from megakaryocytes that play an important role in haemostasis and thrombosis. Kynurenine is a tryptophan metabolite known to participate in different physiological and pathological processes. The documented effect of Kynurenine on blood pressure and the potential relationship between Kynurenine and Nitric oxide pathways led us to investigate the potential effect of kynurenine on platelet function.

Methods: This study was approved by the School of Pharmacy and Pharmaceutical Sciences Research Ethics Committee at Trinity College Dublin. Blood was collected from healthy

volunteers who had not taken any medication known to affect platelet function in the two weeks prior to the experiments. Platelet rich plasma (PRP) and washed platelets (WP) were prepared by centrifugation and platelet concentrations adjusted to 250,000 platelets/µL. PRP and WP were incubated in the presence or absence of kynurenine at various concentrations and its ability to inhibit platelet aggregation induced by different platelet agonists studied by light transmission aggregometry (LTA) and flow cytometry (FC). Both, cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) were measured by enzyme-linked immunosorbent assay (ELISA) and the expression of vasodilator stimulated phosphoprotein (VASP) by immunoblotting. Data were obtained from at least three independent donors and analysed by one-way ANOVA test followed by Tukey's post-test. $P < 0.05$ was considered statistically significant.
Results: Kynurenine was found to significantly inhibit collagen-, adenosine diphosphate-, thromboxane- and arachidonic acid- induced platelet aggregation by LTA. This effect was corroborated by a downregulation in the platelet's expression of GPIIb/IIIa and P-selectin by FC. Kynurenine mechanism of action involves phosphorylation of VASP by cAMP- and cGMP-dependent protein kinases.
Conclusions: In this study, we showed for the first time that Kynurenine inhibits platelet aggregation. The mechanism by which Kynurenine modulates platelet function involves activation of the adenylyl cyclase (AC) enzyme and activation of the reduced and oxidized heme forms of the soluble guanylyl cyclase (sGC) enzyme. Kynurenine may represent a physiological endothelial derived factor that inhibits platelet aggregation activating both, adenylyl cyclase and guanylyl cyclase enzymes.

SOA06-4-AB

CD36 and FasR are responsible for active recruitment of red blood cells to activated platelets during hemostasis and arterial thrombosis

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Background: Recently, a direct interaction of platelets and red blood cells (RBCs) via FasL-FasR interaction was found to be important for the externalization of phosphatidylserine (PS) at the RBC membrane that attributes a direct role for RBCs to thrombin generation and thrombus formation and stabilization upon hemostasis and thrombosis. Currently, the active recruitment of RBCs to adherent platelets and/or the growing thrombus is unknown.
Methodology: Analysis of the recruitment of RBCs to activated platelets under flow conditions and RBC-platelet interactions were performed using blood samples from healthy volunteers.
Findings: The inhibition of integrin aIIb3 with Abciximab resulted in reduced platelet adhesion on recombinant FasR (CD95) and decreased PS exposure on the RBC membrane indicating an interaction between FasR on RBCs and integrin aIIb3 at the platelet surface. This observation was further supported by increased externalization of CD61 at the platelet membrane in the presence of RBCs. Increased externalization of CD61 was blocked by specific anti-CD36 and anti-FasR antibodies. The involvement of FasR was confirmed by FasR knock-out RBCs, demonstrating the importance of RBCs for the upregulation of CD61 at the platelet surface. However, the interaction of RBCs and platelets via aIIb3 and FasR was not important for the recruitment of RBCs to activated platelets. In contrast, first results of dynamic adhesion and thrombus formation using a blocking CD36 antibody imply a contribution of CD36 of RBCs to be involved in the active recruitment of RBCs into the growing thrombus. Furthermore, flow chamber experiments with collagen-adherent platelets provide first evidence for a role of FasR and PS exposure of RBCs and the release of thrombospondin from platelets to be involved in the active recruitment of RBCs to activated platelets.
Conclusion: Taken together, the recruitment of RBCs to collagen-adherent platelets is an active process that is controlled by several surface proteins on the platelet and the RBC membrane including CD36 and FasR on RBCs and the release of thrombospondin from platelets. This initial mechanism might play a prominent role in hemostasis and thrombus formation.

SOA06-5-AB

Tissue factor cytoplasmic tail regulates myelod cell activation and superoxide formation in chronic myocardial infarction

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Rationale: Pathomechanisms driving thrombo-inflammation in subacute myocardial infarction (MI), a clinical condition of high mortality, are incompletely understood.
Objective: We tested the hypothesis that the signaling function of cytoplasmic tail of tissue factor (TF) promotes inflammatory myeloid cell activation in subacute MI.
Methods and Results: We identified increased phosphorylation of cytoplasmic tail of tissue factor (TF) and nuclear factor kappa B (NF-kB) activation in circulating monocytes isolated from subacute MI compared to coronary artery disease control patients. In a mouse model of permanent myocardial ischemia (ligation of left anterior descending artery for 7 days), infarcted myocardium of C57Bl/6 mice revealed accumulation of Ly6C^{high} leukocytes with phosphorylated cytoplasmic tail of TF as well as increased CC-chemokine 2-receptor, interleukin-6 and tumor necrosis factor alpha expression which was attenuated in cytoplasmic tail-deleted (TF$^{\Delta CT}$) mice. Superoxide formation was driven by phosphorylation of cytoplasmic tail of TF with consecutive TF surface expression as well as NF-kB, rac-1 and NADPH oxidase activation in monocytes accumulating in the infarcted myocardium in C57Bl/6, but not in TF$^{\Delta CT}$ mice, with permanent ischemia. In chronic MI (6 weeks of left anterior descending artery ligation), myocardial fibrosis associated with transforming growth factor beta and matrix metalloproteinase 2 activation was increased in C57Bl/6, but not in TF$^{\Delta CT}$ mice. This resulted in partial protection from myocardial dysfunction and remodeling as well as improved survival in TF$^{\Delta CT}$ mice compared to C57Bl/6 controls.
Conclusions: We conclude that phosphorylation of the cytoplasmic tail of TF regulates NADPH-oxidase derived superoxide formation and cardiac fibrosis in a NF-kB dependent manner, representing a putative biomarker or therapeutic target in subacute MI.

SOA07 State of the art & oral communications: Risk factors for ATE/VTE

SOA07-2-AB

Validation of a predictive model for identifying an increased risk for recurrence in adolescents and young adults with a first provoked thromboembolism

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Scientific Research Question: Among risk factors for developing recurrent venous thromboembolism (VTE) in adolescents and young adults with a first provoked VTE were male gender [1 point], inherited thrombophilia (IT) status (none [0 points], single [1 point], combined variants [2 points]), blood group non-O, and age increase per year at first VTE onset. Developing a predictive model for determining patients at increased risk to suffer from a second VTE event would be beneficial in targeting duration and intensity of anticoagulant therapy (AC).
Methodology: To build a risk assessment model 1020 consecutive newly enrolled patients aged 14 to < 60 years (male 32%) with a first provoked VTE were randomized into 2 groups in which predictive variables were incorporated. This model was derived in a cohort (C) of 511 patients (DC) and then validated in 509 subjects (VC). VTE recurrence risk score (4 categories: 0,1,2,3, maximum 3 points) was below or equal two for low-risk group (LRG) and greater than 2 for high-risk group (HRG).
Findings: Within a median time of 3 years after withdrawal of AC recurrence rate in LRG (DC) was 11.8% versus 26% in HRG (p=0.0002). In the VC within 2.2 years the recurrence rate was 9.8% in LRG versus 30.1% in HRG (p < 0.0001). In

multivariate analysis adjusted for age at first VTE onset and blood group (O versus non-O) the recurrent risk in HRG was significantly increased compared with the LRG (DC: hazard/95% confidence interval: 3.7/1.75-7.91; VC: 4.7/2.24-9.81). Model specificity was 78.57% and sensitivity was 51.95% (DC). In addition, in the VC specificity was 78.3% with a sensitivity of 43.04%.
Conclusion: In conclusion, in the prediction model presented here the risk of VTE recurrence was *i)* associated with male gender and combined ITs. On the basis of the specificity *ii)* the model may identify patients with a first provoked VTE not being at risk for recurrence. Of note, whether subjects identified as high-risk patients may benefit from intensified AC or prolonged AC warrants assessment in a larger cooperative clinical trial.

SOA07-3-AB

Prothrombotic variants of von Willebrand factor

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Scientific research question: The multimeric plasma glycoprotein von Willebrand factor (VWF) plays key roles in hemostasis by mediating platelet adhesion to the site of vascular injury and by protecting coagulation factor VIII from rapid clearance. It is well established that dysfunction of VWF, due to mutations in the vwf gene, cause the bleeding disorder von Willebrand Disease (VWD). However, VWF can also be regarded as a prothrombotic factor. A role in stroke and cardiovascular disease has previously been suggested for high concentrations of wildtype VWF in the blood stream. We have investigated if VWF variants exist, which exhibit gain-of-function (GOF) properties that directly enhance VWF's hemostasis activity.
Methodology: We recombinantly expressed VWF variants with missense mutations in the C4 domain, which harbors the binding site for platelet GPIIb/IIIa, and performed functional analysis. GPIIb/IIIa binding was investigated by a static cell-based assay. To determine flow-dependent consequences of the mutations, platelet aggregate formation was measured under shear flow conditions using cone and plate aggregometry and Bioflux setups.
Findings: We have identified the first prothrombotic missense variants of VWF. The frequent polymorphism, p.Phe2561Tyr, plays a significant role in premature and repeated events of myocardial infarction. Our functional studies have indicated an increase in force sensitivity of the VWF-platelet-interaction by the presence of p.Phe2561Tyr that shifts the onset of aggregation to lower shear rates compared to wtVWF. A second variant in the same subdomain of VWF also exhibits an increase in platelet aggregate size and surface coverage in cone and plate aggregometry. However, microfluidic assays revealed that its GOF characteristics are different from p.Phe2561Tyr under flow conditions. While p.Phe2561Tyr forms aggregates at half of the critical shear rate necessary for wtVWF, the new variant forms aggregates at the same shear rate as wtVWF but results in drastically increased aggregate size.
Conclusion: Missense mutations can induce a gain of function in VWF, which results in increased platelet aggregation via different mechanisms. The GOF properties of these variants further underline the prothrombotic character of VWF as a potential target for anti-thrombotic therapy.

SOA07-4-AB

Impact of the compound heterozygosity of Factor V Leiden and Prothrombin G20210A on the manifestation of thromboembolic events

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Scientific research question: To what extent the double heterozygosity of Factor V Leiden (FVL) and Prothrombin (FII) G20210A increases the risk of thromboembolic events is a matter of debate and not well studied because of the rarity of the compound heterozygous carriership. The aim of our study was to investigate whether compound heterozygous carriers of the FVL and FII G20210A mutation have a more severe thromboembolic phenotype compared with single heterozygous, single homozygous, and wildtype carriers.
Methodology: The history of venous and arterial thromboembolism was evaluated in all compound heterozygotes (n=132) who visited the outpatient department of Haemostaseology of the University Hospital Gießen and Marburg between 2008 and 2018. The risk of thromboembolic events was calculated compared with sex-matched FVL heterozygotes (n=132), FII G20210A heterozygotes (n=132), and wildtype carriers (n=132) as well as homozygous carriers of FVL (n=123) or FII G20210A (n=13). Log rang statistics and Cox regression

analyses were performed to estimate the risk of thrombosis in this retrospective cohort study.
Findings: Compared with wildtype carriers, compound heterozygotes had a higher risk of deep venous thromboembolic events (VTE; p=0.002) and developed VTE earlier in life (p< 0.001). However, compound heterozygosity did not significantly increase the risk of VTE compared with FVL heterozygotes (HR 1.21, 95% CI 0.85-1.73, p=0.282), FII G20210A heterozygotes (HR 1.15, 95% CI 0.83-1.60, p=0.391), FVL homozygotes (HR 1.08, 95% CI 0.77-1.52, p=0.641), and FII G20210A homozygotes (HR 2.26, 95% CI 0.88-5.62, p=0.091). The age of VTE manifestation also did not differ between these groups (p=0.310).
9.8% of compound heterozygotes suffered from at least one arterial thrombotic event compared with 7.6% of FVL heterozygotes, 6.5% of FVL homozygotes, 7.6% of FII G20210A heterozygotes, 0% of FII G20210A homozygotes, and 12.1% of wildtype carriers. The risk of at least one arterial thromboembolic event and the age of manifestation did not significantly differ between the groups (all p>0.05).
Conclusion: Although double heterozygous carries of the FVL and FII G20210A mutation have a higher risk of VTE compared with wildtype carriers, compound heterozygosity confers no additional risk of venous or arterial thromboembolic events compared to single heterozygosity or single homozygosity of FVL and FII G20210A.

SOA07-5-AB

Low APC response to *in vivo* thrombin formation is a frequent finding in patients with familial thrombophilia of unknown origin

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Scientific Research Question: A significant share of the patients with unprovoked venous thromboembolism (VTE) does not show any established thrombophilic risk factor. Recently, we have shown that an increased activated protein C (APC) response to *in vivo* thrombin formation reduces the thrombotic risk of factor V Leiden (FVL) carriers but not of prothrombin (FII) 20210G>A mutation carriers. *In vivo* thrombin formation was induced by low-dose administration of recombinant activated factor VII (rFVIIa) followed by hemostasis biomarker monitoring. Aim of the present study was to extend this approach of stimulated hemostasis activity pattern evaluation (SHAPE) to patients with thrombophilia of unknown origin (TUO).
Methodology: The TUO cohort of the study population consisted of 21 subjects with a positive self-history of unprovoked VTE (VTE+) as well as a positive family history, in whom no established thrombophilic risk factor was detectable. A second cohort included 27 VTE+ patients tested positive for FVL (n=14) or FII 20210G>A (n=13). None of the subjects was under anticoagulant treatment at the time of analysis. The control group consisted of 18 healthy volunteers. Blood samples were collected immediately before and during a period of 8 hours following injection of 15 µg/kg rFVIIa. Plasma levels of free thrombin and APC were quantified using oligonucleotide-based enzyme capture assays (OECAs). Prothrombin activation fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT), and D-dimer were measured additionally.
Findings: rFVIIa injections were well-tolerated by all subjects and median D-dimer levels remained within the reference range in all three cohorts. Plasma levels of F1+2 increased after rFVIIa injection without showing significant differences between the groups. A comparable increase of TAT was observed in both TUO patients and VTE+ mutation carriers, from a median of 21.3 to 51.1 pmol/L (*P*=0.022), and from 21.3 to 43.9 pmol/L, respectively (Fig. 1A). Median levels of TAT in the controls and median levels of free thrombin in all cohorts remained below their respective quantifiable limits after rFVIIa injection. APC increased from 0.80 to 3.84 pmol/L (*P*=0.001) in the TUO cohort, from 1.14 to 5.82 pmol/L ($P< 10^{-4}$) in VTE+ mutation carriers, and from 0.87 to 3.39 pmol/L (*P*=0.001) in the control group. The increase of APC levels was significantly smaller in the TUO cohort than in the VTE+ carriers of the thrombophilic mutations FVL and FII 20210G>A (*P*=0.019) (Fig. 1B).
Conclusion: A low APC response to an increased *in vivo* thrombin formation is a frequent finding among patients with thrombophilia of unknown origin. This finding is in line with previous data suggesting that higher APC levels protect FVL carriers from thrombosis development. The results of the present study further suggest that a low APC response might be an independent risk factor of VTE. Further studies are warranted to identify the factors that modulate the APC response.

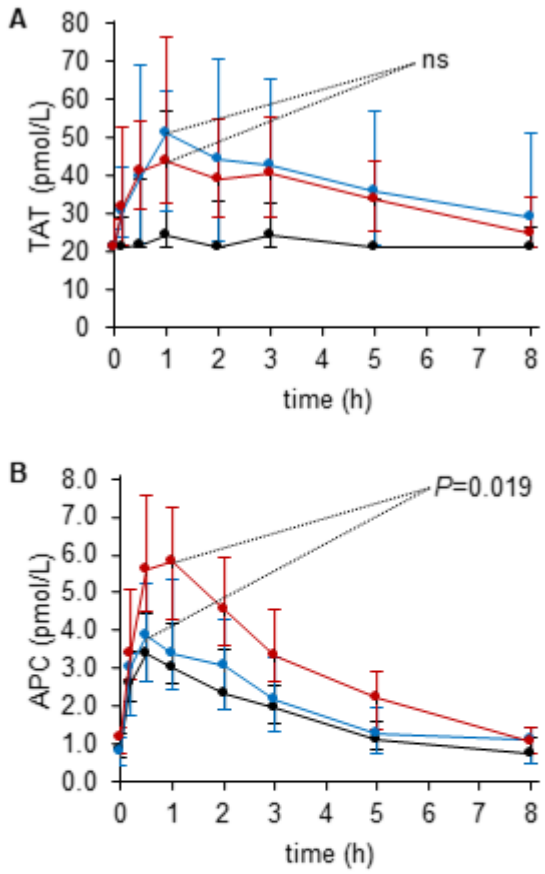

Fig. 1. In-vivo plasma concentrations of A) thrombin-antithrombin complexes (TAT) and B) activated protein C (APC). After t=0 recombinant activated factor VII (15 µg/kg) was administered to patients with thrombophilia of unknown origin (blue symbols, n=21), to thrombophilic patients carrying a factor V Leiden or a prothrombin 20210G>A mutation (red symbols, n=27), and to healthy controls (black symbols, n=18). Data are shown as median and interquartile range. ns, not significant.

OC01 Oral communications: Diagnostics

OC01-1-AB

F8 inversions at Xq28 causing hemophilia A are associated with specific methylation changes: Implication for molecular epigenetic diagnosis

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Diverse DNA structural variations (SVs) in human cancers and several other diseases are well documented. For genomic inversions in particular, the disease-causing mechanism may not be clear, especially if the inversion border does not cross a coding sequence. Understanding about the molecular processes of these inverted genomic sequences, in a mainly epigenetic context, may provide additional information regarding sequence-specific regulation of gene expression in human diseases. Herein, we study one such inversion hotspot at Xq28, which leads to the disruption of F8 gene and results in hemophilia A phenotype. To determine the epigenetic consequence of this rearrangement, we evaluated DNA methylation levels of 12 CpG rich regions with the coverage of 550 kb by using bisulfite-pyrosequencing and next-generation sequencing (NGS)-based bisulfite re-sequencing enrichment assay. Our results show that this inversion prone area harbors widespread methylation changes at the studied regions. However, only 5/12 regions showed significant methylation changes, specifically in case of intron 1 inversion (two regions), intron 22 inversion (two regions) and one common region in both inversions. Interestingly, these aberrant methylated regions were found to be overlapping with the inversion proximities. In addition, two CpG sites reached 100% sensitivity and specificity to discriminate wild type from intron 22 and intron 1 inversion samples. While we found age to be an influencing factor on methylation levels at some regions, covariate analysis still confirms the differential methylation induced by inversion, regardless of age. The hemophilia A methylation inversion "HAMI" assay provides an advantage over conventional PCR-based methods, which may not detect novel rare genomic rearrangements. Taken together, we showed that genomic inversions in the F8 (Xq28) region are associated with detectable changes in methylation levels and can be used as an epigenetic diagnostic marker.

OC01-2-AB

Fully automated inhibitor testing

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Scientific research question: Detection and quantitation of inhibitory antibodies against coagulation factors require time and high technical effort. High interlaboratory variability may in part tied to manual handling of sample mixtures.
In a model of inhibitor binding kinetics, it can be shown that a two-hour incubation time is not required for antibodies against clotting factors

except FVIII. A much simpler mathematical approach for determination of (non-FVIII) inhibitor potency is presented.
Methodology: Samples from patients routinely screened for clotting factor inhibitors (after informed consent) were used in this study as well as commercially available controls or inhibitor samples. Inhibitor potencies were determined custom-made assay procedures on the Atellica COAG360 analyzer (Siemens Healthineers). The assays were tested for FVII (Bethesda-like method), FV (Bethesda; PT and aPTT-based), FIX and FVIII inhibitors (Bethesda- and Nijmegen-like) using different dilutions (1:2 up to 1:256). Factor activity in each mixture as well as the inhibitor potency is calculated by analyzer itself.
One measurement requires about 17 minutes (FIX) to 130 minutes (FVIII); in one case 14 samples (4 dilutions each, anti-FIX) were tested simultaneously within about 90 minutes. The use of reagents is similar in the Bethesda and even reduced in the Nijmegen setup.
Findings: Inhibitor potencies obtained with the fully automated method showed similar results as using classical inhibitor testing. All inhibitor positive samples were found to be positive and deviation in potency was within 30%. All but one inhibitor negative sample was found to be negative. The outlier was caused by a strong lupus anticoagulant. Except for FVIII, the method can be adapted to most automated coagulation analyzers.
Discussion: Fully automated inhibitor testing significantly reduces time and effort. Furthermore, automation contributes to standardization of inhibitor testing and may lead to lower interlaboratory variability.
An adaption to other diagnostic systems (BCS, ACLTOP or STA series) is undergoing. As in the standard inhibitor assay, positive results need to be confirmed by additional lupus testing.

OC01-3-AB

Familial multiple coagulation factor deficiencies (FMCFDs) - analyses of genetic data

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Background: The familial multiple coagulation factor deficiencies (FMCFDs), is a group of rare disorders with reduced plasma activity of more than one coagulation factor. It may arise from co-incidental inheritance of separate coagulation factor deficiencies or from a single defect affecting one gene or part of chromosome. The bleeding symptoms are with different severity, which creates difficulties in diagnosis and treatment. Genetic analyses of such patients improved the diagnostic accuracy and led to better decision making in treatment.
Material and methods: 86 index patients (Ps), with known deficiencies of more than one coagulation factor have been genetically investigated by direct sequencing and MLPA analysis of affected genes.
Results: According to the underlying genetic mechanism the patients were divided into three groups:
Group I-combined defects in two individual genes: 51 Ps were identified and combination either of 2 procoagulation factors (41P) or 2 anticoagulation factors (2Ps) or pro- and anticoagulation factors (8Ps) were defined. Group II summarized only patients (16) with genetic variants in F7 and F10. Majority of Ps present with large deletion affecting the end of the chromosome 13. In remaining 5 Ps different genetic defects were characterized. Group III represents Ps with combined FVIII and FV deficiency (19Ps). Mutations were identified in both *LMAN1* and *MCFD2* genes.
Analyses of our data show that all coagulation factors occur in different combinations, where F7 and F8 were with the highest frequency. The most common combinations in Group I were F8-FG (4Ps), F5-F7 (5Ps), F7-F8 (3Ps) and F8-VWF (3Ps). The largest group of Ps was the one with combined F8 and F5 deficiency. In all Ps genetic alterations were identified. Genetic variants with the highest prevalence were the missense mutations. With a second high prevalence were large deletions (16%). This finding was surprising having in mind that the normal prevalence of this genetic defect is 5%, but can be good explained with high number of Ps in Group II. All other genetic variants showed frequencies, comparable to that of the single gene deficiencies.
Conclusion: The FMCFDs are uncommon compared with the monogenic coagulation factor deficiency

disorders. We presented the largest cohort of patients with FMCFDs. Identification and genetically diagnosing of such Ps will provide improved managements of the bleeding phenotype and better understanding of genotype-phenotype correlation.

OC01-4-AB

Inherited platelet disorders: Identification of novel disease-causing variants using Next Generation Sequencing

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Objectives: Inherited platelet disorders (IPDs) comprise a highly heterogeneous group of disorders affecting platelet number and function. Patients may suffer from life-threatening bleedings. Over the last years we collected data from about 200 patients with suspected IPD (phenotype, platelet functional analyses). For molecular genetic analysis we now established a panel-based next generation sequencing (NGS) approach comprising 95 genes associated with IPDs. In this pilot study we investigated 18 patients with a history of recurrent bleeding episodes and functionally suspected IPD to identify the disease-causing variant.
Methods: 1. Biochemically characterization using platelet aggregometry and platelet flow cytometry. 2. Nextera Rapid Capture Custom Enrichment Kit for targeting enrichment followed by sequencing on a MiSeq (Illumina). For data analysis SeqNext (JSI Medical Systems) and Alamut were used. Variant assessment was performed according to ACMG guidelines.
Results: NGS achieved a mean coverage of 20x for 98% (100x for 91%) of the investigated genes and a mean read depth from 534 per nucleotide. We identified 12 variants which were classified as "pathogenic" or "likely pathogenic" in 7 genes. Correlation with platelet functional analyses (aggregometry and flow cytometry) enabled us to identify class 4 and class 5 pathogenic variant/s for 10 patients. Seven Variants were novel and not listed in HGMD (Human Gene Mutation Database) or disease specific databases.
Conclusions: Panel enrichment is a feasible tool to investigate IPDs in combination with platelet functional analyses. For all patients, the disease causing variant could be identified in 10 out of 18 patients (55%) using panel diagnostic. Interestingly, for those patients for whom the platelet phenotype (aggregometry and flow cytometry analyses) indicated a specific platelet disorder, we could identify the disease causing variant in the suspected candidate gene in all cases (n = 6). For those patients for whom the platelet phenotype did not indicate to a specific platelet disease, we could only identify the disease causing variant in about 33% (n = 4). Patients for whom the disease causing variant could not be identified and who presented with a strong phenotype, will be further investigated using Whole Exome Sequencing.

OC02 Oral communications: Vascular biology

OC02-1-AB

Moderate platelet defects but efficiently restored Thrombopoietin production early after partial hepatectomy via JAK2-STAT3 signaling in mice

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Scientific research question: Side effects of liver injury are characterized by alterations in primary hemostasis including thrombocytopenia and platelet function defects. Moreover, different studies show that hypercoagulability and bleeding complications play a prominent role after liver resection. However, the consequences of liver resection on platelet function are not well defined. The aim of this study is to analyze platelet activity, hemostasis and thrombopoietin (TPO) signaling up to 14 days after partial hepatectomy in mice.
Methodology: *In vitro* and *in vivo* experiments using partial hepatectomy (PHx) in wildtype and interleukin-6 receptor (IL-6R) knockout mice.
Findings: Platelet counts were significantly decreased within the first two days after PHx, whereas platelet counts increase to normal levels three days after PHx in wildtype animals. However, in the first 3 days intrinsic platelet activation was

strongly reduced, due to high plasma levels of nitric oxide, prostaglandin I_2 and bile acids. These factors cause an increased endogenous phosphorylation of VASP resulting in functional platelet inhibition after liver dissection. The activation defects resulted in strongly reduced thrombus formation under flow and prolonged bleeding time in the first 3 days after PHx. Increasing TPO expression in liver tissue and plasma TPO levels were measured in the early phase of regeneration mediated through activation of the Ashwell Morell (AMR) and IL-6 receptor, leading to a JAK2-STAT3 downstream signaling. Interestingly, genetic deletion of IL-6R leads to a compensatory upregulation of the AMR in naive and PHx operated mice resulting in higher TPO expression and plasma levels in knockout compared to wildtype mice. Enhanced TPO production in wildtype mice leads to splenic megakaryopoiesis and moderate splenomegaly of PHx operated animals indicating high platelet turn-over as compensatory event after PHx to overcome decreased platelet counts, defective platelet activation and platelet infiltration into the regenerating liver tissue.
Conclusion: These results indicate that liver dissection affects primary hemostasis and highlights the regulatory mechanisms behind TPO expression in an inflammatory and regenerative *in vivo* model that might contribute to prevent bleeding complications in patients after liver resection.

OC02-2-AB

Inhibition of coagulation factor XI decreases cardiac inflammation and impairs survival in a mouse model of myocardial infarction

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Background: We have recently shown, that inhibition of coagulation factor FXI (FXI) inhibits a vascular coagulation-inflammatory circuit in arterial hypertension. Patients with decreased levels of FXI are at reduced risk of thromboembolic events and cardiovascular diseases. The impact of FXI-inhibition on cardiac inflammation and healing after MI is unknown.
Objective: To investigate the role of FXI and the FXI-thrombin-feedback loop in the inflammatory response post MI.
Methods and results: Male C57BL6/J mice (8-12 weeks) were subjected to permanent ligation of the left anterior descending artery (LAD) to induce myocardial infarction. We effectively reduced coagulation factor FXI (FXI) levels by repetitive injections of FXI antisense oligonucleotide (FXI ASO). Compared to scrambled ASO injected controls, FXI-ASO injected mice had significantly increased mortality 3d post MI (10% vs. 30%) and a worse left ventricular ejection fraction as well as increased wall motion score index measured by high-frequency ultrasound imaging. Cardiac inflammation and especially influx of myelomonocytic cells into the ischemic myocardium was measured by flow cytometry. Compared to controls, inhibition of FXI lead to a decreased cardiac infiltration of $CD45^+CD3^-CD11b^+$ myelomonocytic cells, in particular LyG^-LyC^{high} monocytes, 3d post MI. Accordingly, vascular oxidative stress was significantly reduced in FXI ASO compared to scrambled ASO treated mice in full blood and isolated aortic rings (measured by chemiluminescence, oxidative fluorescence microtopography) 3d post MI. Endogenous thrombin potential (ETP) detected by calibrated thrombin generation assay in platelet rich plasma was significantly reduced in FXI-ASO compared to scrambled ASO treated LAD-ligated mice, whereas the platelet count was not altered, suggesting a role of platelet dependent FXI-thrombin-amplification in cardiac ischemia.
Conclusion: Lowering FXI levels impaired cardiac remodeling with an increase in mortality post MI. FXI and thrombin are a putative link between the coagulation system and the immune response in cardiac remodeling post MI.

OC02-3-AB

Molecular atlas of fetal and adult human liver sinusoidal endothelial cells: a F8 secreting cell

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Introduction: In human, the F8 deficiency leads to hemophilia A, is largely synthesized and secreted by the sinusoidal endothelial cells of the liver. However, the specificity and characteristics of

these cells those make them suitable for this process is not well known.
Method: In this study, we performed genome wide expression (microarrays) and CpG methylation epigenetic profiling (microarrays and whole genome bisulfite sequencing) of fetal and adult human LSECs together with other fetal endothelial cells, from lung (micro-vascular and arterial), and heart (micro-vascular).
Results: Our results reveal plenty of expression and methylation markers distinguishing LSECs at both fetal and adult stages. Differential gene expression of fetal LSECs in comparison to other fetal ECs pointed to several differential regulated pathways and functions in fetal LSECs where EIF2 signaling is down regulated, while antigen presentation pathway and cellular movement are activated. We used targeted bisulfite re-sequencing to confirm selected top differentially methylated regions; between fetal LSECs and other fetal ECs; between fetal and adult LSECs. Methylation markers in two regions provided good markers that showed clear transition of increasing and decreasing methylation respectively, from non-LSECs fetal to fetal LSECs and to adult LSECs. We further designed an assay where we used the selected methylation markers to test the degree of similarity of in house in vitro generated vascular endothelial cells to fetal and adult LSECs; a higher similarity was found to the fetal than to adult LSECs. Our molecular profiling study provides a guide to test the effectiveness of production of in vitro differentiated LSECs that could be used in cellular therapies.

OC02-4-AB

Impact of Von Willebrand Disease on Pro-Angiogenic Factors in the Porcine Female Reproductive Tract

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Objectives: Von Willebrand Factor (VWF) has been shown to be not only essential for haemostasis, but also to influence angiogenesis by itself as well as by its effect on other angiogenic factors. Besides common symptoms of the Von Willebrand Disease (VWD), data show that women with type 1 (T1) or type 3 (T3) VWD are more likely to miscarry or to develop pregnancy related haemorrhagia. We hypothesize an effect of VWD on angiogenesis which is crucial for healthy pregnancy and investigated the expression levels of pro-angiogenic factors Endothelin (EDN1), Endothelin Converting Enzyme 1 (ECE1), and connective tissue growth factor (CTGF) in VWD, which might be connected to the angiodysplasia seen in VWD patients.
Methods: Expression levels of the selected genes were measured in reproductive tissues of a porcine model, which is phenotypically identical to human VWD. Samples from the ovary and uterus were taken from 14 sows, representing T1 (n=12) and T3 (n=2) compared to 10 healthy controls (WT). All of them had been in estrus at time of sampling. Analyses were made via real-time quantitative PCR, applying the ΔΔCT method and using *PECAM* and *PROCR* as reference genes.
Results: The mean expression level of the selected genes mainly showed an upregulation in the affected individuals. *EDN1* was upregulated by 30% (T1) to 40% (T3) in the uterus and more than 3-fold in the T3 ovary (p=0.05) relative to WT. The level of *ECE1* was increased more than 5-fold in the uterus of pigs affected by VWD (T1 and T3; p=0.02). For the ovary, a similar increase was seen in T1 pigs only (p=0.03). The mean expression of CTGF was increased more than 7.5-fold (uterus; p=0.02) and 17.5-fold (ovary; p=0.05) in the mutant pigs (T1 and T3) compared to the WT.
Conclusions: In this study we demonstrate significantly altered expression of several pro-angiogenic factors in the reproductive tract of individuals affected by VWD. The demonstrated upregulation of these factors in VWD-affected pigs might play a role in an increased but low quality blood vessel formation thus resulting in angiodysplasia and contributing to miscarriage in VWD. Further studies involving a larger sample size and further angiogenic factors are warranted.

OC03 Oral communications: Paediatrics

OC03-1-AB

Impact of thrombophilia on risk of perinatal arterial ischemic stroke: a systematic review and meta-analysis of observational studies

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Scientific research question: Recent case-control studies found that inherited thrombophilia (IT) was not associated with the risk of perinatal arterial stroke (PAS). In general, the individual number of patients in the cohorts so far reported were small, furthermore, different stroke/ CSVT entities were grouped together. Therefore, those findings should not be generalized prior to confirmation by other larger perinatal/ neonatal cohorts.The aim of this study was to estimate the impact of IT on a first perinatal/ neonatal PAS through a meta-analysis of published observational studies.
Methods and results: A systematic search of electronic databases (Medline via PubMed, EMBASE, OVID, Web of Science, The Cochrane Library) for studies published from 1970 to 2018 was conducted. Data on year of publication, study design, country of origin, number of patients/ control subjects, ethnicity, stroke type (perinatal/ neonatal arterial ischemic stroke) were abstracted. Publication bias indicator and heterogeneity across studies were evaluated, and summary odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with fixed-effects or random-effects models. We assessed the incidence of the most common prothrombotic polymorphisms in infants with PAS vs. controls. For studies in which paediatric control cohorts were missing, prevalence rates of **IT were reported with respect to ethnic background.**
Findings: 13 of 127 references met inclusion criteria. Thus, 1027 patients (PAS), and 2594 control subjects were enrolled. No significant heterogeneity was discerned across studies, and no publication bias was detected. A statistically significant association with first PAS was demonstrated as described. Summary ORs (fixedeffects model) were as follows: factor V G1691A, 2.8 (95% CI, 1.94 to 4.05); factor II G20210A, 1.7 (95% CI, 0.97 to 2.86); MTHFR C677T, 1.5 (95% CI, 1.14 to 1.92). Whereas prothrombin G20210A mutation yielded no risk of PAS and MTHFR T677T genotype impact was minor, heterozygous factor V G1691A mutation significantly increases risk of PAS. Due to the small number of patients followed-up after PAS, the impact of thrombophilia upon its course or recurrence rate cannot be determined.
Conclusions: The present meta-analysis indicates that inherited thrombophilias serve as risk factors for incident PAS. Overall, the potential association of thrombophilic risk factors and perinatal stroke, notwithstanding, the causality of thrombophilia in the pathogenesis of PAS, clearly deserves further Attention.

OC03-2-AB

A phase III study to assess the efficacy and safety of fibrinogen concentrate for on-demand treatment of acute bleeding and surgical prophylaxis in paediatric subjects with congenital fibrinogen deficiency

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Scientific research question: Determine the efficacy and safety of a human fibrinogen concentrate (HFC, *Fibryga*, Octapharma AG) for on-demand treatment of acute bleeding episodes (BEs) in paediatric patients with congenital fibrinogen deficiency (CFD). Secondary objectives included determining single-dose pharmacokinetics (PK) of HFC.
Methodology: FORMA-04 was a multinational, multicentre, prospective, open-label, uncontrolled, Phase III study in paediatric patients with CFD (afibrinogenaemia or severe

hypofibrinogenaemia). The primary endpoint was efficacy for on-demand treatment of all BEs. Secondary endpoints also included efficacy of prophylactic HFC during and after surgery. Haemostatic efficacy was assessed by investigators/surgeons and final adjudication was provided by an Independent Data Monitoring and Endpoint Adjudication Committee (IDMEAC) using objective 4-point scales. Treatment success was defined as an excellent/good rating. Safety was assessed by monitoring adverse events (AEs).
Findings: Thirteen patients (< 12 years old) received individually dosed HFC for PK. Eight patients were treated for 10 acute BEs (8 minor, 2 major, 5 in age group < 6 years old, 5 in the ≥6-12 years old group, including 4 first BEs in each age group) and 3 patients for surgical prophylaxis (2 minor surgeries and 1 major surgery in the < 6 years old group). Six patients received HFC only for treatment of bleeding episodes, 2 patients for treatment of bleeding episodes and surgeries and 1 patient only for surgery. Haemostatic efficacy for all BEs was rated excellent/good for all 10 BEs (success: 100%; 95% CI [63.06, 100]; Table 1). Overall haemostatic efficacy for surgical prophylaxis was rated as excellent for all surgeries performed (success: 100%; 95% CI [29.24, 100]. Final PK data is under analysis. Ten AEs occurred in 3 patients: 7 AEs were mild, 2 moderate and 1 severe. The severe AE also represented the 1 serious AE that occurred in the study. There were no allergic/hypersensitivity reactions related to HFC treatment.
Conclusion: HFC treatment was efficacious for on-demand treatment of acute BEs and for surgical prophylaxis in paediatric patients with CFD. Furthermore, HFC treatment demonstrated a favourable safety profile in this study population.

OC03-3-AB

Individualized treatment approaches in children with type I plasminogen deficiency and associated complications. Report of two cases and review of the literature

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Introduction: Type I Plasminogen deficiency is a rare autosomal recessive disease. It leads to increased fibrin deposition on all mucous surfaces. Associated complications include bronchial, urogenital or gastrointestinal obstruction, hydrocephalus and ligneous conjunctivitis, which can ultimately cause blindness. Formation of membranes is triggered by infection and injuries. There is no FDA approved treatment available.
Patient A developed ligneous conjunctivitis two months after birth. Despite conservative and surgical treatment, the left eye was destroyed and consequently enucleated. Vision on the right eye is reduced. The patient suffers from recurrent eye infections, gingiva hyperplasia and urogenital pseudo membranes. Until the age of 4 years, the patient had no access to regular plasminogen replacement therapy. After eye surgery the patient developed sepsis, due to central line infection and extensive thrombosis of the upper vena cava and right atrium. He was treated with enoxaparin and Ryplazim™, a human plasminogen product, achieving higher plasminogen levels. Ryplazim™ had to be stopped shortly after, due to unavailability. The boy is now 7 years old and on plasma replacement twice weekly as well as prophylactic anticoagulation with enoxaparin 1mg/kgbw/d. With this treatment regimen plasminogen trough level activity is maintained at 10%. The disease is not progressing. However, the patient reacts allergic to plasma transfusions necessitating premedication with steroids.
Patient B was born at 35 weeks gestational age by caesarian due to hydrocephalus occlusus. A ventriculoperitoneal shunt was implanted 10 days later. Spinal fluid drainage was insufficient due to peritoneal insufficiency, which led to significant ascites. At 3 months, the girl developed ligneous conjunctivitis, which was surgically removed three times in order to avoid amaurosis. Ocular steroids caused glaucoma. At 9 months, she was failing to thrive and started an individual medical treatment with intravenous plasminogen (Ryplazim™). Her condition improved significantly. The ligneous conjunctivitis vanished and she gained weight. Initial treatment frequency of 6,6mg/kg bw (q48h) was reduced to (q5d) after resolution of symptoms. Plasminogen trough levels are maintained at 20%. Treatment intermissions of more than 5d led to recurrence of initial symptoms.
Conclusion: Congenital plasminogen deficiency is a potentially life threatening and painful disease. Early prophylactic substitution of plasminogen either with fresh frozen plasma or with plasminogen products is of paramount importance to avoid long-term morbidity. In clinical situations with high plasminogen consumption like surgery or

sepsis, replacement with plasma may not be sufficient as its use is restricted by possible fluid overload. Treatment with plasmatic plasminogen (Ryplazim™) is highly effective and allows home therapy. We observed resolution of excess fibrin membranes and no adverse side effects.

OC03-4-AB

Caregiver's Burden in parents of toddlers and young children with severe haemophilia

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Introduction and Objective: The diagnosis of a child's haemophilia is life-changing and often related to anxiety. The daily management of haemophilia can cause caregiver's burden. The most feared side effect is the development of inhibitors, which neutralize clotting factor occurring in 25-30% previously untreated patients (PUPs), and usually develop within the first 50 exposure days (EDs). We aimed to evaluate caregiver's burden in parents caring for children with severe haemophilia from a single centre in Germany.
Methods: Caregivers of recently diagnosed PUPs with severe haemophilia from the GZRR were included in this study. Haemophilia-specific caregiver's burden was evaluated using the 'HEMOphilia associated Caregiver Burden scale' (HEMOCAB). Clinical data were collected from patient records.
Results: Twenty-eight caregivers with a mean age of 35.92±8.6 years were enrolled, most of them were mothers (71.4%) and hold the primary responsibility in the management of their children's disease (50%). Eighty-seven percent of the caregivers were married and 95.2% lived with a partner and had a median number of 2 children (range 1-4). Most of them had a high school education (47.6%) and were working full- or part-time (56.5%); 52.9% were not working full-time due to caring for their haemophilic child. Half of the caregivers reported that haemophilia affected their family life and 28.6% had an economic impact. They were caring for 21 children with severe haemophilia with a median age of 2.11 years (range 0.45-8.7). 85.7% of the children had haemophilia A, none had a target joint, reduced range of motion, a concomitant disease or an inhibitor. One third of the children received home treatment (33.3%) and were mainly infused by a physician at the centre (63.2%). In average children had 3.0±3.6 total bleeds in the past 12 months and 0.43±0.8 joint bleeds. 90% of children received low-dose primary prophylaxis with plasma-derived products (median of 60 EDs [range 0-229]). Caregiver's overall burden was 27.95±13.2 with highest impairments in the domains 'emotional stress' (M=39.17±20.0), 'perception of your child' (M=35.85±22.8) and 'work' (M=34.44±36.8). Forty-seven percent 'always/often' asked themselves “if my child's condition will be better in the future”, 40.3% were “afraid that my child could get injured in an accident and I cannot help him” and 30% thought “my child needs more attention and affection”; 33.3% thought that 'quite a bit/very much' “the choice of their job depends on a location close to my child's day care/school”.
Conclusions: Although the critical benchmark of 50 EDs has been exceeded no inhibitors occurred so far and resulted in good bleed protection, while caregivers reported highest burden in the domain 'emotional stress'. Understanding how haemophilia impacts on caregivers living with the daily threat of bleeding and the risk of inhibitor development can help health professionals to provide effective support to those families.

OC04 Oral communications: Platelets

OC04-1-AB

Sodium-calcium exchanger reverse mode is required for generation of procoagulant COAT platelets

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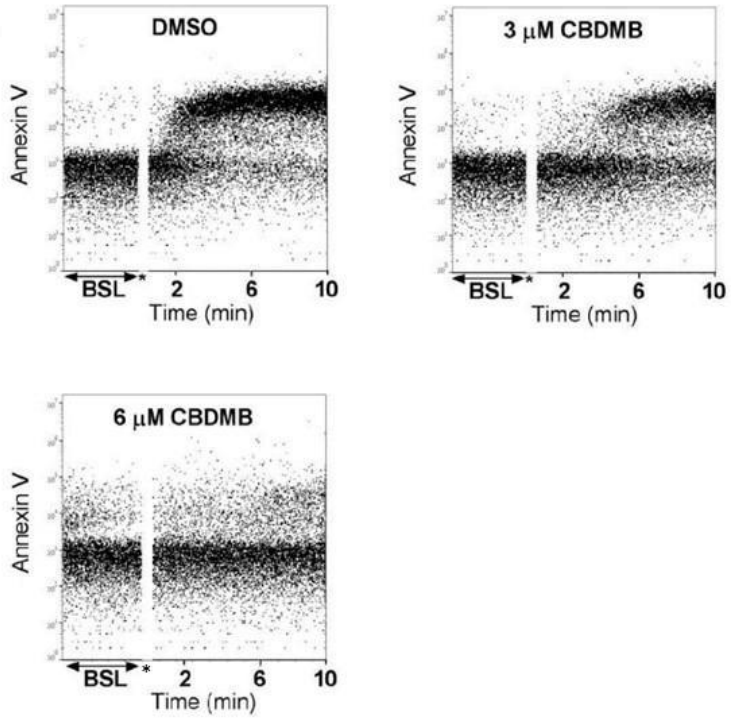
Objectives: The combined activation of platelets with collagen-plus-thrombin induces the formation of procoagulant COAT platelets. Dichotomous intracellular signaling generating a subpopulation displaying procoagulant activity instead of traditional aggregating endpoints is still not fully elucidated. However, it has been demonstrated that secondary messengers such as calcium and sodium play a critical role. Therefore, we investigated calcium and sodium ion kinetics and the role of sodium-calcium exchanger (NCX) during

platelet activation induced by convulxin-plus-thrombin.
Methods: Flow cytometry was used to characterize distinct populations of human platelets forming after activation with convulxin (collagen receptor GPVI agonist) and thrombin. Free cytosolic calcium and sodium were measured with specific ion fluorescent indicators (Fluo-3 and ION NaTRIUM Green-2), and Annexin-V co-staining allowed discriminating procoagulant COAT platelets. NCX function was modulated with NCX inhibitors: 5-(4-chlorobenzyl)-2'-4'-dimethylbenzamil (CBDMB) and 2-[(3,4-Dihydro-2-phenyl-2H-1-benzopyran-6-yl)oxy]-5-nitro-pyridine (ORM-10103).
Results: Specific ion fluxes were observed in distinct procoagulant and aggregating platelet subpopulations. High and prolonged intracellular calcium concentration (>1 µM), and transient sodium increase were observed in procoagulant COAT platelets, whereas aggregating non-COAT platelets rapidly decreased their calcium content and maintained higher cytosolic sodium. Both CBDMB and ORM-10103 dose-dependently diminished (IC50 = 2.85 ±0.3 µM and 9.02 ± 1.1 µM, respectively) and delayed (shown in Figure) generation of procoagulant COAT platelets. Moreover, inhibition of NCX by CBDMB affected ion kinetics: calcium efflux was retarded and reduced in aggregating platelets while sodium efflux was reduced in procoagulant COAT platelets. This suggests that both forward and reverse NCX modes are used in convulxin-plus-thrombin-activated platelets. According to our data, aggregating non-COAT platelets predominantly use forward mode NCX, by pumping calcium out and moving sodium in, while procoagulant COAT platelets mostly rely on reverse NCX function, which exchanges cytosolic sodium with extracellular calcium.
Conclusions: This study demonstrates that NCX is a critical regulator of the convulxin-plus-thrombin platelet response. The present data show that calcium influx from NCX reverse mode is required for achieving an efficient procoagulant signaling.



[Procoagulant COAT platelets (AnnexinV-positive) were generated upon convulxin-and-thrombin activation (*), in presence of DMSO or CBDMB. Baseline, BSL]

OC04-2-AB

Streptococcus pneumoniae toxin Pneumolysin renders platelets non-functional

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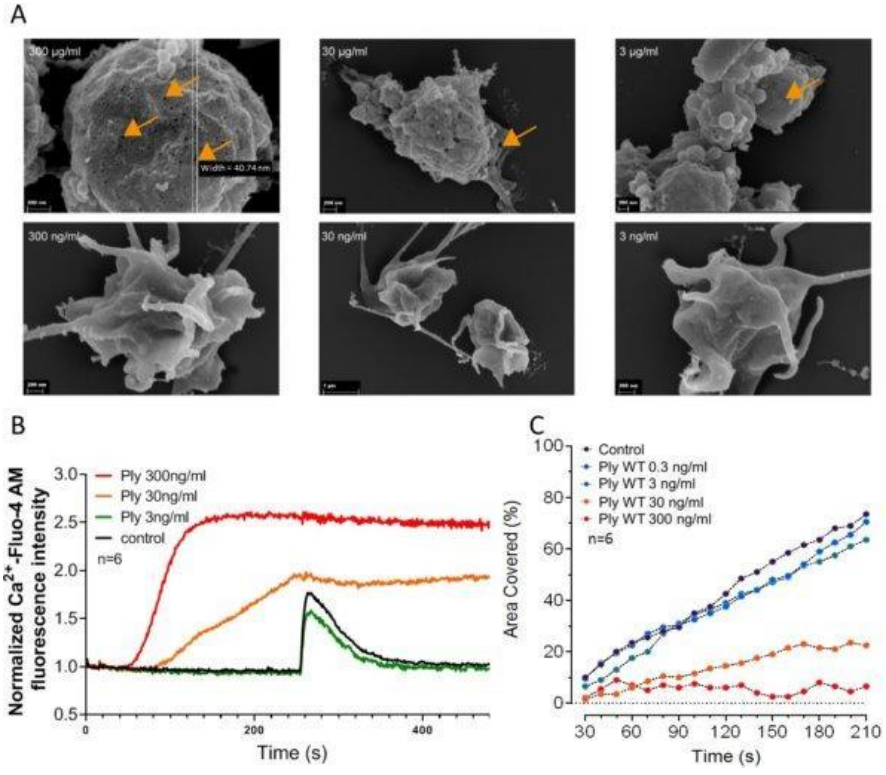
Scientific research question: *Streptococcus pneumoniae* (pneumococcus) is a serious human pathogen and the main cause of community acquired pneumonia. A major complication is respiratory impairment due to diffusion of intravascular fluid and red blood cells from pulmonary vessels into the interstitial compartment. Usually platelets cover endothelial cell damage. *S. pneumoniae* releases pneumolysin (Ply), a pore-forming cholesterol-dependent cytotoxin. Previous studies reported that Ply activates platelets as measured by CD62P expression. In this study we re-investigated the impact of pneumolysin on human platelets by applying complementary methods.
Methodology: Platelets were isolated from healthy human volunteers (n≥3) and incubated with increasing concentrations (3ng/mL-300ng/mL for functional assays, up to 300µg/ml for microscopy) of wild-type Ply and non-functional toxoid variants. Pore-forming was shown using electron microscopy. Cytosolic Ca^{2+} was determined fluorometrically using FLUO-4-AM. Platelet aggregation and activation was assessed using flow cytometry and light transmission aggregometry (LTA). Subsequent TRAP6-stimulation was performed to assess remaining platelet functionality after incubation with Ply. Adhesion to collagen surfaces and thrombus formation was measured in a flow chamber in hirudinated whole blood.

Findings: Ply induced pores in the platelet membrane (Fig 1A). Incubation with 300ng/mL Ply resulted in a rapid and strong increase of free Ca^{2+} in platelets (Fig. 1B) and a decrease of turbidity determined by LTA, which could not be inhibited using RGDS peptide. Ply concentrations >30ng/mL induced a high CD62P signal on platelets (MFI x %gated: 39,511 (25,295-42,747)), but no increase in PAC-1 binding. Ply treated platelets did no longer respond to TRAP6. Three ng/mL of Ply and all concentrations of Ply toxoid variants did not induce pore formation and had no effect on platelet activation and functionality. A Ply concentration of 30ng/mL induced an in-between phenotype with delayed and reduced Ca^{2+} release, and CD62P expression. Concentrations of Ply ≥30 ng/mL caused severely impaired thrombus formation on collagen surfaces (surface area coverage after 210 sec: 70% [control]; 12% [300ng/mL Ply]; 20% [30ng/mL Ply], Fig. 1C).
Conclusion: Perforation of platelet membranes by pneumolysin results in a loss of platelet function. The cytolytic effect of Ply occurs in washed platelets and in whole blood. The increase in CD62P results from intracellular labelling by antibodies passing the pores formed by pneumolysin but not from platelet activation. Impaired platelet function might be a major cause of capillary leakage in pneumococcal pneumonia.



(A) Electron microscopic image of a platelet incubated with Pneumolysin; (B) Increase of free cytosolic Ca2+ after incubation with various concentrations of Pneumolysin; (C) Collagen surface area coverage with various concentrations of Pneumolysin under flow.

OC04-3-AB

Follow-up evaluation of patients with congenital thrombotic thrombocytopenic purpura from the International Hereditary TTP Registry: is frequency and severity of acute disease episodes influenced by gender?

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Scientific research question: Congenital thrombotic thrombocytopenic purpura (cTTP) is an ultra-rare disorder characterized by recurring acute episodes of thrombotic microangiopathy. cTTP is autosomal recessively inherited and results from severely reduced or absent ADAMTS13 activity. Clinically, cTTP presents heterogeneously, and currently little is known about frequency and severity of acute disease episodes in cTTP patients.
Methodology: We analyzed data of 136 confirmed cTTP patients in the International Hereditary TTP Registry. Survival, frequency and severity of acute episodes, and treatment were studied prospectively from enrollment until September 2019.
Findings: Follow-up data (since enrollment median 1.6 years, range 0.1-8.2) were available for 85 of 136 patients. The 44 male and 41 female cTTP patients had a median age at clinical diagnosis and at overt disease onset of 19 years (range 0-70) and 6.9 years (range 0-70) in male, and 17 years (range 0-50) and 4.1 years (range 0-32) in female patients, respectively. 25 (57%) male and 18 (44%) female patients received prophylactic plasma treatment. During follow-up five patients died (causes of death: stroke n=2, asystole and sepsis, heart failure, and sudden death of unknown cause, one each), all were male (age range 39-78 years). In addition, 112 acute episodes were observed in 41 of 85 cTTP patients, 36 episodes occurred in male, 76 episodes in female patients. This resulted in gender-specific annual incidence rates of 0.20 (0.14-0.27) in male, and 0.43 (0.34-0.54) in female cTTP patients (Table).
Infections were the most prevalent trigger and present in 68% (n=13, in male) and 75% (n=40, in female) of acute episodes. Alcohol was the trigger of 5 episodes (male n=3), and 4 episodes occurred during pregnancy.
Of 112 episodes, 77 were documented under regular plasma prophylaxis in 59 patients during 247 patient-years. The calculated incidence rates under plasma prophylaxis were 0.18 (0.11-0.27) in male, and 0.47 (0.35-0.62) in female patients. Patients not under regular plasma prophylaxis experienced 35 episodes in 105 patient-years, amounting to an incidence rate of 0.29 (0.15-0.50) in male, and 0.36 (0.23-0.54) in female cTTP patients.
Conclusion: We provide the first estimate on annual incidence of acute episodes in cTTP based on prospective data, and observed 0.3 episodes per patient-year. Current plasma prophylaxis regimens seem to be insufficient to prevent acute episodes. Female patients had a slightly higher incidence rate, which was not attributable to pregnancy alone. In contrast, mortality was higher in male (n=5) than in female (n=0) patients. Robustness of estimates will improve with the addition of further prospective follow-up and patient-years.

	No. of patients with follow-up	Patient-years	No. of patients with any episodes	No. of prospective episodes	Incidence rate (95% CI)
Overall	85	360	41	112	0.31 (0.26-0.37)
Male	44	185	17	36	0.20 (0.14-0.27)
Female	41	176	24	76	0.43 (0.34-0.54)
Under Prophylaxis*	59	247	29	77	0.31 (0.25-0.39)
Male	30	134	12	24	0.18 (0.11-0.27)
Female	29	112	17	53	0.47 (0.35-0.62)
Not under Prophylaxis*	40	105	16	35	0.33 (0.23-0.46)
Male	17	42	6	12	0.29 (0.15-0.50)
Female	23	63	10	23	0.36 (0.23-0.54)

[Table: Incidence of documented episodes during follow-up]

*numbers do not add up, as some patients changed their treatment regimens during follow-up.

OC04-4-AB

Functionally distinct platelet subpopulations in ST segment elevation myocardial infarction: a response to antiplatelet therapy and clinical significance

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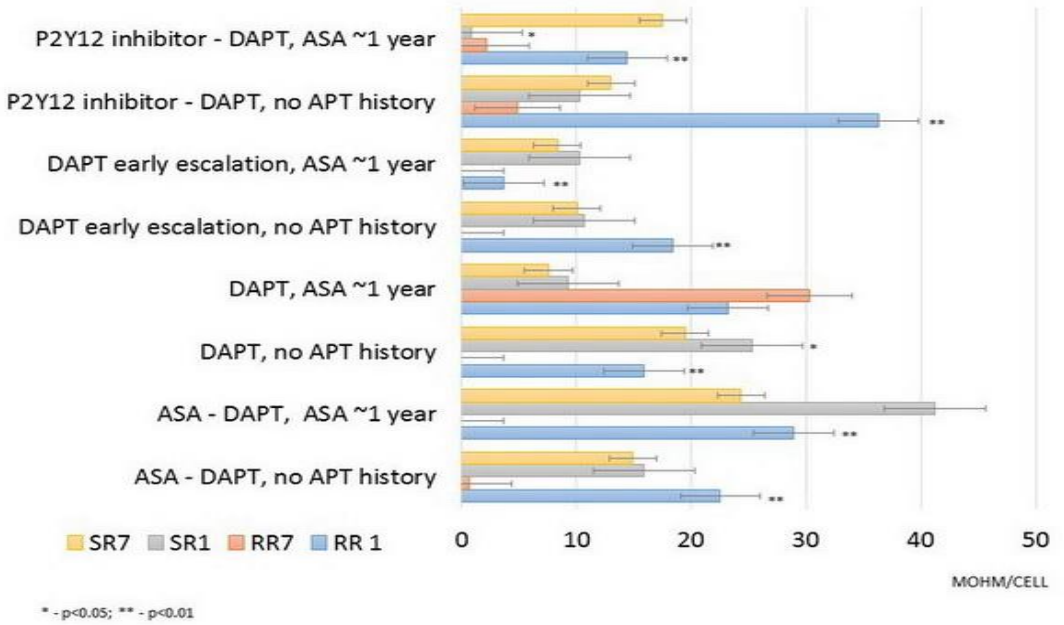
Objectives: To characterize functionally distinct platelet subpopulations in patients with ST-segment elevation under pharmacological suppression of platelet aggregation.

Methods: 587 male patients with STEMI were recruited into the study. Platelet (PLT) aggregation and secretion were assessed by impedance and luminescence aggregometry (Chronolog-700). Double stimulation with collagen (2 mcg/mL) and ADP (10 mcM) was applied to divide a slow and rapid reacting PLT subpopulations (SR and RR). All results were recalculated per cell. PLT derived microparticles (PDMP) and thrombopoietin (TPO) levels were serially measured by ELISA: at admission to cardiac intensive care unit (ICU); on the second and seventh days. The primary endpoint included cases of cardiovascular deaths, readmissions and major bleedings. Minimal follow-up period was 24 months.

Results: According to antiplatelet therapy (APT) scenario (history of APT before STEMI manifestation, APT at FMC and in ICU) we composed 8 study subgroups (Fig.1). RR revealed significant 7 day's decrease in aggregation in all studied subgroups. The only exception were patients treated with acetylsalicylic acid (ASA) for at least 12 months prior to STEMI manifestation, in whom DAPT had been chosen both at FMC and in ICU as an APT. Contrary, SR was characterized by insignificant shift during the observation period in almost all studied subgroups, except the groups treated with P2Y12 inhibitor at FMC following DAPT in ICU. Those groups were characterized by increase in aggregation of SR, which was statistically significant in patients treated with ASA at least 12 months before current STEMI: 1.8 (0.0; 4.2) vs 13.2 (10.6: 28.8) mOhm/cell, $p < 0.01$. SR at admission had a strong positive correlation with PDMP levels on the 2nd day ($R = 0.843$; $p = 0.04$) and TPO at admittance ($R = 0.593$, $p=0.016$). SR correlated with bleeding events ($\tau = 0.467$, $p = 0.045$), while RR- with ischemic ones ($\tau = 0.567$, $p = 0.032$).

Conclusion: Rapid and slow reacting platelet subpopulations demonstrated different responses to antiplatelet therapy in STEMI patients. Received data illustrate strong influence of platelet production and turnover on slow reacting platelet subpopulation. Assessment of functionally distinct platelet subpopulations may be a useful tool for tailoring antiplatelet therapy in STEMI.



[Fig.1. RR and SR in STEMI patients (different APT scenarios)]

OC05 Oral communications: Bleeding disorders

OC05-1-AB

One year data from a phase 2b trial of AMT-061 (AAV5-Padua hFIX variant), an enhanced vector for gene transfer in adults with severe or moderate-severe hemophilia B

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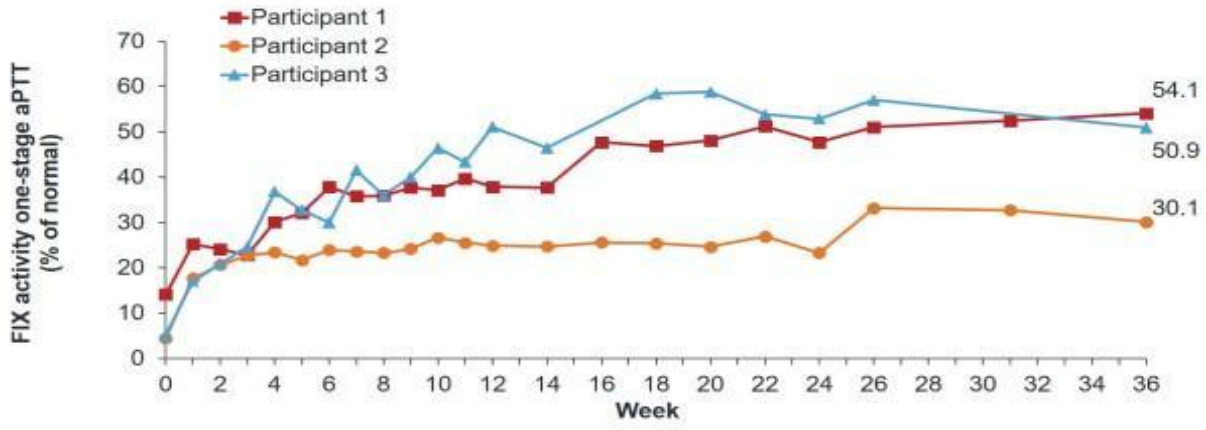
Objectives: Gene therapy for hemophilia offers the possibility of ameliorating the disease severity to a milder or functionally curative state through a single treatment. AMT-061 is an investigational gene therapy for hemophilia B comprising an adeno-associated virus serotype 5 (AAV5) vector containing a codon-optimized Padua variant human factor IX (FIX) gene with liver-specific promoter. The aim of the current study was to confirm that a single dose of AMT-061 will provide a minimum-therapeutic response of FIX activity 6-weeks post-dose in participants with severe or moderate-severe hemophilia B. Here, 1 year of follow-up will be presented.

Methods: Phase 2b, open-label, multi-center trial (NCT03489291) in adult males with FIX ≤2% and without active hepatitis or uncontrolled HIV. Participants were not excluded based on neutralizing antibodies to AAV5. Participants received a single intravenous dose of AMT-061 ($2x10^{13}$ gc/kg) and will be followed for 5-years. The primary endpoint was FIX activity at Week 6. Secondary endpoints include e-diary recordings of bleeds and FIX concentrate use, laboratory parameters, joint health, patient-reported outcomes, and adverse events (AEs).

Results: All participants had FIX ≤1% (severe or moderately-severe FIX deficiency), required routine FIX prophylaxis, and had neutralizing activity to AAV5 at baseline. Following AMT-061 treatment, FIX activity increased rapidly (Figure 1) to a mean of 31% at Week 6. At Week 36, mean FIX activity increased further to 45% with FIX activity levels of 54%, 30% and 51% in participants 1-3 respectively. As of 36 weeks, there were no bleeds post-treatment and no requirement for FIX replacement aside from protocol-specified use for perioperative management in participant 3. There were no clinically significant elevations in liver enzymes and no participants required steroids related to the treatment. One participant experienced 2 mild AEs possibly related to treatment shortly after dosing (self-limiting

headache and slightly elevated CRP). One patient had hip surgery due to worsening of pre-existing avascular necrosis deemed unrelated by investigator to AMT-061 and received FIX per protocol according to standard clinical practice. No participant developed inhibitors to FIX. Updated results to 52 weeks of follow-up will be presented.

Conclusions: Sustained elevation of FIX activity into the mild-to-normative range were observed in all participants 36 weeks after treatment with AMT-061. AMT-061 was safe and well-tolerated with no requirement for immunosuppression. These data support the ongoing Phase 3 study.



aPTT, activated partial thromboplastin time; FIX, Factor IX. No immunosuppression required. *May include activity from exogenous FIX replacement.

OC05-2-AB

First-in-human evidence of durable therapeutic efficacy and safety of AAV gene therapy over three-years with valoctocogene roxaparvovec for severe haemophilia A (BMN 270-201 study)

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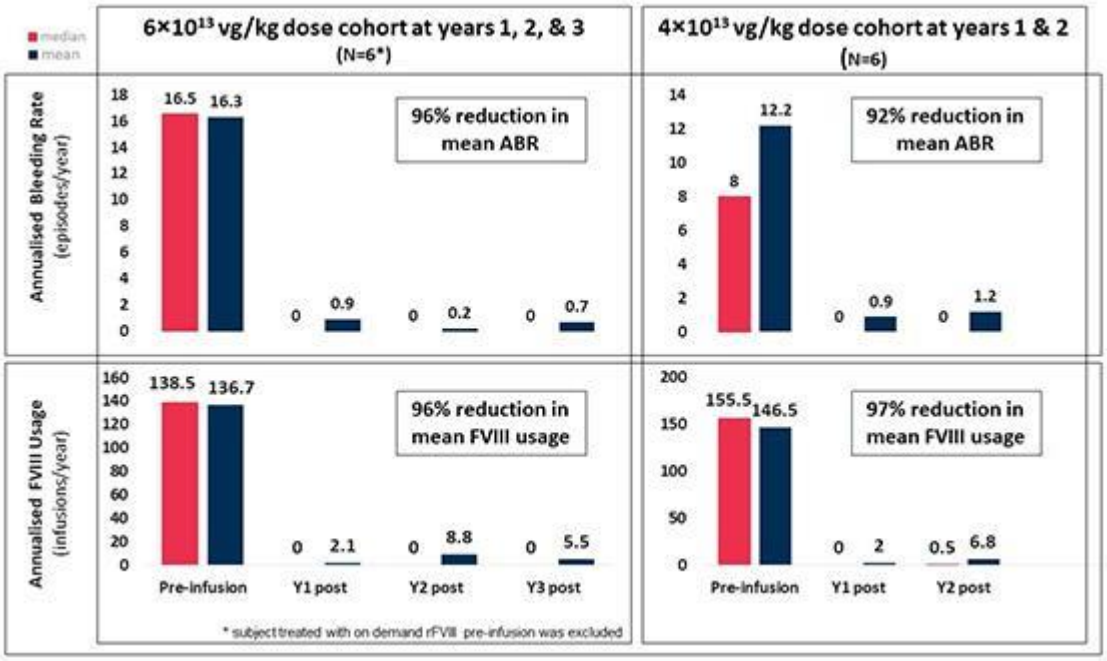
Objectives: Gene therapy is increasingly viewed as a viable treatment option for hemophilia A, using AAV mediated Factor VIII (FVIII) gene transfer. Interim Phase 1/2 data from valoctocogene roxaparvovec (AAV5-hFVIII-SQ) have shown promising results. Outstanding questions for all AAV gene therapies relate to clinical effectiveness and durability. The aim of this study is to assess the long-term safety, efficacy, and durability of AAV5-hFVIII-SQ in a Phase 1/2 clinical study.

Methods: Adult male study participants with severe HA received a single intravenous dose of AAV5-hFVIII-SQ at 6×10^{13}vg/kg (n=7) or 4×10^{13}vg/kg (n=6).

Results: All study participants demonstrated clinically meaningful FVIII activity levels with reductions in bleeds and FVIII usage. Following withdrawal from prophylaxis, annualised bleeding rate (ABR) declined from pre-treatment mean by 96% at year three in 6×10^{13}vg/kg participants, and 92% at year two in 4×10^{13}vg/kg participants. FVIII levels reported by chromogenic assay correspond with the continued absence of target joints and target joint bleeds from years two through three. Expression levels over time are determined to decline as a function of both time post-administration and level of expression achieved, nearing a plateau of expression in year three. The safety profile of valoctocogene roxaparvovec remains favourable and unchanged, with no inhibitor development or ALT elevations beyond year one.

Conclusions: Gene transfer with valoctocogene roxaparvovec has resulted in substantial and sustained FVIII activity levels, clinically relevant reductions in self-reported bleeding episodes, and significant reductions in FVIII replacement infusions for up to three years post-dosing.



[Figure 1: Annualised Bleeding Rates and FVIII Usage by Dose Cohort Over Time]

OC05-3-AB

Bleeding and response to hemostatic therapy in Acquired Hemophilia A (AHA): Results from the GTH-AH 01/2010 study

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Scientific research question: Acquired hemophilia A (AHA) is due to autoantibodies against coagulation factor VIII (FVIII) and most often presents with unexpected bleeding. In contrast to congenital hemophilia, the patient's residual FVIII activity does not seem to correlate with the risk of bleeding as suggested from previous studies. Risk factors for bleeding have not been described.
Methodology: We used data from the prospective GTH-AH 01/2010 study to assess the risk of bleeding and the efficacy of hemostatic therapy. FVIII activity was measured at baseline and weekly thereafter in local laboratories. Bleeding events were assessed, and bleeding treatment was guided by the local physicians

Findings: A total of 289 bleeds was recorded in 102 patients. 141 new bleeds starting after day 1 were observed in 59% of the patients, with a mean rate of 0.27 new bleeds per patient-week before achieving partial remission; 130 (92%) bleeds occurred in the first 12 weeks after diagnosis. Weekly measured FVIII activity was significantly associated with the weekly bleeding rate, but only achieving FVIII ≥50% abolished the bleeding risk. A good WHO performance status assessed at baseline (score 0 vs. higher) was associated with a lower bleeding rate. Hemostatic treatment of bleeds was reported to be effective in 96% of bleeds.
Conclusion: In conclusion, the risk of new bleeds after a first diagnosis of AHA remains high until partial remission is achieved, and weakly measured FVIII activity may help to assess the individual risk of bleeding. These results will help to define future strategies for prophylaxis of bleeding in AHA.

OC05-4-AB

How to predict bleeding in FXI-deficiency

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Objectives: In FXI deficiency, the bleeding tendency does not correlate with the plasmatic level of FXI as measured with aPTT-based clotting test. Therefore, we investigate the utility of thrombin generation (TG) assays [Calibrated Automated Thrombogram (CAT) and Thrombodynamics analyzer system (TD), which analyzes the spatio-temporal propagation of coagulation], in order to discriminate FXI bleeders from healthy subjects and from non-bleeder individuals with FXI deficiency. Furthermore, we investigate the hemostatic potential of patients after replacement therapy with Hemoleven®, a commercial FXI concentrate.
Methods: Using CAT and TD, we measured TG and fibrin formation in a cohort of 23 FXI-deficient patients exhibiting different phenotypes (bleeding, non-bleeding, and prothrombotic) and in 50 healthy subjects. In six patients we could measure *ex vivo* the hemostatic potential at baseline and after replacement therapy with Hemoleven®. Additionally, we analyzed FXI-deficient plasma spiked with different final concentrations of Hemoleven®. A combinatorial analysis of multiple TD parameters (Mazzara et al., DOI:10.1038/srep45477) was carried out to determine the optimal TD marker combinations able to discriminate the FXI bleeding phenotype from controls and from FXI non bleeders (Figure1).
Results: Parameters of TG and fibrin formation describing the amplification phase of coagulation were significantly reduced in FXI-deficient patients with bleeding phenotype compared to non-bleeder FXI-deficient patients and healthy subjects. TG and fibrin formation were strongly improved after administration of Hemoleven® in comparison to baseline. A dose of 0.2 U/ml (which is below the recommended dose) was already sufficient to normalize *ex vivo* hemostasis in patients receiving the FXI concentrate. This was confirmed by spiking experiments *in vitro*. CombiRoc analysis showed that the combination of TD-specific parameters Ast (amplitude of thrombin peak, AU/L), V (rate of clot growth, µm/min) and CS (clot size, mm) had a good performance in discriminating FXI bleeders from control ($AUROC_{Ast+V+CS}$= 0.909, Sensitivity= 100 Specificity= 67.9) and from non-bleeders ($AUROC_{Ast+V+CS}$ = 0.791, Sensitivity= 92.3, Specificity= 71.4).
Conclusions:
1) TD analysis can distinguish FXI-bleeders from healthy subjects and from non-bleeder FXI-deficient individuals.
2) Combining TG parameters with parameters of fibrin formation improves the ability to separate FXI-deficient bleeders from non-bleeders.
3) TD can assess the correction of the hemostatic potential after FXI-replacement therapy, thus offering a monitoring tool for a patient-tailored treatment

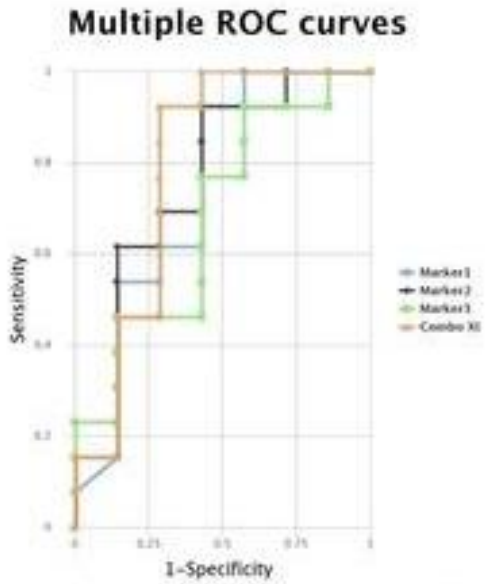

Figure 1: ROC curve comparison of multiple parameters between bleeder and non-bleeder FXI-deficient individuals: true positive rate (SE, sensitivity) is depicted on y-axis as function of false positive rate 1-specificity, SP). Marker 1= rate of clot growth, Marker 2= Ast, amplitude of thrombin peak, Marker 3= clot size. Combo XI= combination of markers 1,2,3.

[Combinatorial analysis of TD parameters]

POSTERS

P01 Posters: Thromboembolic disease

P01-1

Importance of the method of Thrombodynamics (point-of-care) in prognosis of VTEC in patients with thermal injury

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Aim: To identify the possibilities of the method of Thrombodynamics and standard clotting tests in prognosis the progress of venous thromboembolic complications in patients with burn injury.
Materials and methods: The study included 31 patients with thermal injury. TBSA were to 25-65%. Group 1 with the realized thrombosis and group 2 without thrombotic complications. For the study of hemostasis have been used: APTT, PV, TV, fibrinogen and test thrombodynamics (TD). Monitoring the hemostasis system was carried out in points: 1 point - 1 day; 2 - 3 day; 3 - 10±1 day; 4 - 20±2 days; 5 - 30±3 days.
Results: Patients in both groups parameters standard clotting tests as a whole were within the normal range (APTT 27±3,4; PV 92+8,5; TV 18±4,6), the only exception was the level of fibrinogen of 4.02+0,5, which was moderately elevated. Thus APTT is not a statistically significant difference throughout the observation period, with the exception of P. 1 - performance 2 group was significantly displaced in area of weak hypocoagulation. Prothrombin does not have statistically significant differences. The protein has a statistically significant trend to lower values in group 2. The dynamic parameters test TD (Vi, Vst) in both groups are in the area of moderate/significant hypercoagulable state, which captures failure of anticoagulant therapy and keeping the risk of development of thrombotic complications in both groups. Significantly different values of the stationary velocity in the P. 2 and P. 3 in 2 groups - reduced to the upper limit of normal.

The ROC analysis revealed interesting differences between groups: the occurrence of thrombotic accidents is possible when the starting speed ≥ 66,1 µm/min in point 2. For the point 3 values for the starting speed ≥ 59,2 µm/min., fixed speed ≥32 µm/min and density of clot ≥ 32568 also with a probability of 92.3% of the will lead to the development of thrombotic accidents. The density value of the clot in 4 ≥ 29325 while maintaining even moderate hypercoagulation the dynamic parameters test TD (Vi, Vst) settings as well with a probability of 83.3% of may indicate the development of thrombotic complications.
In addition observed a significant difference for Vst and Vi of growth of the clot between points 2 and 3 for the 2 groups, the downward trend in hypercoagulability. With similar values of the stationary velocity of clot growth (Vst) to assess differences between both groups was possible with the help of a specially introduced coefficient of Vst*1000/D.
The ratio of the rate of growth of the clot to the density of the clot is significant difference between 1 and 2 groups at the point of observation 5, where the group with thrombosis (group 1) this parameter is greater than 2 µm/min*u.e.
Conclusions: The parameters of the test Thrombodynamics in contrast the standard clotting tests with sufficient sensitivity and specificity can predict the occurrence of thrombotic accidents, with a certain cutoff criterion.

P01-2

Prevalence of perioperative asymptomatic venous thromboses of the lower extremities in patients undergoing transsphenoidal surgery for Cushing`s disease - a prospective single-center series of 30 cases

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Scientific research question: Cushing´s disease (CD) is a rare endocrinological disorder which is caused by an adrenocorticotropic hormone secreting pituitary adenoma. This condition is usually accompanied by a typical Cushingoid appearance including moon face, central obesity, purple striae, proximal myopathy and other features of the so-called metabolic syndrome.

Besides these features CD patients are prone to cardiovascular events including venous thromboses (VT) and pulmonary embolisms. While there are reports on symptomatic VT in the context of CD, the prevalence of incidental VT of the lower extremities as possible precursors of symptomatic VT or even pulmonary embolisms has never been investigated to date.
Methodology: 30 consecutive adult CD patients (9 male; age 25-77 years) were referred for transsphenoidal surgery after positive endocrinological screening and confirmation testing. All operations were performed by the same senior pituitary surgeon at our tertiary referral center between October 2018 and September 2019. Perioperative thromboprophylaxis with low molecular weight heparin was applied in all cases. Patients were screened for VT by means of whole leg compression ultrasound within one week after surgery (median 2 days; interquartile range 1-4 days). Sonography was performed by a single experienced angiologist in each case. Primary outcome measure was defined as VT of the leg detected by means of compression ultrasound. Preoperative laboratory values including cortisol and coagulation parameters covering the extrinsic and intrinsic pathway were evaluated as secondary outcome measures. Patients with VT were compared with cases lacking thrombotic events regarding clinical characteristics and laboratory findings. Results are presented as percentages with 95% confidence intervals (CI) or mean values ± standard deviation. P values ≤ 0.05 were considered statistically significant.
Findings: 2/30 patients (6.7%; CI 0.8-24.1%) presented with asymptomatic perioperative distal VT of the legs. Patients with and without VT differed not significantly with respect to age, gender, comorbidities and anticoagulation therapy. Preoperative morning plasma cortisol was significantly higher in CD patients with VT compared to patients without VT (421.0±49.5 µg/l versus 188.1±78.2 µg/l; p=0.01). Moreover, von Willebrand factor activity was markedly increased in case of VT (409.0%; only for one patient available) compared to the mean corresponding value obtained from non-VT cases (146.9±60.7%; p< 0.01), which was within the reference range of 48.8-163.4%.
Conclusion: Asymptomatic perioperative lower extremity VT can be found with the aid of compression ultrasound in a considerable proportion of patients undergoing transsphenoidal adenomectomy for CD. This finding emphasizes the great importance of peri- and postoperative thromboprophylaxis in these cases.
Abbreviations:
CD - Cushing`s disease
CI - 95% confidence interval
VT - Venous thrombosis

P01-3

A novel missense mutation (p. Arg510Cys) in *FGA* causes dysfibrinogenemia

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Scientific research question: Inherited dysfibrinogenemia is a qualitative defect of fibrinogen (FG) predicted by various mutations in the *FGA*, *FGB* or in *FGG* genes. Dysfibrinogenemia is associated with an increased venous thrombosis risk. Here we report a thirty-one y/o female with dysfibrinogenemia who experienced sinus venous thrombosis (SVT) after a plane flight and under oral (estrogen) contraceptives.
Methodology: Coagulation tests were done using standard procedures. FG genes were screened using direct genomic DNA sequencing. The structural-functional implications of the Mutation (p. Arg510Cys) was analyzed *in silico*. Since the region on which the mutation occurs is missing in the crystal structure, it was modelled on the *ab initio* Quark server and fitted onto the crystal structure.
Findings: Dysfibrinogenemia was diagnosed in a 31-year-old Caucasian female. She experienced SVT after a flight of > 6 hours and under oral contraceptives. Her father experienced unprovoked pulmonary embolism twice, in his 50s. The patients' FG levels (Clauss method) were mildly decreased (129-138 mg/dl, normal range being 180-355 mg/dl) and the FG antigen being within normal range (276-301 mg/dl). Thrombin time (TT) and reptilase time (RT) were slightly prolonged, 24,9-28,6 s and 22,8-23,4 s (normal ranges for both parameters: < 20,5 s) respectively. Approximately 12 months after the SVT she discontinued warfarin use and switched to s.c. administration of enoxaparin (4000 IU daily) and became pregnant within a few months. Interestingly, in the second trimester functional FG was normalized (178 mg/dl) and the immunologic FG remained stable (278 mg/dl). TT and RT were normalized too. Genetic analysis revealed a heterozygous missense Mutation in the terminal part of the *FGA* gene (c.1528C>T, p. Arg510Cys) encoding the alphaC domain. Our *silico* analysis showed that the affected residue Arg510 was located on a surface exposed randomly ordered loop connecting two helices. The surface exposed character of the wild type Arg510 suggests that the mutated cysteine could form non-native disulfide bonds with surface exposed reactive cysteines

from other plasma proteins like albumin leading to formation of aggregates and possibly thrombosis.
Conclusion: This mutation may play (especially in a combination with other risk factors e.g. long trips, oral contraceptives) a significant role in development of venous thrombosis requiring long term anticoagulation in affected individuals, also in pregnancy.

P01-4

Two novel mutations in the Protein S (PROS1) gene are associated with Protein S deficiency and thrombophilia

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Scientific research question: Protein S (PS) is a vitamin k dependent plasma protein. Main function is to act as cofactor for activated Protein C (PC), but PS also has other anticoagulant skills and thus, deficiency and/or loss of function of PS is associated with an increased risk for thromboembolic complications. Many mutations in the Protein S gene (PROS1) are known. Here we report two novel mutations of the PROS1 in two symptomatic patients.
Methodology: The patients were admitted to our hemostatic consultation due to recurrent thromboembolism. They both were investigated serologically i. a. for deficiency of PC, PS, Antithrombin and resistance against activated PC (APC) as well as for Antiphospholipid-syndrome. Genetical investigation for the Prothrombin G20210A-polymorphism, the Factor-V-A1691A-polymorphism, the Prothrombin G19911A-polymorphism, and the PROS1 were performed by sequencing.
Findings: Patient 1 was a 58-year-old woman. First deep vein thrombosis (DVT) occurred at the age of 19 years, a further DVT post-partum at the age of 25 years. Despite long-term oral anticoagulation (OAC) with a vitamin k antagonist, but due to non-compliance with irregular INR monitoring, she suffered from a third DVT with pulmonary embolism at the age of 56 years. OAC was then switched to Edoxaban 30 mg. Serological and genetical investigation yielded a free PS of 18 % and a PS activity of 13 %. Additional, she had an APC resistance due to a heterozygous Factor-V-A1691A-Mutation and a homozygous Prothrombin G19911G-Mutation. Genetical investigation of the PROS1 showed a heterozygous deletion (c938_945delTAAAATTT) in exon 9, leading to a frameshift and premature stop codon. Patient 2 was a woman. She was 60 years old. First DVT occurred at the age of 34 years, and a second DVT 25 years later. Both DVT´s were triggered. Free PS was 24 %, the PS activity was 42 %. Further risk factors for thrombophilia were not detectable. Genetical investigation revealed a heterozygous missense mutation c.1613C>T in exon 13 of the PROS1. Both patients also had a c.2001A>G polymorphism in exon 15 of the PROS1.
Conclusion: In both patients, we found two mutations in the PROS1, which were unknown at the time of our investigation. Both mutations resulted obviously in the reduced secretion of a truncated or otherwise defective protein, leading to a quantitative and qualitative deficiency of PS, potentially aggravated by the known c.2001A>G polymorphism. The clinical relevance of the two novel mutations was high: Both patients had type 1 PS deficiency and recurrent thromboembolic complications. In patient 1, thrombophilia was increased by further risk factors. Both patients required an indefinite OAC. For this purpose, patient 1 received Phenprocoumon (INR 2,5 - 3) and patient 2 Apixaban 2 x 5 mg. To date, none of the patients had a further thromboembolic event.

P01-5

Platelet serotonin influences thrombus formation in arterial and venous thrombosis

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Objectives: Peripheral serotonin is mainly stored in dense granules of platelets and is well known as an activator of platelets in hemostasis. It has been shown that serotonin plays an important role in inflammation by influencing the recruitment of leukocytes. Because there is a kind of immunothrombosis, our aim was to evaluate the role of serotonin in the development of arterial and deep vein thrombosis.
Methods: We used different mouse models (laser injury in mesenteric arteries, mechanical injury in the abdominal aorta and chemical injury ($FeCl_3$) in the carotid artery) in 4-6 week old C57BL/6 and $Tph1^{-/-}$(Tryptophane hydroxylase 1) mice, lacking peripheral serotonin, to study arterial thrombosis.

Time to occlusion was measured as outcome parameter.
To study the role of serotonin in deep vein thrombosis we used the stenosis model of the vena cava inferior. Therefor we induced a 90% stenosis of the vena cava inferior in 4-6 week-old C57BL/6, SSRI-treated C57BL/6 (WT+Flx; depleted serotonin pools in platelets), SERT$^{-/-}$(serotonin-transporter) and Tph1$^{-/-}$ mice. 48 h later we harvested the thrombus and measured its volume. We analyzed thrombus composition using immunofluorescence microscopy and the circulating immune cell landscape by flow cytometry in peripheral blood.
Moreover, bleeding time of all mouse models was measured to compare coagulation parameters.
Results: In Tph1$^{-/-}$mice arterial thrombi occured later (7.2±1.48 vs. 8.95±3.29 min, p=0.064) and time to occlusion was delayed significantly in mesenteric arteries (14.09±4.91 vs. 19.99±6.14 min, p=0.01), the abdominal aorta (245.9±72.4 vs. 429.6±188.3 sec, p=0.003) and the carotid artery (223.8±30.3 vs. 452.2±176.9 sec, p=0.02).
There was no significant difference in venous thrombus volumes of Tph1$^{-/-}$and WT+Flx compared to WT mice, while Tph1$^{-/-}$showed significantly larger thrombi compared to SERT$^{-/-}$mice (12.0±6.8 vs. 1.33±2.77 mm^3, p=0.03), as well as WT compared to SERT$^{-/-}$mice (13.4±11.6 vs. 1.33±2.77 mm^3, p=0.033). Abundance of circulating neutrophils was significantly reduced in SERT$^{-/-}$ compared to WT mice 48 h after thrombus induction (30.8±12.7 vs. 14.5±4.57% of leukocytes, p=0.004).
So far the results of the bleeding time are pending. We will definitely be able to show these at the congress.
Conclusions: In line with previous observations, the lack of peripheral serotonin in Tph1$^{-/-}$ mice delayed the onset and time to occlusion in arterial thrombus formation, an effect that has been attributed to serotonin effects on platelet reactivity. While arterial thrombotic occlusion was delayed by app. 5 min in the absence of peripheral serotonin, deep vein thrombi developed almost independently of serotonin in Tph1$^{-/-}$mice. However, venous thrombi in SERT$^{-/-}$mice developed less often and were significantly smaller. Since thrombus composition differed among genotypes and neutrophil content was reduced in venous SERT$^{-/-}$thrombi, serotonin appears to modify heterocellular interactions in venous thrombosis.

P01-6

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

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Background: 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 (in shock), it may be considered in a subset of intermediate-risk patients (at risk for shock). The individual bleeding risk must be considered in the decision-making.
Aims: We report single center registry data with 30-day mortality to evaluate guideline-recommended treatment algorithms.
Methods: We followed 119 patients with pulmonary embolism either at high- or intermediate risk (all-comers, 2016-mid 2019). Patients at intermediate risk were treated with alteplase (rtPA), 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: Ninety-eight (82%) of the 119 patients were at intermediate risk. The average age of the patients at intermediate risk was 68.4 years vs. 63.1 years in the patients at high risk. Fifteen of the patients at intermediate risk (15%) received thrombolysis. 7 were treated at full dose, 6 with low dose and 2 with local thrombolysis via the EKOS-catheter. Thirteen of the patients at high risk (62%) received thrombolysis. The survival in the intermediate risk group was 95.9% (94 patients), compared to 42.8% (9 patients) in the high risk group. In the intermediate risk group, relevant bleedings occurred in three patients (3.1%, two cases of epistaxis and one case of retroperitoneal bleeding) while in the high risk group seven patients (33.3%) had relevant bleedings. There was no intracerebral bleeding in patients at intermediate risk compared to one (4.8%) in patients at high risk.
Conclusion: The 30-day survival of patients at intermediate risk PE was 95.9%, after approximately 15% of these patients had received thrombolysis. The risk of relevant bleedings in patients at intermediate risk receiving thrombolysis was relatively low - possibly because

half of them received low-dose alteplase or local lysis. 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 and these data reinforce this approach. Patients with a low bleeding risk and at younger age appeared to benefit from thrombolysis and low-dose alteplase was safe.

P01-7

Improvements in exercise capacity and inspiratory muscle strength in patients with pulmonary embolism after outpatient pulmonary rehabilitation

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Objectives: Patients with pulmonary embolism (PE) may suffer from long-term consequences which decrease their functional capacity, including chronic exercise limitations. Data on the benefits of pulmonary rehabilitation in patients with PE are scarce, and to the best of our knowledge, no data about the effects of outpatient pulmonary rehabilitation (OPR) on PE patients are available so far.
Methods: We retrospectively analyzed data of 22 patients with PE, who attended OPR between 2012 and 2019. The reason for admission to OPR was PE with exertional dyspnea in all patients. Patients with other indications for pulmonary rehabilitation were excluded. Patients underwent a multiprofessional and individualized rehabilitation program 3 times a week for 3-4 hours including endurance, strength and inspiratory muscle training over at least 6 weeks (median (IQR): 6 (6 - 10.5) weeks) according to the ERS/ACCP guidelines. Assessments including six-minute walk (6MWT) in meters (m), cycle ergometer (Wmax) in watt, constant work rate test at 70% of Wmax (CWR70%) in minutes (min), strength of the upper and lower extremity in kilogram (kg) and inspiratory muscle strength (Pimax) in mbar were performed at the beginning and after completing the rehabilitation program.
Results: 22 patients with PE (median age (IQR): 47.5 (42.5 - 54.3) years; median body mass index (IQR): 33.4 (27.7 - 37.6) kg/m2) were included in this analysis. They started OPR in median 19 weeks after the acute PE event. Compared to the baseline assessment, significant improvements after OPR were observed in all exercise parameters, i.e. 6MWT (mean (SD): 556 (105) to 605 (96) m; p< 0.001), Wmax (mean (SD):157 (63) to 188 (57) watt; p< 0.001) and CWR70% (mean (SD): 12.7 (6.7) to 21.2 (7.7) min; p=0.002), strength of upper (mean (SD): 34.9 (12.6) to 44.5 (11.8) kg; p< 0.001) and lower extremity (mean (SD):117 (17.9) to 146.9 (16.5) kg; p< 0.001). Furthermore, a significant increase in Pimax (mean (SD): 94.7 (30.4) to 125.2 (27) mbar; p< 0.001) was detected. All patients included in this analysis finished the rehabilitation program, no adverse events were reported.
Conclusions: OPR in PE patients is feasible and safe. We observed significant improvements in exercise capacity as reflected by improved 6MWT, ergometer and strength tests including inspiratory muscle strength. Further prospective studies are needed to investigate the efficacy of pulmonary rehabilitation after PE and define which patients after PE would benefit most from a structured outpatient pulmonary rehabilitation program.

P01-8

Purified IgG from patients with antiphospholipid antibodies induces tissue factor (TF) in peripheral blood mononuclear cells (PBMCs)

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Scientific research question: Thrombotic events are a major and potentially life-threatening complication in patients with antiphospholipid syndrome (APS), but the underlying pathophysiological mechanisms remain incompletely understood. Preclinical studies have shown that antiphospholipid antibodies (aPL) require additional stimuli, e.g. vascular damage, to exert their thrombogenic effects in vivo. Similarly, the thromboembolic risk of APS patients is particularly high when concomitant pro-inflammatory triggers are present. We therefore asked whether ex-vivo co-stimulation with lipopolysaccharide (LPS) modulates the effect of purified IgG from aPL+ patients on TF procoagulant activity (TF PCA) of PBMCs.
Methods: IgG was purified from controls and aPL+ patients by immunoprecipitation with protein A sepharose. PBMCs, isolated from healthy

individuals by density gradient centrifugation, were stimulated with purified IgG (500 µg/mL) in the presence or absence of LPS (100 ng/mL) for 5 h in RPMI medium supplemented with 20 % FCS and subsequently analyzed for TF PCA and monocyte TF antigen expression by one-stage clotting assay and flow cytometry, respectively. TF-dependent factor Xa generation by released microvesicles (MVs) was also analyzed by a chromogenic endpoint assay.
Findings: Interim results from 21 aPL+ patients and 12 controls are reported. 16 patients (76.2 %) tested positive for IgM aPL and 11 patients (52.4 %) for IgG aPL. Of these, 5 (45.5 %) had only IgG anti-cardiolipin, 2 (18.2 %) had only IgG anti-β_2-glycoprotein I, and 4 (36.4 %) had both antibodies. A lupus anticoagulant was found in 9 patients (42.9 %), and 18 patients (85.7 %) had a history of at least one thromboembolic event. While TF PCA of PBMCs was slightly, but not significantly increased upon incubation with IgG from healthy individuals, IgG preparations from APS patients induced a significant ($p< 0.001$), albeit highly variable response when compared to buffer controls. Consistent with this finding, monocyte TF antigen levels ($p< 0.01$) and TF-dependent Xa generation of released MVs ($p< 0.01$) were also increased following stimulation of PBMCs with patient-derived IgG. Co-stimulation with LPS masked an IgG-specific effect on PBMC TF PCA, although a trend towards higher cellular TF PCA levels was observed in the presence of patient IgG. No significant differences were also observed with regard to monocyte TF antigen expression and MV-associated Xa generation, when experiments were carried out with LPS co-stimulation. However, IgG from 4 patients only exerted a procoagulant response in the presence of LPS, suggesting a synergistic effect in this patient subgroup.
Conclusions: Individual IgG preparations from aPL+ patients induce TF in PBMCs. In a subgroup of patients, this effect was only observed when PBMCs were co-stimulated with a pro-inflammatory trigger, suggesting that aPL-dependent induction of a procoagulant phenotype in PBMCs is context-dependent.

P01-9

Distinguishing a lupus antibody from a coagulation factors deficiency in a patient with atrial fibrillation and ischemic stroke

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Scientific research question: Lupus anticoagulant (LA) owns procoagulant properties in vivo and prolongs phospholipid-dependent clotting times in vitro. The increased in vitro clotting time can be misinterpreted as a bleeding disorder. In some cases, it is necessary to differentiate LA-associated in vitro changes from in vivo coagulation factor deficiency. In this case, we used different laboratory testing in a patient with ischemic stroke and reduced prothrombin time (PT) to identify in vitro effect of LA and exclude in vivo bleeding disorder.
Methodology: The activity of separate coagulation factors was evaluated both with recombinant thromboplastin Innovin (Siemens Healthcare) and reagent tissue extracted thromboplastin Thromborel® (Siemens Healthcare). Moreover, 1:1 plasma mixing test with standard plasma was performed. In order to exclude the interaction of tromboplastin and LA thromboplastin independent global coagulation test, thromboelastography was used. Diluted-Russel-Viper-Venom (dRVVT) assay was applied to detect the presence of LA detection.
Findings: The activity of separate coagulation factors measured with recombinant thromboplastin Innovin (Siemens Healthcare) showed reduced activity of separated coagulation factors: Factor V (20.9%), Factor VII (23.8%), Factor X (19.7%) and Prothrombin Time (PT) (27.6%). Re-assessment of the factor's activity with another reagent tissue extracted thromboplastin Thromborel® (Siemens Healthcare) showed an increasement of PT/INR and factor's activity in comparison to the previously used thromboplastin reagent Innovin: Factor V (77%), Factor VII (45.4%), Factor X (19.7%) and PT (59.1%). Plasma mixing study with 1:1 standard plasma revealed reduced (< 50%) normalisation of PT as well as coagulation factors activity confirming a LA-inhibitor in the patient plasma. Diagnostic LA testing was also performed with dRVVT assay showing significantly prolonged (112.8 Sec) test time. Thromboelastography was without abnormalities.
Conclusion: Different thromboplastin reagents and plasma mixing tests as well as thromboplastin independent coagulation tests may be helpful to differentiate LA and in vitro changes from in vivo factor deficiency in patients with LA.

P01-10

Anti-phosphatidylserine/prothrombin (aPS/PT) in diagnostics APS

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Background: APS/PT antibodies were associated to severe thrombosis, severe pregnancy complications inducing rematurity, and vascular microangiopathy, all generally associated to high risk APS forms requiring strong therapy. Anti-phosphatidylserine/prothrombin (aPS/PT) antibodies have begun to be considered potentional biomarkers for antiphospholipid syndrome (APS). This cohort study investigates the role of aPS/PT antibodies as a laboratory risk factor for APS by evaluating the association between those antibodies and clinical/laboratory profiles of APS.
Methods: Plasma samples from 80 assumed APS patients with autoimmune diseases were collected. Currently reccomended panel of APS diagnostics (lupus anticoagulants, anticardiolipin antibodies and beta-2-glycoperotein I antibodies) was completed with IgG/IgM aPS/PT antibodies were assayed using commercial CLIA (chemiluminiscence antipody assai) kit.
Results: Adding the aPS / PT IgG and IgM tests to the aCL, beta-2-GPI, and LA panel in the group of patients suspected to have acquired APS resulted in an increase in overall positive laboratory screening from 25 to 28.8%.
The actual incidence of aPS / PT IgG and IgM antibodies in our sample was 16.25%. The vast majority of aPS / PT antibody positive findings were already positive for the presence of aCL, beta-2-GPI and LA (76%), however, 3 patients showed an isolated incidence of aPS / PT antibodies. All of them showed positivity in IgM class, suggesting the current state of antibody development, complementing the ongoing course of the disease without clinical manifestation.
Conclusions: Examination of aPS / PT IgG and IgM antibodies appears to be one of the potential tests that could complement the current diagnosis of APS and assist in the detection of patients with other types of antibodies that nevertheless manifest the same type of thrombotic complications in APS as previously known aCL, beta-2 -GPI and LA.
Supported by grant LF-2019-001 and MH CZ - DRO (FNOl, 00098892)

P01-11

Hypoplasminogenemia- a forgotten diagnosis: a case series of genotype and phenotype correlation

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Objectives: Congenital plasminogen (PLG) deficiency is a rare condition caused by mutations in PLG. Homozygous pts have pseudomembraneous lesions,There is a lack of data concerning heterozygous patients with residual activities around 50-60%. We have now characterized 11 PLG deficient patients. All of them dont have any pseudomembrane lesions. We were interested if they have any raised thrombotic Risk which is so far unclear.
Methods: Plasminogen antigen/activity was measured byone-stage clotting assay.
Results: We could include 11 patients (2 male, one female) with a median age of 29 year. Median plasminogen Level was 56 % (70-150% normal range). 4 patients had DVT, 5 pts had missed abortion, 2 had also a FV-Leiden-Mutation, one was asymptomatic.Results : We found a variety PLG gene mutations; p-Leuk715Pro, pVal723Leuk, c.919C>T, c.712G>A, c.1335G>T, c21441>C, c.1748G>A, c.1112C>T, c11aA, (2pts) The phenotypes ranged from asymptomatic DVT.
Conclusion: Plasminogen deficiency may be worth to be tested in pts with thromboembolic events. Like described before, it can be combined with FV-Leiden-Mutation. Further, multicentre studies are useful to collect more data of the heterozygous pts.

P01-12

Management and outcomes of patients with isolated superficial vein thrombosis under real-life conditions (INSIGHTS-SVT)

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Scientific research question: Superficial-vein thrombosis (SVT) is a common acute thromboembolic disease with heterogeneous rates of complications and highly variable treatment patterns. Prospective data on current real-life management and outcomes are sparse. The Investigating SIGnificant Health TrendS in the management of Superficial Vein Thrombosis study (INSIGHTS-SVT; ClinTrials.gov NCT02699151) aims to close this gap.
Methodology: Non-interventional study in patients with objectively confirmed, acute, isolated SVT of the lower extremities managed by hospital-based and office-based physicians. The primary effectiveness outcome was the composite of symptomatic VTE (DVT, SVT, or PE) at 3 months; primary safety outcome was the combined incidence of major and clinically relevant non-major bleeding events at 3 months. Patient characteristics, comorbidities, diagnostic pathways, and medical and nonmedical treatment were collected at baseline, at 10 ± 3 days or 45 ± 3 days (depending on treatment), at approximately 3 months (primary outcome) and at approximately 12 months.
Findings: 1184 patients were enrolled into INSIGHTS-SVT; mean follow-up was 3.3 months, 96.8% completed the study. Mean age at baseline was 60.3 ± 14.7 years, 64.4% were women, mean BMI was 29.3±6.3 kg/m^2. Previous SVT was reported in 30.1%, previous DVT in 14.4%, and previous PE in 2.9%. SVT was located below the knee in 54.5%, above the knee in 26.8%, above and below the knee in 18.8%, respectively. Bilateral SVT was reported in 1.0%. Affected veins were varicose in 75.5%. Mean thrombus length was 14.6 ± 10.7 cm. At baseline, 94.8% of patients received drug treatment (in 1184 patients: 60.0% fondaparinux, 29.7% low-molecular-weight or unfractionated heparin, 4.1% NOAC, 14.1% analgesics), 75.4% compression treatment, and 1.9% surgery for SVT. At 3 months, 62.4% of patients were categorized as cured, 30.2% were improved, 4.9% were unchanged, and 2.5% worsened. Clinical events in patients with at least 1 follow-up within 3 months after the baseline visit were recurrent or extending SVT in 5.9%, DVT in 1.4%, PE in 1.1%, death in 1.0%, and hospitalisation in 2.9%, respectively.
Conclusion: The prospective INSIGHTS-SVT registry shows that SVT bears a substantial risk of clinical events, and opens important insights into current real-world management pathways of SVT.

P02 Posters: Haemophilia I

P02-1

Telehealth in Haemophilia treatment - use and recommendation intentions of patients and physicians

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Objectives: Many patients document bleeding incidents and their usage of replacement factors on paper although apps exist which make this process easier and more reliable. The aim of our studies is to discover the circumstances under which patients consider using the apps and what motivates physicians to recommend the usage of these apps. We also want to find out whether physicians plan to use additional functions afforded by the apps.
Methods: First, a study among haemophilia patients was conducted. Patients were mainly recruited via associations of haemophilia patients. Psychometric survey data was collected inquiring the patients' intention to use telehealth documentation including possible influencing factors like social influence and perceived severity of bleedings. The data was analysed using multiple regression analysis in Stata 14.
Second, a study among physicians who specialise in haemophilia treatment was conducted. They were mainly recruited via haemophilia treatment centres. A model was developed based on physicians' motivation to recommend the use of a designated documentation app. It also included an analysis of their willingness to work with data gathered from patients. The model was analysed using structural equation modelling in SmartPLS 3 and multiple regression analysis in Stata 14.
Results: More than 140 patients completed the patient survey of which about half did not use telehealth documentation. The study indicated that main predictors of their intention to use an app for documentation of their treatment were

enjoyment of using an app and recommendations from their social environment (including their physicians). The model explains 57% of the variance in use intention.
About a third of all haemophilia specialists working in Germany completed the physician survey. Recommendation behaviour was significantly influenced by the expectation of improved work outcome and the intention to use the additional functionalities offered by the telehealth system. The intention to use the app is influenced by the perceived usefulness of the software. Potential barriers like monetary cost and time expenditure do not have a significant influence. The model explains 84% of the variance in recommendation behaviour.
Conclusions: The results show that patients listen to their social environment where physicians play an important role in case of chronic diseases. However, physicians are willing to recommend the use of telehealth technology mainly if they can make further use of the collected data. Of course, this also benefits patients in the medium run. For developers and supporters (e.g., health insurances) of telehealth software this means, that the likelihood of recommendation and adoption of telehealth software probably significantly rises if it not only directly benefits the patient, but also provides functions to support the physicians in their work.

P02-2

Systematic review of efficacy and factor consumption of long-acting recombinant factor VIII products for prophylactic treatment of haemophilia A

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Objectives: To systematically review the evidence from Phase III clinical trials evaluating the use of long-acting (LA) recombinant FVIII (rFVIII) products for prophylaxis in haemophilia A patients. The outcomes considered were total annualized bleeding rate (ABR), spontaneous ABR (AsBR), joint ABR (AjBR) and consumption of rFVIII.
Methods: A systematic literature search was conducted in both EMBASE and PubMed according to PRISMA guidelines. Phase III clinical trials of prophylactic LA rFVIII treatment in previously treated patients aged ≥12 years, diagnosed with moderate to severe haemophilia A (FVIII levels ≤2%) were included. Studies were excluded if they did not meet these criteria, or did not report any of the outcomes mentioned above. Relevant data were then extracted from these studies.
Results: A total of 7,143 articles were identified, with 38 passing the title and abstract screen. Following full-text review, five articles met the inclusion criteria; these studies only included patients with severe haemophilia A. Data were extracted and are summarized in Table 1. Where data was available for more than one prophylaxis schedule, information is presented for the regimen described in the product label. Median ABR ranged from 1.14 with rVIII-SingleChain to 4.1 with BAY 94-9027 (Table 1). Median AsBR was 0.0 in all studies reporting relevant data, including rVIII-SingleChain. Median AjBR ranged from 0.0-0.85. Reported consumption was comparable among all LA products.
Conclusion: This review identified suitable data for an indirect comparison of LA rFVIII products. Within the limitations of potential differences in patient population and study design, a formal indirect statistical comparison may help to understand how these products could compare with respect to the parameters of ABR, AsBR, AjBR and consumption of rFVIII.

Study and regimen	Patients (N) Age (y) mean (SD)	ABR median (IQR)	ABR mean (SD)	AsBR median (IQR)	AsBR mean (SD)	AjBR median (IQR)	AjBR mean (SD)	Consump-tion (IU/kg/year) median (IQR)	Consump-tion (IU/kg/year) mean (SD)
Mahlangu et al, 2016 (rVIII-SingleChain; Every 2nd day, 2-3 × weekly, or other regimens at the investigator s discretion)	N=146 Median (range): 28.0 (12-58)	1.14 (0.0, 4.2)	3.11 (5.05)	0.0 (0.0, 2.4)	2.10 (4.76)	Not reported	Not re-ported	4283	4494 (1778.17)
Mahlangu et al, 2014 (rFVIIIFc, 2 × weekly to start, followed by every 3-5 days)	N=117 Median (min, max): 29 (12, 65) (n=118)	1.6 (0.0, 4.7)	Not re-ported	0.0 (0.0, 2.0)	Not re-ported	Spon-taneous: 0.0 (0.0, 1.7) Trau-matic: 0.0 (0.0, 1.2)	Not re-ported	4050.8 (Calculated from median weekly dose [77.9])	4440.8 (Calculated from mean weekly dose [85.4])
Konkle et al, 2015 (BAX 855, 2 × weekly)	N=120 Median (range): 28 (12-58)	1.9 (0.0-5.8)	3.7 (4.7)	0 (0.0-2.2)	2.1 (3.5)	0 (0.0-2.0)	1.8 (3.0)	4546 (calculated from median dose per infusion [44.6] and number of infusions per week [1.96])	Not reported
Giangrande et al, 2017 (N8-GP, Every 4 days)	N=175 30.6 (12.5)	1.18 (0.00-4.25)	Est. (95% CI): 3.04 (2.45-3.77)	0.00 (0.00-1.82)	Not re-ported	0.85 (0.00-2.84)	Not re-ported	Not reported	4845
Reding et al, 2017 (BAY 94-9027, 2 × weekly)	N=13 (not rando-mized) N=11 (eligible but not rando-mized) 31.4 (11.6)	Not rando-mized: 4.1 (2.0-10.6) Eligible but not rando-mized: 1.9 (0.0-5.2)	Not re-ported	Not re-ported	Not re-ported	Not re-ported	Not re-ported	Not reported	Not randomized: 4497.8 Eligible but not randomized: 3341.1

[Table 1: Details of bleeding rates and consumption in LA rFVIII studies identified by the systematic review]

P02-3

Patient preferences in the treatment landscape of haemophilia A

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Objectives: Haemophilia A is a congenital bleeding disorder that requires careful management. To prevent bleeds and consecutive arthropathy, intravenous Factor VIII prophylaxis is standard of care. Recently emicizumab, a bispecific antibody mimicking Factor VIII, has been licensed for subcutaneous prophylaxis in patients with haemophilia A. Simultaneously, a non-product-specific study was conducted to measure patient preferences for haemophilia treatments, including new alternatives, and to assess the treatment-related benefits and risks. Here, the results of this study will be discussed from the physician's perspective, including how these results are able to support physicians in their decision-making.
Methods: For this clinical debriefing of the results of the non-product specific patient preference study (best-worst scaling), the following patient-relevant endpoints were discussed: the patient's perspective on bleeding frequency per year; route of administration; risk of thromboembolic events; and development of inhibitors. The published results of this study, their implications for the physician's decision and their impact on patient adherence to therapy regimes were analysed by a haematologist.
Results: The preliminary analysis included 57 adult patients with haemophilia A. Bleeding frequency per year and development of inhibitors had the greatest impact on the respondents' decisions. Patients disliked being at risk of inhibitor development more than being at risk of thromboembolic events. The route of administration, either intravenous or subcutaneous, was of less importance in this context. The empirical results of this study match physicians' experience of currently available treatment options.
Conclusion: This study identifies and weighs key decision-making criteria for optimal management of haemophilia A from the perspective of patients. Adult patients value low frequency of bleeding per year and low risk of development of inhibitors more highly than the remaining attributes' levels in the decision context of the study. An increase of inhibitor risk and bleeding frequency would significantly decrease the impact on choices made. The route of administration does not seem very much to influence the choice made compared with the other attributes, at least in adult patients who usually have good venous access. From a physician´s perspective, it is important to understand patients' preferences and to use this information to choose the best available therapy for each patient, to ensure effective treatment and high compliance.

P02-4

A snapshot of a prospective, non-interventional study to evaluate routine practice prophylactic treatment schedules - NIS-Previq

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Objectives: There is still a need to systematically analyze the prophylactic treatment of Haemophilia A in real life routine clinical practice since clinical trials are subjected to certain restrictions. The prospective, observational study NIS-Previq is designed to collect information on prophylactic treatment of Haemophilia A patients treated with coagulation factor VIII concentrates from Octapharma with no inclusion restriction regarding age or residual factor VIII activity.
The primary objective of the NIS-Previq study is the assessment of the influence of the weekly FVIII dose on annualized bleeding rates (ABR). It further documents the prophylactic treatment of haemophilia A patients treated with factor VIII concentrates of the study sponsor as it is done in routine clinical practice. The concentrates are (A) of recombinant origin (simoctocog alfa), (B) plasma derived with von Willebrand factor (VWF) content of up to 0,4 I.U. per I.U. FVIII and (C) plasma derived with a VWF content of 1 I.U. per I.U. of FVIII. With the possibility to evaluate the PK of patients, the practicability of those tools for

individualization of prophylactic schedules can be investigated.
Methods: Patients with haemophilia A of all ages treated prophylactically, without current inhibitor activity and good compliance are eligible to be enrolled after informed consent has been given. All details of bleeding episodes and factor VIII treatments including weekly distribution of injections, activity levels of patients and changes in schedules are recorded. Optional study elements comprise initial and regular assessment of joint scores (HJHS), health related quality of life (SF-36) and a PK-assessment including dosing simulation for potential adaptation of therapy schedules with either the standard approach or the population PK via WAPPS-Hemo (www.wapps-hemo.org).

Age Group (Years)	0-5	6-11	12-17	18-40	>60
Total No	10	6	6	11	1
Presence of at least 1 target joint	3	3	2	10	1
Previous inhibitor activity		3		3	
Non-severe Haemophilia A			2	1	1

[Number of patients by age group and selected demographic information at study entry (n patients)]

Results: Since January 2015, 41 patients have been included by October 2019. The observation time over all patients is 2517.8 weeks (48.4 patient years) with a mean of 61,4 weeks per patient (median 53 weeks). 26 patients are currently treated with concentrate A, 13 with B and 2 patients with the factor VIII concentrate C. Six patients switched from concentrate A to B during the observation period. More than half of the cohort consists of children or adolescents (see table). Adaption of dose and/or dosing interval happens frequently within the paediatric/adolescent group of patients. So far, only one patient has changed his therapy schedule as a result of a full PK assessment.
No inhibitor formation or any other adverse drug reaction occurred during the observation period.
Conclusions: The snapshot on the study data so far confirm the good tolerability and efficacy of the concentrates studied. Such a study allows to further evaluate the real-life application of prescribed prophylactic schedules.

P02-5

Pain and treatment related to joint bleeds - comparison of data between 2017 and 2018 according to electronic diary smart-medication™

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Background: Joint bleeds in patients with haemophilia A/B may cause severe pain leading to immediate or delayed factor treatment, as well as different dosing in home settings.
Methods: Result from 359 patients from 13 haemophilia centers during 2018 were analyzed according to electronic data from smart medication™ and compared to results of the prior year (277 patients, 9 centers). Severity of pain (SP) on a scale of 1 (very mild) to 10 (very severe) was related to the respective initial treatment dose as well as time gap between joint bleed (JB) and treatment.
Results: The annual rate of JBs (AJBR) was 2,20 in 2017 and 1.96 in 2018. The initial treatment dose (IU/kg BW) following JBs was 23.54 - 62.55 (2017) and 24.71 - 33.99 (2018). Severe pain (SP 8-10) was followed by treatment doses of 23.54- 27,94 (2017) and 26.42 - 32,06 (2018). The time between bleeding symptom and treatment ranged from < 1 hour in 36% (2017) and 30% (2018) to >4 hours 13% (2017) and 20% (2018).
Summary: In 2018 a lower AJBR and slightly higher dosing following severe pain compared to 2017 was seen. Whether this reflects a change in treatment pattern or is due to an increasing number of participating centers and patients, needs to be further analyzed.

P02-6

The HaemAcademy - structure and content of a special training programme for physiotherapists to optimize treatment options for haemophilia patients

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Introduction and Objectives: Because Patients with haemophilia (PWH) with regular physiotherapy have better joint health and mobility, more autonomy and a better quality of life, physiotherapists who are well trained in the field of haemophilia should belong to the interdisciplinary team of all Comprehensive Care Centres. PWH need trained physiotherapists not only at their treatment centre for diagnostic purposes, but also in their living surrounding for regular prophylaxis and interventions. The prophylactic physiotherapeutic task is to prevent joint bleeds by training stability and mobility of the musculoskeletal system of PWH. When joint bleeds occur, the curative task is to reconstitute the body equilibrium. In reality, most PWHs have long distances to their treatment centres. The lack of trained physiotherapists in their living contexts demonstrates a need for education of physiotherapists in Germany. The HaemAcademy is a first initiative to solve this issue, initiated by a group of haemophilia treaters - orthopedists who prefer to treat conservatively, physiotherapists for children and adults, as well as sport- therapists with long experience in haemophilia-diagnostics and -treatment in interdisciplinary teams. The aim of this initiative is to educate physiotherapists, to improve the care situation of PWH all over Germany, to raise the awareness of haemophilia treaters for the importance of well-trained physiotherapists and to reduce the anxiety of physiotherapists to manage and to treat PWH.
Methods: The HaemAcademy has a basic and a professional module, both are free of charge and accredited with 15 CME points. The basic module takes place three times a year in the educating Comprehensive Care Centre in Duisburg and Bremen and once a year in a varying haemophilia centre in Germany. The contents of the basic modules are: history and genetics of haemophilia; treatment options; impact of frequent bleeds on joint health and quality of life; role of physiotherapists in the management of haemophilia; orthopaedic interventions and practical exercises with haemophilia patients. The contents of the professional module are: joint bleeds - pathophysiology, arthropathy, pain, synovitis, RSO, inflammation and factor treatment; gait training; physiotherapeutic treatment of bleeding in children and adults; psoas haemorrhage - theoretical and physiotherapeutic basics; orthopaedic methods; gait analysis; thermography; pedography; EMG measurements and development of future action plans.
Results: Since May 2013, eighteen HaemAcademies with altogether 238 participants were conducted. The courses showed great acceptance of the participants (see other abstract: The HaemAcademy - How Participants Evaluate This Haemophilia-Specific Training Programme for Physiotherapists).
Conclusions: The HaemAcademy helps to improve the skills of physiotherapists in order to provide PWH a higher level of independency, better mobility and joint health.

P02-7

Global seroprevalence of pre-existing immunity against various AAV serotypes in the hemophilia A population

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Objectives: Adeno-associated virus (AAV)-mediated gene therapy offers great promise to provide a durable reduction in bleeding frequency and treatment burden in the hemophilia population. However, pre-existing immunity against AAV may limit efficacy and therefore patient eligibility for such treatments. BioMarin has demonstrated via non-human primate studies that an electrochemiluminescent (ECL)-based method to detect antibodies against AAV5 capsid is suitable to identify those subjects most likely to respond to treatment. A comprehensive global study to evaluate the geographic and temporal distribution of AAV capsid seropositivity in hemophilia A patients has not been reported.
Methods: BioMarin employed an identical ECL-based format to develop and validate assays that detect antibodies against AAV2, 5, 6, 8, and rh10 capsids with comparable sensitivity in a central laboratory. To enable direct comparison of the prevalence of pre-existing immunity to each capsid a global seroprevalence study (BMN 270-901) enrolling up to 100 hemophilia A patients (75 adults and 25 adolescents) has been conducted in each of 8 countries (Russia, Germany, France, UK, USA, Italy, Japan, and South Africa). Plasma samples were collected from all patients following ethics approval and informed consent to determine antibody titers to each capsid. Seroprevalence was evaluated longitudinally in 20% of subjects by collecting additional samples at months 3 and/or 6 to assess variation in pre-existing immunity and antibody titer over time.
Results: Data thus far demonstrate varying prevalence of pre-existing antibodies specific for capsid serotype across countries. While AAV5 consistently presents the lowest seroprevalence among the regions studied, the rate of AAV5 antibody positive patients in the US is 20% compared to 47% in Russia. Consistency was observed in both serostatus and antibody titer (for AAV-positive individuals) over 6 months of time.
Conclusions: These data indicate considerable geographic variability in the percentage of hemophilia A patients with pre-existing antibodies to specific serotypes used in gene therapy.

P02-8

Impact of hepatitis C infection on bone microstructure of patients with haemophilia (PWH)

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Scientific research question: Reduced bone mineral density (BMD) is a common finding in patients with haemophilia (PWH). In addition to contributing risk factors for low BMD like orthopaedic joint status (OJS), BMI, mobility, HIV infection, Hepatitis C Virus (HCV) infection has previously been described as a potential risk factor. Most studies have evaluated BMD by dual energy X-ray absorptiometry (DXA), but impact on bone microstructure, assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT), has not been described.
Methodology: Clinical data, OJS, laboratory evaluation of bone metabolism and HCV status, results of DXA and HR-pQCT were captured during routine check-up visits. Additionally, patients completed questionnaires on activity and lifestyle after informed consent.
Findings: 80 male PWH (median age 33 years, range 18-77) were retrospectively analysed, of whom 67 (84%) and 13 (16%) had haemophilia A and B, respectively. 54 (68%), 6 (7%) and 20 (25%) had severe, moderate or mild haemophilia, 35 (44%) were HCV positive. Of all PWH, 39% had an impaired bone microstructure at the distal radius, and 45% at the distal tibia. Comparing PWH based on their HCV status, HCV positive vs. negative patients more often had a reduced volumetric BMD determined by HR-pQCT at the radius (26% vs. 1%, p=0.002) and tibia (56% vs. 7%, p< 0.001) and an impaired bone microstructure at the tibia (68% vs. 34%, p=0.006), with an increased prevalence of combined trabecular and cortical deficits (radius: 23% vs. 2%, p=0.009; tibia: 24% vs. 5%, p=0.018). Univariate analysis revealed significant worse values for trabecular BMD, trabecular thickness, trabecular separation at the radius and trabecular BMD, cortical BMD, trabecular number and trabecular thickness at the tibia. Adjusted for age, BMI, OJS and sporty activity, differences remained significant for trabecular BMD (radius: 85.9±22.1 vs. 100.9±17.7, p=0.027; tibia: 74.8±2.1 vs. 92.8±16.7, p=0.017)

and trabecular thickness at the tibia (78.2±17.2 vs 88.7±14.8, p=0.007). No differences of BMD, as measured by DXA (spine, hip), were observed with regard to HCV status.
Conclusion: Our data suggest an impact of HCV infection on trabecular volumetric BMD and bone microstructure in PWH, which might be caused by the negative impact of chronic inflammation on bone metabolism. HR-pQCT data may contribute to revealing insight into pathophysiological mechanisms involved in the development of osteoporosis in haemophilia.

P02-9

DHR 2.0

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On August 1, 2019, the new version of the German Haemophilia Registry (dhr) was launched with a changed legal framework and extended usage possibilities. The amendments of the German Transfusion Act gave the impetus to completely redesign the dhr.
One of the goals was to adapt the operation and data input in the dhr to modern utilization practices. The data entry forms were streamlined and simplified, and the section on the documentation of previously untreated patients (PUPs) was harmonized with the GEPHARD register and PedNet to ensure comparability of the data. In addition, the new dhr has been adapted to the EMA *Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products* in order to create the best conditions for the international comparability of the collected data.
In addition, innovative therapy options, such as modified factors, monoclonal antibodies or gene therapies, are currently opening up new treatment options. The data set of the new dhr has been modified so that data on these therapies can be collected. Thus, the dhr makes an important contribution to the long-term research of new therapies.

P02-10

Therapy of severe hemophilia A with emicizumab (Hemlibra®)

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Introduction: The greatest advantage of Hemlibra® in the treatment of hemophilia A compared to factor VIII preparations is the subcutaneous administration for prophylaxis (in the sense of preventing bleeding). For this reason, a poorly compliant adult male was switched from Beriate® to Hemlibra®, and a 2-year-old boy first switched to Hemlibra®.
Patients and therapy:
Casuistry I
A. M., b. 17.05.2017, male, height: 93 cm and body weight: 14 kg.
Severe hemophilia A, Factor VIII activity < 1%
On 28.05.2019 for the first time hemorrhage in the left hip after fall, since then slight walking disability for about 3 weeks, no residuals
Therapy and coagulation diagnostics: Hemlibra 40 mg (4x) and 90 mg s.c. (3x)
11.06., 18.06., 25.06., 02.07.2019, 29.07., 27.08.2019
Hemlibra plasma level (µg / ml) controls
18.06.19-**20,8**
25.06.19-**36,5**
02.07.19-**49,3**
07.09.19-**57,6**
29.07.19-**31,9**
08.08.19-**77,0**
27.08.19-**77.7**
Casuistry II
F. S., 10.01.1975, male, height: 175 cm and body weight: 60 kg.
Moderate hemophilia A, Factor VIII act. < 4%
Since 1985, recurrent bleeding in the left hip joint and both hocks, 1999-2001 gastric ulcer with recurrent bleeding, 01/2016 Lip bleeding, from 31.3. until 5.4.2016 rez. Bleeding into the left elbow joint, 07.11.2016 Hip TEP on the left
Therapy and coagulation diagnostics: Hemlibra 210 mg (4x) and 420 mg s.c. (2x) 13.06., 20.06., 27.06., 04.07.2019, 01.08., 29.08., 26.09., 24.10., 21.11., 19.12.2019 Hemlibra plasma level controls (µg / ml)
20.06.19-**21,2**
27.06.19-**36,5**
04.07.19-**52,2**
01.08.19-**36.3**
Results: The desired plasma levels of Hemlibra® are well achieved if the proposed regimen is met. In the case of the 2-year-old boy, substitution was unfortunately carried out 4 weeks too late in the recruitment phase, as a result of which the level

fell to 33 mg / l. Due to the poor compliance on average, only a level of 33 mg / l could be achieved in the adult male.
Discussion: The results reflect the reality in a hemophilia center. The frequency of hemorrhagic complications below 50mg / l and the incidence of bleeding complications will be assessed over the next few years. Experience to date has shown that long-term use is associated with a decrease in the number of bleeds.

P02-11

A single-arm, multicentre, open-label, phase III clinical trial to evaluate the safety and tolerability of prophylactic emicizumab in persons with haemophilia A (PwHA) with FVIII inhibitors (STASEY): interim analysis results

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Objectives: Emicizumab is a bispecific monoclonal antibody administered subcutaneously, which bridges FIXa and FX, thereby replacing the function of missing FVIIIa and restoring haemostasis in PwHA. The aim of this study was to provide interim safety and preliminary efficacy results from the STASEY study of prophylactic emicizumab in PwHA with FVIII inhibitors.
Methods: The primary objective is to evaluate safety (adverse events [AEs], including thrombotic AEs and hypersensitivity). The secondary objective is to evaluate efficacy (number of bleeds over time and quality of life [QoL]). PwHA aged ≥12 years with FVIII inhibitors receive prophylactic emicizumab 3 mg/kg/week for 4 weeks, followed by 1.5 mg/kg/week for the remainder of the 2-year treatment period (NCT03191799). AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA) version 21.1. Multiple occurrences of the same AE in one individual were counted only once except for the total number of AEs, in which multiple occurrences of the same AE are counted separately.
Results: At cut-off (15 October 2018), 88 patients completed 24 weeks on study or discontinued study participation; whichever occurred first. Median age: 28.0 (range 12-80) years; median duration of exposure: 39.2 (range 4.4-57.1) weeks. Emicizumab was well tolerated, with no thrombotic or other AEs of interest reported. In the safety-evaluable population, 66 (75.0%) patients experienced ≥1 AE (total number, 234). In patients with ≥1 AE, one (1.1%) was fatal (head injury, unrelated to emicizumab), 10 (11.4%) were serious, none led to treatment withdrawal/study discontinuation, three (3.4%) led to dose modification/interruption, 14 (15.9%) were grade ≥3. Eighteen (20.5%) patients reported an emicizumab-related AE (one serious AE of catheter site abscess), of which injection-site reactions (ISRs) were the most common (13.6%). In total, 13 (14.8%) AEs were local ISRs. Arthralgia was the most common AE (≥10% of patients) in 12 (13.6%) patients, followed by headache and nasopharyngitis (10; 11.4% each). The rates of treated, all, spontaneous, joint, and target joint bleeds were low (Table). Seventy-one patients had 0 treated bleeds (80.7%). Of 17 patients who received treatment for a spontaneous or traumatic bleed, 16 received recombinant FVIIa, and one received FVIII; no thrombotic events were seen with concomitant bypassing agents or FVIII. Clinically meaningful improvements from baseline in QoL and health status across multiple domains were observed. Of the 80 patients who completed the EmiPref survey, 76 (95.0%) preferred emicizumab to their prior therapy.
Conclusions: No new safety signals were identified. Bleeding rates in PwHA with FVIII inhibitors receiving emicizumab in the STASEY study were in line with previously reported observations from HAVEN 1.

ABR*†	Emicizumab 1.5 mg/kg/week N=88
Treated bleeds‡	
Mean ABR, model-based (95% CI)	**0.5 (0.29-1.00)**
Median ABR, calculated (IQR)	**0.0 (0.00-0.00)**
Patients with zero bleeds, n (%)	**71 (80.7%)**
All bleeds	
Mean ABR, model-based (95% CI)	**1.4 (0.91-2.24)**
Median ABR, calculated (IQR)	**0.0 (0.00-1.31)**
Patients with zero bleeds, n (%)	**56 (63.6%)**
Treated spontaneous bleeds	
Mean ABR, model-based (95% CI)	**0.2 (0.08-0.34)**
Median ABR, calculated (IQR)	**0.0 (0.00-0.00)**
Patients with zero bleeds, n (%)	**79 (89.8%)**
Treated joint bleeds§	
Mean ABR, model-based (95% CI)	**0.3 (0.10-0.84)**
Median ABR, calculated (IQR)	**0.0 (0.00-0.00)**
Patients with zero bleeds, n (%)	**81 (92.0%)**
Treated target joint bleeds	
Mean ABR, model-based (95% CI)	**0.1 (0.03-0.18)**
Median ABR, calculated (IQR)	**0.0 (0.00-0.00)**
Patients with zero bleeds, n (%)	**83 (94.3%)**

***A Bleed and Medication Questionnaire was completed by participants/caregivers via an electronic handheld device. Bleed definitions were based on International Society on Thrombosis and Haemostasis (ISTH) criteria. †Calculated using the negative binomial regression method.**
‡Treated bleeds are defined as a bleed directly followed by a haemophilia A medication reported as a treatment for bleed, without an intervening bleed and irrespective of the time between the treatment and the preceding bleed. If multiple bleeds occurred on the same calendar day, the subsequent treatment was considered to apply to each of these multiple bleeds. Bleeds due to surgery/procedure are excluded.
§Joint bleeds are defined as bleeds with type reported at 'joint' in combination with at least one of the following symptoms: increased swelling or warmth of the skin over the joint, increasing pain, decreased range of motion or difficulty in using the joint compared with baseline.
ABR, annualised bleed rate; CI, confidence interval; IQR, interquartile range.

[Efficacy summary (intention-to-treat population)]

P02-12

Stable FIX expression and durable reductions in bleeding and factor IX consumption for up to 4 years following AMT-060 gene therapy in adults with severe or moderate-severe hemophilia B

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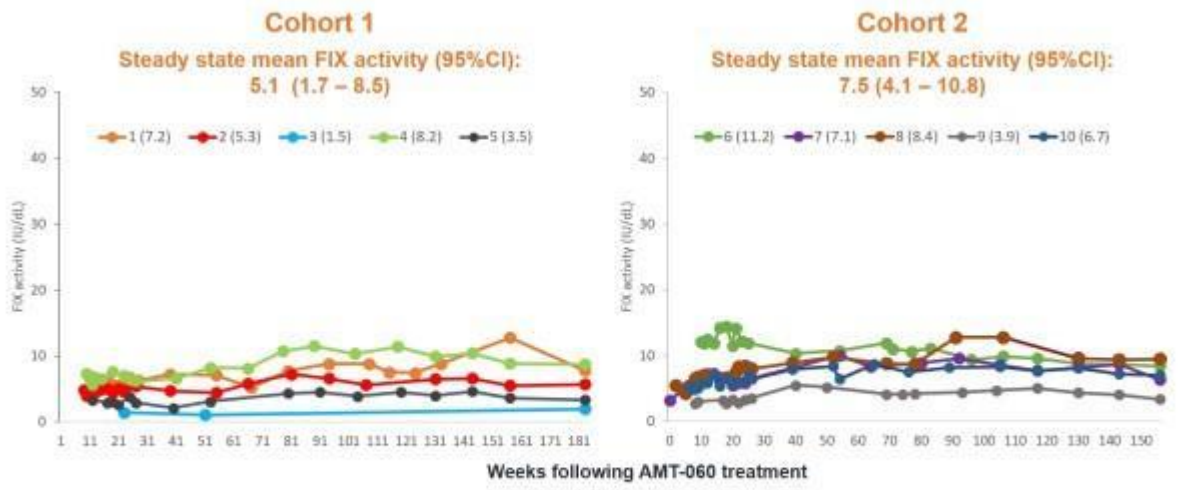
Objective: The aim of gene therapy is to provide long-term therapeutic effect from a single administration, yet information on response durability is currently limited. AMT-060 is an adeno-associated virus serotype 5 (AAV5) vector with a codon-optimized wildtype human factor IX (FIX) gene and liver-specific promoter. AMT-060 is being analyzed in an ongoing study of 10 participants with severe/moderate-severe hemophilia B (Phase 1/2 study, NCT02396342). Here we describe efficacy and safety outcomes from a planned interim analysis at up to 4-years post-AMT-060.

Methods: Adult males with FIX activity ≤2% and a severe bleeding phenotype received a single intravenous infusion of AMT-060 ($5x10^{12}$gc/kg, Cohort 1, n=5) or ($2×10^{13}$ gc/kg, Cohort 2, n=5). Assessments included FIX activity, FIX replacement use, annualized bleeding rate (ABR), treatment-related adverse events (TRAE), immunological and inflammatory biomarkers up to 4 years (Cohort 1) and 3.5 years (Cohort 2).
Results: As of 8 May 2019, for Cohort 1 the mean yearly FIX activity (annualized to 4 years) was 6.0 as compared to 4.4% in the first year, 6.8% in the second year and 7.3% in the third year. Mean yearly FIX activity for Cohort 2 at 3 years was 7.9% as compared to 7.1% in the first year and 8.4% in the second year. Factor IX activity for each patient over the length of follow up is shown in Figure 1. Eight of 9 participants using prophylaxis at baseline were able to discontinue use. During the last 12 months of observation, the mean annualized bleed rate (ABR) was 1.7 for Cohort 1 and 0.7 for Cohort 2. Respectively, these represent a reduction in mean ABR to the year prior to treatment of 88% and 83%. During this same period the consumption of FIX replacement therapy declined 93% and 96% relative to pre-treatment respectively for Cohort 1 and Cohort 2. No participants developed FIX inhibitors or signs of sustained AAV5 capsid-specific T-cell activation. TRAE were mainly reported in the first 3.5-months after treatment, including three participants who experienced transient mild elevations in alanine aminotransferase as previously described. One new TRAE (joint swelling post-exercise) was observed during the last 12 months of observation post-treatment. Updated data, up to 4-years of observation, will be presented.
Conclusions: Long-term stable endogenous FIX activity and reductions in ABR and FIX replacement use were observed following a single treatment with AMT-060. There were no additional safety concerns with longer term follow-up. These findings support the ongoing Phase III study of the enhanced construct, AMT-061, which encodes the highly active Padua FIX variant.



Values in parentheses represent mean FIX activity over time. Only values at least 10 days after last FIX concentrate administration are included. FIX prophylaxis was continued after AMT-060 and tapered between Weeks 6 and 12 *Patient retrospectively tested positive for AAV5 neutralizing antibodies using the luciferase-based assay. 3 patients were presumed cross-reactive matter positive. FIX, factor IX; CI, confidence interval; IU, international units

P03 Posters: Inherited and acquired bleeding disorders

P03-1

Long-term efficacy and safety of recombinant factor IX fusion protein (rIX-FP) in previously treated patients with haemophilia B: Results from a phase 3b extension study

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Objectives: Two phase 3 studies previously demonstrated the efficacy and safety of rIX-FP, a long-acting recombinant FIX fused to recombinant albumin, in prophylaxis with dosing intervals of 7, 10 and 14 days in previously treated patients

(PTPs). This phase 3b extension study aimed to evaluate the long-term efficacy and safety of prophylaxis with rIX-FP, over a range of dosing intervals in previously treated patients (PTPs).
Methods: PTPs with haemophilia B (FIX ≤2%) received rIX-FP prophylaxis every 7 (35-50 IU/kg), 10 or 14 days (50-75 IU/kg). PTPs ≥18 years could switch to a 21-day regimen (100 IU/kg) if well controlled on a 14-day regimen. The primary outcome was the development of FIX inhibitors. Secondary outcomes included annualized spontaneous bleeding rate (AsBR).
Results: Eighty-three PTPs (59 adult/adolescent [13-63 years] and 24 paediatric [2-11 years]) participated in the study (mean duration: 36.2 months). Dosing intervals of 7, 10 and 14 days were maintained in 53 PTPs (64%) while 22 PTPs (27%) extended their dosing interval. Of the 11 PTPs switched to the 21-day regimen, two switched back to a 14-day regimen to reduce their bleeding frequency. At the end of the study, 76% of adult/adolescent PTPs had a dosing interval of 10 (n=11), 14 (n=25) or 21 days (n=9) and 29% of paediatric PTPs had a dosing interval of 10 (n=3) or 14 days (n=4). Two paediatric PTPs who started a dosing interval of 14 days switched back to shorter intervals. Low AsBRs were achieved with all regimens. Mean steady-state trough levels were >5% across all regimens. Seventy-eight (94%) PTPs had ≥100 exposure days to rIX-FP. No PTPs developed inhibitors or antibodies to rIX-FP.
Conclusions: These results demonstrate the long-term efficacy and tolerability of rIX-FP prophylaxis. For selected patients, rIX-FP enables treatment intervals of 21 days in adults and 14 days in children.

P03-2

Factor VIII-poor von Willebrand factor concentrate use for major surgical procedures and major bleeding episodes in von Willebrand disease: Analysis from an observational study

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Scientific Research Question: The efficacy and safety of factor VIII-poor von Willebrand factor (VWF) concentrate in von Willebrand disease (VWD) have been established in clinical studies, although data regarding routine clinical use are limited, especially for prolonged treatment in cases of major surgery or major bleeding. The aim of this study is to investigate these situations in real-life over the first 5 post-approval years in France.
Methodology: This observational study was conducted in 31 centres in patients with inherited VWD who were unresponsive to desmopressin. Patients were followed for up to 3 years.
Findings: Of 155 analysed patients, 50 underwent 57 surgical procedures or childbirths requiring more 5 exposure days (EDs). A majority of surgical patients (72.0%) had a basal VWF:RCo level ≤15 IU/dL. Procedures included 1 neurological, 2 cardiovascular, 12 orthopaedic, 7 digestive procedures and 15 deliveries (10 C-sections). FVIII:C levels were corrected prior to surgery in 40 (70.2%) procedures. This was achieved either by co-administration of a factor VIII concentrate with the VWF preoperative dose (35.1%) or by giving two VWF preoperative doses ~12h apart (35.1%). During the period covering hospitalisation and home treatment, patients received a median daily VWF dose of 44.5 IU/kg (range 17-128) for 10.0 EDs (range 6-45). Haemostatic efficacy in the 56 of 57 evaluated major procedures was rated as excellent (n=40) or good (n=15) (98.2%). One case was a C-section complicated with post-partum haemorrhage rated as moderate. Red blood cell (RBC) transfusions were administered on 10 occasions mainly in digestive surgery. A total of 38 patients (47% type 3, 37% type 2, 13% type 1, and 3% not-documented) required hospitalisation for 67 major bleeding episodes. Almost all (94%) occurred in patients with VWF:RCo ≤15 IU/dL. The most frequent bleeding location was gastrointestinal (GI) tract (34, 50.7%) and musculoskeletal (13, 19.4%). In emergency acts, 13 patients with acute anaemia received concomitant RBC transfusions on 29 occasions, mainly for GI bleeds (86.2%). Efficacy was assessed as excellent/good in 63 episodes (94.0%) and moderate in 4 (6.0%). The median total dose per episode from start to complete resolution was 389.6 IU/kg (range 44-2077) given as 6.0 EDs (range 1-47). A daily dose of 56.9 IU/kg (range 25-138) was required. Median doses and duration were comparable in the three VWD types. A priming dose of factor VIII at the onset of VWF treatment was administered for 34.3% of events, mainly for musculoskeletal episodes (53.8%). Overall, these patients totalized a cumulative number of 1284 EDs. No thromboembolic events were reported.
Conclusions: The study achieved its goal of enrolling patients experiencing severe clinical situations and confirming the efficacy and safety of the product. Repeated administrations were well-tolerated. There were no safety concerns observed in this population of patients with high risk factors.

P03-3

Clinical study to investigate the efficacy and safety of a Von Willebrand factor FVIII concentrate (VWF/FVIII) during prophylaxis in previously treated patients with Von Willebrand disease (VWD)

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Scientific research question: Determine the efficacy of a von Willebrand factor (VWF) FVIII concentrate (VWF/FVIII, *Wilate*, Octapharma AG) in the prophylactic treatment of previously treated von Willebrand disease (VWD) patients with type 3, type 2 (except 2N), or severe type 1 VWD. Secondary objectives are to 1) Assess VWF activity (VWF:Ac) and antigen (VWF:Ag) incremental *in vivo* recovery (IVR) of VWF/FVIII over time, 2) Determine VWF:Ac, VWF:Ag and factor VIII procoagulant activity (FVIII:C) pharmacokinetics (PK) in paediatric patients and 3) Assess the safety and tolerability of VWF/FVIII in this indication. The study will also examine VWF/FVIII efficacy in the treatment of bleeding episodes (BEs) and in surgical prophylaxis, as well as the quality of life (QoL) during prophylaxis with VWF/FVIII. Additionally, the study will examine changes in patient's joint status and in the menstrual bleeding intensity of females of child-bearing potential.
Methodology: WIL-31 plans to enroll 28 patients (≥6 years and with VWD type 1, 2A, 2B, 2M, or 3). Eligible patients must be receiving frequent on-demand treatment with a VWF-containing product, with at least 1 and an average of ≥2, documented spontaneous BEs per month in the preceding 6 months requiring treatment with a VWF-containing product. This will be assessed as part of a run-in observational study (WIL-29) to collect the bleeding profile prior to the start of prophylaxis. In 10 paediatric patients (≥6 to < 17 years old), baseline PK profile will be characterised for VWF ristocetin co-factor activity (VWF:RCo), VWF:Ag, and FVIII:C, based on blood samples taken pre-dose and 1, 3, 9, 24, 48 and 72 hours after dosing. Prophylactic treatment with VWF/FVIII concentrate for 12 months will be initiated at the beginning of the study for adults and after PK for paediatric patients. During the prophylactic period, patients will record all BEs. Based on these data, frequency of BEs and annualized bleeding rate (ABR) under prophylactic treatment will be calculated. Treatment efficacy of BEs will be assessed by the patient (together with the investigator in case of on-site treatment) using a 4-point scale. VWF/FVIII concentrate efficacy will be assessed at the end of surgery by the surgeon and at the end of the postoperative period by the haematologist. Overall efficacy assessment will be made at the end of the postoperative period by the investigator.
Findings: Data will be monitored on an ongoing basis; the study is expected to end in Q2 2021.
Conclusion: Prophylactic treatment in other congenital bleeding disorders is widely accepted as the standard of care to prevent bleeding and preserve QoL in patients, but to date, no sufficient data addresses long term VWD prophylaxis. This study will provide data on the efficacy of prophylactic treatment in reducing the rate of bleeding and on the impact of prophylaxis on the QoL, joint status and severity of menstrual bleeds in VWD patients.

P03-4

A screening algorithm for acquired hemophilia A

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Acquired hemophilia A is a rare but serious disorder for which early diagnosis and intervention may be life saving. Between 2013 and 2018 a total of six patients with acquired hemophilia A were recorded in our tertiary care referral center, which covers a region of 350´000 inhabitants in Southern Switzerland. As the patient number with acquired hemophilia A exceeded the expectations from published epidemiological data, we decided to implement a standardized laboratory algorithm for coagulation testing in order to allow for early diagnosis. The design of our algorithm included a screening of all patients without anticoagulation therapy, who had a prolonged activated partial thromboplastin time (aPTT), and a normal prothombin time (Quick) on a first occasion. If a

second analysis from an additional blood sample confirmed the initial results, and if the anti-Xa activity in this second sample did not exceed 0.05 U/l, aPTT mixing studies with standard plasma were performed. The mixing studies included measurements at baseline, and after 2 hours of incubation in a 37° water bath. The Rosner Index (RI = (aPTT mixed - aPTT standard) / aPTT patient * 100)) was calculated automatically for the results obtained at 2 hours. In patients with a RI ≥11% a senior hematologist had to be informed by the laboratory technician, whereas in patients with a RI < 11% a consultation with a hematologist was suggested but not mandatory.
A total of 133 patients had an aPTT mixing study requested between November 2018 and August 2019. Nineteen (14.3%) patients had erroneously undergone aPTT mixing studies and were excluded from further analysis for the following reasons: six (4.5%) patients had normalization of the aPTT in a second blood sample, five (3.8%) patients had a pathological prothrombin time, and eight (6%) patients had elevated anti-Xa levels. One-hundred and fourteen patients were included in the final analysis. A Rosner Index of ≥11% was present in 27 (23.6%) of these patients, with a median RI of 19% (range 11%-98%). Three quarter of the patients with a RI ≥11%, underwent further testing for factor VIII activity, and lupus anticoagulant. Acquired hemophilia A was diagnosed in four out of 114 (3.5%) patients screened according to the new algorithm, with a median aPTT of 135s (range 71s-200s), a median RI of 30% (range 27%-98%), a median FVIII activity of 1.5% (range 0%-6%), and a median LA of 1.05 (range 1.04-1.07). A lupus anticoagulant was diagnosed in 14 (12.3%) patients, with a median aPTT of 48s (range 40s-80s), a median RI of 20% (range 11%-54%), a median FVIII activity of 108% (range 22%-216%), and a median LA of 1.86 (range 1.5-2.84).
The here proposed laboratory algorithm seems effective in diagnosing acquired hemophilia A, and a lupus anticoagulant. Continuous education of clinical and laboratory personal is mandatory to decrease unnecessary testing, and to improve further laboratory work-up. Updated data will be shown at the conference.

P03-5

How we treat patients with acquired haemophilia A with recombinant porcine factor VIII

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Introduction: Treatment of bleeds in patients with acquired haemophilia A (AHA) with bypassing agents is often difficult and unpredictable. Monitoring of the effect in routine clinical practice is not possible. A recombinant porcine factor VIII B-domain-deleted product (rpFVIII; OBIZUR) is approved for treatment of bleeding episodes in adults with AHA. The recommended high initial dose of 200 IU/kg bodyweight is a matter of debate. We report our approach to the treatment of patients with AHA with rpFVIII.
Methods: A retrospective chart review of all patients with AHA treated with rpFVIII at our institutions from April 2016 to September 2019.
Results: 13 major bleeds in 12 patients (median age 78 years, range 38 to 92) were treated with rpFVIII. In one bleed (gastrointestinal following unsatisfactory bypassing agent therapy) rpFVIII was second-line treatment and in 12 bleeds (mainly muscle and soft tissue bleeds) first line treatment. Good haemostatic efficacy was seen in 12 bleeds. rpFVIII loading doses of 50 U/ kg bodyweight increased FVIII activity to sufficient levels between 55% and 112% within 1 h. Subsequent median doses were 2x 25 - 50 U rpFVIII/kg bodyweight/day for 1 and 7 days to maintain a factor VIII trough level above 30%. Duration of treatment was adjusted the type of bleeding. No rpFVIII-related adverse events were reported. Two patients died in the hospital (one of sepsis and one of pneumonia after aspiration). All patients received 1g tranexamic acid 3 to 4 times daily concomitantly and prednisolone for immunosuppression.
1 patient out of 12 patients received a rpFVIII loading dose of 100 U/kg bodyweight due to a life threatening throat and tongue bleed. In this case we observed no increase of FVIII activity (1% at baseline to 2% after 1h) and treatment was switched to bypassing agents. Measurement of the rpFVIII inhibitor -titer later on in this patient revealed an anti-porcine titer of 13,8 BU (human titre 4,8 BU). All other patients had no relevant cross reactivity and anti-porcine titers between 0,4 and 0,8 BU.

Conclusions: rpFVIII showed good haemostatic efficacy in 12/13 bleeds in 11/12 patients with much lower doses than in the registration study. There was a close correlation between the measured factor VIII Levels and hemostatic efficacy.
We recommend an initial dose of 50 U rpFVIII/kg bodyweight and monitoring of factor VIII levels to trough Levels ofat least 30%. In case of no or low increase of factor VIII levels relevant rpFVIII inhibitor- titres are likely and further treatment with bypassing agents should be considered.

P03-6

Reduced intensity immunosuppressive therapy for acquired hemophilia A

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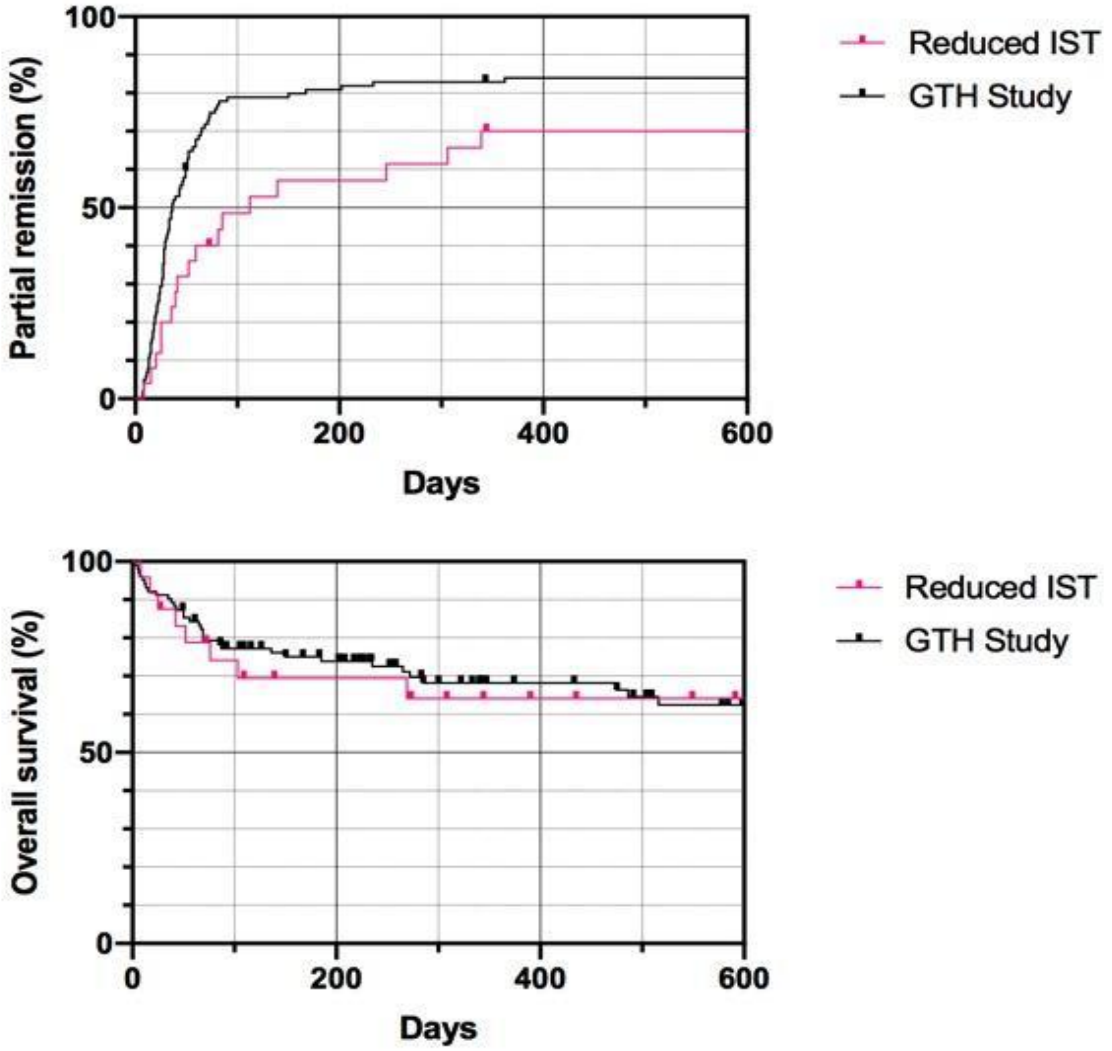
Scientific research question: Acquired hemophilia A (AHA) is an autoimmune bleeding disorder characterized by the formation of neutralizing antibodies against coagulation factor VIII (FVIII). Remission of the disorder can be achieved with immunosuppressive therapy (IST), but this treatment is associated with a high risk of infectious complications in patients with AHA. Here, we studied the feasibility of a reduced intensity IST regimen in a consecutive cohort of patients with AHA.
Methodology: This was an exploratory, monocentric, observational, consecutive cohort study of 25 patients enrolled between 2015 and 2019. IST was planned to consist of (1) in all patients: dexamethasone (40 mg per day on days 1-4, repeated on day 10 and 20, if not responding); (2) in patients with baseline FVIII < 1% or inhibitor >20 BU/ml: 4 weekly cycles of rituximab 375 mg/m^2 starting week 1; (3) in patients with baseline FVIII ≥1% and inhibitor ≤20 BU/ml rituximab 375 mg/m^2 starting week 3 and only if not responding to dexamethasone; (4) in all patients not responding after dexamethasone and rituximab: cyclophosphamide (oral or intravenous). Endpoints were identical to those of the GTH-AH 01/2010 study (Blood 2015; 125: 1091-97). Descriptive statistics and Kaplan Meier analysis were used to describe and compare data.
Results: The median follow-up was 271 days (range 0-1636), thus comparable to the GTH study (262 days). At baseline, patients had a median age of 79 years (range 35-94); the median FVIII activity was 5% (range < 0.3-38%); the median inhibitor titer was 12.8 BU/ml (range 0.9-579). 11 of 25 patients had FVIII < 1% or inhibitor >20 BU/ml. Two patients died before IST was started. IST was given as planned in 16 patients receiving dexamethasone as for 1 to 3 cycles, rituximab (12 patients) and cyclophosphamide (1 patient). The 9 patients with deviations from the planned IST received prednisolone either instead of or in addition dexamethasone, rituximab (4 patients) and cyclophosphamide (4 patients). In total, 9 of the 25 patients died, and the survival rate after 1 year was 68%. Partial remission was achieved by 17 of 25 patients (68%) after a median of 112 days (range 8-344). The rate of achieving partial remission was slower than observed in the GTH study, where 85% of patients after a median of 31 days (range 7-362). Infections occurred in 16 of 25 (64%) patients, compared to 36% in the GTH study, and those infections were always CTC grade 3 or 4, including two events in the patients, who did not receive IST. Results were similar comparing patients treated as planned and patients with deviations from the planned IST.
Conclusions: This cohort of patients received reduced intensity IST as compared with the GTH study. Reduced intensity IST did not appear to reduce severe infections. Partial remission was achieved less frequently and later as compared with the GTH study, but overall survival was similar.



[Kaplan Meier plot of time to partial remission (top) and overall survival (bottom).]

P03-7

Use of rurioctocog alfa pegol in a perioperative setting in patients with mild hemophilia A

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Scientific Research Question: To provide real-world information on the use of rurioctocog alfa pegol (RURIOCT) in a perioperative setting in patients with mild hemophilia A on basis of three patient cases from a single Austrian center.
Methodology: Retrospective analysis of 3 cases from 2019 from a single Austrian center in which RURIOCT - a pegylated recombinant human factor VIII with an extended half-life - was used for perioperative factor VIII replacement in patients with mild hemophilia A who had to undergo major or minor elective surgeries.
Assessment of achieved factor VIII levels with respect to the patients´ baseline FVIII levels and timepoint, number, frequency and dosage of infusions of RURIOCT. Evaluation of intra- and postoperative hemostatic efficacy via assessment of bleedings during surgeries and occurrence of postoperative bleedings.
Findings:
Case 1: Endobronchial biopsy under general anesthetic in a 27-year-old male patient. A loading dose of 4000 IU (49 IU/kg) RURIOCT resulted in an increase of factor VIII levels from 13% to 145% (measured 1 hour after administration). The patient was discharged from the hospital on postoperative day 3 without any bleeding complications. Total perioperative factor VIII consumption was 13 000 IU.

Case 2: Thoracoscopy including biopsy under local anesthetic in an 88-year-old multimorbid male patient. A loading dose of 4000 IU (51 IU/kg) RURIOCT resulted in an increase of factor VIII levels from 9% to 145% (measured 1 hour after administration). Factor replacement was discontinued on postoperative day 3 without any bleeding complications. Total perioperative factor consumption was 10 000 IU.
Case 3: Arthrorisis with a screw for flatfoot treatment of the right food in a 12.5-year-old male patient. A loading dose of 3000 IU (41 IU/kg) RURIOCT resulted in an increase of factor VIII levels from 18% to 129% (measured 1 hour after administration). The patient was discharged on postoperative day 4 without any bleeding complications. Total factor consumption was 11 000 IU.
RURIOCT has shown an immediate and strong increase in FVIII levels following the administration of the preoperative loading dose in all three patients. Intraoperative blood loss was comparable to that in non-hemophilic patients. None of the three patients experienced post-operative bleedings. Postoperative factor VIII replacement was managed with twice daily infusions of RURIOCT in case 1 and case 3 respectively with once daily infusions in case 2.
Conclusion: RURIOCT presents a good and effective option for perioperative factor VIII replacement in patients with mild hemophilia A with the advantage of less frequent infusions due to its extended half-life.

P03-8

Evaluation of GGCX mutations effect on specific VKD proteins responsible for VKCFD1 phenotype

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Introduction: Vitamin K Dependent Coagulation Factor Deficiency type 1 (VKCFD1) is a rare hereditary bleeding disorder caused by mutations in ɣ-glutamyl carboxylase (GGCX) which is often characterized by additional non-bleeding phenotypes including skin hyper-laxity. GGCX ɣ-carboxylates vitamin K dependent (VKD) proteins including coagulation factors f.e. FII, FX, Protein C (PC) and non-hemostatic proteins f.e. Osteocalcin (BGLAP) and Gla Rich Protein (GRP/UCMA).
The aim of this study is to characterize the effect of all reported 26 GGCX mutations on VKD proteins to evaluate the effective dose of vitamin K (K) needed for treatment and to determine the functional binding regions.
Methods: A GGCX knockout HEK293T cell line was generated by CRISPR/Cas9 technology. The cDNAs of GGCX together with F2, F10, PC, BGLAP or GRP were cloned into a bicistronic vector. Cells were transfected with GGCX wild-type and mutants and treated with different K concentrations to determine ɣ-carboxylation by ELISA. GGCX antigen levels were measured to normalize the ɣ-carboxylation. Statistical analyses were performed using GraphPad Prism 8 software to impose dose-response curves in order to identify half maximal effective concentrations (EC50). A GGCX in silico model was established to look for the distribution of mutations in the protein and the cellular localization of the expressed variants was determined by immunostaining.
Results: Elevated K concentration increases ɣ-carboxylation of F2, F10, PC, BGLAP, and GRP for R204C, V255M, S284P, R476H. Mutations like R83W, W157R, L394R shows slight increase in ɣ-carboxylation whereas M174R, S300F shows no recovery. Certain mutants show differential effect on ɣ-carboxylation for different VKD proteins as R485P can restore ɣ-carboxylation for F10, PC, and GRP but not for BGLAP and F2, which is vice versa for G558R. Most of these variants in the GGCX in silico model are located within or close to functional binding domains. This is further validated by immunostaining where these variants co-localize with the ER thus indicating disruption of functional binding rather than protein degradation.
Conclusion: Our data suggests that patients harboring R204C, V255M, S284P, and R476H will show reversible coagulation phenotypes where therapy with K will lead to normal coagulation. Residual clotting factor activities can be achieved for R83W, W157R, and L394R. Patients with mutation M174R, S300F will never reach physiological coagulation and ɣ-carboxylation of other VKD proteins indicating that catalytic activity is abolished. Hence, this study of all GGCX mutations will help to determine specific proteins responsible for particular VKCFD1 phenotype.

P03-9

Five novel mutations resulting in hypofibrinogenemia

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Objectives: Inherited hypofibrinogenemia is the most common form of fibrinogen (FG) deficiency caused by various mutations within (*FGA*, *FGB* and *FGG*) genes, encoding three FG chains. Most of the patients with hypofibrinogenemia are asymptomatic. Nevertheless in stress situations (e.g. surgery) bleeding complications may develop.
Methods: Coagulation tests and thrombin generation assay (TGA) were executed using standard procedures. FG genes were screened using direct genomic DNA sequencing. The structural-functional implications of the missense mutations were analysed in silico.
Results: In total five novel mutations were found comparing two missense mutations (c.742T>A, p.[Trp248Arg];[=] in an *FGA* gene and c.89C>T, p.[Thr30Ile];[=]) in an *FGG* gene, one splice site (c.[490+1G>A];[=]) in an *FGB* gene, one small deletion (c.[637delT];[=]) in an *FGG* gene and one small insertion (c.[1244+2_1244+3insT];[=]) in an *FGB* gene. Silico analysis showed a strong evidence for p. [Trp248Arg] mutation causality for hypofibrinogenemia. All (n=7) affected individuals (five males and two females) showed reduced functional and immunological FG values (below 110 mg/dl) and prolonged thrombin time (TT). No significant differences in the TGA values have been found in a family carrying the splice site mutation in the *FGB* gene. FG concentrate was used to stop bleeding only once in a female with ovarian cyst excision.
Conclusions: All identified mutations except a missense mutation in the *FGG* gene (p.Thr30Ile) may cause hypofibrinogenemia.

P03-10

Genetic background of factor XI deficiency

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Scientific research question: Factor XI deficiency is an autosomal mild bleeding disorder. Bleeding occurs mainly after injury or trauma. Activated Factor XI contributes to the coagulation cascade by activating Factor IX through its serine protease activity. The F11 gene consists of 15 exons and 14 introns. Our aim was to analyse the molecular defect underlying Factor XI deficiency in a cohort of 146 patients.
Methodology: In 146 patients F11 gene was analysed by Sanger Sequencing. In cases without mutation, MLPA was performed to detect large deletions or duplications. The characterisation of the variants was performed with in silico evaluation tools, including Polyhen-2, SIFT, Panther and Pmut. Potential splice site changes were analysed by NetGene2, Human Splicing finder and Fruitfly splicing prediction tool. Potential missense changes were mapped to the X-Ray structure of Factor XI by using molecular imaging tool.
Findings: We detected 59 different mutations in F11 gene. Thereof 43 variants were previously described in the Human Genome Mutation database (HGMD) as disease causing mutations. Twelve variants were novel, 4 were described in the Polymorphism databank of the National Center for Biotechnology Information (dbSNP, NCBI) with very low frequency (0.00001). The 12 novel and the 4 dbSNP variants were further analysed to classify the potential impact for disease. Of these 16 variants 11 were potentially missense changes, 2 nonsense changes, 2 frameshift changes and 1 variant was located in the intron, near to the exon intron boundary (potentially splice site changes). Two of the missense changes were classified as variants of unknown significance, 2 as neutral and 7 as potential missense mutations with pathogenic effect. The variant located near the exon intron boundary was predicted as splice site mutations by using three in silico methods. The 2 frameshift and 2 nonsense changes were classified as pathogenic due to the nature of the mutations (no prediction tools available).
Conclusion: In silico methods and molecular graphic imaging are useful tools to predict the effect of genetic variants on the protein function or structure and further to predict the pathogenic effect of certain variants. Nevertheless these methods cannot replace in vitro analysis by laboratory experiments, phenotypic studies of patients and co-segregation analysis within families.

P03-11

The discriminatory power of bleeding assessment tools in adult patients with a mild to moderate bleeding tendency

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Background: Bleeding assessment tools (BATs) have been developed to quantify bleeding severity. Their ability to predict for the diagnosis of a bleeding disorder has not been thoroughly investigated.
Objectives: To evaluate the ability of the Vicenza BAT and the ISTH BAT to distinguish patients with an established bleeding disorder from those with bleeding of unknown cause (BUC).
Patients/Methods: Three-hundred fifty-nine patients (228 with BUC, 64%) from the Vienna Bleeding Biobank were assessed in this study.
Results: The bleeding scores were similar in patients with an established diagnosis of a bleeding disorder compared to patients with BUC. Both BATs had a low sensitivity and specificity for the diagnosis of a bleeding disorder with areas under the receiver operating characteristic (ROC) curves of 0.53 (95% confidence interval 0.47-0.60) for the Vicenza BAT and 0.52 (0.46-0.59) for the ISTH BAT. In terms of specific diagnoses, both scores were most accurate in diagnosing von Willebrand disease (VWD, areas under the ROC curve; Vicenza BAT 0.67 (0.45-0.90); ISTH BAT 0.70 (0.50-0.90)).
A separate evaluation of different bleeding symptoms in patients who had undergone surgery and tooth extraction revealed that postpartum bleeding and bleeding from small wounds was predictive for diagnosing a MBD in multivariable analysis.
Conclusions: The Vicenza- and the ISTH BAT have a low ability to distinguish patients with an established bleeding disorder from those with BUC.

P03-12

Increased age is associated with a higher bleeding severity in patients with mild to moderate bleeding disorders: results from the Vienna Bleeding Biobank (VIBB)

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Scientific research question: Patients with mild to moderate bleeding disorders (MBDs) present with different clinical symptoms such as epistaxis, easy bruising or bleeding after invasive procedures. Quantification of bleeding severity can be assessed with bleeding scores. A definition for abnormal scores exists for children and adults, respectively. In adults no association of the bleeding score with age was found in healthy individuals (Elbatarny et al., Haemophilia. 2014, 20: 831-835). Data on the influence of increasing age on the bleeding score in adults with a bleeding tendency are scarce. Therefore, we aimed to investigate the association between bleeding severity and age in a large and well characterized cohort of patients with MBDs.
Methodology: Data from the Vienna Bleeding Biobank (VIBB), a prospective single-center cohort study including adult patients with a mild to moderate bleeding tendency, were analyzed. Severity of bleeding was quantified by the standardized Vicenza bleeding score (BS, Rodeghiero F. et al., JTH, 2005, 3: 2619-2626) in all 639 patients. In 359 patients the ISTH-BAT (Rodeghiero F. et al., JTH, 2010, 8: 2063-2065) was additionally available.
Findings: Until April 2019, 639 patients (80.3 % female) were included in the VIBB, of whom in 215 (33.6%) a bleeding disorder was diagnosed and 424 (66.4%) were patients with bleeding of unknown cause (BUC). The most common diagnoses were possible (14.4%) and definite platelet function defects (PFD, 5.6%), followed by low von Willebrand factor (VWF, 5.8%) and definite von Willebrand disease (VWD, 2.8%). The median age [interquartile range] was 40 [28-53] years, the median Vicenza BS and ISTH BAT score was 5 [4-8] and 6 [4-9]. Age, as a continuous variable, correlated only weakly with the Vicenza BS ($r=0.244$, $p< 0.001$) and ISTH-BAT ($r=0.278$, $p< 0.001$).
When we dichotomized patients by age, older patients defined by age (i) higher than the 25th percentile ($\geq Q_{0,25}$), (ii) higher than the median ($\geq Q_{0,5}$) and (iii) higher than the 75th percentile ($\geq Q_{0,75}$) overall had a higher Vicenza BS as well as ISTH BAT score (Table 1a).

Less clinical bleeding manifestations were reported in younger patients (age < $Q_{0,5}$), irrespective of the BS (Table 1a). In women over 40, menorrhagia (56.1% and 67.4%; p=0.013) was more frequently reported.
Moreover, we analyzed subgroups of patients with an established diagnosis and those with BUC. In both groups older patients (≥$Q_{0,5}$) had significantly higher bleeding scores (Table 1b).

Conclusion: Older patients with MBDs have an increase in the bleedings scores, independent of the diagnosis of a bleeding disorder. Most probably this is based on an increased number of hemostatic challenges during life, but might also result from additional factors, which might increase the bleeding severity with age. Thus, also in adults, age should be taken into account when bleeding severity is assessed.

Table 1a Comparison of bleeding severity and number of clinical bleeding manifestations between different age groups.

	Age group A	Age group B	*p value*
	< 28a (<$Q_{0,25}$)	≥ 28a (≥$Q_{0,25}$)	
Vicenza BS, median [IQR]	4 [3-6]	6 [4-8]	***<0.001***
ISTH-BAT, median [IQR]	5 [3-7]	7 [4-9]	***<0.001***
Clinical bleeding symptoms, m [IQR]	2 [1.75-3]	3 [2-5]	***<0.001***
	< 40a (<$Q_{0,5}$)	≥ 40a (≥$Q_{0,5}$)	
Vicenza BS, median [IQR]	5 [3-6.75]	6 [4-8]	***<0.001***
ISTH-BAT, median [IQR]	5 [3-7]	7 [5-9]	***<0.001***
Clinical bleeding symptoms, m [IQR]	3 [2-4]	4 [2-5]	***<0.001***
	< 53a (<$Q_{0,75}$)	≥ 53a (≥$Q_{0,75}$)	
Vicenza BS, median [IQR]	5 [3-7]	6 [4-9]	***<0.001***
ISTH-BAT, median [IQR]	6 [4-8]	7 [4-9.25]	***0.007***
Clinical bleeding symptoms, m [IQR]	3 [2-4]	4 [2-5]	***<0.001***

Table 1b Comparison of bleeding severity and number of clinical bleeding manifestations between different age groups in patients with established diagnosis and those with BUC.

	< 40a (<$Q_{0,5}$)	≥ 40a (≥$Q_{0,5}$)	*p value*
Patients with established diagnosis			
Vicenza BS, median [IQR]	5 [3-7]	7 [5-9]	***<0.001***
ISTH-BAT, median [IQR]	5 [3-8]	8 [6-11]	***<0.001***
Clinical bleeding symptoms, n [IQR]	3 [2-4]	4 [3-5]	***<0.001***
Patients with BUC			
Vicenza BS, median [IQR]	4 [3-6]	6 [4-8]	***<0.001***
ISTH-BAT, median [IQR]	5 [3-7]	7 [5-9]	***<0.001***
Clinical bleeding symptoms, m [IQR]	3 [2-4]	4 [2-5]	***<0.001***

P04 Platelet biology and pathophysiology I

P04-1

Insights into autoimmune Heparin-induced thrombocytopenia

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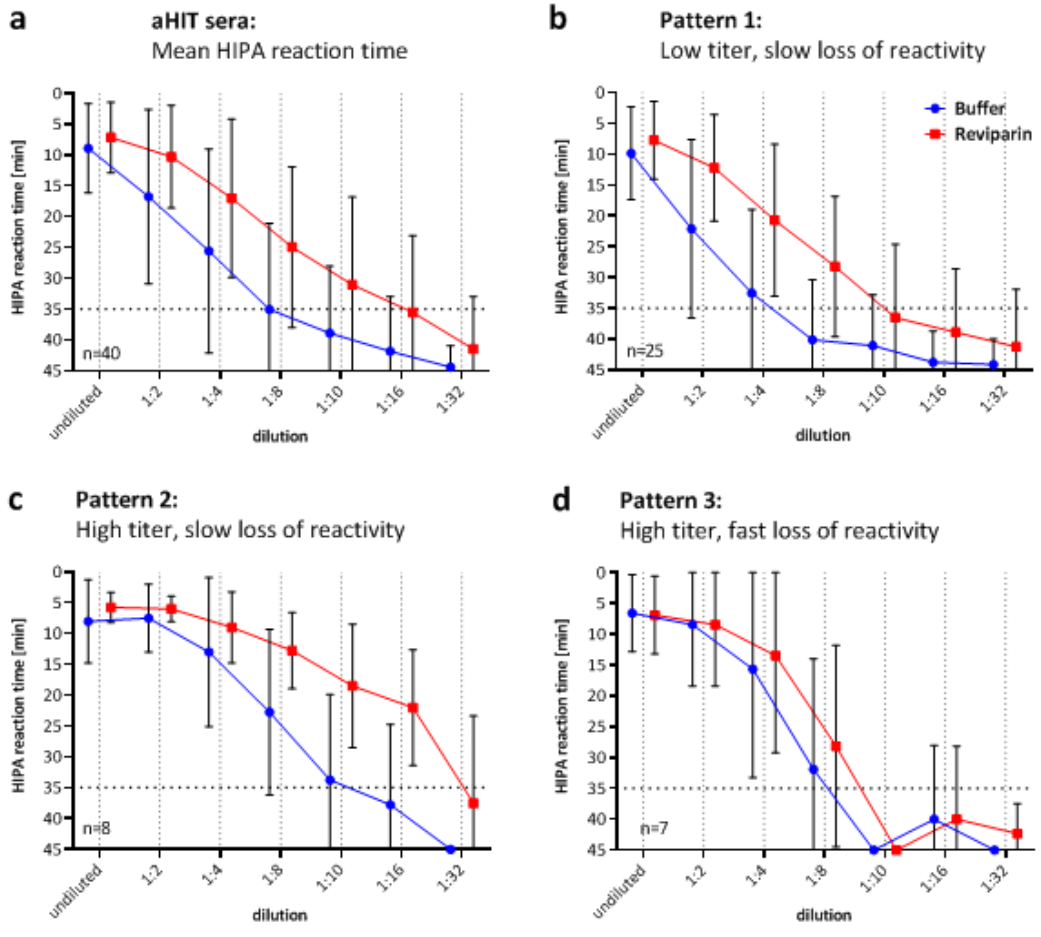
Objectives: Heparin-induced thrombocytopenia (HIT) is an adverse drug effect. HIT antibodies recognize complexes of platelet factor 4 (PF4) and heparin. There are three different groups of HIT-antibodies; group 1 antibodies do not activate platelets in the functional Heparin Induced Platelet Activation test (HIPA). Group 2 antibodies activate platelets only in the presence of heparin (typical clinically relevant HIT antibodies). Group 3 antibodies activate platelets even in the absence of

heparin. They occur in patients with autoimmune HIT (aHIT), which can manifest without heparin treatment. aHIT has only been recently recognized. We assessed the prevalence of aHIT and further characterized group 3 (aHIT) anti-PF4/heparin antibodies.

Methods: The prevalence of sera with aHIT reactivity pattern in 7,510 consecutive patient samples referred to our laboratory with suspected diagnosis of HIT between 2017 and February 2019 was assessed using laboratory files. Patient sera were incubated with washed platelets of healthy donors under different conditions: buffer (control); low dose heparin (reviparin 0.2 aFXaU/mL); high dose heparin (UFH 100 IU/mL). Platelet aggregation was visually detected every 5 min up to 45 min. Confirmed aHIT sera were step-wise diluted with AB-serum of a healthy donor (undiluted, 1:2, 1:4, 1:8, 1:10, 1:16, 1:32) and subsequently analyzed in the HIPA test.

Results: Of 7,510 referred sera, 5,226 (69.6%) were ELISA- & HIPA-; 1,196 (15.9%) ELISA+ & HIPA-; and 998 (13.3%) sera were ELISA+ & HIPA+. 86 sera (1.15% of all referred sera; 3.92% of all ELISA+ sera; 8.62% of all HIPA+ sera) showed the typical aHIT (group 3) pattern in the HIPA. Of the 86 group 3 sera, 66 sera were further assessed. Among 40 of them the group 3 pattern was confirmed during retesting. When these sera were serially diluted, different patterns of platelet activation in the presence or absence of reviparin were found: Serial dilution showed diminished reactivity in buffer compared to reviparin (Fig.1a). In 33 sera, reactivity decreased gradually with each dilution step. Reduced activity in already low dilutions was observed for 25 sera (low titer antibodies; Fig. 1b). In 8 sera reactivity was reduced only in high dilutions (high titer antibodies; Fig. 1c). In 7 sera, however, reactivity diminished quickly after second dilution and similarly for buffer and reviparin (Fig. 1d).

Conclusions: The prevalence of group 3 reactivity pattern is 1.15% for all referred samples; 3.92% for all ELISA+ samples and 8.62% for all HIPA+ samples. This is higher than clinically observed aHIT. Most sera contain a mixture of heparin-independent (group 3) and heparin-dependent (group 2) platelet activating antibodies. Group 3 antibodies are present at lower concentrations/titers than group 2 antibodies and seem to be also occurring in some patients with typical, heparin-dependent HIT.



[Fig.1: Mean HIPA reaction time of serially diluted autoimmune HIT samples]

P04-2

Autoantibody mediated changes in megakaryocytes glycan pattern: Potential impact on thrombopoiesis in immune thrombocytopenia

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Scientific question: The low platelet (PLT) count observed in patients with immune thrombocytopenia (ITP) is due to multiple alterations of the immune system leading to increased PLT destruction as well as impaired thrombopoiesis. Desialylation, the cleavage of sialic acid on PLT has been recently identified to contribute to the increased PLT destruction in ITP. However, the consequences of desialylation on PLT production remain still elusive. In this work, we investigated the impact of autoantibodies (AAbs) induced desialylation on MKs' differentiation as well as PLT production.
Methodology: Sera from well-characterized ITP patients or sera from healthy donors were incubated with CD34-derived MKs and sialylation status was analyzed via flow cytometry. In addition, maturation status and pro-platelet (proPLT) generation were investigated. To elucidate the exact mechanism of impaired thrombopoiesis, adhesion assays on different surface coatings were performed.
Findings: 8 out of 10 ITP sera induced a significant cleavage of sialic acid on the MK surface compared to control sera (mean fold-increase of ß-galactose (FI): 2.18, range: 1.03-3.66, p=0.012; and FI of N-acetylglucosamine: 1.25, range: 1.01-1.7, p=0.027). Next, the proPLT formation of MKs was assessed in the presence of desialylating AAbs. We observed a dramatic reduction of proPLT forming MKs compared to control (% of proPLT forming MKs mean±standard error mean [SEM]: 42±9% vs. 100±0, p=0.004). Interestingly, MKs incubated with desialylating AAbs showed a significant impairment in the adhesion ability on fibrinogen and von-Willebrand factor compared to non desialylating AAbs (% adherent cells/field mean±SEM: 58±8% vs. 88±8%, p=0.049 and 47±4% vs. 79±8%, p=0.023, respectively). Most importantly, the preincubation with a sialidase inhibitor rescued the ability of proPLT formation in the presence of desialylating AAbs compared to buffer (% of proPLT forming MKs mean±SEM: 42±12% vs. 90±9%, p=0.033, respectively).
Conclusion: Our findings suggest that AAbs from ITP patients are able to induce cleavage of sialic acid on the MKs' surface causing an impairment of their ability to interact with different extracellular matrix proteins. Moreover, we demonstrate that ITP AAbs-mediated desialylation interferes with proPLT generation from MKs which is a key step of thrombopoiesis.

P04-3

Emo-test HIT Confirm® - a flow cytometer based functional assay for detection of platelet activating antibodies in heparin induced thrombocytopenia

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Scientific research question: Heparin-induced thrombocytopenia (HIT) is caused by platelet-activating antibodies that recognize platelet factor 4/heparin (PF4/hep) complexes. But only the platelet-activating antiplatelet PF4/hep antibodies cause HIT. Functional assays like the heparin-induced platelet activation assay (HIPA) are technically challenging and limited to specialized laboratories. In contrast, flow cytometers are commonly used in routine laboratories.
In this study we investigated whether a flow cytometry based assay is suitable for the detection of PF4/heparin-dependent platelet-activating antibodies.
Methodology: Blood samples from 390 consecutive patients with suspected were investigated using the EMO-test HIT Confirm® assay (coachrom diagnostica, Maria Enzersdorf, Austria), a PF4/hep IgG enzyme immunoassay (EIA) from (coachrom diagnostica, Maria Enzersdorf, Austria) and compared to the in-house heparin induced platelet aggregation (HIPA) test.
Findings: 390 sera were included in the study, 164 sera tested positive in the IgG EIA and 33 induced platelet activation in the HIPA. In the Emo-test HIT Confirm®assay, 112 sera revealed positive results. The majority (n=89) revealed negative HIPA results. Compared to the HIPA and HIT Confirm® assay revealed consistent results in 23 patients, ambiguous results in 2 patients and in 8 patients sera were false negative in the HIT Confirm® assay. According to the false negative patient sera the HIT Confirm® assay showed a low sensitivity with 69.70% with a slight better specificity of 75.35% compared to the EIA (sensitivity 100%, specificity 63.31).
Conclusion: Functional assays are an important step to distinguish between platelet activating

antibodies and non-activating antibodies. Emo-test HIT Confirm® assay miss a remarkable proportion of sera in diagnosis of HIT and might not improve diagnosis of HIT without additional testing.

P04-4

Potential and limitations of the new $P2Y_{12}$ inhibitor, cangrelor, in preventing heparin-induced platelet aggregation during cardiac surgery: an *in vitro* study

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Background: Heparin-induced thrombocytopenia (HIT) can put cardiac surgery patients at a high risk of lethal complications. If anti-PF4/heparin antibodies (anti-PF4/Hep Abs) are present, two strategies exist to prevent intraoperative aggregation during bypass surgery: firstly, using an alternative anticoagulant, and secondly, using heparin combined with an antiaggregant. The new $P2Y_{12}$ inhibitor, cangrelor, could be an attractive candidate in this setting; several authors have reported its successful use. The present *in vitro* study evaluated cangrelor's ability to inhibit heparin-induced platelet aggregation in the presence of anti-PF4/Hep Abs.
Methods: Platelet-poor plasma (PPP) from 30 patients with functional HIT-Abs was mixed with platelet-rich plasma (PRP) from 5 healthy donors. Light transmission aggregometry was used to measure platelet aggregation after adding 0.5 IU ml^{-1} of heparin (HIT) to the plasma, and this was compared with samples spiked with normal saline (control) and samples spiked with cangrelor 500 ng ml^{-1} and heparin 0.5 IU ml^{-1} (treatment).
Results: Heparin 0.5 IU ml^{-1} triggered aggregation in 22 of 44 PPP-PRP mixtures, with a median aggregation of 85.9% (IQR 69.2-90.9). The median aggregation of these 22 positive samples' respective control tests was 22.1% (IQR 15.9-29.7) ($p < 0.001$). Median aggregation in the treated samples was 28.5% (IQR 19.5-51.9), significantly lower than the HIT samples ($p < 0.001$) but higher than the control samples ($p < 0.05$) (Figure 1). Cangrelor inhibited heparin-induced aggregation by a mean 73.4 ± 34.0%. Cangrelor only reduced heparin-induced aggregation by more than 95% in 10 of the 22 positive samples (45%). Cangrelor inhibited heparin-induced aggregation by less than 50% in 5 of the 22 positive samples (22%) and by less than 10% in 3 samples (14%).
Conclusion: This *in vitro* study found that cangrelor was an unreliable inhibitor of heparin-induced aggregation in the presence of anti-PF4/Hep Abs. We conclude that cangrelor should not be used as a standard antiaggregant for cardiac patients affected by HIT during surgery. Unless cangrelor's efficacy with a particular patient has been confirmed in a pre-surgery aggregation test, other strategies should be chosen.

P04-5

Flow cytometric assessment of AKT signaling in platelet activation - an alternative diagnostic tool for characterization of platelets in antibody mediated diseases like HIT and ITP

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Scientific research question: The assessment of the platelet activation status is essential to predict the clinical outcomes in antibody mediated platelet diseases. Recently, the phosphrylasation of AKT in platelets have been reported to be correlated with platelet activating antibodies in patients with heparin-induced thrombocytopenia (HIT). In this study we analyzed AKT mediated signaling in platelet after stimulation with a special focus on anti-platelet factor 4/heparin (PF4/heparin) mediated activation.
Methodology: Platelet rich plasma obtained by the centrifugation of citrated blood from healthy volunteers was activated with arachidonic acid, Thrombin Receptor-Activating Peptide-6 (TRAP-6), collagen, Adenosine Diphosphate (ADP), collagen related peptide (CRP) and epinephrine. In addition, anti-PF4/heparin-antibodies from patients who were suspected to have HIT were tested. After incubation, samples were fixed and permeabilized before staining with a monoclonal antibody against the phosphorylated variant of Akt. The phosphorylation status of AKT was measured using flow cytometer.
Findings: Healthy volunteers showed a reproducible phosphorylation of AKT. In comparison to non-activated platelets, we documented an increase in pAKT-expression with all agonists within 15 minutes after stimulation. The highest phosphorylation was observed by TRAP-6 and CRP. Sera containing platelet activating anti- PF4/heparin antibodies from clinical confirmed HIT patients induced significant increase in AKT-phosphorylation.

Conclusion: Activation of the AKT-signal pathway by different agonists as well as by platelet reactive antibodies can be detected using flow cytometer. The methods could be used to assess platelet function in patients with antibody-mediated platelet disease and might have diagnostic value in predicting the clinical outcomes.

P04-6

Autoantibody mediated changes in platelets sialic pattern: Potential impact on platelet functionality and lifespan in immune thrombocytopenia

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Scientific research question: Immune thrombocytopenia (ITP) is a bleeding disorder mediated by autoantibodies (AAbs) that are targeting different glycoproteins (GPs) on the platelet (PLT) surface. Desialylation, the loss of sialic acid from PLT GPs, has been reported to contribute to the increased PLT destruction in ITP. In this work, we investigated the impact of human ITP AAb mediated desialylation on functionality and lifespan of human platelets.
Methodology: AAbs from well- characterized ITP patients were first screened for desialylation using a lectin binding assay. After incubation of PLTs with ITP or control sera, glycan changes were analyzed by flow cytometry (FC). To investigate the impact of desialylation on PLTs life- span, the NOD/SCID mouse model was used. The activation of the apoptotic pathway was analysed measuring changes in the inner mitochondrial membrane potential (TMRE assay), by FC. To evaluate the impact of desialylation on PLTs´ function, an adhesion assay with different surface coatings was performed.
Findings: 100 ITP sera were investigated in this study. 28 sera induced a significant increase in the exposure of ß-galactose on the PLT surface compared to control sera (mean Fold-increase (FI): 3.50, range: 1.79- 13.61, p=0.0001). In addition, 21 sera caused higher exposure of N-acetylglucosamine on the surface of PLTs (FI: 2.41, range: 1.54- 5.47, p=0.0001). Increased exposure of ß-galactose and N-Acetylglucosamine mediated by GP IIb/IIIa AAbs was significantly reduced by the use of an anti-CD32 (FcγRIIa) monoclonal antibody (% desialylation mean±SEM: 100±0% vs. 52±11%, p=0.011; 100±0% vs. 60±9%, respectively). Injection of desialylating AAbs resulted in accelerated clearance of human PLTs from the mouse circulation which was significantly reduced by a specific neuraminidase inhibitor that prevents desialylation on the PLT surface (survival of human PLTs after 5h: 28±5%, range 20-46% vs. 45±3%, range 36-54%, p=0.019, respectively). Moreover, ITP AAb induced desialylation led to impaired PLT adherence on fibrinogen and von- Willebrand factor, compared to non- desialylating AAbs (% of adherent cells/field mean±SEM: 34±7% vs. 74±8%, p=0.002; 26±2% vs. 67±5%, p=0.003, respectively). No correlation between the increased exposure of ß-galactose as well as N-acetylglucosamine and the induction of PLT apoptosis was observed (r=-0.33, p=0.351; r=-0.344, p=0.331, respectively).
Conclusion: Our findings suggest that desialylation is not restricted to ITP AAbs of a single GP specificity and that desialylation impairs PLTs survival in an apoptotic independent pathway. Most importantly, our data demonstrate that AAb mediated desialylation contributes to the pathophysiology of ITP beyond thrombocytopenia, namely increased bleeding tendency.

P04-7

Diagnosis and ISTH-BAT of patients with mild platelet function disorders plus type 1 von Willebrand disease

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Scientific research question: Inherited platelet function disorders (PFD) are a heterogeneous group of rare bleeding diseases in terms of frequency, bleeding severity, platelet dysfunction and platelet count/volume. However, the prevalence of PFD is under-estimated due to limited diagnostic potential. In contrast to severe and syndromic PFD, mild and non-syndromic PFD are associated with mild to moderate bleeding diathesis, but may occur with life-threatening complications upon surgery or trauma, and their diagnosis is still challenging. Here, the routinely used platelet function analyzer (PFA) and light transmission aggregometry (LTA) often lack specificity and sensitivity, respectively. In this prospective study, we aimed to identify phenotypically mild non-syndromic PFD with and without type 1 von Willebrand disease (VWD-1) by specialized flow cytometric assays in consideration

of the patient's bleeding-history assessed with the Bleeding Assessment Tool (BAT) of the ISTH.
Methodology: Adult patients (≥ 18 years) from our specialized outpatient clinic were included in this study when diagnosed with "mild-moderate bleeding tendency of unknown origin" or VWD-1, irrespectively of the platelet count and repeated laboratory results of LTA and PFA. Patients with pregnancy, acquired PFD, coagulopathy, type 2, 3, acquired VWD, syndromic PFD and surgery prior analysis were excluded. The patient's bleeding-history was routinely scored using the bleeding score (BS) of the ISTH-BAT. Specialized flow cytometric assays were applied to quantify major platelet surface receptors in citrated whole blood, fibrinogen binding/activation of integrin αIIbβ3, granule exocytosis, mepacrine staining in diluted platelet-rich plasma ex vivo and in response to ADP, epinephrine, thrombin and convulxin in vitro.
Findings: Between September 2018 and February 2019, 54 patients with suspected PFD were included, 20 patients with additional vWD-1 (37%). Patients with VWD-1 [19 females (35%), 1 male (2 %)] presented with a median BS 12, non-VWD-1 [28 females (52%), 6 males (11%)] with a median BS 8. Nine patients (16.7%; 6 females, 3males) were diagnosed as PFD with a median BS 8. 5 patients with PFD and without VWD1 (9.3%) were diagnosed with δ-SPD (+/- thrombocytopenia), Gi-like or isolated epinephrine response defects (median BS 10); 4 patients with VWD-1 (7.4%) were diagnosed with δ-SPD (+/- thrombocytopenia) and isolated ADP-response defect (median BS 7). However, the genetic causes have to be identified for these PFD.
Conclusion: Specialized platelet function methods, such as flow cytometry, are essential for the correct diagnosis of patients with "mild bleeding tendency of unknown origin" including thrombocytopenia or "mild VWD-1". Patients with mild PFD plus VWD-1 do not necessarily present with a higher ISTH-BAT bleeding score than mild PFD without VWD-1, but the diagnosis is important for adequate treatment.

P04-8

Assessment of platelet function after shear stress simulation using an ex vivo model for extracorporeal circulation

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Scientific research question: Extracorporeal circulation (ECC) is used in many intensive care procedures. The management of bleeding and thromboembolic events in ECC patients is still challenging due to the lack of information on the pathophysiological changes in platelet function during the procedure. In this study, platelet activation status was investigated using a novel whole blood flow cytometry-based method after shear stress simulation using an *ex vivo*closed-loop ECC model.
Methodology: A rotation system (chandler loop) was used to mimic shear stress conditions in the ECC procedure. In brief, two polyvinyl chloride tubes coated with heparin were filled with citrated or heparinized whole blood (WB) or platelet rich plasma (PRP) samples from healthy donors. To stimulate shear stress tubes were rotated in a temperature controlled water bath (99 rounds per minute, one hour, 37°C).Samples were obtained before and after rotation for flow cytometer analyses to assess platelet function. They were incubated with buffer, ADP or TRAP and subsequently double-stained for CD41, P-Selectin (CD62P) and CD63, respectively.
Findings: In experiments performed with citrated whole blood samples we observed reduced alpha granule release in response to ADP and TRAP after ECC-simulation compared to steady-state samples stored for the same time (median fold increase [FI] of CD62P: after ADP stimulation 0.96 vs. 1.35, p=0.0006; and after TRAP stimulation 1.14 vs. 2.19, respectively, p=0.0006). Similar results were obtained with PRP samples (median FI in CD62P: after activation with ADP 0.96 vs. 1.22, p=0.0064; after activation with TRAP, 0.93 vs. 1.20, for blood samples obtained from the chandler loop vs. control samples, respectively, p=0.0175). Furthermore, significant decrease in delta-granule response to TRAP was observed in WB (median FI in CD63: after TRAP activation 1.01 vs. 1.23, for samples obtained from the chandler loop vs. control samples, respectively, p=0.0262). The changes in delta granule release did not reach a statistical significance after activation with ADP. Significant reduction was observed in alpha granule release in heparinized blood samples obtained from chandler-loop compared to control samples (median FI in CD62P: after activation with TRAP 1.06 vs. 3.06, p=0.0064). In addition, impaired delta granule release was observed after ECC simulation. However, no significant difference was observed in delta granule release (median FI in CD63: after activation with ADP 1.09 vs. 1.10, p=0.9272; with TRAP 1.12 vs. 1.32 respectively, p=0.0793).
Conclusion: Pathological blood flow patterns and pressure gradients that are simulated using our

closed-loop model mimic shear stress produced during ECC. Our data confirm that increased shear stress conditions can cause dysfunction in delta as well as in alpha granules. Ongoing studies using this model are focusing on the signal transduction pathways leading to these functional changes in platelets.

P04-9

Immature platelet fraction as screening parameter for diagnostics HIT

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Background: Thrombocytopenia is one of the most common abnormalities seen in the blood count. Measurement of immature platelet fraction (IPF) in hematology analyzers is a promising tool to distinguish the cause of thrombocytopenia. The primary goal of using this parameter is to distinguish between bone marrow aplasia and immune forms of thrombocytopenia, which are characterized by increased platelet destruction. However, the results of IPF measurements in patients with suspected HIT are also interesting.
Methods: In this study, we analyzed% IPF in a potential cohort of patients with newly diagnosed thrombocytopenia (Plt below 100 x 10 9 / l). Measurement of immature platelets is based on the principle of flow cytometry. Upon perforation of cell membranes with reagents, nucleic acids are stained with the fluorescent dye oxadine and immature platelets are detected as particles with higher fluorescence intensity due to higher laser beam scattering on the stained nucleic acids.
In a group of 34 patients out of a total of 511 (already explained causes of thrombocytopenia) with the IPF% test, HIT was also considered a possible cause of thrombocytopenia. For this reason, a 4T score was determined, and patients and patients at moderate risk of HIT were screened for antibodies.
Determination of HIT antibodies was performed by aggregation methods on a Multiplate according to the Morell-Kopp method.
Result: In our small group of patients, we found that higher IPF values were (21.8% vs. 13.6%) in patients with HIT antibody positivity than in patients without antibodies.
Conclusions: IPF appears to be a promising parameter to complement the 4T scores prior to anti-HIT screening tests.

P04-10

Platelets express a fully functional proteasome system, which differs from the proteasome in other immune cells

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Scientific research question: The proteasome and especially the immunoproteasome is a major proteolytic component contributing to immune cell function. Platelets are increasingly recognized as part of the immune system and they express at least some proteins of proteasome complexes that are responsible for protein degradation and regulation of signal transduction processes. The aim of this study was to further characterize the proteasome and its functional role in human platelets.
Methodology: On the protein level we identified the catalytically active standard and immunoproteasome subunits in freshly isolated platelets as well as in stored platelets by immunoblot. Proteasome components were purified from washed platelets by cell lysis and their proteasomal activity determined by fluorogenic peptide substrates. Protein transcription in platelets during storage was inhibited by puromycin.
Findings: We identified both, the standard proteasome subunits β1/delta, β2/Z and β5/MB1 and the immunoproteasome subunits β1i/LMP2, β2i/MECL-1 and β5i/LMP7 in platelets of >10 healthy donors. We found chymotrypsin-like, caspase-like and trypsin-like proteasome activity in freshly prepared platelets, which was maintained during 7 days of storage, even when protein synthesis was inhibited.
Conclusion: The standard proteasome and immunoproteasome systems are fully functional in platelets. In contrast to other immune cells, the platelet proteasome has a 20 times longer half-life. The ubiquitin-proteasome system is crucial for maintaining protein homeostasis. The high stability of the platelet proteasome might foster the immunological functions of platelets by stabilization/destabilization of proteins involved in signaling and together with HLA class I molecules expressed on platelets the role of platelet in immune response.

P04-11

Phosphorylation and activation of Pannexin-1 upon platelet activation and thrombus formation

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Scientific research question: Pannexin-1 (Panx1) are transmembrane proteins, which are ubiquitously expressed in many species. They form hexameric structures of single membrane channels, function as ion channels for small molecules and were associated with many inflammatory diseases. Until now, the function of Panx1 channels on platelets remains unclear. Recently it was shown, that platelet activation induces opening of Panx1 channels on platelets and thereby amplifies Ca^{2+} influx and aggregation by activation of the P2X1 channel. The aim of our study is to investigate the activation mechanisms of Panx1 channels in platelets and their impact on haemostasis and thrombus formation.
Methodology: Analysis of activation patterns of Panx1 phosphorylation after platelet activation, Panx1 inhibition by a specific inhibitor Probenecid and *ex-vivo* analysis of blood samples under flow conditions from healthy volunteers to maintain cell-cell interactions.
Findings: Panx1 channels are expressed on human and murine platelets. In western blot analysis we could show, that platelet activation with classical platelet agonists leads to increased phosphorylation of tyrosine residue 198. This is fully dependent on SRC- and partially dependent on PKC kinases. Interestingly, ADP does not phosphorylate Panx1 at Tyr198, but another known phosphorylation side, Tyr308. Here, SRC kinases are only partially involved in phosphorylating Tyr308. These findings indicate other activation mechanisms of Panx1 channels in platelets. Additionally, blockage of Panx1 channels with Probenecid leads to reduced extracellular ATP levels after platelet activation with high and low dose of collagen-related peptide (CRP), Par4 peptide and thromboxane, but not ADP. These data goes ahead with our phosphorylation studies of Panx1 Tyr198. ATP release by Panx1 channels do not significantly alter platelet activation markers as measured under static conditions. Panx1 inhibition leads to reduced thrombus formation at low (450 s^{-1}) and moderate (1000 s^{-1}) arterial shear rates *ex-vivo*. Moreover first experiments revealed, that interaction of platelets and RBCs leads to cleavage of Panx1, known to constitutively open Panx1 channels suggesting that cross talk between cells activate Panx1 channels to support intracellular communication.
Conclusion: Taken together, Panx1 channels function as ATP release channels in platelets and are phosphorylated at different tyrosine residues after platelet activation with classical platelet agonists. Moreover, reduced ATP release by inhibiting Panx1 with Probenecid results in less thrombus formation low and moderate arterial shear rates. These results suggest that Panx1 may play an important role in haemostatis by releasing extracellular ATP to enable cell communication and supporting thrombus formation.

P04-12

NGS for diagnosing inherited bleeding, thrombotic and platelet disorders: Points to consider by reporting results - Primum non nocere, secundum cavere, tertium sanare

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Introduction: Inherited bleeding, thrombotic and platelet disorders (IBTPD) are a heterogeneous group of rare disorders caused by DNA variants in a large number of loci. To date there are close to 100 diagnostic genes associated with coagulation, thrombotic and platelet disorders. A substantial proportion of patients cannot be diagnosed, and, in some cases, the pathogenic mechanisms of certain variants cannot be confirmed. NGS refers to various types of sequencing platforms that can sequence millions of fragments of DNA in parallel. NGS offers opportunities to advance medical diagnostics and treatments, but also raises complicated ethical questions.
Areas considered: 1. Approaches for reporting variants with uncertain significance (VUS). A major challenge, especially in the case of novel variants and VUS, whose role in diseases cannot be ruled out. Attempts to interpret VUS may require affected patients to be informed, which may cause them unnecessary anxiety. 2) Approaches for reporting incidental or secondary findings, taking into account ethical and clinical circumstances. 3) ethical challenges particular in three main ethical areas: privacy, informed consent and return to results. A fundamental question is to what extent information generated by NGS testing should be communicated to patients. Patients should be informed about findings of high clinical importance and that are at least partially correctable, thus balancing risks and benefits. 4) reporting results of children.

Conclusions: NGS is a promising tool for diagnosis of IBTPD but poses ethical, legal, and social questions. It has unrestricted potential to find incidental genomic findings which interpretation is still challenging and requires careful evaluation in order to avoid either a false positive or a false negative result.

P05 Posters: Laboratory diagnostics

P05-1

Thrombin generation as a method to monitor acquired F VIII inhibitor patients independent of their therapy

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Scientific research question: In bleeding patients with acquired hemophilia (AHP), e.g. due to an F VIIIc inhibitor, measuring FVIIIc activity (FVIIIc) is crucial. FVIII activity assays in AHP under therapy however do not always correlate with bleeding. Automated thrombin generation assay (TGA) measures the entire thrombin generation process and shows a good correlation irrespective of the treatment choice ranging from factor replacement therapies to novel non-replacement therapies such as Emizicumab (EMI) including treatment with Anti-Inhibitor Coagulant Complex (AICC; F VIII-Inhibitor-Bypassing activity) or rFVIIa. Aim of this retrospective analysis was to show that TGA parameters are sufficient sensitive in AHP representing in-vitro therapy efficiency.
Methodology: One stage FVIIIc clotting assay (OSA) and a chromogenic FVIIIc assay (CA) using an Atellica Coag 360 analyzer (Siemens, Marburg, Germany) were applied. TGA analysis was performed using standardized reagents (Ceveron TGA assay kit, Technoclone, Vienna, Austria). EMI concentrations were measured using an OSA with an EMI calibrator set (r2 Diagnostics, South Bend, Indiana USA; CoaChrom Diagnostica, Austria) and a specific procedure.
Findings: In AINHP, treated initially by prednisolone 100 mg and AICC, a parallel increase of peak thrombin (PT) and FVIII activity (lowest 2%) was observed. FVIII activity below normal range also the PT was significant shortened in a range from 27 to 196 nM. After elimination of the inhibitor PT showed constant normal results (353 to 375 nM) even when FVIII activity was elevated. In one patient no FVIII restoration could be achieved by classical therapies, therefore EMI therapy was successfully initiated accompanied by Rituximab therapy. PT was observed at a stable level at app. 400 nM at a blood level of EMI 60 µg/ml and after successful elimination of the inhibitor and normalization and even elevation of FVIIIc 170 % (CA).
Conclusion: In this retrospective analysis independently of the patient´s therapy Ceveron TGA was in a good agreement with the clinical and hemostatic situation of the patients. In hemophilia therapy also PT shows a good correlation with the F VIIIc. Switching therapy to EMI, if there is not used a bovine chromogenic assay for FVIIIc testing FVIII in OSA will be overestimated. TGA however shows an earlier and more stable increase of peak thrombin, without overestimation. Automated TGA, in combination with standardized Ceveron TGA kits is an easy to use, sensitive method for monitoring hemophilia therapy at a functional hemostatic level. Thisindependently of the applied therapy and may offer also an additional benefit in detecting individual bleeding risk even during a therapy switch.

P05-2

A pilot external quality assessment survey for the Direct Oral Anticoagulant dipstick point-of-care test

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Currently direct oral anticoagulants (DOACs) are more and more used for the treatment of procoagulant patients. Although laboratory assessment of treatment with DOACs is not necessary, there are circumstances in which it is important to measure the concentration of the DOAC in blood plasma. For this purpose mostly calibrated chromogenic anti-Xa assays are used for Xa-inhibitors and calibrated anti-IIa or diluted thrombin time assays are used for thrombin inhibitors. In emergency situations it is important to quickly know whether a patient is on DOAC treatment or not. For this purpose recently a point-of-care (POC) type of assay was developed. The DOASENSE dipstick is able to detect qualitatively both anti-Xa and anti-IIa type of DOACs simultaneously in urine samples.
To ensure reliable test results an indispensable part of the quality management system in laboratory medicine is the participation in an external quality assessment (EQA) programme. Currently there is no EQA programme available yet for POC DOAC testing. The ECAT Foundation, an EQA provider specialised in blood coagulation, is

setting up a pilot study on EQA for POC DOAC testing. With this pilot study the ECAT Foundation aim to investigate the reliability of DOAC testing with the DOASENSE dipstick by comparing the test results from different participants.
A set of 4 control samples with different levels (0 - 800 ng/mL) of rivaroxaban and dabigatran will be distributed to approximately 35 laboratories. The laboratories will be asked to measure these samples with de DOASENSE dipstick by identification of the colours by naked eye in comparison to a label with printed predefined colours for each result and report whether the test results are negative or positive. In addition the participants will be asked to return also a digital photo of the dipsticks after analysis of each samples. This makes a visual inspection of the returned test results possible.
Results will be tabulated by test outcome for each of the control samples and evaluated according to the expected outcome on the basis of the DOAC concentration in the sample.
This pilot study will be executed in the period November - December 2019 after which data evaluation will be performed immediately.

P05-3

Diagnostic of large genetic defects in blood coagulation genes: NGS vs MLPA

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Background: Large deletions/duplications account for 5% in patients with haemophilia A and B. The conventional method used to detect these large gene alterations is MLPA. In majority of cases this method is good standardized and reliable. Nevertheless, there are cases where the MLPA displays pitfalls in routine diagnostic. NGS (Next Generation DNA Sequencing) is a high-throughput method, which allows not only quick and consistent sequencing results but also overcomes the disadvantages of MLPA in detection of large deletions/duplications.
Aim: Here we report 8 cases in which large genetic variants were successfully detected with NGS, where MLPA was not able to obtain reliable results.
Materials and methods: Large genetic variants were analysed by MLPA and NGS according to the manufacture's recommendation.
Results: Three cases represent the First group, where MLPA gave inconsistent results. In case 1 MLPA diagnosed a false positive large deletion of exon 1 in the F9 due to small insertion in ligation binding site. By 2 other cases large deletions in exon 13 and 18 of the F8 were not detected by MLPA, while the ligation binding site was outside the region of the deletion. All these cases were successfully identified by NGS. The Second group summarises 4 cases, where Klinefelter and Turner syndrome were accidentally identified in the frame of routine F8 and F9 genetic diagnostic. This event was possible only due to the fact by NGS the number of X-chromosomes can clearly be displayed. The Third group comprise of 2 cases. By these female patients the normal sequencing results reveal missense mutations in the F8 gene, which was enough to confirm the carrier status. As both females showed relative low values of FVIII:C the samples were subjected to further investigation with NGS for additional defects. Surprisingly, the data reveal additional large heterozygous deletion in one of the females and heterozygous large duplication in the other, which were not diagnosed by standard sequencing method.
Conclusion: Putting together all these results and our experience, we can conclude that NGS is a reliable method for routine diagnostic not only of small genetic variants, but also for detection of large deletion/duplication in hemi- and heterozygous state. Additionally, this method allows simultaneous detection of aneuploidies. The application of NGS in the routine analyses makes the genetic diagnostic of haemophilia patients safer and quicker.

P05-4

Development of a heparin-insensitive assay to measure activated factor XI

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Scientific research question: Measurement of activated FXI (FXIa) has been implemented to confirm the absence of thrombosis-generating agents in human immunoglobulin G (IgG) preparations used for the treatment of primary immunodeficient patients. Unexpectedly, heparin was found to heavily interfere with the commercially available chromogenic FXIa assay (Rossix). The inhibition, observed even at low levels of heparin, could be reversed by heparin neutralization with protamine sulphate, but excess protamine sulphate itself interfered with the assay. To overcome these limitations, degradation of heparin by heparinase I was established, and the results are presented here.

Methodology: Inhibitory effects of heparin and protamine sulphate on the chromogenic FXIa assay were determined for fixed FXIa concentrations, diluted in buffer and the human IgG preparation. The optimal heparinase I concentration for extensive heparin degradation was determined in buffer and confirmed in an IgG sample, spiked with heparin and purified FXIa. The feasibility of the method developed was confirmed by the measurement of IgG samples, spiked with 1 IU heparin/mL and low levels of purified FXIa.
Findings: Incubation of heparinized IgG samples with heparinase I at concentrations of 5 and 10 U/mL fully reversed heparin's inhibitory effect on the chromogenic FXIa measurement. In particular, at the maximum heparin concentration of 3 IU/mL FXIa recoveries were 92.7% and 90.9% for heparinase I concentrations of 10 and 5 U/mL, respectively in the FXIa-spiked IgG sample (8 mIU/mL). IgG samples spiked with 1 IU heparin/mL demonstrated FXIa recoveries within the acceptable 100 ± 11% range. Intra-run precision (n = 6), determined for these samples, ranged from 6.6% to 7.1%.
Conclusions: Heparinase I pretreatment was added to the commercially available chromogenic FXIa test. This pretreatment was demonstrated to have no effect on assay performance, but to fully remove the inhibitory effect of heparin. FXIa spiked to the IgG preparation was found to have acceptable recoveries even in the presence of 1 IU heparin/mL.

P05-5

Establishment of a competitive enzyme-linked immunosorbent assay for the measurement of heparin in plasma products

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Scientific research question: The potent anticoagulant activity of the sulfated polysaccharide heparin can also be used in plasma fractionation processes to prevent undesired activation of coagulation factors. Consequently, intermediates and the final products have to be measured for their heparin content. Established heparin assays are based on measurement of the accelerated inhibitory action of antithrombin against thrombin (FIIa) or activated factor X (FXa), determining the residual FIIa/FXa activity with a chromogenic substrate. Such assays, however, show limitations, especially at the low sample dilutions required to reach adequate sensitivity. These limitations, which result in invalid results, originate from the specific sample matrix including proteases or protease inhibitors or simply from the color of the test sample. To overcome these shortcomings, we implemented a competitive enzyme-linked immunosorbent assay (ELISA), described so far to measure heparin in plasma only.
Methodology: An ELISA to measure unfractionated heparin in plasma (K-2100, Lifespan) was applied. Briefly, heparin contained in the sample competed with the binding of a peroxidase-labelled heparin binding protein to the wells of a heparin-coated plate. The six-point calibration curve ranged from 0.03 to 7.14 IU heparin/mL and was prepared by diluting the USP reference standard in normal human citrated plasma. Test samples were diluted at least 1/6 in normal human citrated plasma and measured in triplicates. Spike-recovery was carried by adding the heparin standard to obtain a heparin concentration of 0.26 IU/mL in the test dilution of the respective sample.
Findings: The calibration curves obtained by 4-parameter fit showed acceptable accuracy and reproducibility. Spike-recovery experiments resulted in acceptable recovery in all sample types investigated so far (immunoglobulin G final product and process intermediates, albumin and a_1-proteinase inhibitor), while intra-run and inter-run precision data showed relative standard deviations that did not exceed 10%. The ELISA's limit of quantification was 0.18 IU/mL.
Conclusion: The competitive heparin ELISA, described so far for measurement in plasma matrix only, was established and qualified for use in specific plasma protein matrices, which interfere with the FIIa/FXa-based assay.

P05-6

Platelet function assessment after transfusion of platelet concentrates for the treatment of massive bleeding after cardiopulmonary bypass

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Scientific research question: Massive diffuse bleeding is still a problem in cardiovascular surgery. First line treatment is platelet concentrate (PC) transfusion, although there is still insufficient information regarding efficacy, quantity, and timing. The objective of this study was to find out whether the amount of 4 PCs could reduce intraoperative bleeding and improve viscoelasticity and aggregometry.

Methodology: 10 patients were enrolled intraoperatively because of firstly life-threatening diffuse bleeding after cardiopulmonary bypass and secondly an order by the surgeon of 4 apheresis PCs. Each unit was given to each patient in a precise sequence of 5 minutes. After every unit, thromboelastometry (ROTEM®) and aggregometry (Multiplate®) tests were done together with Haematocrit (Hct), Haemoglobin (Hb), and platelet count. For the statistical evaluation the Wilcoxon's test was used.
Findings: Whereas platelet count and viscoelastic MCE-EXTEM (maximum clot elasticity) significantly increased, there was no significant increase in ADP (adenosine diphosphate) or COL (collagen) results. Also bleeding parameters (Hct and Hb) still showed a significant decrease.
Conclusion: Even a series of 4 PCs did not comprehensively improve both components of an adequate haemostasis: viscoelasticity as well as platelet aggregometry. Just the transfusion of PCs alone was not a sufficient course of action to improve haemostasis and reduce intraoperative bleeding.

P05-7

Thrombin formation in platelet-rich-plasma predicts the bleeding risk during radical prostatectomy

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Bleeding is a major and severe adverse affect of surgical procedures associated with need for transfusions, use of haemostatic agents, and significant mortality. Thus it constitutes a priority to develop predictive biomarkers to assess risk of bleeding and blood loss in invasive clinical settings. Here, we used real time thrombin formation with fluorogenic substrates to analyse coagulation in 22 prostate cancer patients during various time points during radical retropubic prostatectomy. Perioperative blood loss was estimated by the volume sucked blood from the surgery site and drop of haemoglobin in the circulation. Total and peak thrombin formation, lag time and time to peak were correlated with blood loss. Patients on anticoagulant regiments, known coagulopathies and impaired liver or kidney functions were excluded in the prospective study.
Consistent with previous reports the procoagulant activity, assessed via the total thrombin generation, largely increased >35% in platelet poor plasma during the surgical interventions. In contrast thrombin formation in platelet rich plasma decreased during the intervention indicating that platelets partially attenuate increased coagulation during radical prostatectomy.
Blood loss and postoperative drop in haemoglobin were associated with the preoperative procoagulant activity in platelet rich but not in platelet poor plasma. Total preoperative thrombin formation in platelet rich plasma was significantly higher in patients with limited bleeding as compared to individuals with severe blood loss (1964 ± 178 versus 1481 ± 86 nM*min).
Together the data suggest use of thrombin formation in platelet rich plasma to assess bleeding risk and outcome in prostatectomy in cancer patients with possible implications for other surgical procedures.

P05-8

Thrombin generation in patients with liver cirrhosis: new insights into a procoagulant state from a modern standardized assay

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Objectives: Cirrhotic patients are at increased thrombotic risk despite prolonged routine *in vitro* coagulation times, *e.g.* prothrombin time and aPTT. This has been proven in clinical studies and *in vitro* using thrombin generation (TG) assessed by Calibrated Automated Thrombogram (CAT). However, *in vitro* studies highlighted some discrepancies, particularly for the results of thrombin generation in absence of thrombomodulin (TM, a protein C/S system activator). These can be explained by a high inter-laboratory methodological variability and by differences in severity and aetiology of liver cirrhosis. In order to reach reproducible results, we initiated a larger study using a standardised assay.
Methods: We have initiated a single-centre prospective study recruiting patients with liver cirrhosis Child-Pugh A to C. TG is measured using ST Genesia® Thrombin Generation System (Stago, Asnières-sur-Seine, France). The analyses were performed without and with TM as protein C/S

system activator. We report here the results of thrombin generation without and with thrombomodulin in the first 172 patients. The endogenous thrombin potential (ETP) represents the total thrombin generated. Its TM-mediated inhibition represents the degree of diminution of thrombin generation after addition of TM and reflects the activity of the protein C/S system. The peak height represents the maximal thrombin concentration and the velocity index, the initial thrombin generation velocity.
Results: Without TM, the ETP was only slightly decreased in cirrhotic patients compared to healthy donors (p=0.0203) and the peak height was not different between the two groups (p = 0.1404). With TM, the ETP and the peak height were increased in cirrhotic patients compared to healthy donors (p=0.0002 and 0.0033 respectively). The ETP with TM also increased significantly between patients with Child A and Child B cirrhosis (p=0.0085). This resulted in a significantly decreased TM-mediated inhibition in cirrhotic patients compared to healthy donors (p< 0.0001) and in patients with Child B cirrhosis compared to Child A (p< 0.0001). Already without TM, the velocity index was significantly increased in cirrhotic patients compared to healthy donors (p=0.0001) and in patients with a Child B cirrhosis compared to Child A (p=0.0086).
Conclusions: Using an automated and standardised assay, we documented that patients with liver cirrhosis exhibit a prothrombotic profile already when measuring TG in absence of TM (increased TG velocity). We also confirmed a decreased TM-mediated inhibition of ETP, which is due to an acquired protein C resistance. The obtained results are robust thanks to the automated and standardised method used and to the number of patients included. They help to solve the discrepancies observed in previous studies that were using non-standardised manual assays and were including less patients.

P05-9

Liver dysfunction biomarkers correlate with a prothrombotic and not with a prohemorrhagic hemostatic profile in cirrhotic patients

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Background: Cirrhotic patients exhibit a prothrombotic hemostatic profile, which increases with liver cirrhosis (LC) severity according to *in vitro* thrombin generation (TG) assays. Different liver dysfunction biomarkers, including prothrombin time (PT), its international normalised ratio (INR) and activated partial thromboplastin time (aPTT) are used to assess the bleeding risk of cirrhotic patients, either on their own or included in bleeding risk assessment scores. We aimed to investigate the relationship between liver dysfunction biomarkers and TG as well as their adequacy for bleeding risk assessment.
Method: We performed a prospective single-centre study including 172 cirrhotic patients. TG was measured using ST Genesia® Thrombin Generation System (Stago, Asnières-sur-Seine, France). The analyses were performed without and with thrombomodulin (TM) as protein C/S system activator. We focused on the velocity index without TM, representing the initial TG velocity, and the TM-mediated inhibition, representing the degree of diminution of thrombin generation after addition of TM. The TM-mediated inhibition reflects the activity of the protein C/S system. Relationships between velocity index without TM and TM-mediated inhibition, and MELD score, PT/INR, aPTT, factor V activity, albumin and total bilirubin were assessed using linear regression or a quadratic function.
Results: The TM-mediated inhibition showed a direct relationship with PT, factor V activity and albumin and an inverse relationship with INR, aPTT, total bilirubin and MELD-score (R^2 = 0.3516, 0.3900, 0.2913, 0.3002, 0.2379, 0.2749, and 0.1984). All slopes were very significantly different from zero (p < 0.0001). The relationship between velocity index without TM and the liver dysfunction biomarkers were coherent with the results of TM-mediated inhibition. All slopes were very significantly different from zero (P < 0.0001). However, the relationship were of lower quality [R^2 = 0.1542 (PT), 0.08599 (INR), 0.1024 (aPTT), 0.2739 (factor V), 0.1315 (albumin) and 0.1276 (total bilirubin)].
Conclusion: We demonstrated an increasing prothrombotic profile with increasing disturbances of liver dysfunction biomarkers in cirrhotic patients. In particular, prolonged PT/INR and aPTT and decreasing factor V activity are related to an increasing thrombotic and not a bleeding phenotype. Therefore, these parameters should not be used to assess bleeding risk of cirrhotic patients. Alternative biomarkers for bleeding risk assessment in cirrhotic patients would need to be developed.

P05-10

The MassARRAY® System: A fast, robust, cost-effective, and highly sensitive tool for the analysis of thrombosis relevant SNPs

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Objectives/Methods: The MassARRAY® System (Agena Bioscience, Inc., San Diego, CA, USA) is a Matrix-Assisted Laser Desorption Ionization - Time Of Flight (MALDI-TOF) mass spectrometer customized for the precise detection of DNA molecules. The molecules are distinguished by their time-of-flight after being ionized in a vacuum chamber. Direct analysis of the mass of the molecules eliminates the need for fluorescence or other labeling. The MassARRAY® System workflow is highly reproducible and generates results within an 8-hour day. Multiplexed analysis reduces per sample cost, targeted content eliminates bioinformatics burden, and scalable through-put optimizes batch and resource requirements.
Results: We developed and validated a single multiplex-PCR for the analysis of the following 10 thrombosis relevant SNPs: AT3 A384S (AT3 *Cambridge II*, rs121909548), Prothrombin G20210A (rs1799963), Factor V R506Q (Factor V *Leiden*, rs6025) and H1299R (rs1800595), F12 C46T (rs1801020), F13 V34L (rs5985), FSAP G534E (*Marburg I*, rs7080536), MTHFR C677T (rs1801133) and A1298C (rs1801131), and PAI-1 4G/5G (rs1799762).
Conclusion: This setup makes it very easy to analyse up to 192 (2 x 96) or 768 (2 x 384) samples per day. Since January 2018, we have tested more than 5.000 patients and found many different constellations of the described SNPs that genetically support the particular clinical situation. In conclusion, the MassARRAY® System combines mass spectrometry, sensitive and robust chemistry, and advanced data analysis software to provide accurate, rapid, and cost-effective analysis (of thrombosis relevant SNPs).

P05-11

Sensitivity for Low Molecular Weight Heparin (LMWH) in ClotPro® using Russell´s viper venom test (RVV-test)

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Introduction: The ClotPro is a novel viscoelastometry analyzer. In addition to the established viscoelastometry assay portfolio ClotPro also provides an assay which uses a direct FXa activation by a snake venom (FXa activator from Daboia Russelli / Russel Viper). We examined the sensitivity of this new assay for the presence of low molecular weight heparin in the sample.
Material and methods: 30 citrated blood samples collected from patients admitted in our general praxis for routine laboratory analysis. Citrated whole blood was used for duplicate measurements on ClotPro using RVV-test (enicor GmbH, Munich). LMWH effects were quantified by the clotting time (CT) (reference range: 48-77 sec). Citrated Plasma was used for determination of anti-Xa activity using a chromogenic one step test (Innovance® Heparin, Siemens) performed on BCS XP.
Results: Anti-Xa-Activity ranged from 0 to 0.9 U/ml. There was a good correlation beweenCT in ClotPro® and Anti-Xa-Activity (r=0.83). A given cut off in ClotPro® of 100 sec. will detect 13 oft 14 samples above 0,2 U/ml Anti-Xa-Activity (sensitivity 93%), however only 2 of 11 samples below 0,2 U/ml Anti-Xa-Activity exceeded CT of 100 sec. (specifity 88%).
Conclusion: RVV-test is capable to detect LMWH at concentrations higher than 0.2 anti-Xa U /ml. This could be a useful tool for situations where a rapid detection of higher LMWH activities is desirable.

P05-12

Sensitivity for Apixaban in ClotPro® using Russell´s viper venom test (RVV-test)

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Introduction: The ClotPro is a novel viscoelastometry analyzer. In addition to the established viscoelastometry assay portfolio ClotPro also provides an assay which uses a direct FXa activation by a snake venom (FXa activator from Daboia Russelli / Russel Viper). We examined the sensitivity of this new assay for the presence of apixaban in the sample.
Material and methods: 44 citrated blood samples colleceted from patients admitted in our general praxis for routine blood control. Citrated whole blood was used for duplicate measurement for ClotPro® using RVV-test (enicor GmbH, Munic). Coagulation time (CT) of RVV-test is given in seconds (sec., Ref.-Range: < 80). Citrated Plasma was used for determination of Anti-Xa-Activity using a chromogenic one step test (Innovance® Heparin, Siemens) performed on BCS XP (Siemens)

using Apixaban Calibrator (Diagnostica Stago, France). Results are given in ng/ml of Apixaban.
Results: Apixaban concentration ranged from 0 to 370 ng/ml. There was a moderate correlation beweenCT in ClotPro® and Apixaban concentration (r=0.77). A given cut off in ClotPro® of 120 sec. will detect 31 oft 33 samples above 45 ng/ml Apixaban concentration (sensitivity 94%), however only 2 of 11 samples below 45 ng/ml Apixaban exceeded CT of 120 sec. (specifity 82%).
Conclusion: RVV-test is capable to detect apixaban at concentrations higher than 45 ng anti-Xa U /ml. This could be a useful tool for situations where a rapid detection of apixaban activities is desirable.

P06 Posters: Haemophilia II

P06-1

Comparison of bleeding rate and factor consumption between extended and short half-life factor VIII in real life according to electronic documentation smart medication™

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Background: With the introduction of extended half-life factor VIII products (EHL) bleeding rate (BR) and factor consumption (FC) may change in comparison to previous treatment with short half-life factor VIII (SHL).
Methods: Joint bleeds and factor VIII consumption was compared between patients receiving EHL and SHL. Included were patients who received at least 12 weeks EHL concentrates within 12 months before August 2019.
Results: 64 patients were treated with EHL with a total number of 7182 entries in their electronic diary. 27 received only EHL (EHL-group), 37 mainly SHL followed by EHL (SHL-group). The schedule of prophylaxis was 2.14 treatments per week in the EHL and 2.83 in the SHL group ($p < 0.01$). Weekly factor consumption (IE/kg BW/week) was 73 in the EHL and 76 in the SHL group. The ratio of FC for prophylaxis vs. bleeding + follow-up was 95/5 in the EHL and 86/14 in the SHL group. Calculated annual joint bleeding rate was 1.13 in the EHL and 1.97 in the SHL group (n. s.).
Summary: Patients on EHL documented mostly twice weekly prophylaxis, compared to nearly three times weekly with SHL. Lower BR and lower FC for bleeding episodes were documented in the EHL group, which was not significant most likely due to small patient numbers. Ongoing real life analysis comparing SHL vs. EHL are required.

P06-2

Five-year follow-up in haemophilia care comparing real life data from electronic documentation smart medication™

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Background: Electronic diary has shown to be a valuable tool for analysis of annual joint bleeding rate (AJBR) and annual factor VIII/IX consumption (AFC) in real life setting. What has changed over a five-year period?
Methods: AFC and AJBR among 358 patients with haemophilia A/B from 13 haemophilia centers in 2018 were compared to data to prior years (277 patients from 9 centers in 2017 and less in prior years) according to electronic documentation smart medication™.
Results: Looking at five consecutive years, the average AFC (IU/kg BW) was 2442, 2701, 2575, 2670 and 2924 the average AJBR 2.1, 2.5, 2.3, 2.2 and 1.9 between 2014 and 2018, respectively. Four

groups, comparing above or below average AFC and AJBR, were compared between 2014 -2018: The majority (45%/40%/44%/45%/40%) had an AJBR of ≤ 2 with less than average AFC, followed by a group (31%/35%/32%/32%/29%) with ≤ 2 AJBR but above average AFC. A minor group (14%/11%/15%/15%/14%) had an AJBR > 2 and more than average AFC. Only few (10%/14%/8%/8%/17%) had an AJBR > 2 but less than average AFC.
Conclusion: In 2018 the AJBR was slightly lower, the AFCR slightly higher compared to prior years. Whether this reflects changes in treatment or is due to an increasing number of participating patients and centers needs to be further analyzed. With the introduction of extended half-life products the future bleeding and factor consumption may change, demonstrating the necessity for ongoing electronic surveillance in haemophilia care.

P06-3

Long-term improvements in bleeding rate and quality of life outcomes for patients who switched from on-demand to prophylactic treatment with BAY 94-9027 in the PROTECT VIII trial and extension

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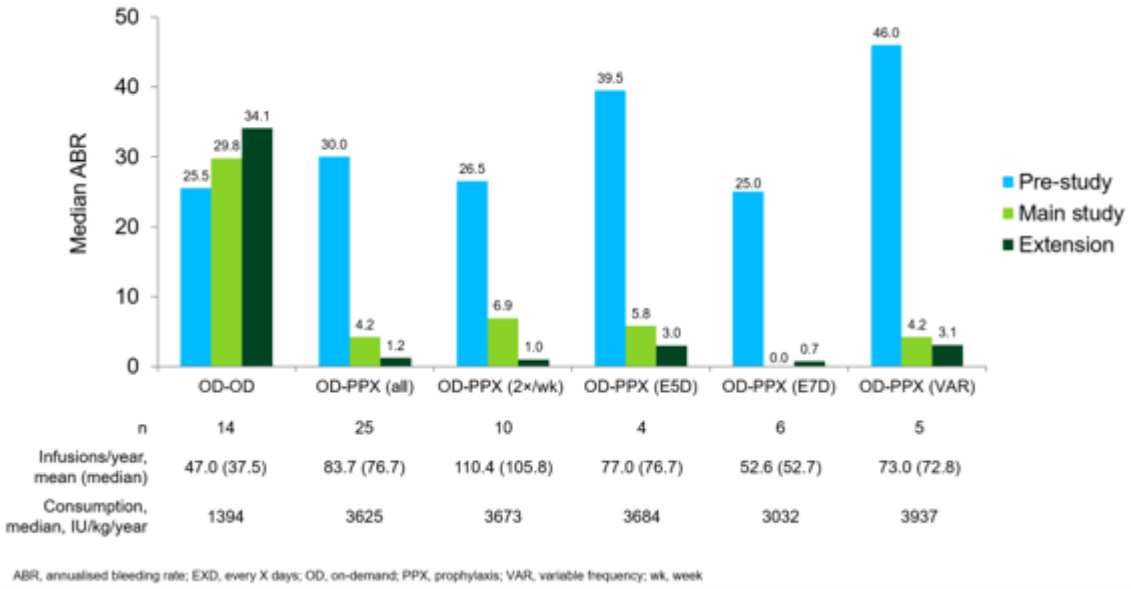
Scientific research question: BAY 94-9027 (damoctocog alfa pegol) is a B-domain deleted recombinant factor VIII (FVIII), site-specifically PEGylated with a 60 kDa polyethylene glycol to extend half-life. Its efficacy and safety as prophylactic (PPX) and on-demand (OD) therapy for previously-treated patients with severe haemophilia A were demonstrated in the PROTECT VIII trial (NCT01580293) and its extension. As individualised PPX regimens reduce bleeding and improve joint health compared with OD therapy, outcomes may also improve in patients who switch from OD to PPX. A *post hoc* analysis of PROTECT VIII extension data (cut-off Aug 2019) aimed to examine outcomes for individuals who switched from OD to PPX treatment.
Methodology: PROTECT VIII was a partially randomised, open-label 36-week trial that examined OD or PPX regimens (2×/week; every 5 days; or every 7 days [E7D]) of BAY 94-9027 in males aged 12-65 years with haemophilia A (FVIII < 1%) and ≥150 FVIII exposure days. Patients completing the main study could enter an extension and continue, or switch regimens. This descriptive analysis of extension data reports long term follow-up outcomes in PROTECT VIII patients who were receiving OD FVIII before study enrolment.
Findings: At the cut-off date, data were available for 25 patients who switched from OD prior to study to PPX (OD-PPX; 22 switched for main study; 3 switched at start of extension) and 14 patients who remained on the OD regimen (OD-OD). Median time in the extension was 3.2 years for both cohorts. At baseline, mean age of OD-OD patients and OD-PPX was 43.5 and 35.7 years, respectively. Disease characteristics were similar in both cohorts with low Haemo-QoL-A scores (Table). OD-PPX patients had marked improvements in median annualised bleeding rate (ABR), irrespective of regimen, while ABR increased over time for OD-OD patients (Figure). During extension, median ABRs were 1.2 and 34.1 for OD-PPX and OD-OD, respectively. Respective median joint ABRs (JABR) were 0.7 and 20.8. Median Haemo-QoL-A score increased from 60 at baseline to 76 at the last visit for OD-PPX patients vs almost no change in OD-OD patients (66 vs 67). In the extension, factor consumption for OD-PPX patients in the E7D regimen was ~2× that of OD-OD patients. Mean (median) number of infusions/year was 47 (38) for OD, 53 (53) for OD-PPX E7D regimen, and 84 (77) for all OD-PPX patients (Figure).
Conclusion: Patients who switched from OD to PPX for PROTECT VIII or its extension experienced robust improvements in ABR and JABR, while ABR increased over time for OD-OD patients. These results were reflected by a clinically important improvement in Haemo-QoL-A in the OD-PPX group. Compared with OD-OD, patients in the OD-PPX (E7D) group showed major improvement in ABR and improved QoL, with only little increase in the number of infusions. The 2× increase in factor consumption could potentially be offset by reduced treatment- and bleed-related costs.

	OD-OD	OD-PPX
Patients, N	14	25
Age at enrolment, years, mean (SD)	43.5 (14.1)	35.7 (11.3)
Patients with target joints, n (%)	11 (78.6)	18 (72.0)
# of target joints, median (Q1, Q3)	2.5 (1.0, 4.0)	2.0 (0.0, 3.0)
# of bleeds in previous 12 months, median (Q1, Q3)	25.5 (12.0, 47.0)	30.0 (18.0, 46.0)
# of joint bleeds in previous 12 months, median (Q1, Q3)	19.5 (10.0, 47.0)	19.0 (11.0, 34.0)
Gilbert score, median (Q1, Q3)	30.5 (19.0, 38.0)	25.0 (18.0, 33.0)
Haemo-QoL-A (total), median (Q1, Q3)	66.2 (44.2, 84.7)	60.2 (55.2, 77.8)

[Table: Baseline characteristics]



[Figure: ABRs during PROTECT VIII and its extension among patients previously treated with on-demand FVIII]

P06-4

Emicizumab treatment is efficacious and well tolerated long term in persons with haemophilia A (PwHA) with or without FVIII inhibitors: pooled data from four HAVEN studies

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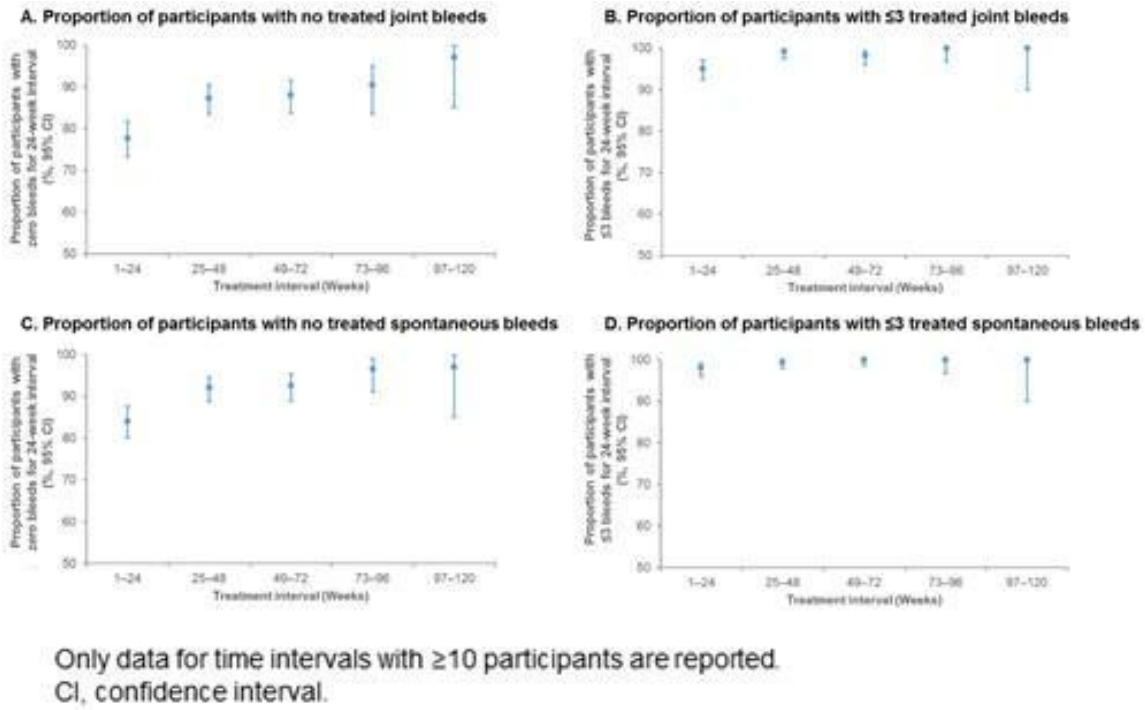
Objectives: Emicizumab is a subcutaneously administered, bispecific monoclonal antibody that restores haemostasis in PwHA. The aim of this study was to present the long-term efficacy and safety of emicizumab in PwHA across the four phase III studies: HAVEN 1 (NCT02622321), 2 (NCT02795767), 3 (NCT02847637) and 4 (NCT03020160).

Methods: The studies enrolled paediatric (< 12 years) and adolescent/adult (≥12 years) PwHA, with or without FVIII inhibitors. Emicizumab prophylaxis was administered 1.5 mg/kg weekly, 3 mg/kg every 2 weeks, or 6 mg/kg every 4 weeks. All participants assigned to receive emicizumab were included, and data were analysed by study or pooled across studies.
Results: Overall, 400 participants in HAVEN 1, 2, 3 and 4 (n=113, 88, 151 and 48, respectively) were included in the efficacy analysis, with a median efficacy period of 82.4 weeks (with 77% of participants treated for ≥74 weeks). The model-based treated annualised bleed rate (ABR; derived via negative binomial regression) was 1.5 (95% confidence interval 1.20-1.84). The mean ABR decreased across consecutive 24-week treatment intervals (Table). Across studies, over 87% of participants had no treated joint bleeds in each interval from Week 25 and over 92% had no spontaneous bleeds (Figure). No death, thrombotic, or thrombotic microangiopathy events occurred in these trials beyond those reported in HAVEN 1 at the respective primary analysis. Emicizumab continued to be well tolerated, with no participants discontinuing due to adverse events beyond the five previously described.
Conclusions: Emicizumab maintained low bleed rates and favourable safety and tolerability in PwHA of all ages, with or without FVIII inhibitors. Mean ABR decreased and the proportion of participants with zero joint or spontaneous bleeds increased across each treatment interval from Week 25.

	1-24 weeks	25-48 weeks	49-72 weeks	73-96 weeks
HAVEN 1 (n=113)				
n	**109**	**101**	**97**	**73**
Mean ABR (95% CI)*	3.1 (0.6-8.8)	0.6 (0.0-4.9)	0.4 (0.0-4.6)	0.4 (0.0-4.5)
% with 0 bleeds (95% CI)	71.6 (62.1-79.8)	84.2 (75.6-90.7)	87.6 (79.4-93.4)	86.3 (76.3-93.2)
% with 0-3 bleeds (95% CI)	89.0 (81.6-94.2)	99.0 (94.6-100)	97.9 (92.8-100)	100.0 (95.1-100)
HAVEN 2 (n=88)				
n	**86**	**64**	**49**	**18**
Mean ABR (95% CI)*	0.4 (0.0-4.5)	0.3 (0.0-4.4)	0.3 (0.0-4.2)	0.1 (0.0-3.9)
% with 0 bleeds (95% CI)	87.2 (78.3-93.4)	87.5 (76.9-94.5)	87.8 (75.2-95.4)	94.4 (72.7-99.9)
% with 0-3 bleeds (95% CI)	100 (95.8-100)	100 (94.4-100)	100 (92.8-100)	100 (81.5-100)
HAVEN 3 (n=151)				
n	**148**	**144**	**129**	**23**
Mean ABR (95% CI)*	1.8 (0.2-7.0)	0.9 (0.0-5.5)	0.9 (0.0-5.5)	0.2 (0.0-4.1)
% with 0 bleeds (95% CI)	62.8 (54.5-70.6)	72.9 (64.9-80.0)	77.5 (69.3-84.4)	91.3 (72.0-98.9)
% with 0-3 bleeds (95% CI)	93.2 (87.9-96.7)	97.2 (93.0-99.2)	96.9 (92.3-99.2)	100 (85.2-100)
HAVEN 4 (n=48)				
n	**48**	**45**	**9**	**0**
Mean ABR (95% CI)*	2.1 (0.3-7.4)	1.5 (0.1-6.4)	NE†	NE†
% with 0 bleeds (95% CI)	64.6 (49.5-77.8)	77.8 (62.9-88.8)	NE†	NE†
% with 0-3 bleeds (95% CI)	91.7 (80.0-97.7)	95.6 (84.9-99.5)	NE†	NE†
Total (N=400)				
n	**391**	**354**	**284**	**114**
Mean ABR (95% CI)*	1.9 (0.2-7.1)	0.8 (0.0-5.2)	0.8 (0.0-5.2)	0.3 (0.0-4.4)
% with 0 bleeds (95% CI)	70.8 (66.1-75.3)	79.4 (74.8-83.5)	82.7 (77.8-87.0)	88.6 (81.3-93.8)
% with 0-3 bleeds (95% CI)	93.4 (90.4-95.6)	98.0 (96.0-99.2)	97.2 (94.5-98.8)	100 (96.8-100)

***Based on the calculated ABR.**
†Only data for time intervals with ≥10 participants are reported.
CI, confidence interval; NE, not evaluable.

[Treated bleeds over time in the HAVEN studies]



[Participants with 0 or ≤3 treated joint/spontaneous bleeds per 24 weeks (N=400)]

P06-5

Obese previously treated patients with severe haemophilia A demonstrated treatment success with rVIII-SingleChain

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Objectives: rVIII-SingleChain is a recombinant factor VIII (rFVIII), designed to have high stability and binding affinity for VWF, and has demonstrated a favourable pharmacokinetic profile in patients on prophylaxis. Obesity rates amongst haemophilia patients are currently very similar to the general population, allowing identification of differences in the response to the rFVIII replacement treatment. This study aimed to evaluate the efficacy of treating obese haemophilia A patients with rVIII-SingleChain.
Methods: A phase 1/3 study (CSL627_1001; NCT01486927) evaluated the efficacy and safety of rVIII-SingleChain in the treatment of bleeding episodes, routine prophylaxis and surgery. Patients were adults/adolescents (≥12 years), with severe haemophilia A (endogenous FVIII < 1%). Participants were treated on-demand or assigned to a prophylaxis regimen with rVIII-SingleChain at the discretion of the investigator.
Results: Of the patients enrolled (n=175), 18 were obese (body mass index [BMI] >30 kg/m^2), with a median (range) age of 37.5 (18-64) years. Fourteen patients received prophylaxis with rVIII-SingleChain, and 4 were treated on-demand. Across all prophylaxis regimens, the median annualized spontaneous bleeding rate (AsBR) was 0.00 (Q1, Q3: 0.0, 3.6) and the median overall annualized bleeding rate (ABR) was 1.75 (Q1, Q3: 0.0, 3.6). Of the 119 bleeds treated and assessed, haemostatic efficacy was rated by the investigator as excellent or good in 89.1% of infusions. Of the treated bleeding events, 109 (91.6%) were successfully treated with 1 infusion, and 9 (7.5%) required 2 infusions. The median total IU/kg per infusion per bleed was 16.9. No patient developed FVIII inhibitors.
Conclusions: In obese previously treated patients with severe haemophilia A, rVIII-SingleChain can be successfully used to treat bleeding episodes, similar to the overall study population based on the currently published data. rVIII-SingleChain demonstrates low AsBR and ABR in patients on prophylaxis, with excellent haemostatic efficacy in the control of bleeding events, and a favourable safety profile.

P06-6

Approximation of emicizumab plasma levels in the absence of dedicated assays. A practical approach

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Introduction: Emicizumab is a bispecific antibody mimicking the function of activated factor VIII (FVIIIa), which is used for the prophylaxis of bleeding events in hemophilia A (HA). Emicizumab - unlike FVIII - does not require activation for its procoagulant activity, which leads to a very strong effect of the drug in the aPTT and the one stage FVIII assay (OSA). Therefore both assays are oversensitive towards emicizumab. To overcome this problem a dedicated emicizumab assay was developed, which is based on the OSA, using a higher sample dilution than the standard FVIII assay and which is calibrated against emicizumab. However this assay is mainly available in hemophilia centers. In situations where patients need medical care in centers other than dedicated hemophilia centers, e.g. during emergency care or when patients need surgical treatments, a method to assess the emicizumab plasma level based on a widely available assay would be desirable.
Aim: A method for approximation of emicizumab based on the OSA assay was developed and validated.
Methods: 28 left-over samples from routine coagulation analysis from HA patients with (n=23) and without (n=5) emicizumab treatment were analyzed. The emicizumab concentration was determined using the commercially available dedicated assay (R2 Diagnostics calibrator and controls (h/l), Haemochrom Diagnostica GmbH, Essen, Germany). In addition FVIII activity was determined using the one stage clotting assay and using the one stage clotting assay following a sample pre-dilution of 1:8 in saline. The FVIII assays were determined in two different laboratories using the Siemens BCS (and respective Siemens reagents) and Werfen ACL TOP (and respective Werfen reagents) analyzer systems.
Results: Emicizumab determination in the patients on emicizumab therapy provided levels of 8-94 µg/ml (mean±SD: 43±25µg/ml). In the patients without emicizumab therapy emicizumab levels of 0-1 µg/ml were reported. Standard FVIII assays revealed > 200 % FVIII in 14/23 (Siemens) respectively in 20/23 (Werfen) samples under emicizumab therapy. The determination of the 1:8 diluted samples provided FVIII activities which correlated excellently to the emicumab levels (Siemens: r=0.99, FVIII%= 0.79*emicizumab level / Werfen: r=0.99, FVIII% = 0.88 * emicizumab level).

P06-7

Practical relevance of bleeding endpoints in haemophilia A - Results of a Delphi survey at German haemophilia centres

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Scientific research question: The therapeutic options in the treatment of haemophilia A have expanded significantly in recent years. Therapeutic efficiency is predominantly determined by bleeding rates (ABR, target joints). However, this does not allow to make any statement on the joints status of the patient. It is not yet known which outcome measures are currently routinely collected in German haemophilia centres to assess the joint status and considered relevant in practice for evaluating the outcome of routine prophylaxis in haemophilia A.
Methodology: 1.) A literature search was conducted to evaluate the evidence from studies on routine prophylaxis of HA with genetically engineered products to address the following questions, among others: Do the publications contain information on spontaneous joint bleedings, target joint bleedings, and all joint bleedings as well as on the definitions of bleeding endpoints or target joints? Selection criteria: prospectively planned interventional studies; randomized controlled or non-randomized comparative; no single-arm studies; study duration at least 24 weeks. Population: Adolescents and adults with severe HA. The intervention to be tested was prophylaxis with a genetically engineered FVIII product or antibody approved in Germany (as of 01.06.2019).
2.) Delphi survey at German haemophilia centres to detect the outcome measures used and considered necessary for the evaluation of study data.

Findings: Based on the selection criteria, 13 studies (12 publications) were identified in the literature search, whose essential characteristics such as study design, study population, the primary endpoint, bleeding types, type of pretreatment and statistical evaluation model were collected. Considerable differences were identified between the studies for the patient populations and bleeding-relevant aspects, the study design with different primary endpoints, duration of observations and number of patients in the respective prophylaxis arms, the spectrum of bleeding-relevant endpoints considered, the statistical analysis and presentation of the results. The results of the literature search were collected in an overview table and will be presented.
The pending results of the Delphi survey will be presented.
Conclusion: The results from studies with recombinant products for routine prophylaxis of HA are only comparable to a limited extent due to considerable differences in the study design, patient populations, and endpoints. Therefore, it is not possible to compare the studies on routine prophylaxis of haemophilia A, and the classification of the study results in clinical practice is difficult. It will be discussed whether a standardisation of outcome measures based on the results of the literature research and the Delfi survey is warranted.

P06-8

Insights into the needs of persons with hemophilia and caregivers: a qualitative research in Germany

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Scientific research question: Hemophilia A and B are rare bleeding disorders caused by deficiencies of factor VIII or IX. Modern treatment options and improved care provide new opportunities for patients and a potential for improved quality of life (QoL).
However, in-depth understanding of the needs of persons with hemophilia (PwH) and their attitudes in relation to their condition is not widely available. A qualitative research with PwH in Germany and their caregivers was conducted to better understand attitudes of PwH towards hemophilia as well as the needs in different age groups.
Methodology: Ten focus group discussions were conducted with overall n=26 PwH and n=14 caregivers (parents and relatives) in five cities across Germany (during Feb - Apr 2019). PwH were evenly distributed into three age groups: 17-35 years (n=9), 36-49 years (n=9), 50+ (n=8) years. Focus group participants were recruited via physicians and hemophilia patient organizations. In moderated discussions of two hours, various methods (e.g.in-depth discussions and creative collages) were used. Key topics were attitudes towards and emotional perceptions of the disease, needs and preferences with regards to improved QoL. Data was analyzed by age group, based on the discussion protocol, the creative work of the patients and the subsequent discussions with the moderator.
Findings: Three of four PwH were male with an average age of 41 years. The gender split in the caregiver group was equal females and males with an average age of 38 years. PwH of 17-35 years only occasionally felt restricted by hemophilia. They maintained an active lifestyle and primarily wished for normality. Respondents of 36-49 years strived for an active and healthy life, although experiencing limitations and joint damage more often than younger patients. PwH over 50 years had learned to cope with the disease, but experienced severe limitations in their daily life. Damaged joints often led to psychological distress and fear of getting old, with more limitations and loss of independence. Across all ages, PwH wished to live a life without limitations, with better joint mobility, to be able to travel and pursue sport activities without significant restrictions. Insights from caregivers indicated that they were often overly protective of their children and felt the burden of hemophilia in their daily lives and activities.
Conclusion: This qualitative research study, one of the largest conducted with PwH in Germany, indicates that PwH wish to live independently without the disease dictating their lives. The experiences and challenges of daily life with hemophilia varied between age groups, however, PwH of all ages expressed the desire to stay active (e.g. travel and pursue sport activities) without significant restrictions and consequently maintain or improve joint health in the long-term.
Key words: hemophilia, persons with hemophilia, caregivers, age, needs, insights, qualitative research, joint health, burden of disease

P06-9

Electronic diary smart medication™ for patients with haemophilia in the context of the Digital Care Act (Digitale-Versorgung-Gesetz, DVG)

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Background: The German government recently launched the Digital Supply Act. The law foresees the use of medical apps on prescription in patient care. Doctors and manufacturers are to receive appropriate remuneration for the use of medical apps. The aim is to promote digitalization in the health care system in Germany and to introduce reasonable digital products in standard care. With the DVG, Germany is breaking new ground in digitization in the health care sector and is thus a pioneer within the European Union.
Method: A large number of criteria must be observed when approving medical apps. For example, the law provides that only medical devices in the low risk classes I and IIa are permitted in the list of prescribable apps. In addition, the intended purpose of the app must support the patient in outpatient care through the service providers (HCPs). The federal agency BfArM is to be responsible for approval and inclusion in the directory for digital health applications (Digitale Gesundheits-Anwendungen, DiGA). The requirements for security, quality, functionality, data protection and data security must be guaranteed. After 12 months, the medical benefit and thus the positive effects of care must be demonstrated.
Results: It could be shown that the requirements of the DVG are met with smart medication™. In particular, the medical benefits have been demonstrated in numerous restrospective and prospective cohort studies conducted since the introduction of smart medication™ in 2012.
Conclusion: DVG requirements for prescription medical apps are discussed in the context of electronic diary smart medication™ for patients with haemophilia and the suitability for inclusion in the DiGA directory is presented.

P06-10

A rare case report of a patient with haemophilia B with age dependent severity known as haemophilia B Leyden

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Haemophilia B is a recessive X-linked bleeding disorder. The mutation causing the disease is located in coagulation factor F9 locus and results on inherited factor IX deficiency. The prevalence rate of haemophilia B is about 1: 30.000 live male births.
The severity of the disease is related to the mutation with reduced to none factor IX activity. We report a patient with severe Haemophilia B diagnosed in early childhood with a factor IX activity of 1%. He suffered severe bleedings during his childhood in the left ankle and left knee joint, which required repeated hospitalizations to hospital for the treatment with factor IX.
Due to frequent joint bleeds prophylactic treatment with plasma derived factor IX concentrate with about 30 IU/kg bodyweight twice a week was initiated at the age of 3 years. With adolescence and the transfer to adult care the patient experienced less bleeds and reduced his prophylaxis regimen to on demand treatment without experiencing any bleeds. The level of factor IX at the age of 18 years was 33% and with 24 years factor IX was nearly normal with 46%. There was no need for any replacement with factor IX concentrate.
Genetic testing revealed a mutation in the F9-gene (gene locus: Xq27.1), c.-29-20T>A, “Factor 9 Type Leyden” (Reitsma PH et al Blood 1988). This mutation was described for the first time in 1970 in Netherlands in a patient with severe symptoms in childhood and with nearly normal factor IX values after puberty. The mutation was located in the promoter region. This region of the promoter resembled an androgen response element. Patients with a mutation at this position respond to higher testosterone levels in puberty because of an intact androgen receptor binding site and increased testosterone in puberty increases FIX levels respectively. In a systematic genetic analysing of Haemophilia B patients another subtype named Haemophilia B Brandenburg was described (Funnell APW at al. Trends in Genetics 2014). The patients with this subtype suffer haemorrhagic symptoms throughout life, with low levels of FIX and without spontaneous recovery after puberty. The Brandenburg mutation is at position -26, in the region where androgen receptor should bind, but this mutation disrupts the binding site with no recovery of FIX. This case report demonstrates how important genetic testing could be in patients with haemophilia.

P06-11

Patient-reported outcomes of patients with bleeding disorders from a single outpatient haemophilia treatment centre in Germany

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Introduction and Objectives: Due to the complexity and high risk of complications in the treatment of blood coagulation disorders, an interdisciplinary care of patients is needed to ensure a high-quality and efficient therapy. The Coagulation Treatment Centre rhein-ruhr (GZRR) follows a holistic treatment concept providing cross-sectoral care through interdisciplinary cooperation. This approach is intended also to improve patients' treatment satisfaction (TS), physical functioning (PF) and health-related quality of life (HRQoL). The aim was to evaluate TS, PF and HRQoL in patients treated at the GZRR.
Methods: Patients > 5 years attending the routine visits at the GZRR completed validated and standardised patient-reported outcomes (PROs). Patients > 12 years filled in the generic SF-36 for the assessment of HRQoL. For the evaluation of TS and PF haemophilia- and age-group specific questionnaires were administered (Hemo-Sat$_{A+P}$, HEP-Test-Q).
Results: 177 patients (range 6-80 years) with bleeding disorders were enrolled, among them 55.9% had haemophilia (Haemophilia A, n=88; Haemophilia B, n=11) and 44.1% had VWD; 57.1% were severely affected. Sixty-eight were children (39%) with a mean age of 11.44±3.4 years and BMI of 18.82±3.7; 109 were adults with a mean age of 38.79±16.7 years and BMI of 25.72±5.5. Patients were relatively satisfied with their treatment, adults reported highest impairments in the domain 'burden', while parents were mainly dissatisfied concerning 'ease & convenience'. Children ($_C$) reported a significantly better ($p < .0001$) subjective physical functioning compared to adults ($_A$). Both age groups showed highest impairments in the domain 'endurance' (M_C=70.69±20.3 vs. M_A=51.48±25.4). Patients reported good HRQoL in the Mental Component Score of the SF-36 50.07±11.1 and showed some impairments in the Physical Component Score 45.26±11.9; significant difference were found between children and adults in all subdomains of the SF-36.
Conclusions: These findings demonstrate that the interdisciplinary approach leads to good results in PROs. Follow-up data will show further improvements.

P06-12

The HaemAcademy - how participants evaluate this haemophilia-specific training programme for physiotherapists

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Introduction and Objectives: The HaemAcademy is a first initiative to educate physiotherapists and to improve the musculoskeletal system of People with haemophilia (PWH) in Germany; it is sponsored by Novo Nordisk. Since PWH who receive regular physiotherapy have better joint health, mobility and greater autonomy, all PWH should have access to physiotherapy. Although haemophilia treatment centers (HTC) often have specialised physiotherapists, most PWH live far away from their HTCs. PWH would need trained physiotherapists also in their living surrounding when joint bleeds occur or to prevent them. There is a need of education of physiotherapists providing haemophilia-specific therapy to all PWH. The overall aim of the HaemAcademy was to educate physiotherapists leading to an improved care situation all over Germany. Furthermore, we wanted to evaluate the training programme (e.g., organisation, structure, content) and its' impact on the routine practice of participating physiotherapists.
Methods: The HaemAcademy offers a basic and an advanced (professional) training programme for physiotherapists which is held by an interdisciplinary team of health care providers (HCP) from the haemophilia field including haematologists, orthopaedics, physiotherapists and sport-therapeutics. The HaemAcademy takes place three times a year alternating in the educating HTCs in Duisburg or Bremen. Additional trainings are organised in other HTCs in Germany, the location of these so-called “Flying

HaemAcademy" varies every year. The content of the basic and professional programme are reported in another abstract (The HaemAcademy - Structure and Content of a Special Training Programme for Physiotherapists to Optimize Treatment Options for Haemophilia Patients) . All participants evaluated the HaemAcademy after the training in a standardized evaluation form; in addition participants of the professional module received a short questionnaire asking them about their experience after the last basic training and their wishes and expectations towards the upcoming professional training.
Results: Since May 2013, 238 physiotherapists have been trained in 18 sessions (6-21 participants each): 16 basic modules including 6 additional "Flying HaemAcademy", and 2 professional modules. 83.6% of the participants were very satisfied with the organisation and the content of the programme (63.1%) and saw their expectations concerning the participation very fulfilled (65.3%). 27% felt completely prepared to treat now PWH appropriately and 89% would like to attend the professional module. 35.7% of the participants of the professional module could very/quite much implement the learned content in their routine practice.
Conclusions: The education programme provided by the HaemAcademy is unique in Germany. It is well accepted by physiotherapist and allows an optimised treatment of PWH. Evaluation of the basic module provided input to modify and improve the professional module.

P07 Posters: Antithrombotic therapy

P07-1

Cochrane review: Non-vitamin K antagonist oral anticoagulants (NOACs) post-percutaneous coronary intervention: A network meta-analysis

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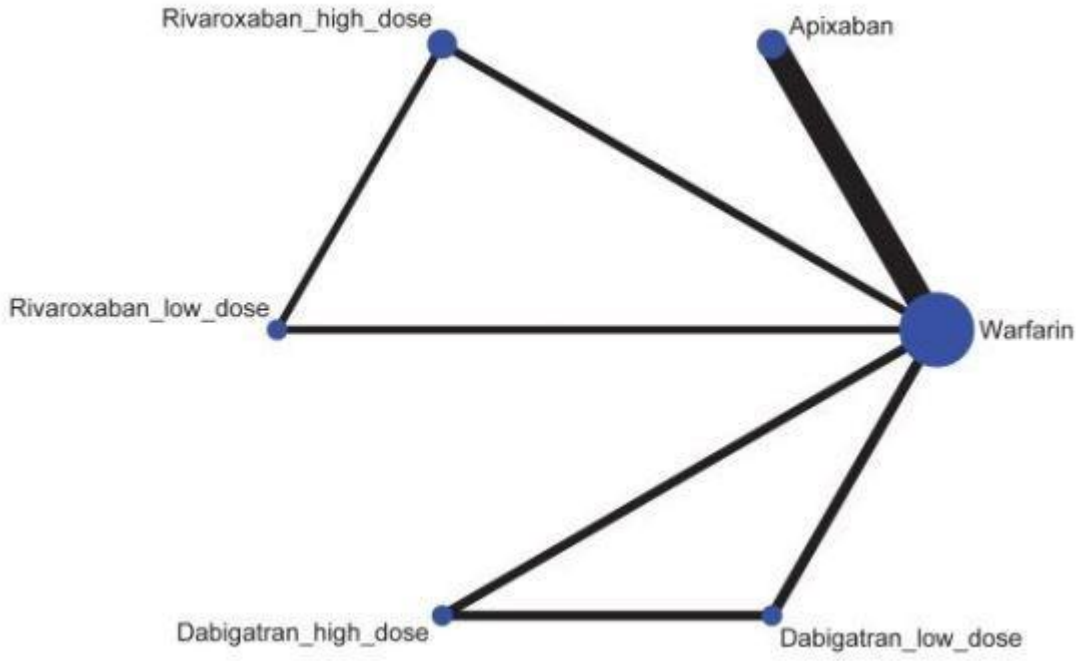
We performed a network meta-analysis (NMA) to review the evidence from randomised controlled trials (RCTs) assessing the efficacy and safety of NOACs post PCI in people with an indication for anticoagulation.
We identified studies by searching CENTRAL, MEDLINE, Embase, the Conference Proceedings Citation Index and two clinical trials registers in February 2019. Two review authors independently checked results of searches to identify relevant studies and extracted study data. We conducted random-effects pairwise analyses and network meta-analyses using the R package netmeta. Competing treatments were ranked by P-scores, which allow ranking of treatments on a continuous 0-to-1 scale and are derived from the P-values of all pairwise comparisons.
9 RCTs met inclusion criteria, but 4 were ongoing trials and not included in the analysis. Therefore, the NMA included 5 RCTs with 8,373 participants. There may be little or no difference between NOACs and VKA in death from cardiovascular causes, myocardial infarction, stroke, death from any cause and stent thrombosis. Apixaban and rivaroxaban probably reduce the risk of recurrent hospitalisation compared with VKA. No studies looked at health-related quality of life. NOACs may be safer than VKA in terms of major bleeding. Dabigatran probably reduces both major and non-major bleeding. Rivaroxaban probably reduces non-major bleeding. Apixaban probably increases rates of non-major bleeding compared to VKA. Our NMA did not show any significant difference between the different NOACs regarding all primary and secondary outcomes, except in terms of non-major TIMI bleeding. Rivoroxaban may be more effective than apixaban in reducing the rate of non-major TIMI bleeding. According to P-score, high-dose rivaroxaban appeared to be the most effective drug for preventing cardiovascular death. Low-dose rivaroxaban appeared to be the most effective drug for preventing myocardial infarction. Apixaban appeared to be the most effective drug for preventing stroke. Low-dose dabigatran appeared to be the most effective drug for preventing major bleeding. High-dose dabigatran appeared to be the most effective drug for preventing death from any cause.
Very low to moderate-certainty evidence shows no meaningful difference in efficacy outcomes between NOACs and VKA post PCI in people with an indication for anticoagulation. However, NOACs probably reduce the risk of recurrent hospitalisation compared with VKA. Low to moderate-certainty of evidence shows that dabigatran is probably safer than VKA in terms of major and non-major bleeding. Rivaroxaban

probably reduces the rates of non-major bleeding compared with VKA. Our NMA did not show superiority of one NOAC over another in all primary and secondary outcomes except in terms of non-major TIMI bleeding, where we found that rivaroxaban may be safer than apixaban. Head to head trials directly comparing NOACs against each other are now required to provide stronger evidence



P07-2

Rates, management and outcome of bleeding complications during edoxaban therapy in daily care: results from the *DRESDEN NOAC REGISTRY* (NCT01588119)

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Objectives: Edoxaban is approved for stroke prevention in atrial fibrillation (SPAF) and treatment of venous thromboembolism (VTE) and widely used in Germany. However, data on the rates, pattern, management and outcome of edoxaban-related bleeding complications are still scarce.
Methods: To evaluate rates, management and outcome of bleeding complications during edoxaban therapy. In the prospective, non-interventional DRESDEN NOAC REGISTRY more than 5000 patients are currently enrolled. In this database, we identified cases of edoxaban-related bleeding complication, which were classified as ISTH minor, clinically relevant non-major (CRNM) or major bleeding. We also evaluated the bleeding sites and the use of blood products and reversal strategies, based on the central adjudication of source data for all events in the registry.
Results: Until December 31st 2018, 951 patients treated with edoxaban were enrolled into the registry (740 SPAF; 203 VTE, 8 off-label). During a mean follow-up of 17.5±10.7 months, 431 patients had 713 bleeding events during edoxaban therapy (excluding events which occurred >72h after last drug intake). Characteristics of overall cohort and patients with bleeding events are presented in Table 1. Overall, patients with major bleeding complications tended to be older and more often had heart failure, diabetes, a history of stroke, coronary or peripheral artery disease, renal impairment or cancer compared to those without major bleeding during edoxaban exposure. Most events manifested as skin/mucosal bleeding (75.7%), followed by gastro-intestinal (11.2%),

genitourinary (9.3%), intracranial (1.1%), pulmonary (0.8%), or other bleeding (1.7%). Bleeding severity was adjudicated as ISTH minor in 53%, clinically relevant non-major (CRNM) in 41.7% and major bleeding in 5.3% (38 cases; classified as major for 22 cases of hemoglobin drop ≥2 g/dl; 18 with transfusion of ≥2 units of blood and 15 with critical organ bleeding). One bleeding was fatal (subarachnoid bleeding followed by symptomatic epilepsy and death due to aspiration pneumonia). The annualized rates of major or CRNM bleeding were 21.7and 21.7/100 pt. years, respectively. The majority of the 38 major bleeding events (76.3%) could be managed conservatively (no treatment, compression or blood transfusion only), 7 cases underwent interventional treatment (18.4%), 2 cases underwent open surgery (5.3%; Table 2). Although 20 cases needed red blood cell transfusions only 2 needed factor concentrates.

Conclusions: In daily care, bleeding complications are frequent also with edoxaban treatment. However, major bleeding is comparatively rare, can usually be managed conservatively and has a very low case fatality rate. However, the difference in patient characteristics between patients with or without major bleeding during edoxaban exposure indicates the complexity of risk-benefit considerations in multimorbid SPAF or VTE patients.

Table 1: Patient baseline characteristics of 951 patients and subgroups of patients with and without major bleeding during edoxaban therapy

	All patients n=951	No major bleeding n=916	Major bleeding n=35
Male, n (%)	533/951 (56.0)	512/916 (55.9)	21/35 (60.0)
Mean Age ± SD (years)	72.5 ± 11.2	72.3 ± 11.2	77.5 ± 7.3
Mean BMI ± SD (kg/m²)	28.8 ± 5.0	28.8 ± 5.0	28.3 ± 4.6
Heart failure, n (%)	247/951 (26)	233/916 (25,4)	14/35 (40)
Hypertension, n (%)	786/951 (82.6)	757/916 (82.6)	29/35 (82.9)
Diabetes, n (%)	277/951 (29.1)	259/916 (28.3)	18/35 (51.4)
TIA/Stroke in Hx, n (%)	102/951 (10.7)	97/916 (10.6)	5/35 (14.3)
CAD/PAD, n (%)	154/951 (16.2)	144/916 (15.7)	10/35 (28.6)
GFR <=50 ml/min, n (%)	136/951 (14.3)	127/916 (13.9)	9/35 (25.7)
Cancer, n (%)	127/951 (13.4)	120/916 (13.1)	7/35 (20)

SD, standard deviation; BMI, body mass index; TIA, transitory ischemic attac; CAD, coronary artery disease; PAD, peripheral artery disease, GFR, glomerular filtration rate

Table 2: Severity and management strategies of edoxaban-related bleeding complications

713 bleeding events in 431 patients	Conservative (no treatment/ compression / tamponade / transfusion)	Intervention	Surgery	RBC	Vit K	FFP	PCC	rFVII
Minor 378/713 (53)	378/378 (100)	0	0	0	0	0	0	0
NMCR 297/713 (41.7)	267/297 (89.9)	27/297 (9.1)	3/297 (1)	0	0	0	0	0
Major 38/713 (5.3)	29/38 (76.3)	7/38 (18.4)	2/38 (5.3)	20/38 (52.6)	0	0	2/38 (5.3)	0
TOTAL	674/713 (94.5)	34/713 (4.8)	5/713 (0.7)	20/713 (2.8)	0	0	2/713 (0.3)	0

FFP, fresh frozen plasma transfusion; NMCR, non-major clinically relevant; PCC, prothrombin complex concentrate; RBC, red blood cell transfusion; rFVII= recombinant Factor VII; Vit K, vitamin K supplementation.

[Patient baseline characteristics and edoxaban-related bleeding complications]

P07-3

Disseminated intravascular coagulation and stroke under DOAC treatment in a patient with homozygous antithrombin Budapest III mutation

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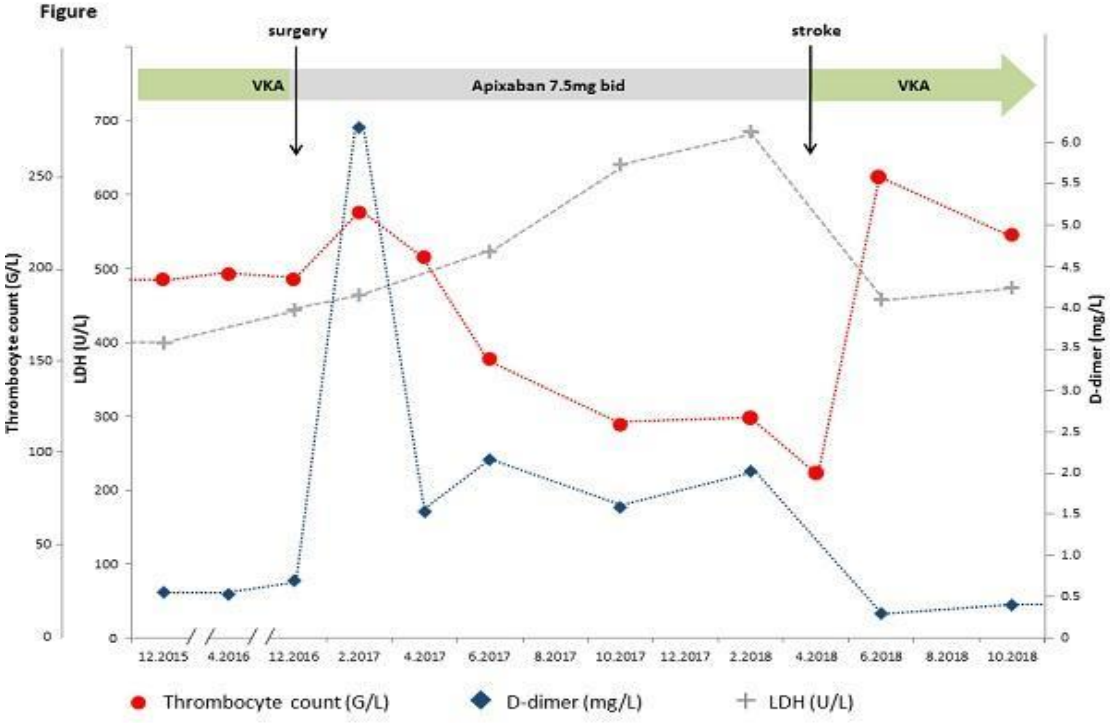
We report the case of a 53-year-old patient with hereditary antithrombin (AT) deficiency, who developed chronic coagulation activation and ischemic stroke while treated with a direct oral anticoagulant (DOAC), and complete normalisation of the clinical picture after switching to a vitamin K antagonist (VKA).
The patient was born in Albania. At the age of 21 he developed a non-provoked near fatal extensive thrombosis of the inferior caval vein. A diagnosis of hereditary AT deficiency was made with an AT activity of 42%. At that time, thrombectomy was performed and long-term anticoagulation with the VKA phenprocoumon was started. Family thrombotic history was unremarkable. He developed a moderate chronic renal insufficiency secondary to vascular damage of the left kidney. In June 2005 the patient had an acute ST-elevation myocardial infarction (STEMI) treated with percutaneous transluminal coronary angioplasty followed by 6 months of dual antiplatelet therapy plus VKA. In January 2006, he presented with a non-STEMI, with occlusion of the proximal stent, which was not re-canalized due to a good coronary collateralization. Aspirin 100 mg/d was continued together with the VKA. In December 2016, anticoagulation was switched to the direct anti-Xa inhibitor apixaban 5.0 mg BID, since the INR ranges were not well controlled. The apixaban dose was increased to 7.5 mg BID, resulting in median peak levels of 168.3 ng/mL. One month after changing from the VKA to a DOAC the patient underwent a surgical intervention for chronic venous insufficiency. Subsequent laboratory results started to show a progressive activation of coagulation with constantly elevated and worsening D-dimer levels and thrombocytopenia, and the patient started to complain of dizziness, and lightheadedness (figure 1). He developed superficial thrombophlebitis of the left leg managed by local treatment. Other causes of chronic disseminated intravascular coagulation (DIC) and thrombotic microangiopathy were ruled out. In May 2018 the patient experienced an acute ischemic cerebral event. Apixaban was then stopped, and the oral anticoagulation with VKA was resumed, with a bridging period of low molecular weight heparin and AT substitution. After one week, the clinical picture improved dramatically with disappearance of all neurological symptoms and resolution of the DIC (figure 1). Genetic testing revealed a homozygous mutation in exon 2 of the AT gene SERPINC1 (c.391C>T, p.Leu131Phe identified by PCR amplification and sequencing), known as a heparin-binding site defect, AT Budapest III.
In conclusion, our patient with a homozygous heparin binding site antithrombin deficiency showed a normal coagulation under phenprocoumon, but a DIC-like picture while treated with apixaban. Further investigations are required to clarify the underlying mechanisms.

Figure



P07-4

Comparison between patients under DOAC or VKA therapy: How to manage these patients before cardiothoracic surgery

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Scientific research question: Due to the progressive use of direct oral anticoagulants (DOACs) as an alternative to vitamin K antagonists (VKA), patients under medication with such a DOAC are also increasingly found in cardiac and thoracic surgery. In recent years, although more and more recommendations for dealing with these patients before surgery in general have been published, for patients specifically undergoing cardiac and thoracic surgery, recommendations are still missing. In our clinic, we have developed a standard operation procedure to fill this gap.
Methodology: Between 04/2014 and 07/2017, we included 14708 patients undergoing major heart surgery in our study. Patients on DOAC therapy were told not to take the drug three days before hospitalization. Depending on the anticoagulation, patients were divided into two groups (patients receiving DOAC or VKA therapy). To make the groups comparable, samples from certain patients, e.g. emergency surgery or the implantation of a TAVI were excluded. Furthermore, we excluded patients with an INR >1.3 from our study, while patients with a DOAC concentration >30 ng/mL did not undergo surgery at all. Measurement of the INR or DOAC concentration was performed on the day of surgery or one day before. This resulted in 239 VKA and 487 DOAC patients who were included in our analysis.
As the primary endpoint, we set the blood loss from the 12h drainage volume. In addition, we also surveyed the number of transfused blood products, 30-day mortality and rethoracotomies.
Findings: For evaluation, the baseline parameters were adjusted containing, among others, the EuroSCORE, age, gender, renal function and preoperative hemoglobin concentration. The primary endpoint was not significant between the DOAC and VKA groups (502 mL/12h VKA group vs. 497 mL/12h DOAC group). Furthermore, there

were no significant differences in the used blood products (red blood cells, fresh frozen plasma, thrombocytes, fibrinogen), the 30-day mortality or the rethoracotomy rate. Also, we did not detect any thromboembolic events in the time between DOAC discontinuation and surgery.
However, we saw, for example, an influence of age and type of surgery on the drainage volume. In addition, we calculated the time between the last DOAC intake and a DOAC concentration measurement of < 30ng/mL, which was 3.8 days in median.
Conclusions: Discontinuation of DOAC medication three days prior to admission and measurement of DOAC concentration on the day before surgery (cut-off: 30 ng/mL) show comparable results in 12h drainage volumes compared to patients receiving VKA therapy. The other endpoints also show no inferiority of the patients under DOAC therapy. Therefore, we recommend that other clinics with a focus on heart surgery integrate a DOAC measurement protocol to increase perioperative safety in heart surgery.

P07-5

Inter-individual variability of peak and trough plasma concentrations of dabigatran, rivaroxaban and apixaban in patients with non-valvular atrial fibrillation

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Scientific research question: Inter-individual variability of both peak and trough plasma concentrations of direct oral anticoagulants (DOACs), dabigatran, rivaroxaban and apixaban, is still insufficiently investigated. Therefore, the aim of our study was to assess inter-individual variability of peak and trough plasma levels of all three DOACs in patients with non valvular atrial fibrillation (NVAF).
Methodology: The study included plasma samples from patients treated with dabigatran (N = 106, 150 mg twice-daily), rivaroxaban (N = 123, 20 mg once-daily) and apixaban (N = 69; 5 mg twice-daily). Blood samples were taken on the same day to obtain both trough (immediately prior the next drug dose) and peak (two hours after drug administration) DOACs concentrations. Both rivaroxaban and apixaban were measured using chromogenic anti-FXa assay (Innovance anti-FXa, Siemens Healthineers, Germany) calibrated with drug specific calibrators (Hyphen BioMed, France). Dabigatran was measured using commercial chromogenic method (Innovance DTI assay, Siemens Healthineers, Germany). All coagulation assays were performed on Behring Coagulation System XP (BCSXP) analyzer (Siemens Healthineers, Germany). Statistical analysis was done using Mann-Whitney test by MedCalc Statistical Software version 11.5.1. The inter-individual variability for trough and peak concentrations was assessed by calculating mean values and standard deviation (SD) for each DOAC concentration measured for all samples. The study was funded as an integral part of the Croatian Science Foundation research project IP-2016-06-8208, entitled New oral anticoagulants: relationship between drug concentration and anticoagulant effect.
Findings: Concentrations for all three DOACs ranged as follows: dabigatran (peak 3 - 473 ng/mL; trough 0 - 292 ng/mL); rivaroxaban (peak 13 - 468 ng/mL; trough 1 - 311 ng/mL) and apixaban (peak 56 - 396 ng/mL; trough 10 - 259 ng/mL) with significant differences between peak and trough concentrations ($P < 0.001$) for all three DOACs (Table 1). Inter-individual variability expressed as coefficient of variation (CV%) for both peak and trough plasma concentrations of all three DOACs was as follows: dabigatran (peak 69.6%; trough 95%), rivaroxaban (peak 50.3%; trough 149%), apixaban (peak 40%; trough 51%), as shown in Table 1.
Conclusion: Our study showed a relatively high inter-individual variability for all three DOACs in NVAF patients. Our results demonstrated that the overall inter-individual variability for all three DOACs to be lower at peak than at trough plasma concentrations. Further, among all three DOACs, apixaban showed the lowest inter-individual variability for both peak and trough concentrations. Relatively high inter-individual variability of peak and especially for trough concentrations of all three DOACs suggests that single measurement of these drugs could not be sufficient for reliable estimation the level of anticoagulation.

	N	Dosing regimen	Median (95%CI) and IQR (ng/mL)	P	Inter-individual variability (CV%)
Dabigatran peak	106	150 mg twice-daily	138 (109.4-172.0) 65 - 246	<0.001	69.6
Dabigatran trough	106	150 mg twice-daily	55 (44.4-67.6) 21 - 107		95.0
Rivaroxaban peak	123	20 mg once-daily	165 (147.4 - 189.3) 118 - 212	<0.001	50.3
Rivaroxaban trough	123	20 mg once-daily	14 (12 - 16) 9 - 29		149.0
Apixaban peak	69	5 mg twice-daily	182 (165.3 - 202.4) 141 - 226	<0.001	40.0
Apixaban trough	69	5 mg twice-daily	100 (82 - 119.6) 58.7 - 136.3		51.0

[Table 1. Peak and trough concentrations and associated inter-individual variability for dabigatran, rivaroxaban and apixaban in NVAF patients.]

P07-6

Thrombin generation in samples with residual plasma levels (< 50 ng/ml) of direct oral anticoagulants

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Scientific research question: Currently, the International Society on Thrombosis and Haemostasis recommends that a direct oral anticoagulant (DOAC) plasma level > 50 ng/mL in patients with serious bleeding might be sufficiently high to warrant administration of an antidote. In patients requiring urgent intervention associated with a high bleeding risk, antidote administration might even be considered at plasma levels > 30 ng/ml. However, additional experimental and clinical data are required to support this recommendation. Especially data on thrombin generation in patients with DOAC plasma levels < 50 ng/ml are scarce.
Methodology: A total of 80 patients (20 for each DOAC - apixaban, edoxaban, rivaroxaban and dabigatran) and 55 healthy blood donors were recruited. Blood plasma was sampled before (baseline) and 3, 6 and 12 hours after intake of the DOAC as well as once in controls. Blood samples with DOAC levels < 50 ng/ml after drug intake were selected. Thrombin generation (TG) was assessed with ST Genesia (Stago) and compared to the TG at baseline before DOAC intake and to that of the controls.
Findings: A DOAC plasma level < 50 ng/ml was detected in 55 of the 240 samples (28 samples with factor-Xa-inhibitors, 27 samples with dabigatran). TG (particularly lag time and peak thrombin) was significantly reduced in samples containing < 50 ng/ml of a factor-Xa-inhibitor when compared to baseline of the patients as well as to that of controls. Dabigatran samples with plasma level < 50 ng/ml showed significant prolongation of the lag time in comparison to baseline and to that of the controls, while other TG parameters did not change significantly (table 1).
Conclusion: Patients with low residual DOAC plasma levels < 50 ng/ml show a significant reduction of TG parameters compared to baseline and to those of healthy controls. These results may aid clinical decision making in urgent surgery, interventions or elective operation in critical areas (e.g. central nervous system, eye). Our data support the current recommendation of antidote administration even at low plasma levels < 50 ng/ml in bleeding patients.

	Drug level ng/ml	Lag time (min)	Peak (nM)	Time to peak (min)	Velocity Index (nM/min)	ETP (nM*min)
Factor Xa-inhibitors						
Baseline	0	1.5 1.3-1.6	452 (401-484)	2.7 (2.3-2.9)	568 (477-622)	1656 (1465-1906)
<50 ng/ml	37 ± 11	2.1* (1.8-2.3)	348* (306-389)	3.9* (3.3-4.5)	282* (205-329)	1716 (1435-1817)
Dabigatran						
Baseline	0	1.5 (1.3-1.6)	452 (295-523)	2.9 (2.5-3.2)	514 (438-679)	1882 (1532-2504)
< 50 ng/ml	23 ±15	1.7* (1.6-2.1)	462 (381-569)	3.1 (2.7-3.3)	527 (414-708)	1916 (1595-2664)
Donors		1.4 (1.3-1.5)	429 (405-465)	2.7 (2.6-2.9)	471 (384-531)	1648 (1482-1855)

Abbreviations: ETP endogenous thrombin potential.
***) p < 0.001 (values were compared to the baseline and control using the Mann-Whitney-U-test).**
Data are expressed as mean ± standard deviation (plasma level) or median (thrombin generation) and interquartile range (25th-75th percentile).

[Table.1 Thrombin generation of DOAC samples with plasma levels < 50 ng/ml compared to baseline and control group]

P07-7

Gender-associated diversity in patients´ DOAC levels

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Objectives: Direct oral anticoagulants (DOAC) like the Xa inhibitors Rivaroxaban (riva) and Apixaban (apix) are increasingly replacing Vitamin K antagonists in the prevention of thromboembolic recurrences. Peak and trough levels in patients blood, however, do overlap to such an extent that concentrations determined by laboratory assessments cannot be identified as such in those patients in who the time point of oral intake is unknown. Narrowing the present range of maximum concentrations according to gender and/or age, which are both usually communicated during laboratory diagnostics, might help to overcome this uncertainty.
Methods: We collected data on peak and trough anti-Xa concentrations of patients treated with either rivaroxaban (n=83) or apixaban (n=49) in a one-center outpatient clinic for hemostaseology. Differences between male (riva n= 42, apix n= 28) and female patients (riva n= 41 and apix n= 21) as well as young (< 60 years, riva n= 44, apix n= 21) and elder (>60 years) patients (riva n= 39 and apix n= 28) were analyzed by student´s t-test.
Results: No gender- or age differences were found in peak levels of apix, but female patients had significantly higher peak concentrations of riva (308.8±178,1 ng/ml) than male individuals (206.4±80 ng/ml, p= 0.013), and patients older than 60 years had significantly higher riva peak levels than those under the age of 60 (293.7±126.7 ng/ml versus 211.7±158,4 ng/ml, p= 1.29x10-8).
Conclusions: Gender and age should be included in the determination of peak blood concentrations of DOACs. Reasons for different findings between patients treated with riva and apix might lie in the different frequency of oral intake (riva once versus apix twice daily).

P07-8

Monocyte activation and acquired protein S deficiency promote disseminated intravascular coagulation in a patient with antiphospholipid antibodies

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Background: Acquired (i.e. antibody mediated autoimmune) protein S (PS) deficiency is a rare and potentially life-threatening disorder. Antiphospholipid antibodies (aPL) are procoagulant and have the capacity for inducing tissue factor (TF) expression in monocytes. To the best of our knowledge, the concomitant occurrence of acquired PS deficiency and aPL-mediated TF expression has not been reported previously in an individual.
Aim: We describe the case of a 76-year-old woman with disseminated intravascular coagulation (DIC) associated with elevated aPL and acquired PS deficiency.
Methods: We measured protein C (PC), PS, activated PC resistance (APC-R), factor V gene mutation Leiden, lupus anticoagulant (LA), and antibodies against cardiolipin (aCL) or beta2-glycoprotein I (anti-β2-GPI). Serum IgM and IgG were purified by immunoprecipitation and analyzed for PS inhibitory activity following addition of normal human plasma (NHP). In addition, a stimulatory effect on monocytes by ex-vivo incubation of peripheral blood mononuclear cells (PBMCs) from healthy donors was investigated. TF-mediated procoagulant activity (PCA) of PBMCs and plasma microvesicles (MVs) was measured by single-stage clotting assay. Furthermore, PBMCs were analyzed for expression of monocyte TF antigen and release of procoagulant MVs by flow cytometry and chromogenic FXa generation assay, respectively.
Results: Initially, the patient presented with a painful reticular erythema indicating microvascular thrombosis and a consumptive coagulopathy: prothrombin time 45.5 % (normal: 80-130 %), fibrinogen 0.5 g/L (1.8-4.0 g/L), D-dimer 34 mg/L (< 0.5 mg/L). Surgical and antibiotic treatment of concurrent sigmoid diverticulitis did not resolve DIC. Overt malignancy was excluded. The coagulopathy was controlled by continuous anticoagulation with heparin or a direct oral FXa inhibitor. Titers of IgM-aCL and IgM-anti-β2GPI were elevated up to 140 U/mL (< 10 U/mL) and 12 U/mL (< 7 U/mL), respectively. Antinuclear antibodies and tests for LA were negative. There was an abnormal APC-R ratio of 1.7 (> 2.0) in the absence of a factor V gene Leiden mutation. Free and total PS antigen were normal, but PS activity was largely reduced to 14 % (55-125 %). Plasma PS activity was inhibited, when NHP was incubated with patient IgG, but not IgM, indicating a function blocking autoantibody against PS of the IgG isotype. In plasma, significant MV-associated TF PCA was detected, and ex-vivo stimulation with purified patient IgG, but not IgM, activated PBMCs from healthy donors with increased TF production and MV shedding.
Conclusion: In this case, activation of monocytes by aPL in combination with acquired APC-R and depletion of protein S activity caused a systemic coagulopathy characterized by microvascular thrombosis, which could only be controlled by uninterrupted anticoagulation.

P07-9

Single-site functionalized heparins for modular immobilization and coagulation responsive release

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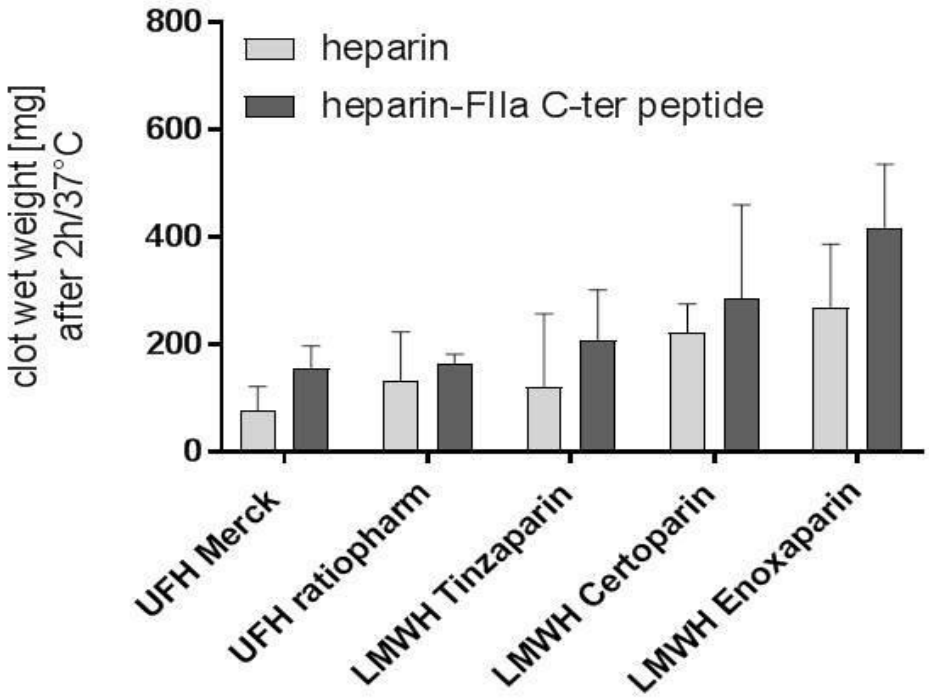
Surface immobilization of heparin is a promising strategy in the development of modern anti-coagulant high performance surface coatings. Here we present an approach for a responsive surface immobilization, based on the use of cleavable linker peptides. The strategy enables the release of single site functionalized heparin triggered by factors of the coagulation cascade, resulting in a local and on demand inhibitory effect without the risk of drug overdosage. Thereby the influence of heparin peptide functionalization on the anti-coagulant activity was investigated and compared for different heparins of varying molecular size. Three different LMWH as well as two UFH were functionalized with N-(2-aminoethyl)maleimide TFA to a final degree of one maleimide group per heparin molecule. The FIIa cleavable peptide NH_2-Gly-Cys-Gly-Gly-(D)Phe-Pip-Arg-↑↓-Ser-Trp-Gly-Cys-Gly-$CONH_2$ should be used as linker peptide in the final system. To model the surface released heparin upon peptide degradation the C-terminal peptide fragment was conjugated by click chemistry. Peptide functionalization was analyzed as chromogenic assay *via* the determination of reactive maleimide moieties. Inhibitory activities of the unmodified and peptide functionalized heparin molecules for FIIa and FXa were measured as chromogenic assay in a buffer system. Besides heparins were incubated with whole blood together with coagulation stimulating surfaces and analyzed for their inhibitory potential and hemocompatible properties.
For all five tested heparin molecules, a functionalization degree of one reactive maleimide group per heparin molecule was achieved. Peptide conjugation decreased the amount of reactive maleimide moieties to the level of detection limit, confirming maleimide saturation by the peptide. The dependence of anti-FIIa activity on the heparin molecule size was confirmed, but anti-FXa and

anti-FIIa activity were not affected by the peptide functionalization. Upon exposure of blood heparinized with comparable anti-FXa units to coagulant conditions, a heparin size dependent clot formation was detected. Peptide functionalized compounds did not induce increased cell activation or inflammatory response, but a loss of activity upon the addition to whole blood and increased clot formation could be detected for those molecules (Fig. 1). However, residing heparin activity was still sufficient to exhibit an anti-coagulant effect.



[Clot formation of heparinized whole blood under coagulant conditions dependent on heparin size.]

The functionalization of heparins with cleavable peptide linkers offers a potential strategy for responsive surface immobilization of both UFH and LMWH. The approach does not affect the heparins potential to inhibit the coagulation factors FIIa and FXa. Loss of activity for the functionalized compounds in whole blood could be counteracted by increased heparin concentration.

P07-10

Sulfated Glycans as direct and C1 inhibitor-mediated inhibitors of factor XIa activity

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Scientific research question: The serine plasma protease factor XI (FXI) and its activated form FXIa are currently attracting attention as targets for novel antithrombotics since FXI deficiency is associated with low bleeding risk and reduced incidence of stroke. Besides inhibition of FXIa, improvement of its regulation represents promising therapeutic strategies. FXIa activity is mainly controlled by the C1 inhibitor (C1-INH), an important endogenous regulator of both complement- and contact system. Previous studies revealed that heparin, heparan sulfate and dextran sulfate potentiate FXIa inhibition by C1-INH and influence the FXIa activity directly. The aim of the study was to evaluate structure-activity relationships by testing well-characterized sulfated glycans (SG).
Methodology: The SG included glycosaminoglycans, two series of semisynthetic linear β-1,3-glucan sulfates (low-molecular weight phycarin sulfates (PhyS) and high-molecular weight curdlan sulfates (CurS)) and other selected SG. Their effects on the FXIa activity in presence and absence of C1-INH were analysed using a chromogenic FXIa activity assay. Additionally, the binding characteristics of heparin, the heparin contaminant oversulfated chondroitin sulfate (OSCS) and the anti-inflammatory drug substance pentosan polysulfate (PPS) were examined using biolayer interferometry (BLI).
Findings: All tested SG enhanced C1-INH-mediated inactivation of FXIa. The extent of their potency

showed to be dependent on their molecular weight (MW) and degree of sulfation (DS). The β-1,3-glucan sulfate PhyS4 turned out as most promising C1-INH-mediated inhibitor of FXIa. In absence of C1-INH, all SG with DS > 1.35 additionally inhibited FXIa activity directly. Both the activity and BLI results revealed a novel mechanism of the APTT prolongation by OSCS and PPS.
Conclusion: The study provided basic information about the structure-dependent potentiating effects of SG on the FXIa activity by C1-INH. Further investigations are needed to evaluate the therapeutic potential of SG as FXIa inhibitors.

P08 Posters: Platelet biology and pathophysiology II

P08-1

Twinfilin 1 and Cofilin 1 synergistically regulate actin and microtubule rearrangements during platelet biogenesis in mice

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Scientific research question: Rearrangements of the actin and microtubule cytoskeleton are essential for platelet biogenesis and function. Hence, defects in actin- or microtubule-regulatory proteins are associated with platelet disorders in humans and mice. Structure and dynamics of the actin cytoskeleton in eukaryotic cells are regulated by the actin-depolymerizing factor homology (ADF-H) domain family of small actin binding proteins. Among the ADF-H domain protein family, Twinfilin 2a (Twf2a) was shown to regulate platelet reactivity and turnover, whereas loss of Twinfilin 1 (Twf1) did not affect platelet biogenesis or function in mice. Recent studies have indicated an interaction between Twf1 and another ADF-H-domain family member, Cofilin1 (Cfl1), in the regulation of actin dynamics.
Methodology: We generated mice lacking both Twf1 and Cfl1 in the MK lineage.
Findings: Twf1/Cfl1 double-deficiency (*DKO*) resulted in a severe macrothrombocytopenia associated with dramatically elevated MK numbers in bone marrow and spleen of the affected animals. *DKO* MKs exhibited defective proplatelet formation in vitro and in vivo as well as impaired spreading and podosome formation on collagen and fibrinogen in vitro. This was associated with increased actin stress fiber formation and microtubule stability, resulting from dysregulation of the actin- and microtubule-binding proteins mDia1 and APC. Surprisingly, the minor functional defects described for Cfl1-deficient platelets, e.g. delayed spreading as well as mild integrin activation and aggregation defects, were only slightly aggravated in *DKO* platelets suggesting that both proteins are largely dispensable for platelet reactivity.
Conclusion: In summary, these findings point to critical redundant functions of Cfl1 and Twf1 for both actin and microtubule dynamics during thrombopoiesis.

P08-2

The impact of hypoxia on FasR-FasL mediated interaction of human platelets and red blood cells

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Background: Platelets (Plts) and red blood cells (RBCs) are able to interact directly by their membrane bound FasR-FasL. This cell-cell-communication was found to be essential for the non-apoptotic externalization of phosphatidylserine (PS) at the RBC membrane and to provide a pro-coagulant surface important for thrombus formation and stabilization. The alterations and consequences of this interaction caused by hypoxia are currently unknown.
Methodology: *In vitro* analysis of FasR-FasL expression of isolated human platelets and red blood cells from healthy donors kept under normoxic (21% O_2) and hypoxic (2% O_2) conditions.
Findings: The incubation of human platelets under hypoxic conditions (2% O_2) leads to an increased adhesion on recombinant FasR indicating a heightened affinity to their interaction. This effect was also found to be consistent by inhibition of FasL using FasL inhibitor. Further alterations of FasR expression were shown at the RBC membrane. Due to a longer incubation time the expression of FasR protein, in detail of one particular cleaved fragment, increased significantly. Recently, it was found that platelet-

RBC interaction facilitates the externalization of PS on the surface of both cell types, which is important for thrombin generation and thrombus formation. As indicated by flow cytometric analysis of platelets and RBCs this effect is more pronounced under hypoxic conditions. Furthermore, the experiments revealed that FasR externalization on the RBC surface in the presence and absence of platelets is augmented under hypoxic conditions compared to normoxic conditions.
Conclusion: In conclusion, these experiments provide first evidence for an augmented protein expression and FasR-FasL mediated interaction of human platelets and RBCs under hypoxic conditions. Since cardiovascular diseases are often associated with local or systemic hypoxia, these findings reflect a high clinical relevance. Thus, these results might be the basis for a conceivable approach of a completely novel antithrombotic strategy supporting the treatment of various cardiovascular diseases in future.

P08-3

Involvement of Glycoprotein VI in platelet mediated amyloid-β fibril formation

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Scientific research question: The progression of Alzheimer's disease (AD) is associated with cerebral amyloid angiopathy (CAA). CAA is characterized by the deposition of amyloid-β peptides (Aβ), mainly Aβ40, in the walls of cerebral vessels. These amyloid deposits can reduce cerebral blood flow, trigger inflammation and lead to cognitive decline. Platelets express high amounts of the amyloid precursor protein (APP) and display all enzymatic activities necessary to produce amyloid β (Aβ) peptides. Recently, we showed that integrin $\alpha_{IIb}\beta_3$ binding to Aβ40 induces outside-in signalling which contributes to Aβ aggregation. In this study, we investigated the involvement of the major platelet-activating collagen receptor GPVI on platelet mediated amyloid β fibril formation.
Methodology: Cell culture experiments and immunoblotting to analyze cell signalling.
Findings: GPVI deficient platelets show reduced platelet aggregation, ATP and fibrinogen release upon stimulation with Aβ40. Moreover, GPVI inhibition by antibody treatment as well as GPVI deficiency decrease platelet mediated amyloid-β fibril formation *in vitro*. Immunofluorescence staining of amyloid β deposits and fibrinogen indicates binding of fibrin (-ogen) to platelets and amyloid-β fibrils. Binding studies confirmed that monomeric Aβ40 binds to GPVI and induces activation of the GPVI signalling cascade.
Conclusion: Binding of Aβ40 to GPVI leads to activation of platelets and consequently to amyloid-β aggregation through binding of Aβ40 to integrin $\alpha_{IIb}\beta_3$.

P08-4

Establishment of a method for the analysis of intracellular platelet proteins

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Scientific research question: Platelets can be activated by a number of mediators (e.g. thrombin, ADP). This induces different signaling pathways within the platelets. Since platelets do not have their own expression system, posttranslational modifications must be used to investigate such pathways. One example is the phosphorylation of proteins, which regulates their activity. The phosphorylation of certain amino acid residues indicates whether the protein is present in its active or inactive form. Such phosphorylations can be detected by Western blot using specific antibodies. Src is a protein that is regulated by phosphorylation/dephosphorylation. By comparing inactivated and activated platelets, Src-induced signaling pathways can be investigated in more detail.
The aim of our study was to establish a sample preparation for the investigation of intracellular platelet proteins before and after platelet activation. Different conditions were tested, including different activators, lysis and activation conditions.
Methodology: In this study, washed blood platelets were first generated, activated with various agonists and thereafter lysed. For the lysis, different conditions were tested, e.g. different chemical and mechanical lyses. Various silicon dioxide spheres and ultrasound were used for mechanical lysis, and frost-thaw cycles were also carried out. Western blots were performed for the subsequent analysis of different proteins.

Findings: The first experiments showed a significant protein loss after activation of the platelets (detected by Western Blot). The main problems were the adhesion of the activated platelets to the laboratory vessel wall and a significantly reduced lysis of the activated and aggregated platelets. Because of the protein loss, activated and inactivated platelets could not be compared.
Therefore different combinations of activators, lysis buffers or lysis conditions were tested. A significant improvement could be observed in several experimental approaches. This was shown by the fact that the protein loss in the activated approaches could be significantly reduced. It was found that platelet activation and subsequent lysis should be performed in the same laboratory vessel. In addition, the use of mechanical lyses in the activated cells showed a significantly higher effectiveness. The best results were achieved with silica spheres and freeze-thaw cycles. The use of different lysis buffers still showed no efficient results with the activated approaches.
Conclusion: In our study we were able to optimize the sample preparation for comparison of inactivated and activated platelets. We could significantly reduce the previously observed protein loss and are now able to compare intracellular signaling proteins, such as Src, of resting and activated platelets.

P08-5

Influence of bile acids on hemostasis and arterial thrombosis

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Scientific research question: Patients with liver disease suffer from bleeding complications and thrombocytopenia but also exhibit hypercoagulability suggesting a bivalent role of platelets in liver disease. Induced cholestatic liver disease through bile duct ligation in mice leads to impaired platelet activation and thrombus formation *in vitro* as well as bleeding complications *in vivo*. It is hypothesized that increased levels of bile acids in plasma affect platelet activation and hemostasis, whereas the underlying mechanisms are not well understood. In this study we investigated the effect of bile acids on platelet activation and hemostasis.

Methodology: Human and murine platelets were analyzed in *in vitro* and *ex vivo* experiments regarding the effect of a variety of common bile acids on platelet activation.
Findings: The incubation of isolated murine and human platelets with bile acids resulted in reduced adhesion on a fibrinogen matrix 5, 20 and 60 minutes after adhesion as well as reduced amount of fully spreaded platelets. Flow cytometric analysis of murine and human platelets revealed a reduced P-selectin expression as well as integrin $\alpha_{IIb}\beta_3$ activation following preincubation with different bile acids whereas bile acids alone had no effect on platelet activation. In aggregation experiments no significant differences in platelet aggregation could be detected. However, diminished platelet activation resulted in reduced thrombus formation on a collagen matrix under arterial flow conditions using human and murine whole blood which were preincubated with different bile acids. Regarding the underlying mechanism, bile acids lead to platelet inhibition through phosphorylation of vasodilator activating phosphoprotein (VASP) on serine residue 157 in a time dependent manner as shown in western blot analysis of isolated murine and human platelets.
Conclusion: These results reveal that bile acids affect platelet activation negatively and impair thrombus formation, indicating a new class of negative regulators of primary hemostasis.

P08-6

Activated platelets kill *Staphylococcus aureus,* but not *Streptococcus pneumoniae* - The role of FcγRIIa and platelet factor 4/heparin-antibodies

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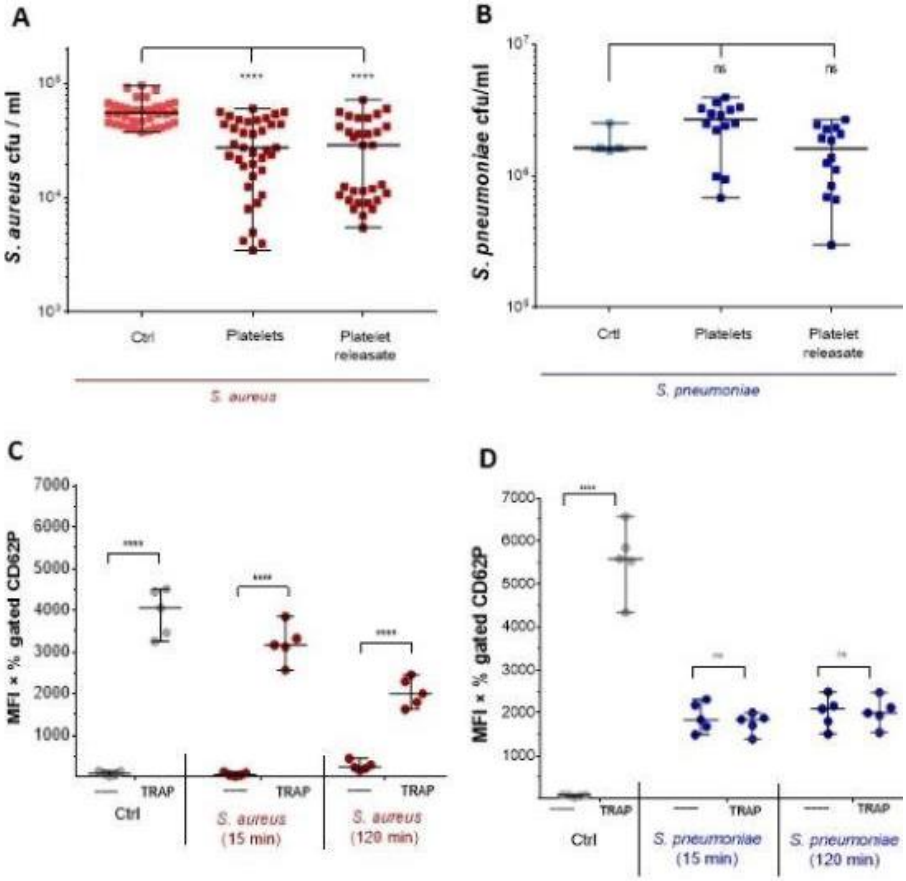
Scientific research question: Heparin induced thrombocytopenia (HIT) is likely a misdirected bacterial host defense mechanism. When Platelet factor 4 (PF4) binds to negatively charged polyanions such as heparin or polyanions on the surface of bacteria, it changes its conformation and exposes neoepitopes to which anti-PF4/heparin

(PF4/H) antibodies bind. Platelets are activated by the resulting immune complexes via FcγRIIA, release bactericidal substances and kill gram-negative *E. coli*. In this study we wanted to assess the role of PF4, anti-PF4/H antibodies and FcγRIIa in killing of gram-positive bacteria by platelets.
Methodology: Binding of PF4 to protein-A deficient *Staphylococcus aureus* (SA113Δ*spa*) and non-encapsulated *Streptococcus pneumoniae* (D39Δ*cps*) and its conformational change were assessed by flow cytometry using monoclonal anti-PF4/H antibodies such as KKO and 5B9 as well as patient derived antibodies. Killing of bacteria was quantified by counting colony forming units (cfu) on blood agar plate after incubation with platelets or platelet releasate. Flow cytometric analysis was used to assess platelet function after incubation with gram-positive bacteria. We measured the activation and reactivity to a TRAP-6 stimulus by CD62P expression or PAC-1 binding and quantified phosphatidyl serine (PS)-exposure via Annexin V on the platelet surface.
Findings: Monoclonal and patient-derived anti-PF4/H antibodies bound in the presence of PF4 to both bacterial strains with different intensities (1.6-fold increased fluorescence signal for human anti-PF4/H antibodies to 24.0-fold increase for KKO). *S. aureus* (5.5×10^4 cfu/ml) was efficiently killed by platelets (2.7×10^4 cfu/ml) or their releasate (2.9×10^4 cfu/ml), reducing cfu by approximately 50 %. Killing was not significantly enhanced by PF4 or anti-PF4/H antibodies. Blocking FcγRIIa had no impact on killing of *S. aureus* by platelets. In contrast *S. pneumoniae* (1.64×10^6 cfu/ml) was neither killed by platelets (2.7×10^6 cfu/ml) nor platelet releasate (1.62×10^6 cfu/ml). Instead pneumococci affected viability of platelets. After incubation with pneumococci, platelets were unresponsive to TRAP-6 stimulation and expressed high levels of PS. Platelets incubated with *S. aureus* showed normal response to TRAP-6 stimulation and exposed no PS.
Conclusions: Anti-PF4/H antibodies seem to have only a minor role for direct killing of Gram-positive bacteria by platelets. *S. aureus* is killed by platelets or platelet releasate. In contrast, *S. pneumoniae* affects viability of platelets.



[Ability of platelets to kill S.aureus and inability to kill S.pneumoniae correlates with activation]

Incubation of *S. aureus* with platelets or platelet releasate led to significant reduction of counted cfu [A] and platelet responded well to stimulation with TRAP-6 after incubation [C]. In contrast, platelets or releasate of platelets failed to kill *S. pneumoniae* [B] and platelets were unresponsive to TRAP-6 stimulus [D].

P08-7

Determination of coagulation factor XIII in different blood products

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Scientific research question: Coagulation factor XIII (FXIII) is in its active form a transglutaminase which mediates the crosslinking of fibrin. After the conversion of fibrinogen to fibrin by thrombin, this is the last step of plasmatic coagulation and essential to achieve stable wound closure. To prevent postoperative bleeding, an adequate activity of FXIII has to be maintained. Transfusion of various blood products e.g. fresh frozen plasma (FFP) or platelet concentrate (PC), might become necessary in case of high blood loss. With respect to FXIII activity, transfusion of FFPs or PCs may result in a possible increase or dilution of transglutaminase activity, depending on the initial FXIII activity of the patient and the volume administered. To gain insight into the effect of FFP and PC transfusions on FXIII activity in patients, the aim of this study was to determine the activity of FXIII in FFPs and plasma of PCs, together with the potential activity of FXIII in platelets of PCs.
Methodology: FXIII activity was analyzed in the plasma of first-time blood donors, FFPs, as well as apheresis or pool PC plasma. In addition, well washed platelets (WWPs) were produced from one to five-day-old apheresis or pool PCs. After freeze-thaw lysis of WWPs, the activity of FXIII in the resulting supernatant was determined. Determination of FXIII took place using Siemens coagulation analyzer CS-5100 (photometric assay).
Findings: The activity of FXIII in plasma of first-time blood donors was 2.40 ± 0.57 U·mL^{-1} (126.2 ± 30.0%) (range 1.51 - 4.52 U·mL^{-1} (79.4 - 237.6%)). The FXIII activity in FFPs was 2.27 ± 0.48 U·mL^{-1} (119.2 ± 25.2%) (range 1.46 - 3.66 U·mL^{-1} (76.7 - 192.0%)). PCs showed a plasma activity of 1.65 ± 0.66 U·mL^{-1} (86.9 ± 34.4%) (range 0.22 - 3.77 U·mL^{-1} (11.7 -197.9%)). In platelet lysates the activity was normalized to the platelet count (10^9 PLT) and showed a very heterogenic distribution between 0.77 U·(10^9 PLT)$^{-1}$ (40.3%·(10^9 PLT)$^{-1}$) and 6.00 U·(10^9 PLT)$^{-1}$ (315.2%·(10^9 PLT)$^{-1}$) (mean 2.36 ± 1.07 U·(10^9 PLT)$^{-1}$ (124.4 ± 56.2%·(10^9 PLT)$^{-1}$)).
Conclusion: The FXIII activity of FFPs almost corresponds to that of healthy donors. In plasma of PCs, transglutaminase activity is lower than in healthy donors. A hypothetical transfusion scenario, e.g. of two PCs, would not significantly affect the FXIII activity of a patient in the normal range (1.90 U·mL^{-1}, 100%) and would only increase a pathological activity of 0.95 U·mL^{-1} (50%) by 7%. In contrast, blood platelets from PCs contain a high amount of FXIII, corresponding to about 550 U per PC. To confirm this, an antigen-based test has to be performed. In addition, in vivo studies would provide insights into the possible release of FXIII by platelets during PC transfusions.

P08-8

Interaction between platelets and *Streptococcus pneumoniae*

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Scientific research question: Community-acquired pneumonia is a frequent major bacterial infection, often caused by *Streptococcus pneumoniae*. Extravasation of fluids and red cells from lung capillaries into the interstitial compartment results in major respiratory distress. Platelets have a major role in vessel integrity. Platelets are also important players in immunity processes and can inhibit growth of some bacterial strains, such as *E.coli* or *Staphylococcus aureus*. Here, we investigated in the interaction of platelets and different serotypes (ST) of *S. pneumoniae*.
Methodology: Platelets were isolated from blood of healthy donors (n≥4). Different *S. pneumoniae* (pneumococci) strains (genomic strains D39 (ST 2) and TIGR4 (ST 4), strains ST 6B and ST 19F) and their pneumolysin deficient mutants (D39Δ*ply*, TIGR4Δ*ply*) were grown in THY-Medium to mid-exponential phase (OD_{600}=0.3-0.4). Platelets and releasate of activated platelets (supernatant of platelets activated with 5 µg/ml collagen and 20 µM TRAP-6)were incubated at a ratio of 5 platelets per bacterium for 2, 3 and 4 hours. Samples were plated on blood-agar plates and colony forming units (CFU) were enumerated. Platelet activation was determined by measuring CD62P expression using flow cytometry.
Findings: All four pneumococcal wild-type strains induced high CD62P expression on platelets within 120, 180 and 240 min. Subsequent TRAP-6 stimulation did not further increase CD62P expression. Pneumolysin deficient mutants induced less CD62P expression compared to their isogenic wild-types. However, CD62P expression

increased over time (CD62P positive cells with TIGR4Δ*ply*24.5% after 2h, 50.3% after 4h; with D39Δ*ply* 36.1% after 2h, 62.2% after 4h). Subsequent TRAP-6 stimulation further increased CD62P expression in platelets incubated with the pneumolysin-mutants (Fig. 1a). Platelets had no impact on growth of D39 (wildtype and *ply* mutant), 6B and 19F, while platelets inhibited the growth of the invasive clinical strain TIGR4. At each time point the CFU was significantly lower when TIGR4 wild-type was incubated with platelets compared to platelet free buffer (Fig. 1b:2h:9.8x10^{6}CFU/ml vs. 13.3x10^{6}CFU/ml, p=0.0293; 3h:5.1x10^{6}CFU/ml vs. 9.59x10^{6}CFU/ml, p=0.0034; 4h: 2.49x10^{6}CFU/ml vs. 7.05x10^{6}CFU/ml, p=0.0002). Incubation with releasate of activated platelets did not decrease the CFU of TIGR4 (p=0.4861).

Conclusion: The interaction of platelets and *S. pneumonia* is dominated by pneumolysin, which leads to rapid CD62P expression and impairment of platelet function, as indicated by the lack of platelet reactivity to TRAP-6. The major complication of community acquired pneumonia, interstitial accumulation of fluid and red cells, is probably caused by pneumolysin induced loss of platelet function.

Figure 1a

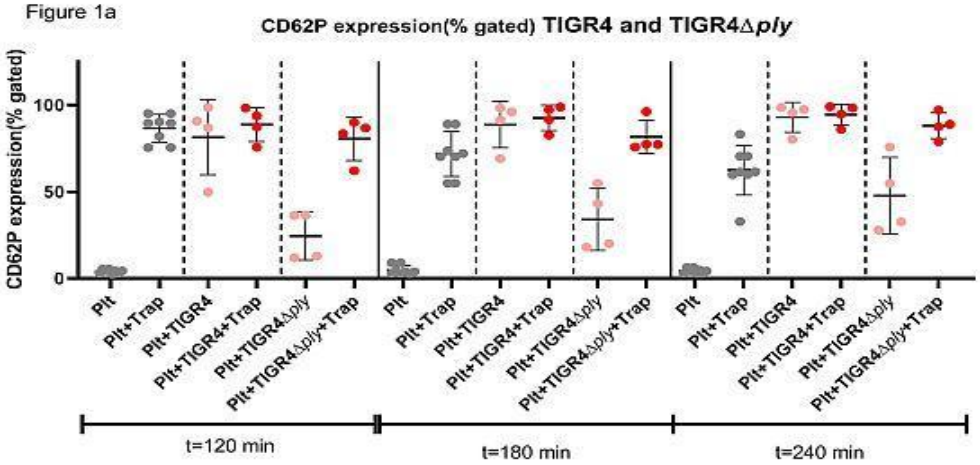
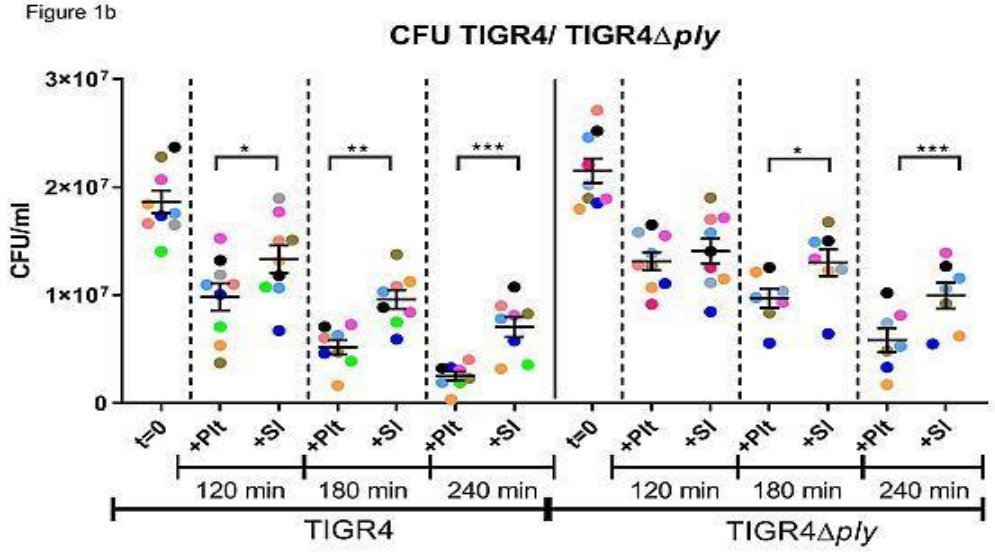


Figure 1b



n ≥ 4

Statistical tests performed: Shapiro-Wilk-Test and paired t test,one-tailed
level of significans: * =0,05; ** = 0,01; *** = 0,001

P08-9

Thromboinflammatory actions of platelets in heparin induced thrombocytopenia are regulated by the chemokine receptor CXCR7

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Scientific research question: Thrombocytopenia and thromembolic complications make HIT a life threatening drug induced thromboinflammatory adversity. On heparin administration during thromboischemic complications, patients may develop PF4/heparin antibodies that activate platelets through FcγRIIA, necessitating alternative therapeutics, but avoiding bleeding risk. Platelet surface expression of the chemokine CXCL12 and its receptors CXCR4-CXCR7 are elevated in coronary artery disease patients, and differentially regulate platelet functions. Our aim was to validate CXCR7-axis in regulating thrombosis and thromboinflammation in HIT.
Methodology: Sera and corresponding IgG fractions were isolated from patients with clinically and serologically confirmed HIT-associated thrombosis (4Ts score 6-8, ELISA and HIPA positive). HIPA, thrombinoscopy, thromboelastography, APTT, PT, flowcytometry, *ex vivo* thrombosis assay, lipidomic (UHPLC-ESI-QTOF-MS/MS) analysis, were performed.
Findings: Platelet surface expression of CXCR7 was significantly enhanced in presence of HIT$^+$ sera/IgG. A specific pharmacological CXCR7 agonist could prevent HIT$^+$ sera/IgG -induced CD62P surface expression, thrombus formation in blood from healthy donors *ex vivo*. Pre-treatment with CXCR7 agonist also counteracted HIT$^+$ serum induced platelet aggregate formation in HIPA assay. Mechanistic basis of the anti-thrombotic action explored in healthy donors show that CXCR7 agonist elevated the levels of platelet inhibitory mediators cGMP-cAMP which caused activation of PKG-PKA, driving phosphorylation of several target proteins including VASP. Moreover, lipidomic analysis of CXCR7 agonist counteracted generation of pro-thrombotic (LPI, LPC, AA, TxA2, 12-HETE, HHT, ceramides) and thromboinflammatory (9/13-HODE) lipid mediators, in thrombin stimulated platelets. Inhibition of PLC activity downstream of CXCR7 ligation, reduced diacylglycerol formation and subsequent activation of PKC, PI3K, Akt, intracellular calcium mobilization. Being a prosurvival mediator, CXCR7-agonist decreased activation-induced externalization of phosphatidylserine and relative percentage of procoagulant platelets. As a consequence CXCR7 agonist checked platelet dependent (in PRP) but did not affect plasma (PPP) dependent thrombin generation, or the time (R), kinetics (K) and amplitude (MA) of clot formed. CXCR7-agonist administration *in vivo* did not affect bleeding time or coagulation profile (APTT, PT) in mice, suggesting that inhibitory actions of CXCR7 agonist do not compromise haemostasis. CXCR7 agonist regulated thromboinflammatory platelet-neutrophil aggregate formation in presence of HIT$^+$ sera/IgG, also counteracted the release of inflammatory mediators IL-1β, IFN-γ and TNF-α from HIT$^+$ IgG activated platelets.
Conclusion: CXCR7 might interfere with thrombotic and thromboinflammatory platelet functions in HIT without enhancing bleeding risk.

P08-10

Mechanophenotyping of single platelet activation induced by bacterial proteins

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Scientific research question: Gram-negative and Gram-positive bacteria and their secreted proteins can interact directly and indirectly (through serum proteins) with human platelets. Previously, we have identified several proteins from *Staphylococcus aureus* capable of activating platelets *in vitro*. Here we aimed to quantify changes in viscoelastic properties of platelets induced by *S. aureus* proteins using high-throughput mechanophenotyping.
Methodology: For single cell mechanical analysis of platelets we used real-time 1D-imaging fluorescence and deformability cytometry (RT-FDC) which screens > 1000 single cells/second. 50 µL of washed platelets (300,000/µL) were labelled with anti-CD41-FITC and incubated for 10 minutes with *S. aureus* proteins: phospholipase C (Plc, 4 µM, internal negative control), major autolysin (AtlA-1, 2 µM), extracellular adherence protein ($EapD_3D_4$

domain, 4 µM), chemotaxis inhibitory protein of *S. aureus* (CHIPS, 4 µM) and formyl peptide receptor-like 1 inhibitory protein (FLIPr, 4 µM). Buffer and TRAP-6 (20 µM) served as platelet function controls. Using RT-FDC, platelet activation was detected by anti-CD62P-AlexaFluor647 in combination with platelet deformation in microfluidic chips with a cross-section of 15 µm. **Findings:** Except for FLIPr all tested proteins lowered the deformability (i.e. increased stiffness) significantly compared to the internal negative control (Plc) (Figure 1, A). Our mechanophenotyping approach revealed $EapD_3D_4$ and CHIPS lowered platelet deformation and induced CD62P release simultaneously, whereas in the presence of AtlA-1 platelets showed lower deformation in the absence of CD62P release (Figure 1, A & B). Thus RT-FDC can be used to reveal viscoelastic changes in platelets induced by *S. aureus* proteins even in the absence of CD62P expression in some donors (especially for AtlA-1). **Conclusions:** RT-FDC allows for high-throughput biomechanical fingerprinting of changes in platelets' viscoelastic properties based on their deformation and activation status. Viscoelastic changes in platelets in the presence of *S. aureus* proteins reveal heterogeneity in platelet activation response in individual donors. Biomechanical changes in viscoelastic properties of platelets (e.g. increased stiffness) can be used as a label-free activation marker in place of labelling antibodies (e.g. anti-CD62P).

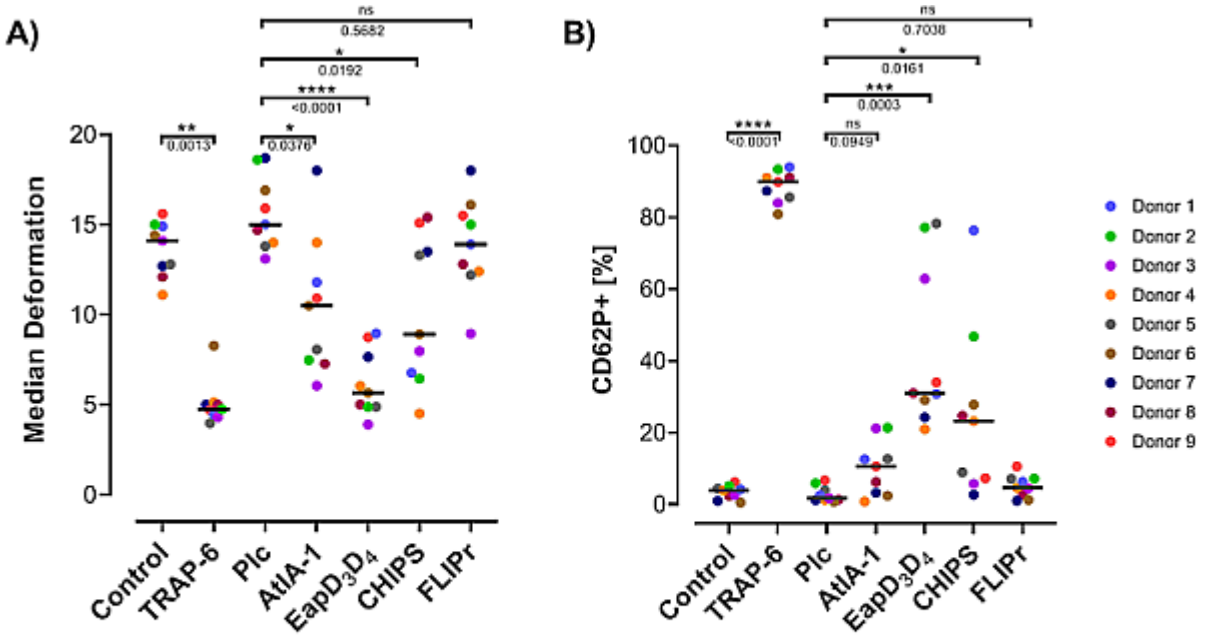

Figure 1: Platelet deformation and activation induced by *S. aureus* proteins in RT-FDC. A) Median platelet deformation based on $CD41^+$ gated events (decreased deformation means increased cell-stiffness). B) Platelet activation as median percentage of $CD62P^+$ events from $CD41^+$ platelet gate. PBS served as negative control, 20 µM TRAP-6 served as positive control. Data representative of n=9 healthy donors. Statistical analysis: Friedman test followed by Dunn's multiple comparison test.

P08-11

The role of NMDA receptors in platelet specific hemostasis

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Scientific research question: NMDA receptors (NMDA-R) are well-known players in the development and function of neuronal cells. However, little is known about non-neuronal NMDA-R and their functional properties. Especially, the role of NMDA-R in platelets is controversially discussed. Some studies propagate an anti-aggregatory effect of NMDA-R activation,

while others reported an amplifying role of NMDA-R in platelet activation and aggregation. The aim of the study is to decipher the impact of NMDA-R on platelet activity, thrombus formation and hemostasis by genetic means.
Methodology: *In vitro* and *in vivo* experiments using platelet specific NMDA-R knockout mice (*Grin1$^{fl/fl}$ - PF4-Cre+*) as well as the NMDA-R specific inhibitor MK-801 for NMDA-R inhibition in human platelets.
Findings: Platelet specific NMDA-R knockout mice were generated and platelet activation was analyzed. While we found significantly reduced activation of the integrin $\alpha_{IIb}\beta_3$ in NMDA-R deficient platelets, no differences were detected in P-selectin exposure or ATP release, suggesting no impact of NMDA-R in platelet degranulation. However, the activation defect of integrin $\alpha_{IIb}\beta_3$ in NMDA-R knockout platelets led to reduced thrombus formation under high and moderate shear rates under flow. *In vivo*, platelet-specific deficiency of the NMDA-R provokes prolonged bleeding times as measured by tail bleeding experiments as well as extended occlusion times in an arterial thrombosis model when compared to wildtype mice. In human platelets, blocking of the NMDA-R with MK-801 resulted in decreased integrin $\alpha_{IIb}\beta_3$ activation and thrombus formation but no alterations in platelet degranulation, thus confirming the results with murine NMDA-R knockout platelets.
Conclusion: The NMDA-R contributes to the activation of human and murine platelets in hemostasis and arterial thrombosis. Thus, blocking of the platelet NMDA-R might be a new anti-thrombotic target in the prevention of cardiovascular diseases.

P08-12

Influence of anticoagulants on biomechanical phenotype of platelets

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Objectives: Biomechanical properties of platelets are increasingly recognized as highly relevant for platelet function. Real-time fluorescence and deformability cytometry (RT-FDC) is a high-throughput technique for mechanophenotyping of individual platelets. Using RT-FDC, we aimed to quantify at a single platelet level, whether different anticoagulants influence actin cytoskeleton dependent intrinsic viscoelastic properties of blood platelets.
Methodology: Blood from healthy donors was collected by venipuncture in BD Vacutainer® Blood Collection Tubes containing following anticoagulants: anticoagulant citrate dextrose solution - Solution A (ACD-A), 3.2% buffered sodium citrate (Citrate), Lithium-Heparin (Li-Heparin), Hirudin and dipotassium ethylenediaminetetraacetic acid (K_2EDTA). Platelet rich plasma was obtained by centrifugation at 120xg. Mechanophenotyping in PRP was performed using RT-FDC (Figure 1a-c) in the absence and presence of soluble platelet agonist (TRAP-6, 20µM). Platelets were labeled with anti-CD61-PE and anti-CD62P-AlexaFluor 647 was used to quantify platelet activation. 50µL PRP was suspended in 450µL RT-FDC Carrier Fluid B. In parallel, using flow cytometry, we quantified the effect of the different anticoagulants on changes in F-actin amounts in platelets using Phalloidin labelling in the absence and presence of TRAP-6 as agonist.
Findings: Unstimulated platelets in PRP (n=6 donors, ≥1500 single platelets/donor, see Figure 1d for single donor) showed a median deformation (i.e. softer platelets) of 0.1225 (in ACD-A); 0.123 (in Citrate); and 0.102 (in Hirudin). Median platelet deformation was lower (i.e. stiffer platelets) with the other anticoagulants: 0.0694 (in Li-Heparin) and 0.0338 (in K_2EDTA). TRAP-6 stimulation of platelets resulted in up to three fold decreased deformation (i.e. increased stiffness) of 0.0434 (in ACDA-A), 0.044 (in Citrate) and 0.0421(in Hirudin). In contrast only minimal differences in platelet deformation where observed in Li-Heparin (0.0434) and K_2EDTA (0.0314) upon TRAP-6 stimulation. Basal F-actin content in unstimulated platelets (n=6 donors) was up to threefold higher in K_2EDTA (median 2.94 and 3.33), Li-Heparin (2.2 and 2.5) and Hirudin (1.64 and 1.87) in comparison to ACD-A and Citrate respectively. TRAP-6 (n=6 donors) resulted in F-actin fold increase (median) of 4.56 (in ACD-A); 4.53 (in Citrate); and 2.9 (in Hirudin) while minor changes in total F-actin content of 1.86 (in Li-Heparin) 1.183 (in K_2EDTA) were observed in comparison to unstimulated platelets.
Conclusions: Anticoagulants such as K_2EDTA and Li-Heparin influence intrinsic viscoelastic properties by decreasing platelet deformability (i.e. increase stiffness) by an increase in actin polymerization resulting in higher F-actin content in unstimulated platelets. This shows that biomechanical properties of platelets strongly depend on the anticoagulant used for blood collection.

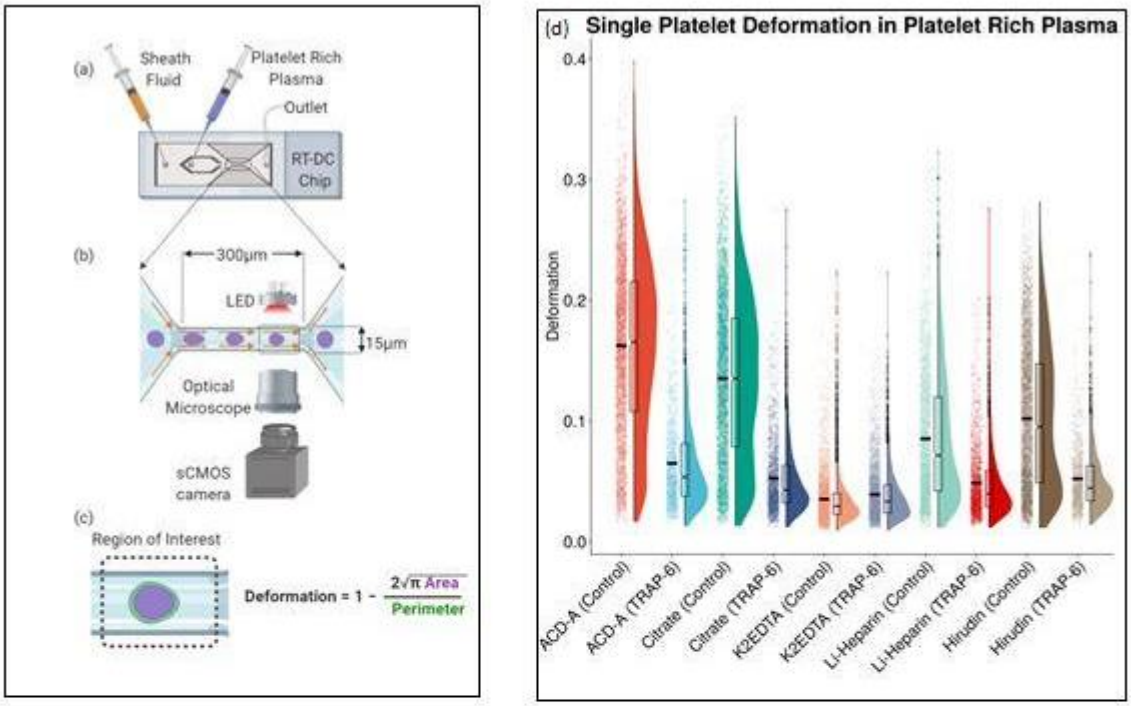

Figure 1: Schematics of RT-FDC microfluidic chip (a) consist of inlets for sheath fluid and for cells/platelets, which combine into to a channel with narrow constriction zone (b) where cell/platelets undergo deformation due to hydrodynamic compression brought about by sheath fluid. A high- powered light emitting diode (LED) is used to illuminate the samples. Images are recorded by a scientific complementary metal–oxide–semiconductor (sCMOS) camera working at high frame rates (≈ 2000 images/second) that is synchronized with the frequency of illumination time. This combined together with microfluidic flow, RT-FDC allows for mechanoprofiling of >1000 unique platelets per second. Platelet deformation (c) is determined on-the-fly in real time by computational image processing taking into account the area and perimeter of the deformed platelet as it passes through the region of interest. (d) Comparison of viscoelastic deformation of single platelets from a single donor in platelet rich plasma, measured by RT-FDC in the presence of different anticoagulants and upon stimulation with platelet agonist TRAP-6. The half-violin plots show distribution of platelet population based on their deformation, notched-box plot represent median deformation while horizontal line shows mean deformation. (n≥ 1500 single platelets for each condition).

[Schematics of RT-FDC and Single Platelet Deformation in Platelet Rich Plasma]

P09 Posters: Paediatric and women's health issues

P09-1

Hemostatic management of severe hemophilia B and paraneoplastic coagulopathy in a child with surgery, high-dose chemotherapy and autologous stem cell transplantation for stage 4 neuroblastoma

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Introduction: The co-occurrence of childhood cancer and severe hemophilia B is a rare condition, in which professional hemostatic management is of paramount importance for successful therapy. Experiences with half life proloinged factor IX concentrates children with cancer have not been reported so far.
Case report: An 11 months old boy with severe congenital hemophilia B was diagnosed with metastatic neuroblastoma. He developed serious paraneoplastic coagulopathy with life-threatening tumor hemorrhage. Management consisted of factor IX replacement with albutrepenonacog alpha, a half-life extended factor IX concentrate, which was adjusted to the current bleeding risk and to the changing pharmacodynamics during cancer treatment. Additional therapeutic interventions included supplements of factor XIII, fibrinogen, fresh frozen plasma, tranexamic acid and platelet transfusions to control bleeding complications. This closely monitored treatment allowed high-dose chemotherapy, autologous stem cell transplantation and subsequent immune therapy with a monoclonal GD2-antibody without major bleeding or thrombosis.
Conclusions: Even in large paediatric cancer centers experience with hemophilic patients is very limited. There are no treatment guidelines for factor replacement during chemotherapy or tumor surgery available. We present the hemostatic management of hemophilia B with albutrepenonacog alpha in a severely ill patient. Moreover we present a general management guideline to inform clinicians treating cancer in patients with hemophilia and herewith allowing optimal cancer treatment in these patients.

P09-2

Deep Vein Thrombosis in children - different therapeutic approaches according to clinical presentation and morphological imaging

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Objectives: Deep Vein Thrombosis (DVT) is a rare disease in children. In children most of the time unfractionated heparin (UFH) and low molecular weight heparin (LMWH) has been used for therapy. In recent years multidisciplinary approaches with interventional procedures have become a therapeutic option if heparinization fails to improve perfusion.
Methods: We report on four female patients under the age of 18 who were treated for a DVT in our hospital.
Patient I (f) developed a thrombosis of the external iliac vein at the age of 13. She received treatment with therapeutic dosage of LMWH. MRI after five months of therapy showed continuous thrombosis of the right external iliac vein, but very good collateralisation. The patient did not show any clinical symptoms regarding the thrombosis. Thus, the patient did not receive any catheter intervention.
Patient II (f) who is carrier for a heterozygous factor V Leiden mutation developed a thrombosis of the left common and external iliac vein at the age of 16. After seven months of therapy with enoxaparin, MRI did not show any improvement of venous flow. Therefore, interventional diagnostics was indicated, but complete recanalization was not possible, due to chronic thrombotic closure. The patient received LMWH treatment for further 5 months until labaratory findings showed no signs of activated coagulation anymore. To date the patient reports no sign of claudicatio venosum.
Patient III (f) developed thrombosis of the left common femoral vein and left common iliac vein at the age of 13. Thrombophilia screening was positive for anti-phospholipid-antibodies and a heterozygous factor V Leiden mutation. After 2 months of therapeutic heparinisation, ultrasound investigations showed only minimal improvement. Since the patient still suffered from typical symptoms a catheter intervention was initiated. The patient received ballon-dilatation and stenting of the left common iliac vein and has not reported any signs of claudicatio venosum since the intervention.
Patient IV (f) developed DVT of the right iliacal vein at the age of 16. Thrombophilia screening showed a heterozygous factor V-Leiden mutation. Under therapeutic treatment with LMWH, MRI showed stable disease after 12 months of therapy. Thus, the patient received balloon dilatation and partial stenting of the right common iliac vein. In short-term, restored drainage was clinically noticeable immediately. Re-intervention was performed 9 months later due to suspected residual stenosis and re-dilatation was necessary because of neo-intimal proliferation.
Conclusion: In paediatric patients suffering from DVT a multidisciplinary therapeutic approach is important. According to the extent of the thrombosis, risk factors, course of the thrombosis under anticoagulation and the clinical symptoms catheter intervention may be necessary in addition to anticoagulation to achieve good results in perfusion.

P09-3

"Coagulin Project": Improvement of quality of life and clinical safety in children treated with oral anticoagulants for thrombosis

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Scientific research question: Anticoagulated children require successive clinical and analytical monitoring, requiring multiple hospital visits, which causes a deteriory in their quality of life and their families.
The "Coagulín" project combines an innovative process of diagnosis and treatment, maintaining a more humane relationship and giving voice to patients, evaluating the reported health and experience results based on *PROMS "Measures of results reported by the patient" and PREMS "Measures of experience reported by the patient"*
Methodology: From May / 2018, we include in the project all children under 16 who require oral anticoagulation. After an initial evaluation in consultation, parents are instructed in the management of the program and in the control of coagulation at home with portable coagulometers. The results obtained are sent via web through a personal link of the patient with the hospital. The hematologist receives and validates the result and sends the new treatment schedule and the date of the next control. Due to ethical considerations, all recommendations are given to parents and analytical data and clinical complications are collected in the medical record
As an additional security measure, a direct mail or telephone is provided for technical / analytical doubts or complications. We evaluate quality of care results (efficacy and clinical safety) and humanization (degree of family satisfaction through anonymous quality of life surveys).
Findings: 17 patients included (14♂ / 3♀). Age: 1-14 years. 80% the reason for anticoagulation is thrombotic events.
Clinical and safety results: 93% of controls in range. There are no emergency visits due to problems derived from anticoagulation. 100% of doubts resolved (82% by phone and 18% by mail). There are no problems related to the reliability of controls, devices or software.Quality of life results: 100% report improvement in the quality of life of their children / families with a 98% satisfaction with the program, highlighting the time gained by their children for other activities (avoiding trips to the hospital) and without loss of school days or parent work. 100% would recommend it to other families. The perceived improvement in family conciliation, personalized follow-ups and the children most involved in their illness were the best qualified elements.
Conclusions: The "Coagulín" Project is an innovative project that unites technology and health. It prioritizes the quality of life, humanizes analytical control and favors efficient and sustainable management of resources. The measured results based on the patient´s experience (PREMS and PROMS) demonstrate a high degree of satisfaction with the program, obtaining adequate and safe controls, without relevant complications, and with an evident improvement in family reconciliation and the life of the patient's children.

P09-4

The role of hereditary elevated lipoprotein a as a thrombophilic risk factor in two siblings with cerebral vein thrombosis

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Background: Increased lipoprotein a (Lp(a)) is a known independent risk factor for the development of arterial and venous thromboembolisms in children as well as in adults. Lp(a) > 30 mg/dl increases the risk of venous thromboembolic events in children by factor 7.2. It has structural homologies to plasminogen which explains its antifibrinolytic and prothrombotic characteristics. Lp(a) levels show an interindividual variability and are genetically determined.
Case report: We describe a Caucasian family with five children. Two of them suffered from cerebral venous thrombosis. In 2013 the first born twin-brother was delivered prematurely at 30+5 weeks of pregnancy. At the age of 14 days he developed seizures and a large thrombosis of the sinus sagittalis superior was detected in the cranial sonography and confirmed in the following MRI. Because of this event the boy suffers from spastic cerebral palsy and developmental disability to this day.

In April 2019 the oldest daughter of the family was diagnosed with a long-distance cerebral vein thrombosis at the age of 16 years. In her case the thrombosis could be resolved completely via systemic anticoagulation without any remaining defects.

Results: A thrombophilia screening was performed in the family and revealed considerably elevated levels of Lp(a) in several family members (Table 1). The highest values were detected in the two children suffering from cerebral venous thrombosis (girl 16, twin 1).

Family member	Lpa (< 30 mg/dl)
mother	53.3 mg/dl
father	166.3 mg/dl
girl, 16 years	**140 mg/dl**
girl, 12 years	30.7 mg/dl
girl, 7 years	8.3 mg/dl
boy, 6 years (twin 1)	**154.6 mg/dl**
boy, 6 years (twin 2)	9.8 mg/dl

[Table 1: Lp(a) levels in the described family.]

The values for protein C, protein S and antithrombin were within the normal range in all family members. In the two siblings suffering from cerebral venous thrombosis as well as in the mother heterozygous mutations of the prothrombin gen (G20210A) were detected. The older girl was taking estrogen-containing oral contraceptives and was smoking about 10 cigarettes a day at the time of thrombosis. The other twin with normal levels of Lp(a) is developed according to age.

Looking at the medical history of the two non-consanguineous parents, one finds that the father also suffering from elevated Lp(a), was diagnosed for times with deep vein thrombosis of the leg. Furthermore there is a history of thromboembolisms in the paternal family. In contrast a thrombotic event has not yet been observed in the mother, although she also suffers from a slightly elevated Lp(a) and an additional prothrombin mutation.

Conclusion: Increased Lp(a) concentration is an important, independent risk factor for venous thromboembolisms, especially in family-related clustering events. Therefore, it should be included in thrombophilia screening in children. To this day no specific Lp(a)-lowering therapy is available. Thus the management of these patients can be challenging.

The evolution of antibody response during immune tolerance induction in patients with severe hemophilia A

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The eradication of inhibitors in patients with severe hemophilia A during immune tolerance induction (ITI) is a great challenge. Treatment success is evaluated by inhibitor titers, FVIII recovery, and half-life. The change of IgG subtypes during ITI has not been evaluated in detail.

Methods: We report the course of two boys with severe hemophilia A who were monitored long-term for coagulation, pharmacokinetic and immunological parameters during ITI. We analyzed FVIII specific antibodies, isotypes, IgG subclasses (IgG 1-4), inhibitor titers and FVIII affinity during ITI for more than 4 years.

Patient 1, a 7 years old boy had a high titer inhibitor of 82 BU before and >7000 BU after start of ITI with a recombinant rFVIII preparation according to a modified Bonn Protocol. The inhibitor decreased slowly but was boosted after each danger signal. Therefore, treatment was switched to rFVIIIFc after 9 months ITI. After 21 months ITI the inhibitor titer became negative, with normal recovery but still shortened half-life (actually 4 hours).

Patient 2, a 5 years old boy developed a high titer inhibitor (150 BU), increasing to 881 BU during ITI. After 10 months of ITI the inhibitor was 0.6 BU, increased after CVL infection to 7 BE and decreased very slowly without reaching < 2 BU. A change to rFVIIIFc resulted in only temporary improvement. After 3 years of ITI, treatment was switched to emicizumab and the ITI Atlanta protocol (rFVIIIFc 100IE/kg 3x/week). After initial increase to 12.4 BU, inhibitor dropped down to 0.7BU.

Immuological findings: Initially, the FVIII specific antibodies of patient 1 belonged mainly to subclass IgG4, in patient 2 to IgG1 together with IgG3 and IgG4 at high levels. In patient 2, IgG1, 3 and 4 increased after infection and vaccination.

After switch to rFVIIIFc the IgG4 levels of patient 1 initially dropped sharply, but still remained detectable after reaching negative BU, while IgG1 and 3 were negative. After one further year IgG4 became finally negative.

In Patient 2, IgG1, 3 and 4 did not decrease further but reached a plateau during ITI. After switch to

emicizumab and a low dose ITI protocol all 3 subclasses IgG1, IgG3 and 4 increased again with a predominance of IgG4. The IgGs bound to the HC, A2, LC C1 and C2 domains of FVIII. Over time IgG1 and 3 decreased, while IgG4 reached a plateau. After 1 year only IgG4 was highly positive, IgG1 was barely detectable. They bound only to LC and C1.
Conclusion: rFVIIIFc can be an effective preparation to induce tolerance for the purposes of ITI (Pt.1) and low-dose ITI can be effective in the presence of emicizumab (Pt.2). Antibody titers mirrored the clinical courses.
All together, this may suggest that not a single subclass alone but a combination of subclasses may indicate successful or unsuccessful ITI.

P09-6

Systemic thrombolysis for acute pulmonary embolism during pregnancy and postpartum: analysis of data of 1,839 women from the German Nationwide Registry, 2005-2016

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Objectives: We investigated the incidence, use of systemic thrombolysis, and fatality of acute pulmonary embolism in women during pregnancy or postpartum included in the German Nationwide Inpatient registry from 2005 to 2016.
Methods: In Germany, all diagnoses referring to hospitalised patients are coded according to the International Classification of Diseases and Related Health Problems, 10th Revision with German Modification (ICD-10-GM). The diagnosis of pulmonary embolism was confirmed based on two main codes: I26 (pulmonary embolism) and O88.2 (obstetric thromboembolism). The analysis was restricted to women aged 18-50 years at delivery or postpartum. The primary outcome was all-cause in-hospital death.
Results: A total of 8,271,327 births were registered in Germany from 2005 to 2016. During this 12-year time period, pulmonary embolism occurred in 1,839 pregnant women aged between 18 and 50 years, corresponding to 2.2 cases every 10,000 pregnancies. A total of 135 (7.3%) women were classified as having haemodynamically unstable pulmonary embolism. A total of 63 deaths have been reported from 2005 to 2016, corresponding to an in-hospital fatality rate of 3.4% (95% CI: 2.7-4.4%) and to a pulmonary embolism-related mortality rate of 0.8 per 100,000 pregnancies (95% CI: 0.6-1.0%). Of 63 deaths, 50 occurred among patients with high-risk pulmonary embolism (case fatality rate: 37.0%) and 13 among haemodynamic stable patients (case fatality rate: 0.8%). Systemic thrombolysis was administered to 67 (3.6%) of 1,839 pregnant women with pulmonary embolism and in 51 (37.8%) of 135 patients with high-risk pulmonary embolism.
Conclusion: Pulmonary embolism-related fatality remains substantial in pregnant women with high-risk pulmonary embolism. Systemic thrombolysis is used in a minority of women with high-risk pulmonary embolism.

P09-7

Management of heavy menstrual bleeding in adolescents with immune thrombocytopenia. Retrospective single center cohort study

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Objectives: Adolescent girls with immune thrombocytopenia (ITP) and low platelet counts often present with or develop heavy menstruation bleeding (HMB) during the course of disease. Patients frequently develop anemia, that may necessitate emergency management. To avoid hospitalization and complications in these patients current treatment strategies need to be evaluated to optimize the management of HMB in adolescents with ITP .
Methods: We performed a retrospective analysis of patients < 18 years at the time of diagnosis of HMB, treated at the Children's Hospital of the Charité between 2003 and 2017. We included 19 adolescents with ITP and HMB and compared them to 37 adolescents with HMB with either inherited bleeding disorders (IBD, n=12) or with no known underlying disease (n=25). To this end we measured iron deficiency, hemoglobin values, erythrocyte transfusions to measure bleeding severity in these patients and evaluated the use of specific treatment strategies.
Results: Adolescents with ITP showed more severe anemia compared to those with inherited bleeding disorders and those with no underlying disease.((*p=0.005,* hemoglobin values in ITP median 7.9 g/dl, range 4.0 -12.4 g/dl, versus no ITP median 11.1 g/dl, range 4.4-4.5 g/dl in patients

with no underlying disease). 26 % of patients with ITP versus 18% with no ITP developed severe anemia defined as a hemoglobin value below 7 g/dl. Treatment strategies differed according to the underlying disease. The majority of patients with ITP received immunoglobulins (IVIG) and corticosteroids (15/19) or one of both treatment options (each 1/19) to treat acute bleeding. Additionally 72% of patients with ITP were treated with hormonal therapy, which was significantly higher compared to adolescents with IBD (42%) or with no underlying disease (60%). 52% of ITP patients received antifibrinolytic therapy,compared to 80% in IBD patients. Only three patients received transfusions. In our center almost all adolescents with ITP associated HMB were treated with first line treatment for ITP associated bleeding according to current guidelines.However in addition the majority of adolescents were treated with hormone therapy to control bleeding. On the other hand supportive therapies with antifibrinolytics or iron replacement therapy were not applied consistently, despite significant anemia and iron deficiency in these patients.
Conclusion: Based on this retrospective analysis we propose a systematic treatment algorithm for adolescents with ITP and HMB. Early therapeutic interventions with second line therapies for ITP (eg. anti CD20 antibodies or TPO agonists) to prevent from or treat HMB and associated complications need to be weighed against hormonal therapy. Treatment decisions need to be customized to age and underlying autoimmune disease.

P09-8

D-dimer levels and thrombin generation parameters using the ST Genesia system in untreated pregnant women at risk for thromboembolic events

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Objectives: The increase in D-dimer levels during pregnancy is well described and this parameter is commonly used for the monitoring of women at high risk for thromboembolic events during pregnancy. The aim of this study was to compare changes in thrombin generation (TG) and D-dimer levels during pregnancy and to analyse whether TG could be a better parameter to predict the risk of thrombosis during pregnancy.
Methods: We investigated changes in D-dimer levels and TG parameters during pregnancy in 24 women at risk for thromboembolic events not receiving prophylaxis for venous thromboembolism. Underlying diagnosis were thrombophilia without a history of thrombotic events, family history of thromboembolic events, recurrent pregnancy loss, history of stroke or systemic inflammatory disease.
D-dimer levels were analysed using the HS-500 assay (HemosIL Werfen). TG was assessed using the ST Genesia system (Stago) with the DrugScreen and ThromboScreen (±Thrombomodulin) application.
Findings: D-dimer levels increased significantly during pregnancy. There were no significant changes in TG using either the DrugScreen or ThromboScreen (±Thrombomodulin) application during pregnancy and no significant correlation between D-dimer levels and thrombin generation parameters in our cohort.
Conclusion: In our rather small and inhomogeneous cohort of pregnants, TG did not seem to be significantly influenced during pregnancy in contrast to D-Dimer levels. Further investigation with a larger cohort is necessary to evaluate the significance of the automated ST Genesia system in the monitoring of pregnant women at high risk for thromboembolic events.

P10 Posters: Thrombo-inflammatory disorders

P10-1

Membrane attack complex formation correlates with neutrophil extracellular trap mediated blood vessel occlusion in small vessel vasculitis

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Vasculitis describes the inflammation of the blood vessel. In the skin, vasculitis is frequently manifested in postcapillary venules and thus defined as small vessel vasculitis. Immune complex deposition at the blood vessel wall is considered as one of the main mediators of inflammation. However, the development of small vessel vasculitis is based on a complex cross-talk between

the complement and the coagulation system, neutrophils, platelets and endothelial cells.
To better understand the synergy between proinflammatory and procoagulatory mechanisms, we performed immune histological analysis of skin biopsies of patients suffering from IgA and non-IgA vasculitis. Next to the deposition of immune complexes at the vessel wall and the initiation of the complement cascade as indicated by the generation of the complement factor C3b, we discovered the formation of the membrane attack complex (MAC) on neutrophils. MAC associated leucocytoclasia, denoting the disintegration of the nucleus of neutrophils, was accompanied by an increased vascular permeability and intravascular thrombus formation.
To further support our ex vivo investigations, we used microfluidic devices to investigate the underlying pathophysiological mechanisms in vitro. Human umbilical vein endothelial cells were perfused with whole blood containing hirudine as a direct thrombin inhibitor. Vasculitis was mimicked by inflammatory phorbol 12-myristate 13-acetate and heat aggregated immunoglobulins. The activation of neutrophils and the interplay with platelets followed by clot formation was visualized by live cell fluorescence microscopy. In line with our ex vivo results, we found that membrane attack complex positive neutrophils disintegrate and release neutrophil extracellular traps (NETs). At static or low shear rates with a wall shear stress below 5 dyn/cm^{2}we detected only marginal thrombus formation, whereas large blood clots were formed at wall shear stresses above 5 dyn/cm^2. Fluorescence microscopy revealed the shear force dependent elongation of NETs and the trapping of platelets. In future experiments, different low molecular weight heparins will be tested to investigate their ability to attenuate the procoagulatory impact of the complement system. Antagonists of NETs such as DNAse or blockage of NET release by peptidyl arginine deiminase 4 inhibitors will be used to further clarify the shear force dependent blood clot promoting activity of NETs.
In conclusion, we established a microfluidic based in vitro system to investigate the pathophysiology of small vessel vasculitis. First results indicate that the complement system together with NETs foster blood vessel occlusion at elevated shear stress conditions.

P10-2

Heparan sulfate dependent binding of melanoma cells to plasmatic von Willebrand factor attenuates adhesion to endothelial cells

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Objectives: The high metastatic potential of malignant melanoma is responsible for the bad prognosis and high mortality rate of patients. One of the hallmarks of hematogenous metastasis is the intravasation of the disseminating melanoma cell into the vascular system. Once in the circulation, these melanoma cells can interact with different blood components such as platelets, immune cells or plasma proteins. Among a series of plasma proteins, elevated plasma levels of the multimeric glycoprotein von Willebrand factor (VWF) have been associated with tumor progression. We hypothesized that circulating tumor cells can interact with plasma VWF and the interaction affects metastasis formation.
Methods and Results: Here, we show that different human and murine melanoma cell lines have distinct abilities to interact with the plasmatic von Willebrand factor (VWF). Human recombinant VWF mutants lacking the integrin-binding motif or the heparan sulfate binding site bound less to the surface of melanoma cells. Additionally, Fluorescence microscopy and super-resolution microscopy further indicated that the binding of VWF to melanoma cells was synergistically dependent on integrins (αvβ3, αvβ5) and heparan sulfate. Heparan sulfate is a highly sulfated glycosaminoglycan exposed at the plasma membrane of all mammalian cells. However, the length and the composition of the heparan sulfate chains depend on the cell type and are often changed in tumor cells. A series of different enzymes are involved in the complex heparin sulfate biosynthesis process. Among those is exostosin-1 (EXT1) which is necessary for the polymerization of heparan sulfate chains. The knockdown of EXT1 by shRNA or gene deletion by CRISPR/Cas9 prevented the synthesis of heparan sulfate in human and murine melanoma cells. By

functional assays, we found that the absence of heparan sulfate on the cell surface prevented VWF binding and thus attenuated VWF induced tumor cell aggregation under static conditions. Importantly, in microfluidic experiments mimicking melanoma cell extravasation, the presence of VWF decreased the binding of flowing melanoma cells to the endothelial cells. Moreover the lack of heparan sulfate and thus abolished binding of VWF was associated with a significantly increased binding of melanoma cells to the endothelial surface under flow conditions.
Conclusions: In conclusion, our data suggest that plasmatic VWF has an anti-metastatic effect. This is also in line with an increased rate of lung metastasis in VWF knockout mice upon intravenous injection of melanoma cells. Future in vivo experiments applying melanoma cells with a modulated heparan sulfate biosynthesis will allow further insight into the pathophysiological relevance of heparan sulfate and VWF for tumor progression.

P10-3

Pro-coagulant polyphosphate regulates T cell differentiation

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Scientific research question: Inorganic polyphosphate (polyP) is abundantly found in every cell in nature and acts intracellularly as energy storage molecule while its exposure to the extracellular plasma has the potential to initiate factor XIIa-driven contact activation. We analyzed polyP derived from CD4+ T cell subsets and analyzed its effect on T cell differentiation and contact activation.
Methodology: We quantified and compared polyP levels from different CD4+ T cell subsets using negative DAPI staining and malachite green assays. In vitro differentiation of CD4+ T cells and selective inhibition of polyP metabolism were utilized to show interactions between polyP levels and T cell responses.
Findings: We found that pro-coagulant polyP was increased in pro-inflammatory CD4+CD25- T cells compared to anti-inflammatory CD4+CD25+ T cells. Moreover, T cell stimulation increased, whereas TGF-beta decreased polyP levels. PolyP metabolism depends on oxidative phosphorylation and inositol hexakisphosphate kinase 1 (IP6k1). Mice deficient in IP6k1 had lower polyP levels in CD4+ T cells and impaired T cell differentiation. Furthermore PolyP isolated from CD4+ T cell initiates contact activation in vitro and preliminary data suggest that T cells may release polyP upon activation.
Conclusion: The presence of polyP in the CD4+ T cell compartment and the ability to secrete polyP indicates a potential contribution of activated CD4+ T cells for polyP-mediated contact activation.

P10-4

The p21-mediated and senescence-associated hyperglycemic memory in diabetic nephropathy is therapeutically amendable via coagulation protease aPC

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Scientific research question: Diabetic nephropathy (DN) is a major cause of end-stage renal disease. A major therapeutic obstacle in DN is the failure of renal recovery upon improved blood glucose levels. The mechanisms underlying this phenomenon, known as the metabolic memory, remain unknown. We aimed to identify mechanisms contributing to the metabolic memory in DN.
Methodology: Two mouse models with established DN (16 weeks after STZ-induced persistent hyperglycemia or 16 weeks old db/db mice) were used in the study. Blood glucose was normalized for 6 weeks using an SGLT2-inhibitor. An unbiased approach (mRNA-seq) was used to evaluate pathways involved in metabolic memory. Candidate genes were studied in human diabetic patients and mice after lowering blood glucose. In vitro and in vivo studies were conducted to determine mechanistic and translational relevance.
Findings: Despite a marked reduction of blood glucose levels, albuminuria and glucose induced changes in renal gene expression persisted, enabling us to study mechanisms contributing to the metabolic memory. PI3-kinase-Akt signaling, cellular proliferation and senescence, and complement and coagulation cascades were linked with metabolic memory. Sustained tubular expression of p21 - a senescence-associated cyclin-dependent kinase inhibitor -was confirmed in humans (histology, urinary p21) and mice (histology, RNA, protein) despite blood glucose lowering. Sustained p21 expression was linked with demethylation of its promoter and reduced DNMT activity and DNMT1 expression. In silico and in

vitro analyses identified miR-148a as a potential regulator of DNMT1. The nephroprotective zymogen protein C was among the genes persistently repressed in DN. Increased tubular senescence, interstitial fibrosis, and albuminuria was confirmed in diabetic mice with a superimposed genetic deficiency of protein C activation. Substituting the protease activated protein C (aPC), mimicking biased aPC-signaling (parmodulin-2), or reducing miR-148a in addition to normalizing blood glucose reversed sustained tubular p21 expression, senescence, and renal damage in DN.
Conclusion: Epigenetically sustained p21-expression and associated senescence contribute to the metabolic memory in DN. This pathogenic mechanism can be targeted by inhibiting miR-148a or by mimicking cytoprotective aPC-signaling

P10-5

Cytoprotective function of podocyte tissue factor in diabetic nephropathy

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Objectives: Tissue factor (TF), the principle coagulation cascade initiator, is known for its cytodisruptive function and proinflammatory effects. Hence, TF is believed to aggravate diabetic nephropathy (dNP). TF is expressed by podocytes, highly specialized renal epithelial cells modulating the filtration barrier. But the role of TF signaling in podocytes and its contribution to the progression of diabetic kidney disease is not established.
Methods: Human TF mRNA expression levels were obtained from the Nephroseq database. Diabetes in mice was induced by streptozotocin (STZ) injections, while non-diabetic mice received sodium citrate injections. We obtained blood, urine and kidney samples after 26 weeks of hyperglycemia in diabetic mice. Immunoblotting was used to analyze protein expression. The kidney sections were stained by PAS, immunohistochemistry and immunofluorescence. Mice urine albuminuria was measured by ELISA. Transmission electron microscopy was used to analyze the glomerular basement membrane (GBM) and foot-process width (FPW).
Results: Analyses of the Nephroseq database revealed that renal glomeruli constitutively express TF at higher levels than the renal tubulointerstitium. Immunohistochemistry revealed TF expression in podocytes. Unexpectedly, the glomerular TF expression is significantly reduced in wild type (WT) diabetic mice compared to WT control mice. Likewise, human dNP kidney sections showed reduced TF staining in the glomeruli compared to healthy controls. To investigate the pathophysiological role of TF in podocytes, we employed STZ-induced hyperglycemia in $TF^{LoxP/LoxP}$ and podocyte-specific TF knockout ($TF^{LoxP/LoxP}Pod^{Cre}$) mice. Surprisingly, the non-diabetic and diabetic $TF^{LoxP/LoxP}Pod^{Cre}$ mice have significantly elevated albuminuria, extracellular matrix accumulation, GBM and FPW compared to the non-diabetic and diabetic $TF^{LoxP/LoxP}$ mice, respectively. Furthermore, significant loss WT1 positive podocytes from the glomeruli of non-diabetic and diabetic $TF^{LoxP/LoxP}Pod^{Cre}$ mice was observed compared to non-diabetic and diabetic $TF^{LoxP/LoxP}$ mice, respectively.
Conclusions: Our study suggests that TF confers a cytoprotective effect in basal and diabetic conditions in podocytes. These data identify an unexpected cytoprotective role of TF. However, the molecular mechanism by which TF regulates podocytes function remains elusive.

P10-6

ADAMTS13 mutations in five patients leading to congenital thrombotic thrombocytopenic purpura (cTTP)

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TTP is caused by a severe deficiency of the plasma enzyme ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motifs 13), either due to mutations in the *ADAMTS13* gene in case of congenital TTP (cTTP), or resulting from autoantibodies inhibiting or clearing the protease in autoimmune TTP (iTTP). The differentiation between cTTP and iTTP is not always clear, especially when no ADAMTS13

inhibitor can be detected. Sequencing of the *ADAMTS13* gene is needed to confirm cTTP.
We report five cases, four females and one male, with repeatedly confirmed severe ADAMTS13 deficiency (< 10%) and without detectable ADAMTS13 inhibitor. Investigation of the *ADAMTS13* gene showed that severe ADAMTS13 deficiency was caused by pathogenic *ADAMTS13* mutations.
The four investigated female patients had a late onset cTTP associated with pregnancy complications. In two patients a missense mutation in exon 24 (c.3178C>T, p.Arg1060Trp) which is associated with some residual ADAMTS13 activity (~5-10%) and late-onset, often pregnancy-induced cTTP (Kremer Hovinga et al. 2017) was found.
The following compound heterozygous mutations were found in the four female patients:

1. A frame shift-mutation (c.4143insA; p.Glu1382Argfs*6) in exon 29 and an amino acid exchange (c.2085C>G; p.Cys695Trp) in exon 17.
2. A frame shift-mutation (c.4143insA; p.Glu1382Argfs*6) in exon 29 and a splice site mutation (c.2862-1G>C) in IVS 23. The second mutation is not yet described in the databases known to us.
3. A premature stop codon (c.130C>T, p.Gln44*) in exon 2 and a missense mutation (c.3178C>T, p.Arg1060Trp) in exon 24 (Falter et al 2014).
4. A c.304C>T, p.Arg102Cys mutation in exon 3 and a missense mutation (c.3178C>T, p.Arg1060Trp) in exon 24.
5. The male patient with disease-onset in early childhood showed a homozygous mutation in exon 3 (c.290A>G, p.Gln97Arg) and exon 26 (c.3655C>T, p.Arg1219Trp).

Genetic analysis of *ADAMTS13* is important in TTP patients of all ages if an ADAMTS13 inhibitor has been excluded, especially in women with first TTP manifestation during pregnancy. The installation of regular plasma infusions in these cases as therapy and for prophylaxis may be life-saving. A correct and early diagnosis of cTTP is instrumental for guiding therapy. In the future recombinant ADAMTS13 may simplify management of cTTP patients.

P10-7

Subgroup analysis of aTTP patients treated with Caplacizumab in the HERCULES study according to baseline disease severity

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Scientific Research Question: Acquired thrombotic thrombocytopenic purpura (aTTP) is a rare, life-threatening autoimmune blood clotting disorder potentially resulting in multiple organ dysfunction. Certain risk factors for death and/or refractory disease have been identified including older age, lactate dehydrogenase (LDH) levels >10x the upper limit of normal (ULN), cerebral involvement, and increased levels of cardiac troponin-I. In the randomized, double-blind, placebo-controlled phase 3 HERCULES study, patients with aTTP received placebo or caplacizumab plus daily therapeutic plasma exchange and immunosuppression. The aim of this analysis was to determine the efficacy of caplacizumab

according to baseline disease severity in patients from HERCULES.

Methodology: In the HERCULES study, very severe disease was defined as:

1. a French severity score ≥3, or
2. severe neurological involvement (i.e. coma, seizures, focal deficit), or
3. cardiac involvement (cTnI >2.5xULN)

All of these factors have independently been associated with worse outcomes and higher mortality. The French severity score is a discrete score from 0 to 4, involving evaluation of 3 parameters:

- Cerebral involvement: yes=1; no=0
- LDH: >10xULN=1; ≤10xULN=0
- Age: >60 years=2; >40 and ≤60 years=1; ≤40 years=0

Scores ≥3 indicate very severe disease.

A subgroup analysis for efficacy outcomes was performed for patients participating in HERCULES according to baseline disease severity (i.e., less severe/very severe disease).

Findings: Patients with less severe disease at baseline had a similar risk of mortality, exacerbations and refractoriness compared to patients with very severe disease. Generally, outcomes were improved in the caplacizumab vs. placebo group, irrespective of disease severity. Caplacizumab treatment resulted in faster platelet count normalization and a lower proportion of patients experiencing the composite endpoint of TTP-related death, exacerbation of TTP, or treatment-emergent major thromboembolic event during the double-blind treatment period. In the caplacizumab group no deaths occurred, while in the placebo group 1 patient (2.1%) with less severe and 2 patients (8.0%) with very severe baseline disease died. Refractory disease developed in 0 patients receiving caplacizumab compared with 1 (2.1%) and 2 placebo-treated patients (8.0%) with less severe and very severe disease, respectively.

Conclusion: Although some risk factors for unfavorable outcomes have been identified, aTTP can be unpredictable. Our results suggest that risk of death, refractoriness and exacerbations is similar in patients with less severe - and very severe disease at baseline (although this analysis is based on a small patient population). Irrespective of baseline disease activity, treatment with caplacizumab improved outcomes, thus highlighting the importance of starting therapy early in all patients with aTTP.

	Less severe		Very severe	
	Caplacizumab (n=42)	Placebo (n=48)	Caplacizumab (n=30)	Placebo (n=25)
Time to platelet count response, HR (95% CI)	1.59 (1.02 to 2.47)		1.69 (0.94 to 3.04)	
≥1 event of the composite of TTP-related death, exacerbation of TTP, or treatment-emergent major thromboembolic event during double-blind treatment, n (%)	2 (4.9)a	24 (50.0)	7 (23.3)	12 (48.0)
TTP-related death, n (%)	0	1 (2.1)	0	2 (8.0)
Exacerbation of TTP, n (%)	0	20 (41.7)	3 (10.0)	8 (32.0)
Treatment-emergent major thromboembolic event, n (%)	2 (4.9)	3 (6.3)	4 (13.3)	3 (12.0)
Refractory TTP, n (%)	0	1 (2.1)	0	2 (8.0)
a. 41 patients were assessable for this event **CI, confidence interval; HR, hazard ratio; TTP, thrombotic thrombocytopenic purpura**				

[Overall efficacy outcomes according to baseline disease severity in the HERCULES study, during the double-blind treatment period]

P10-8

Seasonal patterns of anemia, hemolytic markers, healthcare resource utilization, and thromboembolic events in cold agglutinin disease

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Scientific Research Question: Cold agglutinin disease (CAD) is a rare form of autoimmune hemolytic anemia in which circulating IgM autoantibodies preferentially bind to the I antigen on red blood cells at low temperatures, resulting in chronic complement-mediated hemolysis. Patients with CAD have an increased risk of thromboembolic events (TEs). Although it is established that cold weather can elicit some of the circulatory symptoms of CAD (eg, acrocyanosis), its association with other CAD manifestations is not well understood. We therefore compared hemoglobin (Hgb), markers of hemolysis (bilirubin and lactate dehydrogenase [LDH]), healthcare resource utilization (HRU), and TE rates between seasons for patients with CAD.
Methodology: Patients with CAD were identified from the Optum Humedica database. Hgb, bilirubin, LDH levels, HRU measures (inpatient days, outpatient visits, and emergency room visits), and number of transfusion days were evaluated. TEs were identified using diagnostic codes. Data were compared between seasons using logistic regression adjusted for age, sex, race, region, year, Charlson Comorbidity Index, and clustering within patients.
Findings: 808 patients with CAD were identified (63% female; 66% aged ≥65 years). The median minimum Hgb for winter as compared with summer was decreased by 0.54 g/dL (P< 0.001). The median maximum bilirubin and LDH increased by 0.12 mg/dL (P=0.005) and 42.1 U/L (P< 0.001), respectively, in winter vs summer. No significant differences in HRU measures or transfusion days were observed when stratified by season. One or more TE (n=287) occurred in 204 CAD patients (25%). Of these, 56 (19.5%) were in summer, 57 (19.9%) in fall, 79 (27.5%) in winter, and 95 (33.1%) in spring. Compared to summer, the adjusted TE risk was higher in spring (odds ratio [95% confidence interval]: 1.60 [1.09-2.33]; P=0.016), but not fall (1.06 [0.70-1.61]; P=0.785) or winter (1.42 [0.96-2.12]; P=0.082).
Conclusion: Patients with CAD had evidence of persistent hemolysis across all seasons. Variations in median Hgb, bilirubin, and LDH between winter and summer were not associated with differences in clinical outcomes, as there were no significant changes in HRU or transfusion days. Additionally, there was no association between colder weather and TE risk. The lack of seasonal variability in this cohort suggests that treatment considerations and monitoring of complications such as TEs in patients with CAD should be season independent.

P10-9

Inflammation related protein modifications, leading to bona-fide misfolded protein aggregates, have an impact on the plasminogen activation system

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Scientific research question: Recently is was shown by Coen Maas et al. that several protein aggregates were able to induce plasminogen activation to plasmin. The aim of our study was to test the influence of post-translational modifications like citrullination and modification by the myeloperoxidase/HOCl system on proteins (IDEA-proteins) like the abundant proteins albumin, fibrinogen, IgG on t-PA dependend plasmin formation. Exposure of proteins to HOCl or citrullination can result in the loss of protein structure because of unfolding. The effect on fibrinolysis was compared to that of amyloid-beta 42(Abeta42), serum amyloid protein (SAP) and the C-terminal thrombospondin-1 peptide RFYVVWMK.
Methodology: Purified plasma proteins were modified by adding HOCl, to mimic the action of the myeloperoxidase/H2O2/HOCl system. Remaining HOCl was completely separated by gel-filtration. Citrullinated was performed by human rec. peptidylarginine deiminase 2. Protein aggregation induced by modification was visualised by Nanoparticle Tracking Analysis. Plasmin formation was measured using the chromogenic substrate S-2403™ and the reaction was started by t-PA. T-PA/plasminogen alone served as negative control, with addition of fibrin as positive control.

Findings: Citrullinated albumin and citrullinated fibrinogen were much stronger enhancers of the t-PA dependent plasmin formation as the native mother proteins. IDEA-proteins, we tested IDEA-albumin and IDEA-IgG, enhanced plasmin formation at low concentration and inhibited it at higher concentrations. The optimal cofactor concentration for IDEA-albumin was 1µg/ml and 10µg/ml for IDEA-IgG. The thrombospondin-1 peptide showed a similar curve with an optimum at 20µM. The optimal concentration for beta Amyloid1-42 peptide (1-20µg/ml) induced plasmin formation was 5µg/ml. SAP enhanced t-PA dependend plasmin formation in a dose dependend manner up to the highest tested concentration (100µg/ml).
Conclusion: Protein modifications like those by the myeloperoxidase/HOCl system or by citrullination are often connected with inflammatory diseases like, CVD, stroke or rheumatoid arthritis. The capability to act as cofactor or inhibitor for the plasminogen activation system might be of clinical relevance.

P10-10

Platelets and monocytes are involved in the development of experimental chronic cholangitis in rats

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The blood cells are involved in the development and the recovery of different type of inflammatory and neoplastic disorders. Presence of platelet-derived growth factor inside the granules of platelets and transforming growth factor-alpha in the monocytes induce proliferation of connective tissue cells (include fibroblasts) and production of extracellular fibers during healing wounds. Development of chronic cholangitis associated with replacement of epithelial cells (cholangiocytes) by fibroblasts. The aim of this work was to analyze state of blood cells during development of experimental chronic cholangitis in rats and impact on them of inhibitor of PDK1, VEGF-R1,2,3, Src(h), Syk(h) and other protein kinases maleimide derivative (MI-1) compare with prednisolone. MI-1 (1-(4-Cl-benzyl)-3-Cl-4-(CF3-phenylamino)-1H-pyrrole-2,5-dione) was synthesized in Taras Shevchenko National University of Kyiv (Ukraine). MI-1 possesses anti-inflammatory and antitumor activity.
Method: The white outbred male rats with the median weight of 198±10 g were randomly divided into four groups (8 rats per group) and treated with: I (control) - 0.1 ml sunflower oil *per os* and intraperitoneal 0.1 ml saline solution; II - α-naphthyl isothiocyanate (ANIT, Sigma, USA), 100 mg/kg, once a week for 4 weeks, intraperitoneal (experimental chronic cholangitis); III - ANIT+MI-1 2.7 mg/kg in 0.1 ml sunflower oil, *per os* daily for 4 weeks; III - ANIT+presnisolone 0.7mg/kg, intraperitoneal daily for 4 weeks. Blood cells and myelograms were determined by conventional methods. The difference between the parameters was evaluated by Dunnett´s test.
Results: α-naphthyl isothiocyanate-induced chronic cholangitis associated with increased number of platelets by 49% ($p< 0.001$), monocytes in 5.5 times ($p< 0.001$), lymphocytes by 35% ($p< 0.05$) and eosinophils by 26% in blood compare with control group. MI-1 reduces the number of platelets to control group range ($p>0.05$), but number of monocytes ($p< 0.001$), lymphocytes and eosinophils are increased compare to the control group. Prednisolone reduces the number of eosinophils, lymphocytes and monocytes by 24% compare to the chronic cholangitis group. But number of monocytes and platelets are still increased compare to the control one ($p< 0.05$).
Conclusion: Platelet and monocyte number are increased in blood after α-naphthyl isothiocyanate-induction of chronic cholangitis that reflect their involvement to the pathological process development. The MI-1 reduces involvement of platelets to the development of chronic cholangitis, but does not modified the level of inflammation.

P10-11

Signaling selective modulation of the protease activated protein C using exosite inhibiting aptamers

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Scientific research question: Activated protein C (APC) is a serine protease with anticoagulant and cytoprotective activities. The interaction of APC with its various macromolecular substrates is tailored by two exosite structures flanking the

active center of APC. To study, if biased APC signaling can be achieved by exosite binding ligands, the aptamer technology has been used.
Methodology: For aptamer selection different randomized single-stranded DNA-libraries including a G-rich library were screened using a homogenous capillary electrophoresis-systematic evolution of ligands by exponential enrichment procedure. Selected aptamers were characterized in terms of binding affinity, specific binding site and their influence on functional activities and catalytic half-life of APC. Modulation of anticoagulant activity of APC was tested using a prothrombinase assay, a tenase assay, and by coagulation testing. A human endothelial cell-based staurosporine-induced apoptosis assay was used to measure the impact of aptamer binding on the antiapoptotic effect of APC. The effect of aptamers on the catalytic life of APC in plasma was determined using kinetic analysis and determining APC-protein-C-inhibitor formation rates.
Findings: Using CE-SELEX, three aptamers were selected, named NB1, NB2 and G-NB3. Among all, a G-quadruplex forming aptamer, G-NB3, binds to the basic exosite of APC with a KD of 0.2 nM and showed no binding to the zymogen protein C or to APC related serine proteases. G-NB3 inhibits the inactivation of FVa and FVIIIa with IC50-values of 11.6 and 13.1 nM, respectively, without inhibiting the cytoprotective function of APC. In addition, G-NB3 prolonged the plasma half-life of APC to more than 120 min through partial inhibition of APC-serine protease inhibitor (PCI) complex formation.
Conclusion: Our data demonstrate that exosite inhibition by high affinity binding DNA-aptamer can be used to inhibit the anticoagulant effect and prolong the plasma half-life of endogenously generated and exogenously administered APC. These features qualify G-NB3 as a promising therapeutic agent usable to enhance the cytoprotective functions of APC without increasing the risk of APC-related hemorrhage.

P10-12

Intracranial haemorrhage after intravenous thrombolysis with rtPA in patients with ischaemic stroke

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Scientific research question: Intravenous thrombolysis with rtPA is the standard treatment of ischaemic stroke under defined conditions. In 2-6% of treated patients, intracranial haemorrhage occurs as a complication of thrombolysis. Depending on the size and localisation, the bleeding has a major impact on the symptoms and the prognosis of the patient. Thus, it is necessary to identify those patients who have an increased risk of bleeding. Even though the plasma half-life of rtPA is rather short, it might exert a longer-lasting impact on the haemostatic system. The goal of our study was to identify relevant changes in haemostatic parameters and to delineate constellations of laboratory values that are associated with a higher risk of secondary bleeding in rtPA-treated stroke patients.
Methods: This retrospective study included a cohort of patients with ischaemic stroke who were treated on our intensive care unit (n=140). We analysed all available laboratory parameters and vital signs during a period of three days and compared them between the following three groups: patients treated with rtPA thrombolysis who suffered from intracranial bleeding, patients who did not have a bleeding, and patients who had an ischaemic stroke, but did not receive thrombolysis. Most of the patients were additionally treated with mechanical thrombectomy. We performed a correlation analysis of single parameters over time. We used linear modelling, considering individual dynamics, to state differences between aforementioned groups.
Findings: We found significant differences in some haemostatic parameters between the three groups of patients. Patients who were treated with rtPA and suffered from a bleeding showed a significantly longer aPTT and a lower haemoglobin level during the entire observation period. The correlation between the level of fibrinogen and the thrombin time differed significantly between the patients who were treated with rtPA and those who did not receive intravenous thrombolysis (p= 0,021).
Conclusions: We found some different dynamics in haemostatic parameters, suggesting that there might be a direct effect of rtPA on fibrinogen, which could result, in some cases, in a direct and persistent reduction of plasma fibrinogen levels. This effect might increase the risk of secondary bleeding. With our approach to analyse high dimensional data it will be possible to reveal further underlying risk factors for bleeding after treatment of ischaemic stroke with rtPA.

P11 Posters: Various topics

P11-1

Monitoring of inactivation kinetics of activated factor XI in healthy individuals and thrombophilic patients

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Scientific research question: The serine protease factor XI (FXI) is part of the intrinsic pathway of coagulation and known to cause a variable bleeding phenotype in patients with FXI deficiency. On the other hand elevated levels of FXI e.g. after substitution are associated with thromboembolic events. Although these findings demonstrate the crucial role of FXI and of activated FXI (FXIa) in the regulation of thrombin formation, the knowledge about physiological inactivation mechanisms of FXIa is limited.
Methodology: To evaluate the impact of the SERPINs antithrombin (AT) and C1-inhibitor (C1-INH) on the catalytic life of FXIa, we analyzed inactivation patterns of exogenously added FXIa in normal human plasma, in plasma deficient for C1-INH and AT, and in a purified system containing different levels of these inhibitors. FXIa was added to the respective sample matrix with a final concentration of 10 ng/mL and FXIa inactivation was stopped by addition of benzamidine after 30 seconds, 1, 2, 5, and 10 minutes. Subsequently, the residual amount of FXIa was quantified by an enzyme capture assay using a monoclonal antibody to capture FXIa. To further evaluate the clinical impact of FXIa inactivation kinetics on the thrombophilic phenotype, inactivation of exogenously added FXIa was analyzed in plasma samples obtained from 90 patients with thrombophilia and compared with matched healthy controls. Plasma levels of C1-INH and AT, and of the FXIa inhibitors α1-antitrypsin and α2-antiplasmin were measured additionally in both cohorts.
Findings: The FXIa inhibition assay demonstrated coefficients of intra- and inter-assay variation of 13.2% and 15.2%, respectively. The catalytic half-life of FXIa in normal human plasma was 133.8 ± 18.8 s (mean ± SD). FXIa half-life times prolonged to 251.6 ± 29.4 s in plasma with decreased activity levels of C1-INH of 25% and to 175.7 ± 3.7 s in AT deficient plasma. After addition of plasma purified C1-INH or AT, FXIa half-life times shortened in a concentration-dependent manner. The observation that AT and C1-INH additively control the catalytic life of FXIa prompted us to measure FXIa inhibitor levels in thrombophilic patients, as a combination of critically low inhibitor levels might constitute a thrombotic risk factor. However, plasma levels of more than one inhibitor below the normal range were not observed in the thrombophilic patients of our study population, and inactivation patterns of exogenously added FXIa did not differ from those in the control group.
Conclusion: The data obtained demonstrate the significant and synergistic contribution of AT and C1-INH to FXIa inactivation in plasma. Critically decreased FXIa inhibitor levels or impaired FXIa inactivation rates are not a frequent finding in thrombophilic patients.

P11-2

Influence of ABO blood group on factor VIII and factor V levels in fresh frozen plasma

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Scientific research question: What is the influence of the ABO blood group on factor V and factor VIII levels in fresh frozen plasma?
Methodology: We conducted a survey of the results of coagulation factor V and VIII determinations of fresh frozen plasma (FFP) produced from whole blood from fit donors from January 2015 to December 2018 by the Service Quality Control Laboratory of Hemotherapy at São Vicente de Paulo Hospital in Passo Fundo - RS - Brazil. Samples were randomly selected monthly, one out of every 50 units produced, with the 50th segregated for evaluation. Coagulation factors were measured in the Fibrintimer equipment (Siemens). FFP was frozen within 8 hours after collection in a -80 ° C freezer. Statistical analysis was performed using independent T-test and as tool BioEstat 5.0 system was used.
Findings: A total of 779 FFP were analyzed: 272 (34.9%) from group A, 84 (10.7%) from group B, 21 (2.7%) from group AB and 402 (51.6%) The average Factor VIII was 1.13 IU / mL in group A with compliance was 96.0%, 1.36 IU / mL in group B and compliance was 98.8%, 1, 26 IU / mL in group AB with 100% compliance and 0.98 IU / mL in group O with 89.5% compliance. Alharbi, et. al., 2018 reported mean factor VIII activity in blood donors of 96.9% (36-157) in group A, 111.8% (45-178) in group B, 127.6 (86-170) in group AB and 81.2% (35-128) in group O. Findings were similar to those

obtained in our study, the mean factor VIII was higher in group B and AB donor plasmas and the lowest mean was observed in group O donors, as well as most nonconforming results were observed in group O donors. Regarding Factor V, we found an average of 1.07 IU / mL in group A with 95.6% compliance, 1.10 IU / mL in group B with 94.0% compliance, 1.07 IU / mL in group AB with 100% compliance and 1.07UI / mL in group O with 97.0% compliance. In case of Factor V were similar in the different blood groups, as well as the percentage of compliance. For statistical analysis, we separated the samples from group O (402) and non-O (377), and only for Factor VIII the difference between both groups was statistically significant ($p < 0.0001$) (Table 1). For the results of altered Factor VIII, the result of aPTT was evaluated, of the 42 nonconforming cases of group O, in 6 (14.2%) the aPTT was also altered and in the 12 nonconforming cases of the non-O group, 3 (25%) presented alteration in aPTT.

Conclusion: We found that mean factor VIII values are lower in group O donors when compared to non-O group donors, and non-compliant results were more present in group O. Regarding Factor V, the means were similar in different blood groups and the compliance of the results was also similar. Altered factor VIII and aPTT may be related to hereditary coagulation disorders, such as mild hemophilia or von Willebrand disease, but it is extremely important to do complementary exams to confirm the results.

	Factor VIII	Factor VIII	Factor V	Factor V
Blood Group	Average (UI/mL)	Compliance (%)	Average (UI/mL)	Compliance (%)
Non-O	1,19	96,8	1,07	95,4
O	0,98	89,5	1,07	97,0
	p <0,0001		p = 0,4843	

[Results comparation of factor V and factor VIII in blood group O and non-O]

P11-3

Dynamics of antithrombin III and protein C in patients with burn injury, depending on the outcome

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Massive tissue damage during a burn can cause both hypercoagulation and hypocoagulation, creating a high risk of life-threatening complications, such as venous thromboembolism and clinically significant bleeding.

Aim: To evaluate the indicators of the anticoagulation system of hemostasis in patients with burn injury, depending on the outcome of the course of burn disease.

Material and methods: 63 patients who were treated in the burn center were prospectively examined. The total area of thermal damage in all the victims exceeded 30% of the body surface. All were divided into 2 groups: group 1 - "survivors" (41 injured), group 2 - "dead" (22 patients). The examined parameters of the hemostasis system were determined: activated partial thromboplastin time (APTT), Quick prothrombin (PT), international normalized ratio (INR), Clauss fibrinogen (FG), protein C (Pr C), antithrombin III (AT III) and D - dimer. The study was carried out on days 1, 3 and 10 from the moment of injury. Statistical analysis was performed using Statistica 10.0 software. Comparison of the studied groups with the control group was performed using the Mann-Whitney U-test. Data were presented as median and interquartile range (25th and 75th percentiles).

Results: Indicators of the coagulation system (APTT and PT) in the group of "survivors" and "dead" did not statistically significantly differ. The level of FG significantly increased relative to the norm in all groups, starting from 1 day. However, it should be noted that FG was significantly higher in the group of “deceased” 3.76 (3.06-4.01) g / l than in the group of “survivors” 2.83 (2.45-3.89) ($p < 0.05$). Marker of thrombosis D-dimer, was significantly higher than normal in all groups at all periods of the study ($p < 0.05$). However, in the group of "dead" it was also significantly higher at 1 day than in the group of "survivors" ($p < 0.05$). When assessing the anticoagulant system of hemostasis, namely, indicators of AT III and Pr C, it was found that in the group of "dead" already at 1 day there was a significant decrease in the activity of AT III - 68.9 (50.6-76.7)% ($p < 0.05$), the norm is 104.8 (98.1-107.7) than in the “survivors” group. Activity Pr C significantly decreased in the group of “deceased” during all periods of observation. On day 3, in the “dead” group, Pr S -75.8 (72.2-89.0) was significantly lower than in the surviving group 98.1 (88.6-11.8) ($p < 0.05$). The activity of Pr C in the “survivors” group remained within normal values. It should also be noted that a decrease in the activity of Pr C on the 1st and 3rd day of burn injury in the "dead" was noted in 50% of cases, while among the "survivors" - only 5%.

Conclusion: Thus, the data obtained indicate that burn patients with an unfavorable outcome show a significant decrease in anticoagulants, which leads to thrombosis, impaired microcirculation, hypoperfusion and, ultimately, to multiple organ failure and death.

P11-4

Indicators of the fibrinolysis system in patients with burn injury

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Dysfunction in the hemostatic system in burn injuries is characterized by activation of the procoagulant link, increased fibrinolytic activity and weakening of the endogenous anticoagulant system. The relationship between the indicators of the hemostatic system and the volume of burn damage was established.
Aim: To study the state of the fibrinolysis system in patients with severe burn injury.
Material and methods: A prospective study was conducted on 26 patients who were treated in the burn center of the Research Institute for Emergency Medicine, Moscow, Russia. All patients had burns TBSA of 22 to 75% of the II-III degree. Burn injury was regarded as severe: the Frank index was over 30 units, the abbreviated burn severity index (ABSI) exceeded 6 points. All patients content of von Willebrand factor (VWF), plasminogen, plasminogen activator inhibitor (PAI-1) and α2-antiplasmin. Statistical analysis was performed using the programs Statistica 10.0
Results: VWF was significantly higher than the norm of 3.6; 3.7 and 3.5 times on 1-3, 7 and 14 days, respectively ($p < 0.05$). At 21 days, there was a tendency to a decrease in VWF by 2 times relative to 1-3 and 7 days. Consequently, a marked increase in the content of VWF in burned patients also increases the risk of thrombosis. Plasminogen in patients with burn injury was significantly higher than normal by 1.3 times on day 14 ($p < 0.05$). Also on days 14 and 21, plasminogen was significantly higher than on days 1-3 ($p < 0.008$). The PAI-1 values did not significantly differ from the norm throughout the study day. α2-antiplasmin was significantly higher than normal by 1-3, 7, 14, 21 days, respectively ($p < 0.05$). On day 21, a downward trend in α2-antiplasmin was observed compared to 14 days. From the above data it is seen that in patients with burn injury, violations in the fibrinolysis system occur. In the examined patients, the plasminogen level and PAI-1 activity did not significantly differ from the norm, which, however, does not exclude the risk of developing thrombosis in them. α2-antiplasmin is the fastest "reactive" plasmin inhibitor; it does not allow the presence of plasmin in the blood in free form. α2-antiplasmin, in addition to plasmin, inhibits plasminogen activators. A statistically significant increase in the content of α2-antiplasmin in the examined patients with burn injury may indicate thrombosis.
Conclusion: The formation of microthrombi in the immediate vicinity of burn injury is necessary to maintain the integrity of the microcirculatory part (vascular bed) surrounding the burn wound. Although this phenomenon serves as a protective mechanism for bleeding from blood vessels damaged by a burn, generalized systemic formation of microthrombi can lead to a decrease in organ perfusion and the appearance of disseminated intravascular coagulation, which should be taken into account during the treatment of burned patients.

P11-5

Buffy coat, a potential source of hematopoietic stem cells for in vitro platelet production

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Scientific research question: Transfusion of platelets (PLTs) is a standard medical therapy used to treat different bleeding disorders. However, this clinical approach is severely limited by the dependency on donor-derived PLTs. In this context, a reasonable solution may be the in vitro PLT production from hematopoietic stem cells (CD34+). In the present study, we investigated the possibility to use buffy coat (BC), obtained during routine blood donation process, as an alternative source of CD34+ cells for in vitro PLT formation.
Methodology: CD34+ cells were magnetically isolated from peripheral blood (PB) or BC and differentiated in vitro into megakaryocytes (MKs). Flow cytometry (FC) analyses were performed to investigate MK and PLT function. We quantified the number and percentage of isolated CD34+ cells, number and maturation status of MKs, PLT generation as well as their function.
Findings: Similar purity and quantity of CD34+ cells were found after isolation from both cell sources, BC and PB (%CD34+ mean±standard error mean (SEM): 77%±5 vs. 68%±5, p=0.35; number CD34+ $1.29±0.15x10^5$ vs.$1.17±0.14x10^5$, BC vs. PB, respectively, p=0.66). However, after 6 days of in vitro expansion enhanced proliferation ability was observed for CD34+ cells isolated from BC compared to PB (CD34+ number mean±SEM: $5.33±0.64x10^6$ vs. $3.02±0.45x10^6$, BC vs. PB, respectively, p=0.026). A slightly higher ratio of MKs/CD34+ cell was observed in BC- compared to

PB-derived CD34+ cells (cell number MKs/CD34, mean±SEM: 7.21±1.48 vs. 5.57±0.60; BC vs. PB, respectively, p=0.380). Interestingly, despite a comparable MK nuclear maturation, the yield of PLTs released from BC-derived MKs was significantly higher than from PB cells (PLT yield mean±SEM: 7.23±1.26 vs. 2.70±0.48, BC vs. PB, respectively, p=0.0078). Most importantly, the functionality of in vitro produced PLTs was significantly higher in BC-derived cells (fold increase of CD62P mean±SEM BC vs. PB: 1.41±0.05 vs. 0.94±0.06, p=0.003).
Conclusion: Our results indicate that CD34+ cells isolated from BC may have higher proliferation ability, increased yield of PLTs released from mature MKs and enhanced in vitro functionality, compared to PB-derived cells. This work suggests that BC is a promising source of MKs for in vitro PLT production.

P11-6

Localization and functional interactions studies of VKORC1 and VKORC1L1 in a native cell environment: a CRISPR/Cas9 approach

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Aim: Generation of two fluorescent-tagged reporter cell lines to study the localization and functional interactions of the two VKOR proteins in a native cell environment.
Vitamin K epoxide reductase complex 1 (VKORC1) and VKORC1-like1 (VKORC1L1) are two paralogue proteins which possess vitamin K oxidoreductase (VKOR) activity and share 50% identity in proteins´ primary sequences. In vivo, VKORC1 drives the reduction of vitamin K to support γ-carboxylation of vitamin K dependent proteins. VKORC1L1 is believed to support γ-carboxylation in extra-hepatic tissues and mediate vitamin K dependent intracellular antioxidation functions.
Given the absence of specific antibodies able to discern between the two paralogues, studying their individual interactions with the cell environment at the endogenous level is yet not feasible.
CRISPR/Cas9 technology allows for simple and efficient genome editing of virtually any sequence in the genome. We exploited the homology direct repair (HDR) mechanism following a double strand DNA break to introduce a different fluorescent reporter sequences at the C-terminus of each VKOR gene.
Methods: Two plasmids were transfected by nucleofection into the HEK 293 cells to produce HDR gene knock-in of fluorescent reporter proteins. The first plasmid encodes for spCas9 and a gRNA targeting the stop codon while the second one is composed of the fluorescent reporter in-frame with an antibiotic resistance sequence in between two homology arm of 800 bp each. Treatment with antibiotic allowed for selection of edited cells and resistant colonies were picked two weeks after selection started. Genotyping was performed by insert specific PCR followed by sanger sequencing and microscopy to confirm the presence of the reporter gene.
Results: Two distinct reporter cell lines were obtained: VKORC1-eGFP and VKORC1L1-mCherry. The sequencing of the clones confirmed the in-frame insertion of the fluorescent tags with the genes of interest while immunostaining and live-cell imaging confirmed the expression of the two reported genes within the ER.
Conclusions: Our reporter cell lines will allow us to understand how both VKOR proteins localize and interact within the cell at endogenous level and how extracellular factors and stimuli affect their expression.

P11-7

Absence of FVIII protein in vascular endothelial cells with *F8* methionine missense mutation (c.[2T>G];[0]), differentiated from a patient specific IPS cell model

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Introduction: Hemophilia A (HA) is a common, X-linked, recessive disorder caused by deficiency or dysfunction of coagulant factor VIII. In about 20% of patients with severe HA, treatment with replacement FVIII is complicated due to the development of inhibitory antibodies against FVIII. The intracellular cross-reactive material (CRM) status of a specific mutation is believed to affect the inhibitory risk. *F8* null mutations, like large deletions, nonsense mutations and intron 22 inversions (I22I), have the highest risk for inhibitor formation, and had been grouped so far as CRM-negative. Missense mutations that still result in some protein synthesis have the lowest risk for inhibitor development. Here we present one HA-patient (P_{Met}) with a *F8* missense mutation located in the start codon methionine (c.[2T>G];[0]), and one HA-patient with a large deletion (ex7-9). The

index patient P_{Met} shows FVIII:C_{Chr} of < 1% and suffered from high inhibitor titers. His sister is a hemophilia carrier and gave birth to a hemophilic son in June 2018 also showing FVIII activity levels of < 1%. Here we analyzed the CRM-status (F8 mRNA & protein) of both patients in IPS differentiated vascular endothelial cells (vEC) and compared the data to wild type vEC.
Material and methods: Isolated PBMCs from P_{Met} and P_{Del} were expanded for erythroid progenitors (EPCs) and reprogrammed into stable IPS clones. After proof of pluripotency IPS cells were differentiated into day9 old vascular endothelial cells and analyzed for intracellular F8 mRNA using overlapping RT-PCR. Protein was localized with an ApoTome2 microscope by immunofluorescent staining with a monoclonal antibody targeting FVIII heavy chain (GMA-012), co-stained with anti-PDI (ER-marker) or anti-TGN46 (trans Golgi-marker).
Results: P_{Met} presents complete F8 mRNA, while P_{Del} presents the expected gap between ex7-9. FVIII protein is not detectable in differentiated vEC from P_{Met} and P_{Del}, while 90% of wild type vECs present the majority of FVIII located in the ER.
Conclusion: Our cellular model enables us to detect intracellular FVIII in wild type differentiated vEC, while both P_{Del} and P_{Met} are missing any intracellular FVIII. We propose that the missense mutation c.[2T>G];[0] can be classified as null mutation not able to produce intracellular truncated protein (alternative start codon exon1 aa37).

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IMPRINT
Organiser and publisher

GTH Gesellschaft für Thrombose- und Hämostaseforschung e. V.
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