Thieme

GTH Congress 2023 – 67th Annual Meeting of the Society of Thrombosis and Haemostasis Research – The patient as a benchmark

Date/Venue:
21.–24. February 2023, Frankfurt, Germany

Congress Presidents
Prof. Dr. med. Wolfgang Miesbach; Prof. Dr. med. Dr. h.c. Erhard Seifried

Welcome

It is our great pleasure to welcome you to Frankfurt for the 67th Annual Meeting of the Gesellschaft für Thrombose- und Hämostaseforschung e. V. (GTH).

In recent years, there has been a groundbreaking increase in knowledge in haemostaseology, both preclinically in basic research and clinically in the development of innovative diagnostic and treatment strategies. In line with the congress motto, "The Patient as a Benchmark," we aim to highlight the latest developments in research in the field of haemostaseology with regard to their impact on the current and future care of our patients.
We are particularly delighted to welcome renowned researchers and physicians - leaders in the field of thrombosis and haemostasis - to present their latest data at the 67th GTH Annual Meeting.

To emphasize the interdisciplinary nature of the Congress, we have planned joint sessions with the following organisations: the Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie (DGHO), the Deutsche Gesellschaft für Chirurgie (DGCH), the Deutsche Gesellschaft für Transfusionsmedizin und Immunhämatologie (DGTI), and the Deutsche Gesellschaft für Klinische Chemie und Laboratoriumsmedizin (DGKL).

Another important aspect of this congress is the promotion of young haemostaseologists. We are pleased that last year's young GTH awardees will be able to present and discuss their work in a separate scientific symposium.

In our exhibition area, you will learn about new developments from the industry.

We hope that you will enjoy the congress and take advantage of the many opportunities to exchange ideas and make contacts. We also hope that you will notice our efforts towards sustainability throughout the congress, which we have further developed in continuity with last year's congress.

Let us make the 67th Annual Meeting of the GTH in Frankfurt a success together!

INV | Invited Talks

INV-01 Inherited platelet disorders – a short introduction

Authors Zieger B, Boeckelmann D
Institute Medical Center, Faculty of Medicine, University of Freiburg, Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Freiburg, Germany
DOI 10.1055/s-0042-1760451

Introduction Inherited platelet disorders (IPD) are a very heterogeneous group of disorders that are phenotypically and biochemically diverse. Platelet dysfunction (thrombocytopathy) can be accompanied by a decreased number of platelets (thrombocytopenia). The extent of the bleeding tendency can vary greatly. Symptoms include mucocutaneous bleeding, (epistaxis, hematoma, petechiae, gastrointestinal bleeding and/or menorrhagia). Life-threatening bleeding can occur after trauma or surgery. IPD are divided into defects of platelet receptors, cytoskeleton, granule secretion, signal transduction, of membrane phospholipids, megakaryopoiesis, and enhanced platelet clearance. However, the differentiation criteria can overlap so that a classification can change depending on the focus, e.g. thrombocytopenia can be associated with granule secretion defects. Comprehensive biochemical investigations (platelet aggregometry, flow cytometry) are necessary to characterize a congenital platelet defect. In addition, molecular genetic analysis using high throughput sequencing is helpful to unravel the underlying genetic defect.

Methods Platelet count and blood smear. Platelet aggregometry according to Born (LTA) and flow cytometry (FC) to investigate platelet receptor expression and function or secretion of α- and δ-granules. Molecular analysis by NGS panel. Segregation analysis (family genotyping) using direct sequencing.

Results In the last years, we successfully identified among others patients with receptor defects (e.g. Bernard-Soulier syndrome, Glanzmann thrombasthenia, P2Y12 (ADP)-receptor defect), as well as different types of the heterogeneous Hermansky-Pudlak syndrome. In addition, we identified patients with signal transduction defects (CalDAG-GEFI deficiency), defects of megakaryopoiesis, and enhanced platelet clearance (GNE-defect).

Conclusion Biochemical analyses of platelets combined with NGS are successful tools to unravel genetic defects in patients with inherited platelet disorders. In addition, the combination of comprehensive platelet characterization and genetic analysis helps to better understand platelet physiology and identify the risk of bleeding for individual patients.

Conflict of interest None

T-01 | Atherosclerosis and Inflammation

T-01-01 Air pollution impacts on in-hospital case-fatality rate of ischemic stroke patients

Authors Keller K[1, 2], Rastguye Haghi SH[1], Hahad O[1], Schmidtmann I[4], Chowdhury S[5, 6], Lelieveld J[5], Münzel T[1, 3], Hobohm L[1, 2]
Institutes **1** University Medical Center of the Johannes Gutenberg-University Mainz, Department of Cardiology, Mainz, Germany; **2** University Medical Center of the Johannes Gutenberg-University Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **3** German Center for Cardiovascular Research (DZHK), Partner Site Rhine Main, Mainz, Germany; **4** University Medical Center of the Johannes Gutenberg-University Mainz, Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), Mainz, Germany; **5** Max Planck Institute for Chemistry, Mainz, Germany; **6** CICERO Center for International Climate Research, Oslo, Norway
DOI 10.1055/s-0042-1760452

Introduction The United nations (UN) have called upon all governments worldwide to regulate air pollution emissions more strictly and develop plans to improve air quality, based on strong evidence that air pollution exposure is associated with development of cardiovascular disease including stroke. Besides typical, established risk factors, increasing evidence suggests that air pollution is an important and growing risk factor for stroke events, estimated to be responsible for approximately 14 % of all stroke-associated deaths. However, real-world data regarding the impact of air pollution long-term exposures on stroke mortality are sparse.

Method The German nationwide inpatient sample was used to analyse all cases of hospitalized patients with ischemic stroke in Germany 2015-2019, which were stratified according to their residency (source: RDC of the Federal Statistical Office and the Statistical Offices of the federal states, DRG Statistics 2015-2019, own calculations). Data of the German Federal Environmental Agency regarding average values of air pollutants were assessed 2015-2019 at district-level. Data were combined and the impact of different air pollution parameters on in-hospital case-fatality was analysed.

Results Overall, 1,505,496 hospitalizations of patients with ischemic stroke (47.7 % females; 67.4 % ≥ 70years old) were counted in Germany 2015-2019 of whom 8.2 % died during hospitalization. When comparing patients with residency in federal districts with high vs. low long-term air pollution, enhanced levels of benzene (OR 1.082 [95 %CI 1.034-1.132], P = 0.001), ozone (O3, OR 1.123 [95 %CI 1.070-1.178], P < 0.001), nitric oxide (NO, OR 1.076 [95 %CI 1.027-1.127], P = 0.002) and PM2.5 fine particulate matter concentrations (OR 1.126 [95 %CI 1.074-1.180], P < 0.001) were significantly associated with increased case-fatality independent from age, sex, cardiovascular risk-factors, comorbidities, and revascularization treatments. Conversely, enhanced carbon monoxide, nitrogen dioxide, PM10, and sulfur dioxide (SO2) concentrations were not significantly associated with stroke mortality. However, SO2-concentrations were significantly associated with stroke-case-fatality rate of > 8 % independent from residence area-type and area-use (OR 1.518 [95 %CI 1.012-2.278], P = 0.044).

Conclusion The air pollution constituents O3, benzene, NO, SO2 and PM2.5 were associated with increased stroke mortality, based on our analysis of more than 1,500,000 hospitalizations of patients with ischemic stroke in Germany between 2015 and 2019. In particular SO2 was independently associated with a considerably increased risk for in-hospital mortality. Our study results support the strong and urgent need for air quality control measures with the aim to reduce stroke morbidity and other health outcomes in future.

Conflict of Interest KK, SHRH, OH, ISc, SC and JL report no conflict of interests. TM is PI of the DZHK (German Center for Cardiovascular Research), Partner Site Rhine-Main, Mainz, Germany. LH received lecture/consultant fees from MSD and Actelion, outside the submitted work.

T-01-02 Hypercoagulability impairs plaque stability in diabetes-induced atherosclerosis

Authors Ambreen S, Fatima S, Elwakiel A, Rana R, Singh K K, Gupta A, Isermann B, Shahzad K
Institute Universität Leipzig, leipzig, Germany
DOI 10.1055/s-0042-1760453

Introduction Diabetes mellitus, which is largely driven by nutritional and behavioral factors, is characterized by accelerated atherosclerosis with impaired plaque stability. Atherosclerosis and associated complications are the major cause of mortality in diabetic patients. Efficient therapeutic concepts for diabetes-associated atherosclerosis are lacking. Atherosclerosis among diabetic patients is associated with reduced endothelial thrombomodulin (TM) expression and impaired activated protein C (aPC) generation.

Method Female ApoE-/- or TMPro/Pro ApoE-/- mice (age 6 to 8 weeks) were fed a normal chow diet and were made diabetic (DM) by injecting streptozotocin (once daily for five consecutive days) reflecting type 1 DM. Control mice were injected with an equal volume of 0.05 M sodium citrate for 5 days. After 22 weeks of age, mice were sacrificed and analysed for different parameters.

Results Here, we demonstrate that atherosclerotic plaque stability is reduced in hyperglycemic mice expressing dysfunctional TM (TMPro/Promice), which have a pro-coagulant phenotype due to impaired thrombin inhibition and markedly reduced aPC generation. The vessel lumen and plaque size of atherosclerotic lesions in the truncus brachiocephalic were decreased in diabetic TMPro/ProApoE-/- mice compared to diabetic ApoE-/- mice. While lipid accumulation in lesions of diabetic TMPro/Pro ApoE-/- mice was lower than that in diabetic ApoE-/- mice, morphometric analyses revealed more prominent signs of instable plaques, such as a larger necrotic core area and decreased fibrous cap thickness in diabetic TMPro/Pro ApoE-/- mice. Congruently, more macrophages and fewer smooth muscle cells were observed within lesions of diabetic TMPro/Pro ApoE-/- mice [1–10].

Conclusion Thus,it can be concluded that impaired TM function reduces plaque stability, a characteristic of hyperglycemia-associated plaques, thus suggesting the crucial role of impaired TM function in mediating diabetes-associated atherosclerosis.

Conflict of Interest There is no conflict of interest.

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T-01-03 Investigating the interaction of circulating von Willebrand factor with polymorphonuclear leukocytes ex vivo

Authors Yadegari H, Kuanyshbek A, Hamedani N S, Oldenburg J

Institute University Clinic Bonn, Institute of Experimental Hematology and Transfusion Medicine, bonn, Germany

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Introduction Von Willebrand factor (VWF) is a plasma glycoprotein that is best known for its crucial roles in hemostasis. Recently, the involvement of VWF in inflammation has been suggested. It is previously proved that anchored VWF on the endothelium binds to the polymorphonuclear leukocytes (PMNs) through the β2 integrin. In this study, we intended to investigate the interaction of free VWF with PMNs ex vivo, its fate following binding into the PMNs, and its consequence on cell functions.

Method PMNs were isolated from the whole blood of healthy volunteers using EasySep Direct Human Neutrophil Isolation Kit. The purity of the isolated cells was determined by flow cytometry (CD66 + and CD16 +) on the CD45-positive cells. The isolated PMNs were either left unstimulated under static or shear conditions, or they were stimulated with either PMA (10 ng/ml), TNFα (5 ng/ml), or IL-8 (0.7 ng/ml), in the presence of the purified plasma-derived VWF. Subsequently, the PMNs were immune-stained to visualize VWF, CD11b, EEA1, and Rab7. The Z-series images were acquired by Carl Zeiss Apotome.2 microscope. The Mean Intensity Value (MIV) of the VWF signal and co-localization (Pearson's correlation coefficient, PCC) of VWF with PMNs markers were calculated. The impact of VWF on the function of PMNs was evaluated by analysis of the expression of surface receptors CD45 and CD66b by flow cytometry.

Results The immunofluorescence image analysis demonstrated that the obtained MIV of the VWF signal was significantly increased after activation of PMNs with PMA, TNFα, IL8, and shear stress, indicating a MIV value of 517.3 ± 29.6, 494.8 ± 23.9, 463.2 ± 15.5, and 443.3 ± 19.9 respectively vs. 313 ± 10.9 for resting PMNs (p-value < 0.0001). Furthermore, we showed that colocalization (presented by PCC)of VWF with EEA1 and Rab7 (early and late endosome markers respectively) was increased with incubation time (VWF/EEA1 and VWF/Rab7 PCC of 0.11 ± 0.01 and 0.13 ± 0.02 at time point 0 to 0.27 ± 0.02 and 0.29 ± 0.02 after 60 minutes, respectively). Additionally, we observed a significant reduction of VWF antigen levels in the supernatant of stimulated cells (by 2- 3 %, p-value < 0.001), compared to negative control and resting cells. Furthermore, exposure of neutrophils to shear flow with the presence of VWF resulted in up-regulation of surface expression of CD45 and CD66b.

Conclusion Our data showed that circulating VWF interacts/binds to PMNs upon activation with inflammatory modulators or shear flow. Furthermore, we confirmed that VWF is internalized following interaction with PMNs, contributing to the elimination of VWF from cells supernatant. In addition, we demonstrated a regulatory effect of VWF on PMNs' inflammatory functions.

Conflict of Interest The authors declare no conflict of interest.

T-01-04 IRE1α induced senescence promote endothelial barrier dysfunction in diabetes-induced atherosclerosis

Authors Fatima S, Ambreen S, Gupta A, Singh K K, Isermann B, Shahzad K

Institute Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik, Universitätklinikum, Leipzig, Germany

DOI 10.1055/s-0042-1760455

Introduction Diabetes mellitus is hallmarked by accelerated atherosclerosis, which is the major cause of mortality in diabetic patients. Efficient therapeutic concepts for diabetes-associated atherosclerosis are lacking. Accelerated atherosclerosis in diabetic patients is associated with reduced endothelial thrombomodulin (TM) expression and impaired activated protein C (aPC) generation.[1–10]

Method To gain insights into pathomechanisms of diabetes induced atherosclerotic plaque development we cultured human coronary artery endothelial cells (HCAECs) under hyperglycemic (HG) or hyperlipidaemic (oxLDL) conditions for 48 h. ApoE-/- mice (age 8 weeks) was made either diabetic by streptozotocin injections (A mouse model of type 1 diabetes) or fed them HFD to induce hyperlipidemia. Mice were analyzed after 20 weeks of treatments.

Results High glucose induced more pronounced responses in regard to maladaptive unfolded protein response (UPR), senescence, and vascular endothelial cell barrier disruption. Ex vivo, diabetic ApoE-/- mice revealed increased expression of senescence and UPR markers within atherosclerotic lesion as compared with nondiabetic ApoE-/- mice. Activated protein C restored barrier integrity and reduced glucose induced expression of senescence and UPR markers in vitro. Inhibition of IRE1α (inositol-requiring enzyme 1 alpha), a key UPR activator, prevented glucose induced endothelial barrier disruption and cellular senescence.

Conversely, an activator of IRE1α's RNAase domain recapitulated hyperglycaemia-induced effects, suggesting that hyperglycaemia-induced IRE1α RNAse activity is sufficient to induce senescence and vascular dysfunction.

Conclusion These data suggest that high glucose induced maladaptive UPR and associated senescence promote vascular endothelial cell dysfunction, which —however—can be reversed by aPC. This suggests that reversal of glucose-induced vascular endothelial cell dysfunction is feasible.

Conflict of Interest There is no conflict of interest.

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T-01-05 Liver damage promotes thromboinflammatory T-cell responses

Author Mailer R

Institute University Medical Center Hamburg-Eppendorf, Clinical Chemistry and Laboratory Medicine, Hamburg, Germany

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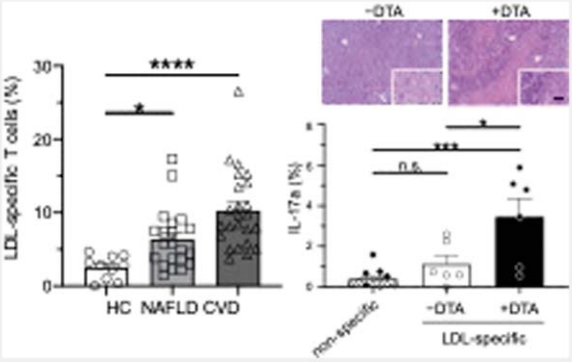
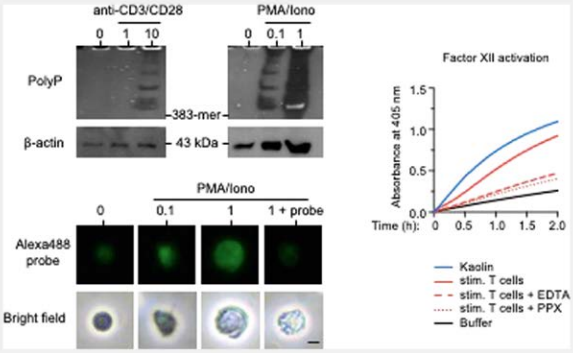
Introduction Cardiovascular disease (CVD) is related to an activation-induced gene expression profile and increased antigen-driven stimulation of CD4+ T cells by elevated plasma cholesterol levels. Hypercholesterolemia promotes T-cell differentiation in the liver and hepatic T cells relocate to the chronically inflamed vasculature upon adoptive transfer into atherosclerotic mice. In CVD patients, pro-inflammatory T cells that are reactive against the protein component of low-density lipoprotein (LDL) particles, apolipoprotein B-100 (ApoB100), contribute to the development of atherosclerotic lesions. Clinically, CVD is a major mortality risk for non-alcoholic fatty liver disease (NAFLD) patients and T-cell stimulation in response to (auto-)immune reactions is associated with an increased risk for thrombotic events. However, T-cell reactivity against LDL in a transient liver damage model or NAFLD patients have not been characterized and pro-thrombotic functions of stimulated CD4+ T cells have not been analysed so far.

Method We expressed diphtheria toxin A via hydrodynamic tail vein injection in hepatocytes of human ApoB100-transgenic mice to assess the induction of a systemic LDL-specific T-cell population in vivo. We used activation-induced marker expression to identify splenic LDL-specific T cells following transient liver damage. In a cross-sectional study we compared population size and differentiation pattern of LDL-specific T cells from healthy controls, NAFLD and CVD patients and correlated these results to the lipid profile and liver damage parameters. To analyse the effect of T-cell responses on thrombosis we quantified pro-coagulant polyphosphate (polyP) levels in stimulated T cells and assessed their contribution to initiate factor XII-dependent contact activation using chromogenic substrate and real-time thrombin generation assays (▶ **Fig. 1**).



▶ **Fig. 1** Pro-coagulant polyphosphate accumulates in stimulated CD4+ T cells.; Quantification of polyP in stimulated T cells by negative DAPI staining and fluorescence microscopy (left). Stimulated T cells promote FXII activation in a chromogenic substrate assay (right).



▶ **Fig. 2** Increasing populations of LDL-specific T cells upon liver damage.; LDL-specific CD4+ T-cell populations increase in blood from NAFLD and CVD patients (left) and transient liver damage in mice promotes LDL-specific Th17 cells (right).

Results We found that transient liver damage in mice increases the LDL-specific T-cell population in the spleen. In line with this, CD4+ T cells specific for ApoB100-derived peptides were more abundant in NAFLD patients and dis-

played a shift towards pro-inflammatory differentiation in comparison to healthy control samples. Dyslipidemia and liver damage parameters in blood correlated with reduced regulatory T cells and elevated Th17 cells among ApoB100-specific T cells, respectively. Moreover, we found that polyP accumulates in response to signal strength and duration of T-cell stimulation. In a polyP-dependent manner stimulated CD4+ T cells were able to promote FXII activation and thrombin generation in vitro.

Conclusion Our results show that liver damage enhances the generation of pro-inflammatory LDL-specific T cells with implications for CVD risk in NAFLD patients. FXII activation through increasing polyP levels of stimulated T cells suggests that T-cell responses may also contribute to thrombus formation (▶ **Fig. 2**).

Conflict of Interest The author declares no conflict of interest.

T-02 | Pathological Mechanisms of Thrombosis

T-02-01 Mansoor's self-report tool for cardiovascular risk assessment predicts adverse in-hospital events in patients with pulmonary embolism

Authors Keller K[1, 2, 3], Schmitt VH[1], Ostad MA[1], Münzel T[1, 4], Hobohm L[1, 2, 3], Espinola-Klein C[1]

Institutes **1** University Medical Center Mainz (Johannes Gutenberg-University Mainz), Department of Cardiology, Mainz, Germany; **2** University Medical Center Mainz (Johannes Gutenberg-University Mainz), Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **3** University Hospital Heidelberg, Medical Clinic VII, Department of Sports Medicine, Heidelberg, Germany; **4** German Center for Cardiovascular Research (DZHK), Partner Site Rhine Main, Mainz, Germany

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Introduction Pulmonary embolism (PE) is a life-threatening acute disease accompanied by high morbidity and mortality. In patients with acute PE it is well established that right ventricular dysfunction (RVD) during the acute phase (assessed by echocardiography or computed tomography), myocardial injury as well as hemodynamic status, are important predictors of adverse in-hospital outcomes. In addition, several approaches with different scores were tested and some of them established for risk stratification of PE patients in order to identify patients at higher risk to develop adverse events and especially those, who should be treated with reperfusion treatments. The recently published Mansoor's Self-Report Tool for Cardiovascular Risk Assessment is an interesting and novel simple risk score to predict cardiovascular events in adults and might also be helpful for risk stratification in PE patients.

Method The nationwide German inpatient sample of the years 2005-2018 was used for this present analysis (source: Research Data Center [RDC] of the Federal Statistical Office and the Statistical Offices of the federal states, DRG Statistics 2005-2018, own calculations). Hospitalized PE patients were stratified according to Mansoor's Self-Report Tool for Cardiovascular Risk Assessment class and the performance of this score was evaluated to predict adverse in-hospital events.

Results Overall, 1,174,196 hospitalizations of PE patients (53.5 % females; 56.4 % ≥ 70 years) were registered in Germany between 2005 and 2018. According to the Mansoor's self-report tool for cardiovascular risk assessment, 346,126 (29.5 %) PE patients were classified as high risk.

Higher Mansoor's Self-Report Tool for Cardiovascular Risk Assessment class was predictive for in-hospital death (OR 1.129 [95 %CI 1.117-1.141], P < 0.001), shock (OR 1.117 [95 %CI 1.095-1.140], P < 0.001), cardiopulmonary resuscitation (OR 1.109 [95 %CI 1.092-1.126], P < 0.001), right ventricular dysfunction (OR 1.039 [95 %CI 1.030-1.048], P < 0.001), intracerebral bleeding (OR 1.316 [95 %CI 1.275-1.358], P < 0.001) and gastro-intestinal bleeding (OR 1.316 [95 %CI 1.275-1.358], P < 0.001).Systemic thrombolysis was not associated with lower in-hospital mortality in high-risk class (OR 5.139 [95 %CI 4.961-5.323], P < 0.001).

Conclusion The Mansoor's Self-Report Tool for Cardiovascular Risk Assessment was helpful to identify PE patients at higher risk for bleeding events and partly those, who were at higher risk for in-hospital mortality and decompensated status, but was not able to identify those patients at high-risk, who benefit from systemic thrombolysis.

Conflict of Interest There are no conflicts of interest regarding the presentation.

T-02-02 Elevated plasminogen activator inhibitor-1 is not associated with impaired plasmin formation and thrombotic risk after low-grade coagulation activation in vivo

Authors Schwarz N, Reda S, Müller J, McRae H L, Shahidi-Hamedani N, Oldenburg J, Pötzsch B, Rühl H

Institute University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany

DOI 10.1055/s-0042-1760458

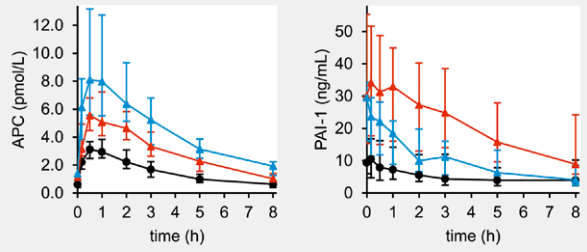
Introduction Elevated levels of plasminogen activator inhibitor-1 (PAI-1) have been shown in patients with venous thromboembolism (VTE) or prothrombotic disorders such as factor V Leiden (FVL) and the prothrombin mutation 20210G > A (PTM). Therefore, it has been hypothesized that an elevated PAI-1 level constitutes a thrombophilic risk factor, but clinical data remain inconclusive. In this study we investigated the impact of PAI-1 on the fibrinolytic response to in vivo coagulation activation by low-dose recombinant activated factor VII (rFVIIa).

Method Our study population consisted of 35 healthy individuals, 38 FVL carriers (thereof 19 with a history of VTE, VTE+), 36 PTM carriers (17 VTE+), and 20 VTE+ patients with a family history of VTE in whom no established risk factor was found. Plasma levels of thrombin-antithrombin complex (TAT), activated protein C (APC), tissue-type plasminogen activator (tPA), plasmin-α2-antiplasmin (PAP), and PAI-1 were monitored before and during eight hours after administration of 15 µg/kg rFVIIa. Differences between cohorts were evaluated using the Mann-Whitney test. Differences in changes of plasma concentrations over time were assessed by comparing the area under the curve (AUC).

Results Median TAT and tPA lay below their respective limits of detection (21.3 pmol/L, 1.71 ng/mL) in all cohorts. After rFVIIa-induced coagulation activation, TAT increased to 30.9 pmol/L in healthy controls and, at a greater extent, in the three thrombophilic cohorts (up to 56.1 pmol/L, p < 0.013). APC consecutively increased in all cohorts (from 0.63-1.25 to 2.94-6.60 pmol/L), whereas tPA levels remained unchanged. At baseline PAI-1 was 9.4 ng/mL in healthy controls. Consistent with previous studies, PAI-1 was 2.5-3fold higher (p < 3.0x10-4) in FVL/PTM carriers (29.8/28.2 ng/mL) and familial thrombosis patients (22.5 ng/mL). After rFVIIa administration, PAI-1 decreased in all cohorts and fell below baseline levels after 8 hours (3.9-6.5 ng/mL). Corresponding with a lower APC response in VTE+ vs VTE- FVL carriers (p = 0.002), PAI-1 decreased at a significantly lower extent (p = 0.010) in the FVL VTE+ cohort (▶ **Fig. 1**). Changes in APC and PAI-1 did not differ in VTE+ vs VTE- PTM carriers. PAP increased in all cohorts, indicating plasmin formation at similar extent in healthy controls and FVL carriers, but at a greater extent (p < 0.019) in PTM carriers and familial thrombosis patients.

Conclusion Opposite changes in plasma levels indicate that proteolysis by APC is involved in the decrease of PAI-1 after coagulation activation. Even low-grade coagulation activation was sufficient to equalize baseline differences rapidly and consistently, making it unlikely that elevated PAI-1 is a clinically relevant

thrombophilic risk factor in the general population. However, under conditions of endothelial impairment, persisting high levels of PAI-1 might possibly contribute to the increased thrombotic risk associated with reduced APC formation rates.



▶ **Fig. 1** rFVIIa-induced changes in APC and PAI-1 levels in FVL carriers; APC (**a**) and PAI-1 (**b**) were measured after administration of 15 µg/kg rFVIIa in healthy controls (n = 35, black), FVL carriers with (n = 19, red) and without a history of VTE (n = 19, blue).

Conflict of Interest JO has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai Pharmaceutical Co., Ltd., CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda. All other authors report no conflict of interest.

T-02-03 Hemolysis-derived heme interacts with components of the blood coagulation system

Authors Hopp M-T, Imhof D
Institute University of Bonn, Pharmaceutical Biochemistry and Bioanalytics, Bonn, Germany
DOI 10.1055/s-0042-1760459

Introduction Hemolysis results in an accumulation of labile heme, which leads to proinflammatory and prothrombotic complications. As a regulatory molecule, heme can affect the function and/or stability of proteins through binding to short, surface-exposed amino acid stretches. As such, the stimulation of the complement and coagulation system through direct heme binding to participating proteins (e.g., C3, fibrinogen, and APC) was described.

Method In order to characterize respective heme-binding sites in select coagulation proteins in more detail, the potential candidates were screened for potential heme-binding motifs by using the webserver HeMoQuest. Subsequently, these motifs were synthesized as nonapeptides and analyzed for heme binding using UV/vis spectroscopy. Promising sites were further evaluated by molecular docking simulations of the respective heme-protein complexes.

Results Heme binding to select procoagulant proteins (e.g., FVIII) is demonstrated by applying a combination of biochemical and spectroscopic approaches.

Conclusion The results provided extend our understanding of hemolysis-derived heme as a regulator in the blood coagulation system on the molecular level, which will support the knowledge on the progression of thrombosis under hemolytic conditions. This research is funded by the Society for Thrombosis and Haemostasis Research e.V. (GTH) and the German Research Foundation (DFG).

Conflict of Interest The authors declare no conflict of interest.

T-02-04 Heme-triggered effects on blood coagulation: a bioinformatics approach based on experimental data

Authors Hopp M-T[1], Mubeen S[2], Domingo Fernandez D[2], Imhof D[1]
Institutes 1 University of Bonn, Pharmaceutical Biochemistry and Bioanalytics/Pharmaceutical Institute, Bonn, Germany; 2 Fraunhofer SCAI, Bioinformatics, St. Augustin, Germany
DOI 10.1055/s-0042-1760460

Introduction A considerable amount of heme is released under hemolytic conditions and thus causes pathological states such as thrombosis, by either binding to plasma proteins or interaction with distinct cells. The huge number of publications describing these effects, including the apparently contradictory observations, cannot be managed anymore in a traditional way to close knowledge gaps and deepen our understanding of heme-driven coagulopathies with the aim to develop suitable and specific treatment strategies. A computational approach and exploration of the complex network of heme-triggered effects in the blood coagulation system is therefore useful.

Method Herein, the knowledge available so far concerning heme-triggered effects on blood coagulation was curated and modeled in a mechanistic interactome. The data were incorporated in an earlier established heme knowledge graph, "HemeKG", to better comprehend the existing data on heme biology.

Results A pathway enrichment analysis of these data provided insights into hitherto unknown connections and novel experimental targets within the blood coagulation cascade and platelet activation pathways.

Conclusion This curated knowledge will support further investigation of the prothrombotic nature of heme in the future, since our study allows, for the first time, a detailed network analysis of the effects of heme in blood coagulation.

Conflict of Interest The authors declare no conflict of interest.

T-02-05 Bridging the gap between coagulation in experimental research and clinical hemostaseology – a modified rotational thromboelastometry assay to show the pro-coagulatory effect of senescence on human blood

Authors Kausche L E[1, 2], Mairhofer M[2], Yu Y[4], Wöß C[4], Pammer M[2], Lee S[3], Schmitt C A[1, 2]
Institutes 1 Kepler Universitätsklinikum, Universitätsklinik für Hämatologie und Internistische Onkologie, Linz, Austria; 2 Johannes Kepler Universität Linz, Universitätsklinik für Hämatologie und Internistische Onkologie, Linz, Austria; 3 Johannes Kepler Universität Linz, Institut für Tumorbiologie, Linz, Austria; 4 Johannes Kepler Universität Linz, Tumor Epigenetik, Linz, Austria
DOI 10.1055/s-0042-1760461

Introduction Clinical interpretation of findings gained in experimental research is a known challenge in translational research in the field of hemostaseology. Global hemostasis assays used for research are mainly elaborate and need to be carried out by experienced operators.

The cellular status of senescence is associated with massive secretion of pro-coagulatory and pro-inflammatory factors, as we confirmed by multiplex bead-based protein analysis of the supernatant (SN) of senescent cells. [1] Clinical conditions in which senescence occurs prematurely, like viral infections or hematologic malignancies, are associated with an increased risk of thrombosis. However, evidence of the effect of senescence on clotting in human blood was lacking.

The ROTEM device provides global hemostasis assays as diagnostic point-of-care tests to guide acute hemostatic treatment. The aim of our analysis was to investigate applicability of these widely used rotational thromboelastometry assays in translational research.

Method In this monocentric pilot study we focused on evaluation of modified ROTEM assays to facilitate clinical interpretation of our experimental findings.

To test the influence of elevated pro-coagulatory factors detected in SN of senescent cells on coagulation of human blood modified INTEM and EXTEM assays were performed on a ROTEM delta device. The assays were carried out using citrated whole blood of male and female healthy donors between 18 - 50 years of age. Inclusion criteria were absence of known coagulation disorders as well as INTEM and EXTEM tests without abnormalities. Participants must not take any medication known to affect coagulation. INTEM and EXTEM assays were performed spiking each blood sample with 15, 25 and 35 µl SN of senescent cells at the moment of starting the INTEM and EXTEM assays. Simultaneously INTEM and EXTEM assays with spiking of each blood sample with 15, 25 and 35 µl SN of non-senescent cells were run.

Results Clotting times in INTEM and EXTEM assays were significantly shorter when whole blood of healthy donors was spiked with SN of senescent cells compared to clotting times measured in blood spiked with SN of non-senescent cells. No significant difference between spiking with SN of senescent or SN of non-senescent cells was observed for Maximum Clot Firmness.

Conclusion Our data indicate a pro-coagulatory effect of the SN of senescent cells on human blood, as suspected based on the prior molecular findings. The design of the ROTEM delta device allows to modify assays with little effort. Therefore, the modified INTEM and EXTEM assays provide useful tools to test effects on coagulation suspected based on molecular findings in a whole blood assay. Further improvement of the assays is planned to use modified rotational thromboelastometry assays as widely available and easy to handle tools in translational research.

Conflict of Interest The authors have no conflicts of interest to declare.

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T-02-06 Pharmacological targeting of ACKR3/CXCR7 enhances anticoagulant acylcarnitine levels in platelets and modulates thrombotic response to lipids

Authors Thomas S[1], Dittrich K[2], Fu X[2], Cebo M[2], Lackner M[3], Bouzabia B[1], Geisler T[4], Nürnberg B[1], Beer-Hammer S[1], Schäffeler E[3], Hofmann U[3], Schwab M[3], Haag M[3], Lämmerhofer M[2], Chatterjee M[1]

Institutes **1** University Hospital Tübingen, Department of Pharmacology, Experimental Therapy and Toxicology, Tübingen, Germany; **2** University of Tübingen, Institute of Pharmaceutical Sciences, Tubingen, Germany; **3** Robert-Bosch-Hospital, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology (IKP), Stuttgart, Germany; **4** University Hospital Tübingen, Department of Cardiology and Angiology, Tübingen, Germany

DOI 10.1055/s-0042-1760462

Introduction Pharmacological targeting of chemokine receptor CXCR7 restricts generation of atherothrombotic, while favoring anti-thrombotic lipids that impose platelet inhibition ex vivo in coronary artery disease (CAD) patients with an atherogenic platelet lipidome. CXCR7 ligation induces the platelet inhibitory cAMP dependent signaling cascade in coordination with the prostacyclin receptor. Current investigation explores the impact of the non-canonical CXCR7 in modulating platelet thrombotic response and procoagulant potential.

Method Lipidomics analysis by UHPLC-QTOF-MS/MS; metabolomics analysis by LC/MS; lipid (LDL/oxLDL) uptake, platelet activation, mitochondrial superoxide generation (MitosOxRed), intraplatelet lipid (per)oxidation (C-11-BODIPY581/591), mitochondrial membrane integrity (Δψm) by flow cytometry; aggregation by impedance aggregometry; thrombotic response over collagen and collagen-tissue factor-(TF) coated surfaces by T-TAS; thrombin generation by calibrated automated thrombinoscopy; phosphorylation of adenosine monophosphate-dependent kinase (AMPKSer-172) and acetyl-CoA carboxylase (ACCSer-79) by immunoblot analysis.

Results Pharmacological CXCR7-agonist (VUF11207) decreased LDL/oxLDL instigated platelet degranulation, αIIbβ3-integrin activation, aggregation and thrombotic response. VUF11207 also reduced intraplatelet lipoprotein (DiI-LDL/oxLDL) uptake by decreasing the surface availability of scavenger receptors-(ApoER2, CD36). Moreover, VUF11207 induced the generation of Factor-Xa inhibitor acylcarnitines (ACars) in platelets from healthy subjects and CAD patients ex vivo, and prompted its release into the platelet supernatant. Inhibitory effect of VUF11207 on platelet dependent thrombin generation was partially counteracted by carnitine palmitoyltransferase-1 inhibitor etomoxir that prevents generation of ACars. Metabolic profiling of resting and thrombin activated platelets revealed significantly elevated levels of long-chain (16:0, 18:1, 18:2) ACars, that are utilized in mitochondrial fatty acid β-oxidation, but not short and medium-chain ACars in the presence of VUF11207. However, accumulation of ACars was not due to breakdown of mitochondrial metabolism since CXCR7-agonist preserved mitochondrial membrane integrity (Δψm), decreased mitochondrial superoxide generation, also prevented nonenzymatic lipid oxidation (intraplatelet oxLDL), and peroxidation in activated platelets. Besides, the platelet energetic status, assessed by ATP measurements and adenylate energy charge, remained at high levels suggesting sustained anabolic pathways. CXCR7-agonist triggered downstream activation of AMPKSer-172, and subsequently AMPK-mediated phosphorylation and thereby inhibition of ACCSer-79, a pathway which fosters lipolysis over de novo lipogenesis.

Conclusion Therefore, CXCR7 may modulate thrombogenic platelet-lipid associations, sustain lipid catabolism, and generate a stockpile of intraplatelet anticoagulant ACars which may influence platelet-assisted coagulation.

Conflict of Interest None

T-02-07 Telomere length is associated with increased risk of cardiovascular events in hemodialysis patients

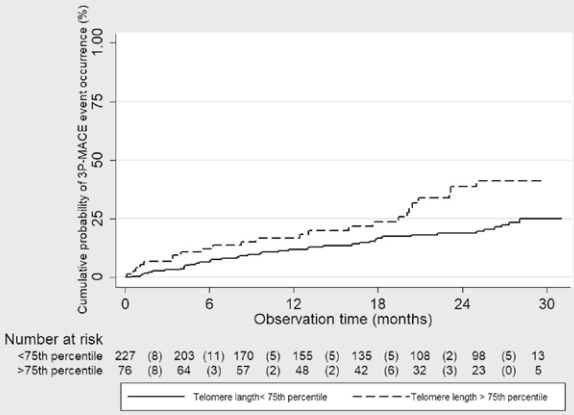
Authors Vostatek R[1], Hohensinner P[2, 3], Schmaldienst S[4], Lorenz M[5], Klauser-Braun R[6], Pabinger I[1], Säemann M[7], Ay C[1], Königsbrügge O[1]

Institutes **1** Medical University of Vienna; Vienna, Austria, Clinical Division of Hematology and Hemostaseology, Department of Medicine I, VIenna, Austria; **2** Medical University of Vienna; Vienna, Austria, Center for Biomedical Research, VIenna, Austria; **3** Medical University of Vienna; Vienna, Austria, Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria; **4** Clinic Favoriten, Department of Medicine I, Vienna, Austria; **5** Vienna Dialysis Center, Vienna, Austria; **6** Clinic Donaustadt, Department of Medicine III, Vienna, Austria; **7** Clinic Ottakring, Department of Medicine VI, Vienna, Austria

DOI 10.1055/s-0042-1760463

Introduction Inpatients with end-stage kidney disease (ESKD) on hemodialysis (HD), cardiovascular disease (CVD) is a common complication and the leading cause of death. Traditional CVD risk factors in these patients are highly prevalent and risk evaluations are difficult to make due to the saturated CVD risk profile in the ESKD population. Our aim is to elucidate the role of biological aging in this patient population, expressed by telomere length and its association with cardiovascular outcomes.

Method The Vienna Investigation of Atrial Fibrillation and Thromboembolism in Hemodialysis (VIVALDI) study is a prospective population-based cohort study of prevalent HD patients. Adult patients were followed up for a maximum of 1350 days and the occurrence of the outcome 3P-MACE, defined as ischemic stroke, myocardial infarction, and cardiovascular death, was recorded. The DNA from whole blood, sampled at baseline, was isolated and analyzed for average telomere length via qPCR-based method. The VIVALDI study was approved by the local ethics committees and all patients consented to participate in written form. Statistical analysis was performed using regression analysis with consideration for competing risk of all-cause mortality (▶ **Fig. 1**).



▶ **Fig. 1** Cumulative probability of 3P-MACE event occurrence.; The solid line denotes the cumulative incidence over time in patients with telomere lengths below the 75th percentile, the dashed line patients above the 75th percentile.

Results In 303 patients with ESKD (113 women, 190 men, median age 67 years), the median telomere length was 1.5 kb (25th to 75th percentile (0.58 – 3.07kb). There was no relevant correlation between telomere length and age (rho-0.102, p = 0.077). The 3P-MACE outcome occurred in 65 patients (incidence rate 9.6 per 100 patient-years). After multivariable adjustment for age, etiology of ESKD, presence of atrial fibrillation, and prior history of myocardial infarction or stroke, telomere length was significantly associated with the occurrence of the composite 3P-MACE outcome (subdistribution hazard ratio [SHR] per 1kb increase in telomere length 1.10, 95 % confidence interval [CI] 1.01-1.19, p = 0.030). Patients with telomere length in the highest quartile had a 2.1-fold increased risk of 3P-MACE outcome (95 %CI 1.20 to 3.79, p = 0.010) (figure 1).

Conclusion In a heterogeneous cohort of patients with ESKD on HD at a high baseline risk of cardiovascular events, telomere length was, contrary to expectation, associated with increased risk for cardiovascular outcomes.

Conflict of Interest The authors declare that they have no competing interests.

T-02-08 Factor V Leiden paradox in Factor V Leiden homozygotes – a retrospective cohort study

Authors Luxembourg B[1], Miesbach W[2], Gall H[3]

Institutes **1** Giessen University Hospital, Department of Thrombosis and Haemostasis, Gießen, Germany; **2** University Hospital Frankfurt a.M., Department of Haemostaseology, Medical Clinic 2, Institute of Transfusion Medicine, Frankfurt, Germany; **3** Gießen University Hospital, Department of Internal Medicine, Universities of Giessen and Marburg Lung Center, Gießen, Germany

DOI 10.1055/s-0042-1760464

Introduction In heterozygous carriers of the Factor V Leiden (FVL) mutation, the risk of pulmonary embolism (PE) is lower than the risk of deep vein thrombosis (DVT), a phenomenon that has been described as the FVL paradox. Whether FVL homozygotes exhibit the FVL paradox is a matter of debate. It has been hypothesized that the second FVL allele counteracts the FVL paradox. To evaluate the FVL paradox in FVL homozygotes, we investigated the odds to suffer from PE for FVL homozygotes compared with FVL wildtype carriers and FVL heterozygotes.

Method In a retrospective cohort study, we recruited 100 consecutive FVL homozygotes with venous thromboembolism who visited the Departments of Haemostasis of the University Hospital Gießen & Marburg GmbH and the University Hospital Frankfurt a.M. between 2007 and 2020. Age- and sex-matched FV wildtype carriers (n = 100) and FVL heterozygotes (n = 100) were enclosed as controls. Logistic regression analysis was applied to calculate the Odds Ratio (OR) for PE.

Results The cohort encompassed more females (60 %) than men and the median age of the participants was about 46 years. FVL homozygotes suffered significantly less often from PE compared with FV wildtype carriers (16 % vs. 44 %, p < 0,001) and FVL heterozygotes (16 % vs. 39 %, p < 0,001). By contrast, isolated DVT was more common in FVL homozygotes than in wildtype carriers (84 % vs. 56 %, p < 0,001) and heterozygotes (84 % vs. 61 %, p < 0,001). The odds to suffer from PE was 76 % lower for FVL homozygotes compared with wildtype carriers (Odds Ratio, OR, 0,24, 95 % CI 0,13-0,47, p < 0,001) and 70 % lower than for FVL heterozygotes (OR 0,30, 95 % CI 0,15-0,58, p < 0,001). In stratified analyses, we found no effect modification for the DVT localisation (proximal vs. distal leg vein thrombosis), a high BMI (≥ 30 vs. < 30), and hormone-associated (oral contraceptives, pregnancy, puerperium) vs. non-hormone-associated VTE in women. Proximal DVT was not significantly more common in FVL homozygotes than in FV wildtype carriers (48,9 % vs. 43,5 %, OR 1,24, 95 % CI 0,66-2,34, p = 0,502) and FVL heterozygotes (48,9 % vs. 63,3 %, OR 0,55, 95 % CI 0,30-1,03, p = 0,062).

Conclusion FVL homozygotes suffer more often from isolated DVT but less often from PE compared with FVL wildtype carriers and FVL heterozygotes. FVL homozygotes exhibit the FVL paradox and the paradox is even more pronounced in FVL homozygotes than in heterozygotes.

Conflict of Interest none

T-02-09 Participation of T helper cell-mediated negative regulation of coagulation in human arterial thrombosis

Authors Luo J[1], Langheinrich T[1], Radev M[1], Sachs N[2], Kempf W[2], Maegdefessel L[2], Engelmann B[1]

Institutes **1** Ludwig-Maximilians-Universität (LMU) München, Vascular Biology and Pathology, Institute of Laboratory Medicine, Munich, Germany; **2** Technische Universität München(TUM), Department for Vascular and Endovascular Surgery, Klinikum rechts der Isar, Munich, Germany

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Introduction Cardiovascular ischemic events caused mainly by arterial thrombosis after rupture/erosion of atherosclerotic plaques are the most common cause of death and disability worldwide. Arterial thrombi contain myeloid and lymphoid immune cells that could be implicated in the regulation of intrathrombotic coagulation. We here focused on immune cells expressing negative coagulation regulators.

Method Arterial thrombosis samples from 14 patients with peripheral arterial disease (PAD) were fixed, embedded and sectioned. Thrombi were analyzed by Carstairs' staining and expression of coagulation regulators in thrombus-associated immune cells by IHC. Plaques were homogenized and analyzed for their ability to regulate whole blood coagulation. RNA sequencing analyses were performed to detect coagulation-relevant genes in immune cells associated with stable or unstable atherosclerotic plaques. CD4 + T cells were isolated from peripheral blood of healthy donors and activated in vitro with anti-CD3/anti-CD28. The fibrinolytic activity of CD4 + T cells was measured by their ability to promote plasmin formation.

Results By Carstairs' staining and IHC of the arterial thrombosis samples, we detected platelet-rich areas consisting of dense fibrin structures packed with platelets and immune cells. Platelet-poor areas consisted mainly of red blood cells, few platelets, and sparse fibrin. CD4 + T cells mostly accumulated around fibrin in platelet-rich areas. RNA seq analysis indicated that unstable plaques accumulated more immune cells including T helper cells expressing negative regulators of coagulation (PLAUR, PLAU, PLG, PROCR) than stable plaques. Negative regulators of coagulation were highly expressed in thrombus-associated T helper cells. T helper cells in arterial thrombi were mostly activated

(CD69 + , CD38 +) and expressed uPAR. Activated isolated human T helper cells showed higher fibrinolytic activity than non-activated T helper cells.

Conclusion T helper cells accumulate in fibrin- and platelets-rich areas of human arterial thrombosis, are activated, express fibrinolytic mediators, and negatively regulate coagulation.

Conflict of Interest The other authors declare that they have no competing interests.

T-02-10 Gut microbiota promotes arterial thrombus formation in hyperlipidemic Ldlr-/- mouse model

Authors Khuu MP[1], Kiouptsi K[1], Pontarollo G[1], van der Vorst E PC[3, 4], Döring Y[3, 4], Müller M[6], Schäfer K[5], Reinhardt C[1, 2]

Institutes **1** University Medical Center Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **2** Partner Site RheinMain, German Center for Cardiovascular Research (DZHK), Mainz, Germany; **3** Ludwig-Maximilians-University, Institute of Cardiovascular Prevention, Department of Medicine, Munich, Germany; **4** Partner Site Munich Heart Alliance, German Center for Cardiovascular Research (DZHK), Munich, Germany; **5** University Medical Center Mainz, Department of Cardiology, Cardiology I, Mainz, Germany; **6** University Medical Center Mainz, Department of Psychiatry and Psychotherapy, Mainz, Germany

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Introduction The gut microbiota has been identified as a dynamic ecosystem impacting the vasculature and remote organ functions. Emerging research demonstrated that gut microbes are involved in modulating cardiovascular diseases (CVD). However, underlying molecular pathways that causally explain the role of the gut microbiota in thrombosis still remain obscure. Thus, we took advantage of established gnotobiotic low-density lipoprotein receptor-deficient (Ldlr-/-) mice to provide new insights on how the commensal gut microbiota, which is influenced by diet, modifies arterial thrombosis.

Method The Ldlr-/- atherosclerosis mouse model was kept at germ-free (GF) or conventionally raised (CONV-R) housing conditions. Following 16 weeks of feeding with chow diet (CD) or atherogenic high-fat Western diet (HFD), the role of the gut microbiota in late atherosclerosis and atherothrombosis was studied using the ultrasound-induced plaque rupture model. Adhesion of rhodamine B-stained platelets to the injured vessel wall was visualized in real-time with high-speed intravital microscopy. Further, the lipoprotein levels and aortic root lesion size was quantified. To investigate the influence of the microbiome at early time points, the effect of 8 weeks of feeding with CD or HFD on arterial thrombus formation was analyzed in the carotid artery ligation model.

Results At 16 weeks CD feeding conditions, we could demonstrate reduced total cholesterol, VLDL and LDL levels in CONV-R mice relative to GF counterparts. This effect was abolished at 16 weeks of HFD. While 16 weeks of HFD did not affect aortic root lesion size, females had an increased lesion size compared to male mice. Decreased plaque rupture-induced thrombus formation was observed in the carotid artery of GF Ldlr-/-mice, suggesting that the presence of gut microbiota enhances arterial thrombus growth. The carotid artery ligation model revealed that platelet deposition to the injury site after 8 weeks of HFD feeding is enhanced by the presence of gut microbiota. Interestingly, GF Ldlr-/- mice receiving CD or HFD showed a decreased platelet deposition compared to CONV-R mice on HFD.[1–2]

Conclusion Here, we report that the gut microbiota is a relevant modifier of arterial thrombus growth under hyperlipidemic conditions.

Conflict of Interest The authors declare that they have no conflict of interest.

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T-03 | Diagnosis and Therapy of Acquired and Congenital Thrombotic Disorders

T-03-01 Recombinant ADAMTS13 treatment in a pregnant patient with hereditary thrombotic thrombocytopenic purpura

Authors Asmis L M[1, 8], Serra A[8], Krafft A[3, 8], Licht A[4, 8], Leisinger E[5], Henschkowski-Serra J[2], Ganter M T[5, 8], Hauptmann S[6], Tinguely M[6, 8], Kremer Hovinga J A[7, 9]

Institutes **1** Zentrum für perioperative Thrombose und Hämostase, Zürich, Switzerland; **2** Klinik Hirslanden, Nephrology und Dialysis, Zürich, Switzerland; **3** Klinik Hirslanden, Obstetrics, Zürich, Switzerland; **4** Klinik Hirslanden, Emergency room, Zürich, Switzerland; **5** Klinik Hirslanden, Institute for Anthesiology and Intensive Care, Zürich, Switzerland; **6** Pathologie Institut Enge, Pathology, Zürich, Switzerland; **7** Inselpital – University Hospital, Dept Hematology and Hematologic Central Laboratory, Bern, Switzerland; **8** University of Zurich, Medical faculty, Zürich, Switzerland; **9** University of Berne, Medical faculty, Bern, Switzerland

DOI 10.1055/s-0042-1760467

Introduction Thrombotic thrombocytopenic purpura (TTP) can be difficult to diagnose. Gold standard treatment to date is plasma-based with or without immunosuppresssion depending on the absence or presence of autoantibodies in hereditary and acquired forms. We describe the first compassionate use case of recombinant ADAMTS13 (rADAMTS13) therapy in a pregnant patient with hereditary TTP (hTTP) who had previously suffered 2 ischemic strokes and one intra-uterine fetal death.

Method Diagnostic tests were performed in the Swiss reference laboratory. Diagnosis was elaborated at the Center for perioperative Thrombosis and Hemostasis as a second opinion. Treatment was provided at the Hirslanden Klinik in Zurich.

Results The hTTP diagnosis was based on the current and previous clinical events, the evolution of platelet count, and ADAMTS13 activity levels of < 5 %, the absence of an inhibitor and the presence of two previously described mutations. Plasmapheresis was performed as an emergency procedure and on a daily basis. The pregnant patient suffered relevant side effects. Despite this therapy, ADAMTS13 activity and platelet count did not adequately rise. R-ADAMTS13 was requested under a compassionate use indication. The request was granted by Takeda. Following cantonal approval rADAMTS13 could be imported and administered at the Hirslanden Klinik in Zurich.

Under weekly application of 40 IU/kg of bodyweight, ADAMTS13 activity could be restituted with favorable development of the platelet count. The fetus' body weight had dropped below the 3rd percentile. Maternal and fetal conditions were stabilized under rADAMTS substitution. After a single booster dose of 20 IU/kg bw, Cesarean section in the 37th week of gestation delivered a low for gestational age boy who subsequently developed very favorably. Current substitution at 40 IU/kg bw continues every other week. Mother and child are currently very well.

Conclusion We hypothesize that it was the possibility to administer larger amounts of ADAMTS13 with the recombinant drug than by plasmapheresis which was crucial to overcome the acute TTP episode and prevent imminent fetal demise in our patient. The close interprofessional and interdisciplinary interaction of nursing teams, midwives, different medical specialists as well as the team at the pharmaceutical company producing rADAMTS13 were vital to

the case and permitted a fast diagnosis, emergency treatment and eventually compassionate use of rADAMTS13. The case represents the successful treatment of an acute, persistent and plasma-refractory episode with rADAMTS13 in a hTTP patient.

Conflict of Interest LMA has received travel support and honoraria for advisory boards from Takeda. JAKH is a Takeda study investigator.

T-03-02 Current diagnostic and therapeutic standard of iliac vein compression syndrome (May-Thurner syndrome) in children, adolescents and young adults. A survey among national thrombosis experts

Authors Cuntz F[1], Gebauer B[2], Greiner A[3], Hagedorn N[1], Reschke M[1], Eberl W[4], Zieger B[5], Holzhauer S[1]

Institutes **1** Charité-Universitätsmedizin Berlin, Campus Virchow-Klinikum, Department of Paediatric Oncology and Haematology, Berlin, Germany; **2** Charité-Universitätsmedizin Berlin, Campus Virchow-Klinikum, Department of Radiology, Berlin, Germany; **3** Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Clinic of Vascular Surgery, Berlin, Germany; **4** Städtisches Klinikum Braunschweig, Department of Pediatrics, Braunschweig, Germany; **5** Medical Center, Faculty of Medicine, University of Freiburg, Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Freiburg, Germany

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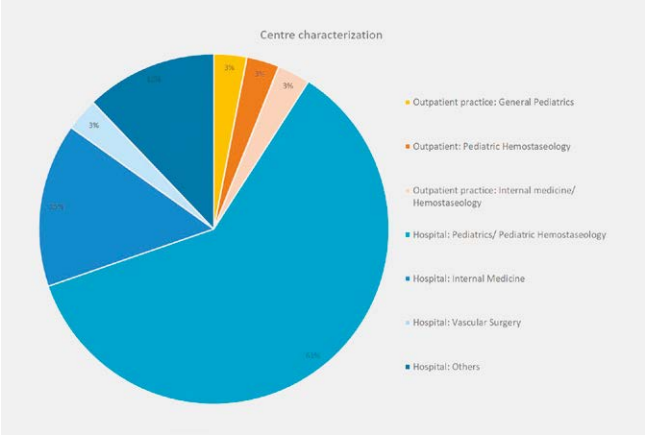
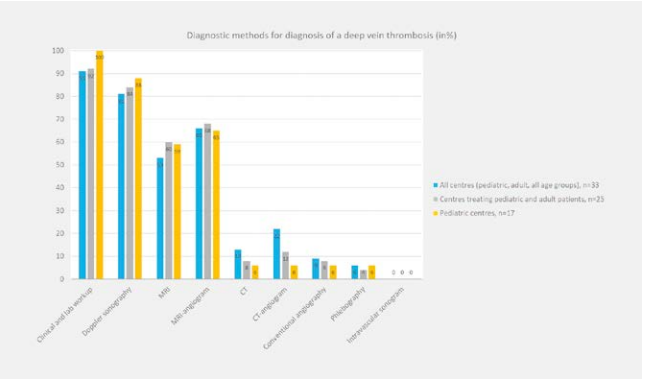


▶ **Fig. 1** Centre characterization; Characterization of centres participating in the survey in %; most centres are hospitals (pediatrics/ pediatric hemostaseology, internal medicine/ angiology, vascular surgery) and outpatient practices (general practice, pediatric practice including hemostaseology/ pediatric oncology and hematology, general practice including hemostaseology) along with a few others (not shown: pediatric cardiology, transfusion medicine/ hemostaseology, internal medicine/ rheumatology).

Introduction Iliac vein compression syndrome (May-Thurner Syndrome) is an anatomic variant with chronic pulsatile compression of the left common iliac vein by the overlying right common iliac artery against lumbar vertebrae. Reduced venous backflow and endothelial damage result in a predisposition for left-sided deep vein thromboses. The prevalence of iliac vein compression syndrome may be underestimated as the sensitivity of colour doppler sonography to screen for iliac compression is low. Therapeutic options comprise anticoagulation, thrombectomy and intravascular stent. The aim of this survey was to assess the current practice in Germany to develop a standardized diagnostic and therapeutic approach.

Method Our interdisciplinary team (pediatric hemostaseology, radiology, vascular surgery) designed an online survey consisting of 11 questions which we distributed via the mailing list to the members of the German Society of Thrombosis and Hemostasis.

Results Between July and October 2022, 33 questionnaires were returned. Most participating centres are hospitals (▶ **Fig. 1**). 52 % treat patients < 18 years, 21 % adults and 27 % all age groups. Numbers of annually treated thromboses in patients < 25 years range from 1-5 (26 %) to > 30 (13 %). Centres treating adult patients reported higher patient numbers. Main diagnostic tools used for deep vein thrombosis (DVT) are clinical/ laboratory workup and doppler sonography, only 53 % of centres report the use of MRI (▶ **Fig. 2**). DVT is treated by therapeutic anticoagulation (84 %), systemic fibrinolysis (13 %), interventional thrombectomy (39 %), catheter lysis/ ultrasound directed methods (32 %) or surgical thrombectomy (10 %). Screening for iliac vein compression syndrome is performed by 25 % of centres (always 7 %, in case of typical clinical signs 4 %, in case of left-sided DVT 4 %, in case of iliac vein compression 7 %). Treatment for iliac vein compression syndrome are anticoagulation (65 %), balloon angioplasty (13 %) or stent/ AV fistula (32 %). Choice of treatment is highly individualized and centre specific. Interventional treatment is administered depending on thrombus size (68 %), age of thrombosis (65 %), and thrombus localization (71 %), for lack of contraindications (35 %) or to avoid post-thrombotic syndrome (35 %). Half of participating centres identified as specialized treatment centres, in 36 % patients are referred to the centres [1–3].



▶ **Fig. 2** Diagnostic methods for diagnosis of a deep vein thrombosis (%); Diagnostic tools used for diagnosis of DVT in all centres (blue bars), centres treating adult and pediatric patients (grey) and pediatric centres (yellow), shown in %. Main diagnostic methods are clinical/ laboratory workup and doppler sonography. Only 53 %-60 % chose MRI and 65-68 % MRI-angiogram. CT and CT-angiogram are done less frequently, and very rarely in pediatric centres. Conventional angiography and phlebography are used by 4-9 %. None of the centres opt for intravascular sonography.

Conclusion The participating centres use various diagnostic and therapeutic options for young patients with iliac vein compression syndrome. As expected, approaches differ between centres reflecting the limited experience with this patient group. Only 25 % of centres systematically screen for iliac vein compression. To optimize care it is crucial to compare different therapeutic options currently used and follow up on outcome. Next steps are to develop a standardized diagnostic approach and therapeutic algorithm within our interdisciplinary team and the working group Pediatrics of the German Society of Thrombosis and Hemostasis.

Conflict of Interest The authors declare no conflict of interest.

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T-03-03 Evaluating the individual risk of venous thrombo-embolism in patients with liver cirrhosis: Usefulness of in vivo and ex vivo thrombin generation parameters

Authors Zermatten M G[1], Fraga M[2], Bertaggia Calderara D[1], Aliotta A[1], Pereira Portela C[1], Mdawar-Bailly É[2], Artru F[3], Moradpour D[2], Alberio L[1]
Institutes **1** Lausanne University Hospital (CHUV), Division of Hematology and Central Hematology Laboratory, Lausanne, Switzerland; **2** Lausanne University Hospital (CHUV), Division of Gastroenterology and Hepatology, Lausanne, Switzerland; **3** Institute of liver studies, King's College Hospital, London, UK
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Introduction Venous thrombo-embolic events (VTE) and especially splanchnic vein thromboses (SVT) are common complications in patients with liver cirrhosis. SVT can worsen portal hypertension and cause ascitic decompensation. Identification of high-risk patients and consecutive targeted primary prophylactic anticoagulation could prevent these complications, preserving favorable conditions for liver transplantation and improving patient quality of life and prognosis. In this study, we aimed to investigate the role of in vivo and ex vivo thrombin generation (TG) parameters in the prediction of VTE in patients with liver cirrhosis.

	No VTE	VTE	*p*-value
	278 (94.6%)	16 (5.4%)	
ETP [nM*min]	998 (241)	1261 (204)	0.15
Peak height [nM]	222.9 (61.2)	246 (34.3)	0.19
Velocity index [nM/min]	140.8 (75.6)	180.8 (60.8)	**0.0198**
ETP normalized [%]	77.6 (15.6)	85.2 (13.3)	0.13
Peak height, normalized [%]	77.6 (21.7)	85.7 (11.3)	0.06
Velocity index, normalized [%]	84.6 (41.7)	124.6 (34.9)	**0.0027**
TM-mediated Inhibition [%]	37.8 (21.5)	17.0 (19.2)	**0.0044**
TAT [µg/l]	2.5 (3.2)	3.2 (1.5)	**0.0499**
F1+2 [pmol/l]	157.3 (127.6)	233.0 (166.0)	**0.0019**
D-dimers [ng/ml]	459 (2513)	1323 (3046)	**<0.0001**
aPTT [s]	33 (6.7)	38.5 (5.5)	**0.0048**
Prothrombin time [%]	80 (17.8)	65 (12.5)	**0.0028**
Fibrinogen [g/l]	2.6 (1.0)	2.4 (0.7)	0.11
Factor V [%]	80 (30.2)	58.5 (21.8)	**0.0119**
Child-Pugh score	5 (1.4)	6 (1.6)	**0.0075**

▶ **Tab. 1** Comparisons between groups with and without venous thrombo-embolic events; Data are number (%), respectively median (standard deviation) and results of Mann-Whitney tests. VTE, venous thrombo-embolic events; ETP, endogenous thrombin potential; TM, thrombomodulin; aPTT, activated partial thromboplastin time.

Method We performed a prospective single-centre study at Lausanne University Hospital (CHUV) including patients with liver cirrhosis. The main outcome was VTE including deep venous thrombosis, pulmonary embolism and SVT. We analysed in vivo TG parameters (prothrombin fragments 1 and 2 [F1 + 2], thrombin-antithrombin complexes [TAT], D-dimers) and ex vivo TG. Ex vivo parameters were measured using the ST Genesia Thrombin Generation System (Stago, Asnières-sur-Seine, France). The analyses were performed with an intermediate concentration of tissue factor, as well as without and with thrombomodulin (TM) as protein C/S system activator. We focused on the velocity index without TM (representing the thrombin generation velocity), peak height (representing the maximal thrombin concentration), and TM-mediated inhibition (which is the degree of diminution of TG after TM addition, reflecting the activity of the protein C/S system). We also analysed routine hemostatic tests (activated partial thromboplastin time [aPTT], prothrombin time [PT], fibrinogen, factor V activity). Comparisons between groups were assessed by Mann-Whitney test (▶ **Tab. 1**).

Results We included 294 non-anticoagulated patients. Median age was 59 years (range, 18-81 years) and 68 patients (23 %) were female. Regarding cirrhosis severity, 235 (79.9 %) had a Child-Pugh score A, 49 (16.7 %) B and 10 (3.4 %) C. Comparisons of the parameters between groups with and without VTE are presented in Table 1. The velocity index, TM-mediated inhibition, TAT, F1 + 2 and D-dimers as well as aPTT and PT were significantly associated with the risk of VTE.

Conclusion In vivo and ex vivo TG parameters are associated with the risk of VTE in patients with liver cirrhosis. The integration of these parameters with clinical (e.g. etiology of liver cirrhosis, history of VTE, or presence of cancer) and paraclinical variables (in particular, parameters of portal hemodynamics such as portal vein flow) is a promising approach to identify high-risk patients who could benefit from primary prophylactic anticoagulation. Analyses are ongoing to develop a VTE risk prediction score in patients with liver cirrhosis.

Conflict of Interest Stago (Asnière-sur-Seine, France) supported the study with discounts for ST Genesia reagents.

T-03-04 Neonatal exchange transfusion: hereditary thrombotic thrombocytopenic purpura (hTTP) should be in the differential diagnosis

Authors Tarasco E[1, 2], von Krogh AS[3, 4], Knöbl P[5], Matsumoto M[6], Hrachovinova I[7], Friedman K D[8], George J N[9], Schneppenheim R[10], Lämmle B[1, 11], Kremer Hovinga J A[1, 2]
Institutes **1** Bern University Hospital, Department of Hematology and Central Hematology Laboratory, Bern, Switzerland; **2** University of Bern, Department of Biomedical Research (DBMR), Bern, Switzerland; **3** Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Trondheim, Norway; **4** St Olavs Hospital Trondheim University Hospital, Department of Hematology, Trondheim, Norway; **5** University of Vienna, Division of Hematology and Hemostasis, Departmentof Medicine, Vienna, Austria; **6** Nara Medical University, Department of Blood Transfusion Medicine, Kashihara, Japan; **7** NRL for Hemostasis, Institute of Hematology and Blood Transfusion, Prague, Czech Republic; **8** Medical College of Wisconsin, VersitiBlood-Center of Wisconsin, Division ofHematology and Oncology, Milwaukee, USA; **9** Hudson College of Public Health, University of Oklahoma, Department of Biostatistics & Epidemiology, Okhahoma City, USA; **10** University Medical Center Hamburg-Eppendorf, Department of Pediatric Hematology and Oncology, Hamburg, Germany; **11** University of Mainz, Center for Thrombosis and Hemostasis, Mainz, Germany
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Introduction Transition from intra- to extra-uterine life is complex and abnormalities in this adaptive process may require resuscitation and/or intensive treatment in the first days of life, which seems to be particularly true for neonates later in life diagnosed with hTTP. These neonates may show severe hyperbilirubinemia, profound anemia and thrombocytopenia, requiring emergency treatment including neonatal exchange transfusion (NExT) in the more complicated cases. Severe congenital ADAMTS13 deficiency is the cause of hTTP, a rare autosomal recessively inherited disorder rarely diagnosed at birth despite of indicative symptoms and laboratory parameters.
The most common indication for NExT is severe hyperbilirubinemia due to allo-immune hemolytic disease that has steadily decreased since the introduction of anti-D immunoglobulin prophylaxis in Rhesus-negative women. Retrospective analyses of the national hTTP cohorts in Japan1,2 and Norway3 showed that 35-43 % of patients had required NExT in the first days of life.

We investigated the prevalence of severe neonatal hyperbilirubinemia, neonatal thrombocytopenia, the use of phototherapy or NExT and age at hTTP diagnosis in patients of the International Hereditary TTP Registry (www.ttpregistry.net).

Method Confirmed hTTP patients enrolled until October 2022 with information on their neonatal period were eligible for study. We collected information from the patients, their parents whenever possible, the treating physicians, and all available patient charts. We used the following age group definitions: neonatal period birth to 3 months of age; early childhood >3 months to ≤6 years; late childhood >6 to ≤18 years; and adulthood >18 years.

Results The International Hereditary TTP Registry has enrolled 178 confirmed hTTP patients eligible for this study, 52 of them are ≤18 years of age. Disease onset in the neonatal period was documented in 81/178 (45.5%) patients, their most prevalent symptoms were jaundice and/or thrombocytopenia, present in 69/81 (85.2%). Phototherapy was required in 16 (19.8%) and NExT in 42 (51.9%) patients. Information whether or not NExT was performed was not retrievable for 33/178 (18.5%) patients, 29 of them were born before year 2000. In patients with neonatal disease-onset, hTTP was diagnosed immediately in 13 (16%; 12 had received NExT), during early or late childhood in 28 (35%) and 18 (22%), respectively, and in 21 (26%) in adulthood. For one patient exact time of diagnosis is missing.

Of 67 patients with a delayed diagnosis, at least 16 experienced arterial thrombotic events (mainly strokes) before hTTP diagnosis; half of them had required NExT [1–3].

Conclusion Differential diagnosis of severe neonatal hyperbilirubinemia with or without thrombocytopenia should nowadays include hTTP. ADAMTS13 activity has to be determined in any newborn requiring NExT for severe hyperbilirubinemia, followed by molecular analysis of the ADAMTS13 gene when ADAMTS13 activity is <10% to prevent delayed hTTP diagnosis.

Conflict of Interest NA

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T-03-05 The phenotypic and genetic assessment of hereditary antithrombin deficiency in 215 patients from the Rhein-Ruhr area in Germany

Authors Halimeh S[1,2], Rott H[1], Kappert G[1], Günther M[1,2]

Institutes **1** Gerinnungszentrum Rhein-Ruhr, Hämostaseologie, Duisburg, Germany; **2** Universität Duisburg-Essen, Kinderheilkunde, Essen, Germany

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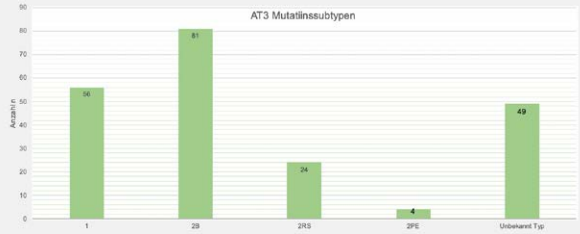
Introduction Hereditary antithrombin (AT) deficiency is a genetic disorder with an increased risk of venous thromboembolism (VTE), especially deep vein thrombosis. It is caused by a decreased level of AT (Typ 1) or a function loss (Typ 2A, B or PE).

A genetic testing of the type of antithrombin deficiency allows reliably the subtyping of the variant of antithrombin deficiency. The exact diagnosis helps deciding about therapy in pregnancy or if a longtime anticoagulation is indicated.

Our cohort currently contains 215 patients with a genetic confirmed AT-deficiency

Method Retrospectively, we analyzed 215 patients with hereditary antithrombin deficiency and their clinical outcome.The Following parameters were collected: Age, age of first thrombotic event, gender, AT-activity using the concentration of factor Xa including reference range, AT-activity using the concentration of factor IIa (Thrombin) including reference range, Typ of AT-deficiency (I, IIA, IIB, IIPE), the SERPINC1 mutation, patient's anamnesis, other diseases, current medication, pregnancies or miscarriages (▶ **Fig. 1**).



▶ **Fig. 1** Likelihood of venous thromboembolism in patients with hereditary AT3 deficiency; This figure illustrates the probability of venous thromboembolism in patients with hereditary Antithrombin deficiency, depending on the subtype (type 1, type 2RS, type 2PE, type 2B).

We investigated the influence of molecular subtype of AT-deficiency on occurrence and recurrence of VTE as well as the age at which first VTE occurred.

Results So far 72 patients of the cohort suffered VTE whereas 59 patients did not. In patients with Typ 1 AT deficiency, VTE occurred in 69,7% (n = 33) of patients, whereas in patients with type 2B AT deficiency, VTE occurred in 36,96% (n = 46) (▶ **Tab. 1**).

	Alle Subtypen	Typ 1	Typ 2B	Typ 2 RS	Typ 2 PE
Pat. die eine VTE erlitten	72	23	17	8	3
Pat. die keine VTE erlitten	59	10	29	7	1
Summe der Pat.	131	33	46	15	4
Wahrscheinlichkeit einer VTE	54,96%	69,70%	36,96%	53,33%	75,00%

▶ **Tab. 1** Subtypes of genetic confirmed hereditary antithrombin deficiency; This diagram emphasizes the amount of subtypes in our cohort for type1, type 2RE, type 2B, type 2PE or unknown type.

Conclusion Currently the recommendation after VTE in patients with AT-deficiency is a lifelong anticoagulation. Our data show, that in patients with subtypes of AT-deficiency, the VTE-risk differs. Further studies regarding the risk of recurrent VTE in different subtypes of AT- deficiency are needed.

Conflict of Interest No conflicts of interest.

T-03-06 Characteristics, management, disposition, and outcome of patients with pulmonary embolism in a tertiary care setting

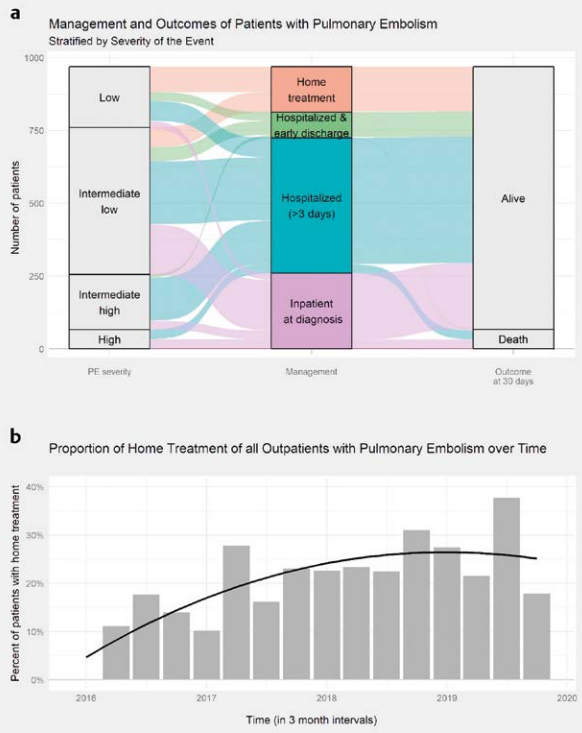
Authors Nopp S, Bohnert J, Mayr T, Steiner D, Lang I, Behringer W, Janata-Schwatczek K, Ay C

Institute Medical University Of Vienna, Vienna, Austria

DOI 10.1055/s-0042-1760472

Introduction Acute pulmonary embolism (PE) is a life-threatening disease. Current guidelines suggest risk-adapted management and disposition of patients. Hospitalization is required for intermediate and high-risk patients. Early discharge and home treatment are considered safe in the majority of low-risk patients. In this study, we describe characteristics, management, disposition, and outcome of patients diagnosed with PE at a tertiary care center.

Method All in- and outpatients undergoing computed tomography pulmonary angiography or a ventilation/perfusion lung scan between 01.01.2016 and 31.12.2019 at the Vienna General Hospital, Austria, were screened for a PE diagnosis. Electronic patient charts were used to extract characteristics, clinical course, and outcome of patients with PE.



▶ **Fig. 1** a) Management and Outcomes of Patients with Pulmonary Embolism b) Proportion of Home Treatment of all Outpatients with Pulmonary Embolism over Time

	Total cohort (n = 969)
Demographics & Setting	
Age, years	63 (50-74)
Female sex	487 (50.3%)
Outpatient at diagnosis	709 (73.2%)
Comorbidities	
Heart failure	47 (4.9%)
Chronic lung disease	129 (13.3%)
Chronic kidney disease	108 (11.2%)
History of or active cancer	312 (32.2%)
Previous VTE	233 (24.1%)
PE characteristics	
Site - Bilateral	563 (58.1%)
Location - Central	232 (23.9%)
Severity of PE according to ESC guidelines	
Risk stratification	
- Low risk	209 (21.6%)
- intermediate-low risk	505 (52.1%)
- intermediate-high risk	189 (19.5%)
- high risk	66 (6.8%)
Right ventricular dysfunction	303 (31.3%)
Infarct pneumonia	110 (11.4%)
Risk factors for PE	
Major transient risk factor	245 (25.3%)
Minor transient	117 (12.1%)
Persistent	298 (30.8%)
None	369 (38.1%)
Management & Outcomes	
Treatment management	
- Home treatment	156 (16.1%)
- Hospitalized & early discharge (≤3 days)	99 (10.2%)
- Hospitalized (>3 days)	454 (46.9%)
- Continued inpatient treatment	260 (26.8%)
Death at 30 days	67 (6.9%)

Values are presented as median (interquartile range) or number (percentage)

▶ **Tab. 1** Characteristics and outcomes of patients diagnosed with pulmonary embolism (PE) at a tertiary care center between 2016-2019.

Results In total, 969 patients (median age: 63 years, 50 % women) were diagnosed with PE within the 4-year period (Table 1). At diagnosis, 709 (73 %) were outpatients and 260 (27 %) were inpatients. Sixty-six (7 %) patients were classified as high-risk, 189 (20 %) as intermediate-high risk, 505 (52 %) as intermediate-low risk, and 209 (22 %) as low risk PE according to the European Society of Cardiology risk stratification. After 30 days, 67 (7 %) patients had died. Of all outpatients at diagnosis, 553 (78 %) were hospitalized, with a median length of hospitalization of 7 days (IQR, 5-11). Half of low-risk outpatients and 20 % of intermediate-low risk outpatients were discharged for home treatment. All low-risk patients and 99 % of patients discharged for home treatment (▶ **Fig. 1a**) survived the first 30 days. The proportion of patients with home treatment increased from ~10 % to ~25 % during the study period (▶ **Fig. 1b**).
Conclusion In our study population, the majority of PE patients were classified as intermediate-low risk. Early discharge and home treatment increased over time and seem to be safe. Notably, half of low-risk PE outpatients were hospitalized, but 20 % of intermediate-low risk outpatients were discharged (▶ **Tab. 1**).
Conflict of Interest The authors declare no conflict of interest.

T-03-07 Measurement of procoagulant platelets in platelet-rich plasma by flow cytometer for the diagnosis of heparin-induced thrombocytopenia

Authors Pelzl L[1], Karakuyu A[1], Jaffal H[1], Marini I[1], Zlamal J[1], Althaus K[1], Hammer S[1], Bakchoul T[1, 2]
Institutes **1** University Hospital of Tuebingen, Institute for Clinical and Experimental Transfusion Medicine, Tuebingen, Germany; **2** University Hospital of Tuebingen, Centre for Clinical Transfusion Medicine, Tuebingen, Germany
DOI 10.1055/s-0042-1760473
Introduction Heparin-induced thrombocytopenia (HIT) is caused by anti- PF4/Heparin IgG antibodies, which activates platelets and leads to thrombocytopenia and thrombosis. The diagnosis of HIT can only be confirmed by using functional assays such as the Heparin-Induced Platelet Activation assay (HIPA assay). However, functional assays are technically demanding and routinely available only in specialized laboratories. The aim of the current study was to establish a flow cytometer-based method to detect procoagulant platelets using platelet-rich plasma (PRP) for the diagnosis of HIT.
Method Sera samples from patients with HIT were incubated with PRP from healthy donors for different durations (30, 60, 90 minutes). Procoagulant platelets were determined by double expression of P-selectin (CD62P) and phosphatidylserine (PS) externalization by flow cytometry. CD32a-mediated cross-linking and platelet stimulation with TRAP-6 and Convulxin were used as positive controls.
Results Sera from HIT-diagnosed patients but not from the control-group induced a significant increase in the procoagulant platelet subpopulation in the presence of 0.2 U/mL heparin, compared to 100 U/mL treated cells (% double positive CD62P/Annexin, 100 U/mL vs. 0.2 U/mL Mean ± SEM: 1.2 ± 1.1 vs 18.5 ± 8.1, p = 0.0021). The optimal incubation time was detected after 60 minutes. A donor dependency of the flow cytometric method was not observed (% double positive CD62P/Annexin, control vs. HIPA + , Mean ± SEM: 0.7 ± 0.59 vs. 19.0 ± 3.2, p = 0.0129). In addition, the use of washed platelets and PRP with HIT-sera showed comparable results in the flow cytometric analysis (% double positive CD62P/Annexin, PRP vs. washed platelets Mean ± SEM: 34.2 ± 6.3 vs 30.1 ± 2.2, ns).
Conclusion Our data suggest that flow cytometry-based protocol using PRP can be suitable to detect the ability of HIT antibodies to induce procoagulant platelets by flow cytometry. An ongoing study is currently investigating the clinical implementation of this protocol in the diagnostic of HIT.
Conflict of Interest I have no potential conflict of interest to report.

T-04 | Anticoagulation and Antiplatelet Therapy

T-04-01 Polymer anchors with responsive heparin release for the anticoagulant decoration of hemodialysis membranes

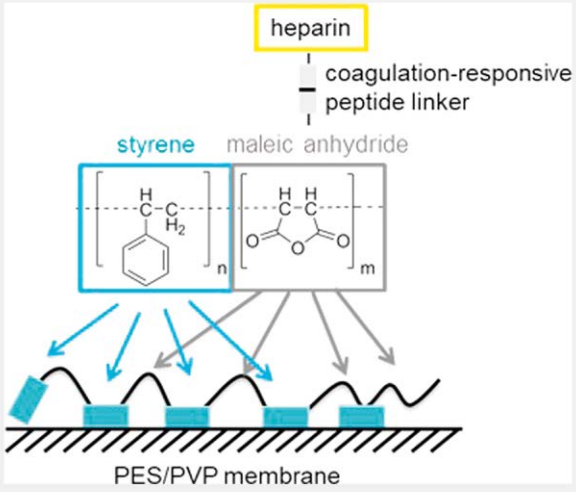
Authors Helmecke T[1], Tsurkan M[1], Hahn D[1], Ruland A[1], Krug H[1], Maitz M F[1], Werner C[1,2]

Institutes **1** Leibniz-Institut für Polymerforschung Dresden e.V., Institut Biofunktionelle Polymermaterialien, Dresden, Germany; **2** Technische Universität Dresden, Center for Regenerative Therapies Dresden (CRTD), Dresden, Germany

DOI 10.1055/s-0042-1760474

Introduction Extracorporeal hemodialysis is a standard treatment of terminal renal insufficiency. The intense contact of blood with the dialysis membrane requires systemic anticoagulation, associated with bleeding risks and side effects. Surface modifications for improved hemocompatibility are requested that allow reduced systemic anticoagulation.

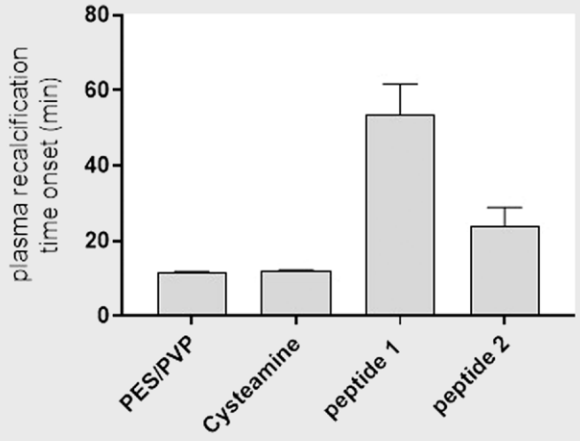
Method Styrene-maleic acid anhydride copolymers were systematically conjugated with poly(ethylene glycol) (PEG) to form amphiphilic polymer anchors that adsorb to the surface from aqueous solution. The anticoagulant heparin was functionalized to an anchor polymer-PEG conjugate or directly to the polymer backbone. The system was extended for feedback-control properties by heparin-anchor polymer conjugation via a linker peptide, which is selectively cleaved by the coagulation factor thrombin (▶ **Fig. 1**).



▶ **Fig. 1** Scheme of the anchor polymer structure and adsorption to the membrane.

Anchor polymer-heparin conjugates were analysed for their adsorption on PES surfaces and PES/PVP membranes. Besides, the thrombin responsive heparin release and the anticoagulant effect of the coating were tested in a buffer system and recalcified citrate plasma.

Results Anchor polymers formed a homogeneous and stable coating on a PES film in an aqueous solution, with tunable density by modification of the PEG:Styrene ratio and the molecular weight of the PEG. Still, removal was possible by a detergent or by self-replacement, allowing regeneration of the coating [1].



▶ **Fig. 2** Coagulation time of citrate plasma on coated flat membranes.

Heparin conjugation had only minor effects on the adsorption characteristics of the anchor polymer. The heparin surface density on hemodialysis membranes was comparable to established heparinized products. Thrombin-antithrombin complex formation on coated hollow fibers proved the catalytic activity of the immobilized heparin.

The heparin release by thrombin substantially delayed plasma coagulation compared to stable immobilization; however, there was high variation between different peptides (▶ **Fig. 2**).

Conclusion Anchor polymers present a versatile method for the biofunctionalization of polymer surfaces such as hemodialysis membranes from aqueous solutions. The integration of selectively cleavable linker peptides allows for the formation of feedback-controlled responsive systems. The modular set-up of the anchor polymers provides flexibility to exchange and combine drugs with different functionality.

Conflict of Interest none

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T-04-02 Understanding the biomolecular corona formation at the nano-bio interface

Authors Londhe V, Werner C, Weiss A CG, Besford Q A

Institute Leibniz-Institut für Polymerforschung Dresden e.V., Institut Biofunktionelle Polymermaterialien, Dresden, Germany

DOI 10.1055/s-0042-1760475

Introduction Engineered polymeric nanoparticles (NPs) are promising candidates in controlled and targeted therapeutic drug delivery applications. However, formation of a biomolecular corona in physiological environments alters the synthetic identity of the NPs.[1] This influences cell-NP interactions, often leading to immune recognition and rapid clearance of the NPs from circulation.[2] In this context, NPs composed of protein-resistant and immune-modulating materials are of interest.

Method Surface-initiated atom transfer radical polymerization (SI-ATRP) and surface-initiated reverse addition fragmentation chain-transfer (SI-RAFT) within mesoporous silica template particles allows the fabrication of polymeric replica NPs with predetermined composition and monodisperse size distribution. To impart a low-fouling and stealth character to the NPs, polyethylene glycol (PEG)-based monomers were used as backbone monomers. Further-

more, the additional incorporation of end-functionalised monomers such as 2-hydroxyethyl methacrylate (HEMA) for a hydroxyl functionality and ethanolamine methacrylate (EAMA) for an amine functionality, enabled decoration of the NPs with fluorescent labels as well as immune-associated biomolecules such as heparin. The additional incorporation of heparin has the potential to further improve the NPs bioavailability. The NPs were characterised by dynamic light scattering (DLS) and electron microscopy. The performance regarding their biocompatibility was evaluated through in vitro and ex vivo assessments in human plasma and blood.

Results Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analyses revealed the spherical morphology and monodispersity of PEG-NPs. DLS measurements indicated a particle size of ~230 nm and neutral zeta potential of ~1 mV. Sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated a reduction in plasma protein adsorption on PEG-NPs (indicative of a low-fouling nature) as compared to silica template particles. Confocal laser scanning microscopy analyses and enzyme-based activity assays confirmed incorporation of fluorescent labels and heparin respectively in the NP architecture. Furthermore, cell-NP association studies in human whole blood were performed to characterize the NPs stealthiness.

Conclusion PEG-based monomers confer a low-fouling character and the additional monomers impart functional versatility to the NPs. The PEG-NPs present a platform for investigating the link between biomolecular corona formation and downstream immune responses. Decoding the immunological aspects of the biomolecular corona, and establishing an immunogenic link towards NP clearance, will further assist in understanding the possibilities of immunoevasive NPs. This may ultimately be employed in the development of advanced long circulating NPs for the controlled delivery of drugs for the therapeutic treatment of disease.

Conflict of Interest none

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T-04-03 A hierarchical network of Src, Syk, Btk and PKC controls GPVI-dependent human platelet activation

Authors Zhang P[1, 2], Solari F A[2], Heemskerk J WM[4], Kuijpers M[3], Sickmann A[2, 5, 6], Walter U[1], Jurk K[1]

Institutes **1** Johannes Gutenberg University Mainz, Center for Thrombosis and Haemostasis (CTH) of University Medical Center, Mainz, Germany; **2** Leibniz Institute for Analytical Sciences - ISAS-e.V., Dortmund, Germany; **3** Maastricht University, Department of Biochemistry, CARIM, Maastricht, Netherlands; **4** Synapse Research Institute Maastricht, Maastricht, Netherlands; **5** Ruhr-Universität Bochum, Medizinische Fakultät, Medizinisches Proteom-Center, Bochum, Germany; **6** University of Aberdeen, Department of Chemistry, College of Physical Sciences, Aberdeen, UK

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Introduction Src family kinases (SFKs), spleen tyrosine kinase (Syk) and Bruton´s tyrosine kinase (Btk) play central roles in the activation of immune cells and platelets. Glycoprotein VI (GPVI) stimulation activates platelet SFKs, Syk and Btk resulting in phospholipase Cγ (PLCγ) and protein kinase C (PKC) activation. However, their functional hierarchy and cross-talk in platelets are not well understood. Recently, selective Syk and Btk inhibitors showed potency to treat thrombosis, cancers and immuno-inflammatory diseases. Using such inhibitors, we investigated hierarchy of Syk, Btk and PKC with respect to their downstream effectors in GPVI-stimulated human platelets.

Method Aggregation of washed human platelets was monitored by light transmission aggregometry in response to the GPVI agonist convulxin (cvx). Syk, Btk and PKC were inhibited by PRT, acalabrutinib and GFX, respectively. Site-specific antibodies were used to quantify phosphosites of Syk (S297, Y352, Y525/526), Btk (S180, Y223, Y551), PLCγ2 (Y759, Y1217), LAT (Y220), Akt (T308, S473) and MAPKs (Erk T202/Y204, p38 T180/Y182) in platelets, which were time-dependently (10-300s) stimulated by cvx under stirring.

Results Cvx induced a strong platelet aggregation which was abolished by 1 µM PRT or 5 µM acalabrutinib. Cvx induced a rapid, transient upregulation of multisite Y/S-phosphorylation with a clear kinetic hierarchy of Syk, Btk, LAT, PLCγ2, PKC, MAPKs and Akt. Tyrosine phosphorylation (pY) of Syk, Btk, PLCγ2, LAT preceded serine phosphorylation (pS) of Syk and Btk, Erk, p38 and Akt. PRT did not affect Syk pY352, but inhibited all other cvx-induced Y-phosphosites studied. Acalabrutinib inhibited cvx-induced Btk pY223 (autophosphorylation), PLCγ2 pY759/pY1217, Erk pT202/Y204, p38 pT180/Y182, Akt pT308/S473, Syk pS297 and Btk pS180, but not Syk pY352, pY525/526 and LAT pY220. GFX did not reduce, often enhanced pY of Syk, Btk, LAT and PLCγ2, abolished Syk and Btk pS and inhibited Erk but not p38.

Conclusion This kinetic analysis of cvx-induced phosphorylation in combination with the inhibitor effects indicate a hierarchical order of Y-/S-/T- protein kinases phosphorylation during GPVI-induced platelet activation. Stimulation of SFKs, Syk, Btk results in a strictly Btk-dependent activation of PLCγ2, PKC and Erk, whereas p38 and Akt activation are Btk- but not PKC-dependent. There is substantial cross-talk, such as the feedback inhibition of Syk and Btk by PKC-mediated phosphorylation (Syk pS297, Btk pS180). Importantly, specific phosphosites can be well used as markers for certain kinase activities: Syk pY352 as SFK marker; Syk pY525/526, LAT pY220, Btk pY551 as Syk marker; Btk pY223, PLCγ2 pY759/ p1217 as Btk marker; Syk pS297/Btk pS180 as PKC marker. The functional implications of differential inhibition of the Syk-Btk-system and their downstream effectors in human platelets are currently investigated [1–4].

Conflict of Interest The authors declare no conflicts of interest.

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T-04-04 Platelet hyperactivation and neutrophil extracellular traps promote thrombo-inflammation and glomerular endothelial dysfunction in diabetic kidney disease

Authors Gupta A, Singh K K, Fatima S, Ambreen S, Zimmermann S, Isermann B, Kohli S

Institute Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik,Universitätsklinikum, Leipzig University, Leipzig, Germany

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Introduction Diabetic kidney disease (DKD) is the cause of end-stage renal failure and contributes to morbidity and mortality worldwide. However, therapeutic options that ameliorate or reverse the progression of DKD are limited. Endothelial dysfunction, platelet hyperactivity, immune cell infiltration and glomerular filtration barrier disruption are associated with DKD. Therefore, we

aimed to scrutinize the mechanistic interplay between platelets and neutrophil extracellular traps (NETs) and ensuing renal thrombo-inflammation. These insights may identify new avenues to prevent or reverse DKD.

Method Streptozotocin induced type 1 diabetic mice were used to evaluate renal function, platelet activation and NET formation. Therapeutic interventions (ASA, Anakinra, Solulin, GSK484, Ticagrelor, PSGL blocking) were performed in sub groups of mice with drugs starting at 16 weeks of diabetes until 24 weeks to study disease reversal. In vitro studies were performed using glomerular endothelial cells (GENC), platelets and neutrophils exposed to high glucose.

Results Experimental DKD in C57Bl6 mice resulted in albuminuria, increased fractional mesangial area, activated platelets (CD62P) and neutrophil extracellular traps (NETs; CitH3, NE, PAD4, MPO) within glomeruli. In parallel, increased expression of inflammasome markers (NLRP3, IL1β) and reduced expression of coagulation regulator thrombomodulin (TM) was observed. In vitro, platelets exacerbate NETs mediated inflammasome markers (IL1β, NLRP3), reduce endothelial function markers (p-eNOS, KLF2, KLF4 and TM) in GENC under high glucose stress. Furthermore, high glucose induced platelet-neutrophil interaction and resulting NET formation disrupted the glomerular filtration barrier In vitro (enhanced FITC-dextran leakage, disoriented VE cadherin). Under flow condition, exposure to high glucose resulted in enhanced NET formation on GENC monolayers which got accelerated in the presence of platelets. Inhibition of platelet activation (ASA or Ticagrelor), amelioration of NETs by inhibition of histone citrullination (PAD4 inhibition;(GSK484), inhibition of platelet-neutrophil interaction via P selectin ligand blocking antibody, IL-1 receptor inhibition (anakinra), restoring TM expression (solulin), ameliorated these effects. The treatment with these inhibitors in diabetic mice after the onset of DKD resulted in disease reversal [1–5].

Conclusion Taken together, hyperglycemia promotes platelet-neutrophil interactions resulting in NETs, activation of clotting system, endothelial sterile inflammation, glomerular endothelial dysfunction, barrier disruption and cell death. This results in aggravated disease course and impaired renal health in DKD. Inhibition of platelets or NETs is a promising therapeutic strategy for DKD.

Conflict of Interest The authors do not declare any conflict of interest.

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T-04-05 Prevention of coronary and peripheral arterial events in patients with end-stage kidney disease on hemodialysis: prospective results of the VIVALDI study

Authors Königsbrügge O[1], Lorenz M[2], Schmaldienst S[3], Klauser-Braun R[4], Pabinger I[1], Säemann M[5], Ay C[1]

Institutes **1** Medical University of Vienna, Medicine I, Vienna, Austria; **2** Vienna Dialysis Center, Vienna, Austria; **3** Clinic Favoriten, Medicine I, Vienna, Austria; **4** Clinic Donaustadt, Medicine III, Vienna, Austria; **5** Clinic Ottakring, Medicine VI, Vienna, Austria

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Introduction Antithrombotic agents are frequently used medications in patients with end-stage kidney disease (ESKD) on hemodialysis (HD), because indications including atrial fibrillation, coronary heart disease, and peripheral artery disease are highly prevalent in ESKD, although evidence on efficacy and safety is scarce. Vitamin-K-antagonists (VKA) are, however, suspected of accelerating vascular calcification and increasing the risk for arterial cardiovascular outcomes. We aimed to investigate the incidence of arterial outcomes in HD patients and the association with antiplatelet agents and VKA treatment.

Method A cohort of 625 patients with ESKD on HD was prospectively observed for a median observation time of 3.4 years (25th to 75th percentile 2.9-3.5 years) and occurrence of arterial outcomes were recorded and independently adjudicated. Participation in the study was confirmed with informed consent and with the approval of the local ethics committee. The occurrence of death was considered in competing risk regression.

Outcome	Absolute frequency (% of cohort)	Incidence rate, per 100 patient-years
Myocardial infarction (MI	38 (6.1%)	2.8
Composite of coronary artery revascularizations and MI	66 (10.6%)	5.0
Acute limb ischemia	4 (0.6%)	0.3
Major limb amputation	16 (2.6%)	1.1
Any limb amputation	25 (4.0%)	1.8
Composite of peripheral artery revascularizations, acute ischemia, and all amputations	83 (13.3%)	6.3

▶ **Tab. 1** Antithrombotic use and risk of arterial outcomes respective to indication.

Results Absolute frequency of coronary and peripheral artery outcomes and incidence rates are presented in (▶ **Table 1**).

Use of antiplatelet agents in secondary cardiovascular disease (CVD) prevention (N = 276 out of 367 patients with prior cardiovascular disease) was not statistically significantly associated with occurrence of arterial outcomes compared to no antithrombotic treatment (▶ **Table 2**).

	Vitamin K Antagonists In AF group (N = 238)		Antiplatelet agents In secondary CHD prevention (N = 367)	
Outcome event	**Univariable risk regression**	**Multivariable risk regression**	**Univariable risk regression**	**Multivariable risk regression**
Myocardial infarction	1.71 (0.54-5.40)	1.59 (0.53-4.74)	0.62 (0.30-1.27)	0.52 (0.24-1.14)
Composite of coronary artery revascularizations and MI	1.38 (0.58-3.28)	1.29 (0.56-2.96)	0.99 (0.53-1.87)	0.82 (0.43-1.56)
Acute limb ischemia	No events in VKA group	No events in VKA group	0.97 (0.10-9.49)	0.97 (0.09-10.25)
Major limb amputation	2.52 (0.78-8.12)	2.20 (0.64-7.57)	0.99 (0.32-3.06)	0.77 (0.23-2.53)
Any limb amputation	2.14 (0.85-5.39)	1.75 (0.67-4.61)	1.05 (0.42-2.61)	0.89 (0.35-2.23)
Composite of peripheral artery revascularizations, acute ischemia, and all amputations	**1.87 (1.10-3.17)**	**1.81 (1.04-3.16)**	1.23 (0.73-2.09)	1.00 (0.57-1.77)

▶ **Tab. 2** Frequency and incidence of coronary a peripheral artery outcomes.

Use of VKA in AF patients (N = 99 out of 238 patients with AF) was associated with increased risk of the composite outcome of peripheral artery revascularizations, acute limb ischemia, and any amputations (HR 1.81, 95 % confidence interval 1.04-3.16, p = 0.035) after adjustment for prior ischemic stroke, coronary artery disease, and peripheral artery disease compared to no antithrombotic treatment (table 2).

Conclusion Coronary and peripheral artery events are common cardiovascular events in HD patients. Neither VKA in AF patients nor antiplatelet agents in

secondary CVD prevention was associated with reduced risk of outcomes, but use of VKA may be associated with increased risk of peripheral artery outcomes, albeit a potential confounding by indication remains.

Conflict of Interest The authors declare that there are no conflicts of interest.

T-04-06 Aspirin resistance and higher risk of pump thrombosis in patients with ventricular assist device – a 7-year follow up study

Authors Flieder T[1], Blaesen S[1], Schulte K[1], Blasberg J[2], Wolny M[1], von Bargen K[1], Hohbein A[1], Knabbe C[1], Schramm R[3], Gummert J[3], Birschmann I[1]

Institutes **1** Herz- und Diabeteszentrum Nordrhein-Westfalen Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany; **2** Universitätsklinikum Jena, Institut für Psychosoziale Medizin, Psychotherapie und Psychoonkologie, Jena, Germany; **3** Herz- und Diabeteszentrum Nordrhein-Westfalen Universitätsklinik der Ruhr-Universität Bochum, Klinik für Thorax- und Kardiovaskularchirurgie, Bad Oeynhausen, Germany

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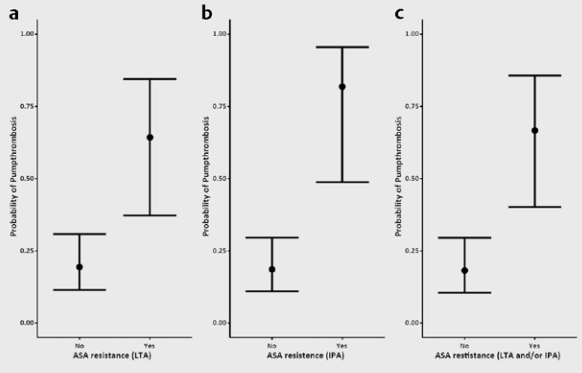
Introduction In patients with terminal heart failure, implantation of a ventricular assist device (VAD) is often the last therapeutic option as a bridge therapy to heart transplantation or as a destination therapy. Since a VAD has a large artificial surface and high shear forces are generated by the pump, concomitant medication with anticoagulants is necessary. Vitamin K antagonists and, in most cases, a platelet aggregation inhibitor are used. Despite this medication, however, many patients experience thromboembolic events. Whether laboratory testing of the efficacy of antiplatelet drugs is helpful has long been discussed, as has the correct method for this detection. In our study, we evaluated 81 patients with a VAD for the efficacy of aspirin (ASA) therapy in a routine ambulatory setting. After a follow-up, we compared the incidence of thrombosis between patients with ASA resistance and ASA efficacy.

Method Between August 2013 and March 2014, we initially evaluated patients with VAD for the efficacy of ASA therapy. For this purpose, we assessed platelet function by impedance aggregometry (IPA) and light transmission aggregometry (LTA). An ASA resistance was defined when the results of the IPA was below the reference interval and when the aggregation in LTA was below 30 % (at the end of aggregation). The patients were followed-up until April 2021, and the observation period was terminated if: 1) the LVAD was explanted because of e.g. heart transplantation or cardiac recovery 2) the patient died 3) the ASA was discontinued or it was switched to clopidogrel.



▶ **Fig. 1** Predicted probability of pumpthrombosis; Predicted probability of pumpthrombosis with 95 % confidence intervals given no ASA resistence (left) or ASA resistence (right) measured via LTA (**a**), IPA (**b**) or either IPA or LTA (**c**).

Results The median age in our collective was 52.5 (sd 14) and there were 16 % women (n = 13). Patients had the following systems implanted: 57 HeartWare, 22 HeartMate II, 1 BVAD, 1 Total Artificial Heart. The median time the devices were implanted to the end of the follow-up period (April 2021 or an event mentioned above) was 42 months (IQR 43 months). Using IPA we detected 14 patients and using LTA we detected 11 patients with ASA resistance, of whom 9 each developed pump thrombosis. Using the logistic regression models there was a significant effect of ASA resistance with LTA on the odds to show a pump thrombosis (OR = 7.48, CI [2.22 – 28.08], p = .002, 1-β = 0.93, R^2-Tjur = 0.15) as well as for ASA resistance with IPA (OR = 19.73, CI [4.46 – 140.16], p < .001, 1- β = 0.98, R^2-Tjur = 0.24) (▶ **Fig. 1**). In the group of 66 (81.5 %) patients in whom ASA resistance was not detected by either method, 12 patients had at least one pump thrombosis.

Conclusion We were able to show in our study that the risk of pump thrombosis is increased in patients who have been identified as ASA-resistant (whether with LTA or IPA) compared with patients in whom ASA efficacy could be measured. Whether these thromboses could have been prevented by switching from ASA to, for example, clopidogrel cannot be answered by our study and should be investigated in follow-up studies. Therefore, routine monitoring of ASA efficacy in this population should be considered.

Conflict of Interest Ingvild Birschmann received speaker's honoraria from Aspen Germany GmbH, Bristol-Myers Squibb/Pfizer, Siemens Healthcare and CSL Behring and reimbursement for congress traveling and accommodation from aspen and performed contract research for Siemens Healthcare. Ingvild Birschmann is a member of the advisory board of LFB biomedicaments, Siemens Healthcare and CSL Behring. All other authors have no competing interests.

T-04-07 Comparison of closure time and whole blood impedance aggregometry to light transmission platelet aggregometry for the assessment of platelet response to aspirin and P2Y12 inhibitor therapies: a large-scale study in the outpatient population

Authors McRae H L, Klee J, Müller J, Oldenburg J, Pötzsch B, Rühl H, Reda S

Institute University Hospital Bonn, Institute for Experimental Hematology and Transfusion Medicine, Bonn, Germany

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Introduction Light transmission platelet aggregometry (LTA) is considered the clinical gold standard for measuring platelet response to aspirin and P2Y12 inhibitor therapies. However, LTA is cumbersome and time-consuming to perform, is not routinely available in all clinical laboratories, and is highly sensitive to pre-analytical variables, making it difficult to perform in all patient populations. Whole blood-based methods are logistically more feasible and commonly available, but there is currently no consensus on whether these methods are more efficacious in measuring anti-platelet therapy response, and if so, which are the most appropriate and under what conditions.

Method Platelet response to aspirin therapy was measured via closure time with collagen and epinephrine (PFA-100 [Siemens]), and response to P2Y12 inhibitors was measured using whole blood impedance aggregometry (WBA, Multiplate [Roche]) in the outpatient population. Both PFA and WBA were compared against the gold standard LTA. The study population included 1,069 patients taking antiplatelet therapy. Patients were stratified by aspirin monotherapy (ASA, n = 714), P2Y12 monotherapy (P2Y12, n = 59), and aspirin and P2Y12 dual antiplatelet therapy (DAPT, n = 230). Low response was denoted by a total platelet aggregation of less than 70 %, PFA < 161 seconds, WBA with ADP > 533 U, and WBA with ADP/prostaglandin E1 (PGE1) > 310 U. Statistical analysis was performed using the two-tailed McNemar test. A p-value of < 0.05 was considered statistically significant.

Results Age and sex were comparable between groups and average platelet counts across the three groups ranged between 243-257x109/L. In the ASA group, 62.3 % were found to have a low platelet response on PFA-100 compared

to 70.4 % with LTA-epinephrine, 88.7 % with LTA-arachidonic acid, and 79.5 % with LTA-collagen ($p < 0.0001$ each). In the P2Y12 group, WBA revealed low response in 84.7 % (ADP reagent) and 86.4 % (ADP/PGE1) of patients as compared to 78.0 % when tested with LTA-ADP. These results were not statistically significant, indicating that WBA and LTA have comparable efficacy in measuring the platelet response to P2Y12 therapy. In the DAPT group, PFA-100 was again found to be less sensitive than LTA, revealing a low response in 78.7 % of patients as compared to 81.7 % (LTA-epinephrine, $p > 0.05$, ns), 97.8 % (arachidonic acid $p < 0.0001$), and 97.4 % (collagen, $p < 0.0001$). In the same group, WBA with ADP and ADP with PGE1 again revealed comparable if not better detection of low responders (both 87.0 %, $p < 0.0001$ each).

Conclusion Our data reveal that PFA-100 is not reliable to assess ASA response, either in monotherapy or DAPT. By contrast, WBA was comparable to LTA in detecting P2Y12 inhibitor response in monotherapy as well as in DAPT. Surprisingly, LTA was not superior to WBA in detecting P2Y12 responders especially in the group of patients on DATP, which might be explained by the overall more compromised platelet function in these patients.

Conflict of Interest JO has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai Pharmaceutical Co., Ltd., CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda. All other authors report no conflicts of interest.

T-04-08 Platelet responsiveness in the post-acute phase of pulmonary embolism

Authors von Ungern-Sternberg S[1, 2], ten Cate V[1, 2], Panova-Noeva M[1, 2], Dahlen B[2], Prochaska JH[1, 2], Heitmeier S[3], Gerdes C[3], Konstantinides SV[1], Münzel T[4, 5], Espinola-Klein C[1, 6], Lackner KJ[4, 7], ten Cate H[8, 9], Leineweber K[3], Wild PS[1, 2], Jurk K[1, 2]

Institutes **1** University Medical Center of the Johannes Gutenberg-University Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **2** University Medical Center of the Johannes Gutenberg-University Mainz, Preventive Cardiology and Preventive Medicine, Department of Cardiology, Mainz, Germany; **3** Bayer AG, Division Pharmaceuticals, Wuppertal, Germany; **4** German Center for Cardiovascular Research (DZHK), Partner Site Rhine-Main, Mainz, Germany; **5** University Medical Center of the Johannes Gutenberg University Mainz, Department of Cardiology, Cardiology I, Mainz, Germany; **6** University Medical Center of the Johannes Gutenberg University Mainz, Center for Cardiology, Cardiology III – Angiology, Mainz, Germany; **7** University Medical Center of the Johannes Gutenberg-University Mainz, Institute for Clinical Chemistry and Laboratory Medicine, Mainz, Germany; **8** Maastricht University Medical Center, Laboratory for Clinical Thrombosis and Hemostasis, Department of Internal Medicine, Cardiovascular Research Institute Maastricht (CARIM), Maastricht, Netherlands; **9** Maastricht University Medical Center, Thrombosis Expertise Center, Heart and Vascular Center, Maastricht, Netherlands

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Introduction Enhanced platelet activity is associated with an increased risk of venous thromboembolism, in particular acute pulmonary embolism (PE) and less so for deep vein thrombosis. This study aimed to investigate platelet function during and after partial cessation of antithrombotic therapies in the post-acute stage of PE.

Method A subgroup of 206 confirmed PE patients from the GMP-VTE project (Genotyping and Molecular Phenotyping of Venous ThromboEmbolism), a multicenter prospective cohort study of 693 confirmed VTE cases, was analyzed. Platelet fibrinogen binding, CD62P, CD63 surface expression and procoagulant activity (annexin-V) were assessed in citrated whole blood by flow cytometry as well as PFA-200; light transmission platelet aggregometry (LTA) and calibrated automated thrombinography (CAT) capacity were assessed in platelet-rich plasma at the acute PE event (BL) and at follow-up at 3/6, 12, and 24 months. Repeated-measures ANOVA was used to examine changes over time, and paired t tests were used for comparisons between time points (e.g., Δt1-t0).

Results Within the follow-up period the percentage of anticoagulant users declined (BL: 84.2 %; 12 months: 72.8 %; 24 months: 35.9 %). In contrast, antiplatelet therapy was stopped immediately after confirmation of PE in most cases (BL: 37 %; 3/6 months: 11.1 %). Ex vivo platelet fibrinogen-binding (Δ12m-BL = -11 %, $p < 0.0001$), tissue factor (Δ12m-BL = -12 %, $p = 0.028$) and CD63 (Δ12m-BL = -3 %, $p < 0.0001$) surface expression were stably downregulated after 12 months independent of antiplatelet and anticoagulant therapies. In comparison, the PFA coll/epi but not the coll/ADP closure times showed a stable shortening at 3/6 months follow-up (Δ3/6m-BL = -35 sec, $p = 0.0033$) independent of anticoagulant therapy. However, reduced levels of CD63-positive platelets and shortened PFA closure time were still within established normal reference ranges. Impaired arachidonic acid-induced platelet aggregation in the acute phase had increased after 3/6-month follow-up for non-antiplatelet drug users (Δ3/6m-BL = + 17 %, $p = 0{,}0021$). Impaired collagen-induced platelet aggregation (Δ3/6m-BL = + 18 %, $p < 0.0001$) and tissue factor-triggered endogenous thrombin potential (ETP) (Δ3/6m-BL = + 696nM * min, $p = 0.015$) in the acute phase had normalized at 3/6-months follow-up for non-antiplatelet drug and non-anticoagulant users, respectively.

Conclusion Platelet activation and reactivity are impaired in acute PE and recover over a period of 3 to 12 months after the acute event. Both antiplatelet and anticoagulant therapies are associated with platelet hypo-reactivity in post-acute PE.

Conflict of Interest Three coauthors are employees of Bayer AG (no role in the design or conduct of the research). One coauthor has received research funding and honoraria outside the present study from Boehringer Ingelheim, Sanofi-Aventis, Bayer Healthcare, Daiichi Sankyo Europe, Novartis, Evonik, AstraZeneca and Sanofi-Aventis.

T-04-09 Direct oral anticoagulants cause placental vascular abnormalities and epigenetic reprogramming in placenta and the offspring

Authors Singh K K, Gupta A, Fatima S, Ambreen S, Isermann B, Kohli S

Institute Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik, Universitätsklinikum, Leipzig, Germany

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Introduction Direct oral anticoagulants (DOACs) are increasingly used as antithrombotic agents in non-pregnant individuals. Their use is discouraged in pregnancy due to their potential teratogenic effects. Several reports ask for better monitoring and reporting in cases of inadvertent DOAC use during pregnancy questioning whether DOACs may be safe to use during pregnancy. In the current study we aimed to study whether DOAC exposure during pregnancy impairs placental function and may have long-lasting effects in the offspring.

Method To study the effect of DOACs during pregnancy, mice received factor IIa inhibitor (fIIai, dabigatran) alone or in combination with procoagulant extracellular vesicles (EVs, to induce placental thrombo-inflammation). Placenta was evaluated for morphological alterations (H&E) and expression of trophoblast differentiation marker GCM-1. In vitro, trophoblast cells were treated with fIIai to study trophoblast differentiation and epigenetic regulation. Similarly, placenta, neonatal brain and kidney were studied for global epigenetic marks (DNMT1, HDAC3, H3K9me3, H3K9ac) using immunoblotting.[1–5]

Results EV-induced pregnancy loss was prevented by fIIai treatment. However, the embryos showed growth restriction suggesting embryopathy. fIIai treatment on days 9.5 and 11.5 post-coitus resulted in altered placental morphology at day 13.5 post-coitus reflecting persistent impaired placental vascularization. fIIai reduced GCM-1 expression and altered epigenetic marks in

vitro and in vivo. Remarkably, even neonatal brains and kidneys showed dysregulated epigenetic marks suggesting that fIIai, which can cross the placenta, can epigenetically re-program the offspring.

Conclusion These results suggest that fIIai, while preventing thrombo-inflammatory effects during pregnancy, has severe consequences on placental and embryonic development. These effects may be partially epigenetically programmed and can persist in the offspring. This may affect the offspring health. Further mechanistic studies are required to evaluate whether these effects are thrombin dependent, the mechanism underlying the altered epigenetic marks and their relevance. In line with current recommendations these results warrant caution regarding the use of fIIai in pregnancy.

Conflict of Interest The authors do not declare any conflict of interest.

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T-04-10 In vitro efficacy of direct oral anticoagulants in plasma from patients with liver cirrhosis

Authors Pereira Portela C[1], Zermatten M G[1], Fraga M[2], Bertaggia-Calderara D[1], Aliotta A[1], Veuthey L[1], Shehwar D[1,3], Moradpour D[2], Alberio L[1]

Institutes **1** Lausanne University Hospital (CHUV) and University of Lausanne, Division and Central Laboratory of Hematology, Lausanne, Switzerland; **2** Lausanne University Hospital (CHUV) and University of Lausanne, Division of Gastroenterology and Hepatology, Lausanne, Switzerland; **3** Quaid-i-Azam University, Department of Biochemistry, Islamabad, Pakistan

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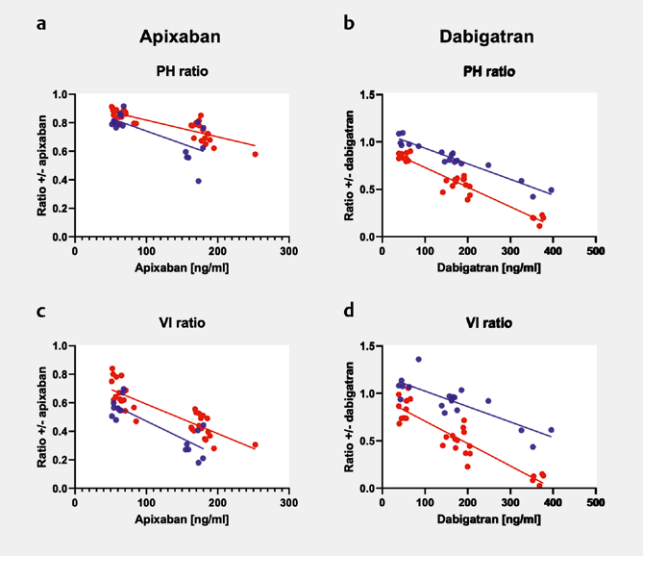
Introduction Liver cirrhosis (LC) is a complex pathology which confers a prothrombotic state. The anticoagulation of LC-patients remains challenging because of the unknown efficacy of heparins and direct oral anticoagulants (DOAC), monitoring difficulty (particularly for coumarins), and alterations in drug metabolism. Here, we aimed to analyse the in vitro efficacy of DOAC (apixaban, edoxaban, rivaroxaban, and dabigatran) in LC-patients' plasma.

Method We included 22 LC-patients and 9 healthy donors. Plasma samples were spiked with either Owren's veronal buffer or DOAC (final target concentrations: 50, 150 ng/ml; additional concentration of 300 ng/ml for dabigatran). We have chosen these concentrations because they represent peak and through levels observed in clinical studies of a therapeutic anticoagulation with these drugs. After spiking, apixaban and dabigatran concentrations were verified and ex vivo thrombin generation were analysed using ST Genesia with DrugScreen reagents (Stago, Asnières-sur-Seine, France). Analysis for edoxaban and rivaroxaban has just started at the time of the writing of this abstract. Ratios for velocity index and peak height assessed without and with anticoagulants were calculated and compared between LC-patients and healthy donors for each target concentration using Mann-Whitney test (▶ **Fig. 1**).

Results Ratios for velocity index and peak height according to apixaban and dabigatran concentrations are presented in Fig. 1. At a target concentration of 150 ng/ml, in apixaban treated samples the peak height ratio was slightly but significantly higher [median 0.74 (LC, red) vs. 0.60 (control, blue), p-value 0.0329] and the velocity index ratio significantly higher (0.42 vs. 0.27, p-value 0.0076) in LC-plasma compared to controls. With dabigatran, both ratios were significantly lower (0.58 vs. 0.80, p-value <0.0001; 0.51 vs. 0.94, p-value <0.0001) in the LC-group compared to controls.

Conclusion We demonstrated a slightly lower anticoagulant efficacy of apixaban and a clearly higher efficacy of dabigatran in LC plasma compared to control samples. Based on these preliminary data and on DOAC metabolism, apixaban appears to have a safer profile than dabigatran for LC-patients. First data obtained with edoxaban and rivaroxaban seem to confirm discordant anticoagulant efficacies of DOAC in normal versus LC-plasma.



▶ **Fig. 1** Ratios for velocity index and peak height after spiking with DOAC; Ratios for velocity index and peak height according to apixaban (**a**, **b**) and dabigatran (**c**, **d**) concentrations in patients with liver cirrhosis (red) and healthy donors (blue). The lines represent the linear regression lines. PH, peak height; VI, velocity index.

Conflict of Interest Stago (Asnière-sur-Seine, France) supported the study with discounts for ST Genesia reagents. No other relevant conflict of interest to disclose.

T-05 | Perinatal and Paediatric Hemostasis, Anticoagulation in Children

T-05-01 Treatment of VTE with rivaroxaban in adolescents – long-term results from the prospective Dresden NOAC Registry (NCT01588119)

Authors Tittl L, Marten S, Naue C, Köhler C, Beyer-Westendorf J

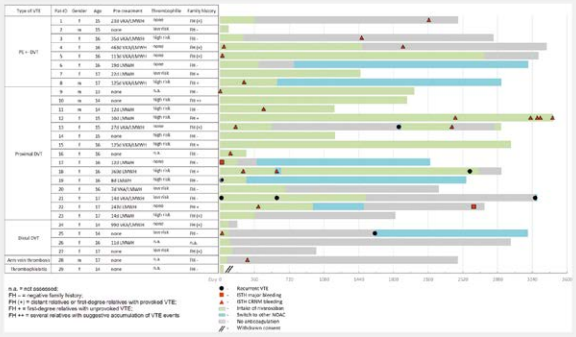
Institute University Hospital "Carl Gustav Carus", Thrombosis Research Department of Medicine I, Division Haematology, Dresden, Germany

DOI 10.1055/s-0042-1760484

Introduction The effectiveness and safety of acute venous thromboembolism (VTE) treatment with rivaroxaban in adolescents, demonstrated in phase-III trials, needs to be confirmed in daily care.

Method Since 2012 (even before rivaroxaban was approved for under-aged patients), a prospective cohort of adolescent VTE patients treated with rivaroxaban was enrolled into the Dresden NOAC Registry. All patients and their legal representatives provided informed consent to participate in the registry and for off-label treatment with rivaroxaban. In our registry, all patients receive quarterly phone visits by the registry office. All suspected outcome events are centrally adjudicated using standard scientific definitions.

Results Until 31st August 2022, 29 adolescents receiving rivaroxaban for VTE were enrolled (23 female, 6 male, mean age 15.6 ± 1.1 years, mean BMI 22.3 ± 3.7 kg/m²). None of the patients reported a previous VTE. 11 patients (37.9 %) had a family history for VTE. 22 of 23 female patients received hormonal contraception at time of VTE. 16 patients were carriers of thrombophilia, 8 were tested negative and the remaining 5 patients were not tested so far.



▶ **Fig. 1** Time-frequency-plot of anticoagulation status; Time-frequency-plot of anticoagulation status, recurrent VTE, ISTH major and clinically relevant non-major bleeding

Median time between VTE diagnosis and initiation of rivaroxaban was 12 d (inter-quartile range [IQR] 6/35 d); a total of 9 patients started rivaroxaban within the first week after VTE diagnosis.

Median rivaroxaban exposure was 18 months (range 6.2-39.8 months). During follow-up (median 82.4 months; IQR 48.7/100.5 months), 8 recurrent VTE occurred in 5 patients (▶ **Fig. 1**). Patient 13 experienced a late VTE recurrence (no anticoagulation) as distal DVT. Patient 18 experienced a late recurrence of an iliac DVT (due to incompliance) in the context of May-Turner syndrome and Type I protein C deficiency. Patient 19 experienced two early thrombotic stent occlusions after scheduled May-Turner Stenting 18d after index event (both events occurred during heparin bridging following catheter thrombolysis). Patient 21 experienced 2 recurrent thrombotic iliac vein stent occlusions (due to incompliance) and a late distal DVT (no anticoagulation). Patient 25 experienced a late VTE recurrence (popliteal vein DVT; no anticoagulation).

19 patients reported a total of 54 bleeding events. Of these 37 were ISTH minor and 16 were ISTH non-major clinically relevant bleeding. One major bleeding occurred (enteral bleeding with haemoglobin drop in acute ulcerative colitis).

Conclusion Rivaroxaban treatment for VTE seems feasible and effective also in adolescent patients, in whom the prevalence of thrombophilia and a positive family history for VTE is high. However, bleeding complications are common with rivaroxaban exposure and recurrent VTE events are predominantly ssen in complex iliac vein thrombosis, in incompliant patients and after stopping anticoagulation, indicating a complex patient selection. As a consequence, VTE treatment of under-aged patients should ideally be performed in specialized anticoagulation clinics.

Conflict of Interest J.B.-W. has received honoraria and research support from Bayer, Boehringer Ingelheim, Daiichi Sankyo, Pfizer, Alexion, Norgine, DOASENSE and Sanofi. L.T. has received honoraria and travel support from Daiichi Sankyo and Bayer. S.M. has received honoraria from Daiichi Sankyo and Bayer. None of the other authors declared a conflict of interest with regard to the NOAC registry or this manuscript.

T-05-02 Ontogeny of peripheral blood platelet GPIIb/IIIa activation and granule release in preterm and term neonates

Authors Weiss L J[1, 2], Drayss M[1, 2], Beck S[2, 3], Mott K[2], Speer C P[4], Härtel C[4], Schulze H[4], Andres O[2]

Institutes **1** University Hospital Würzburg, Department of Internal Medicine I, Würzburg, Germany; **2** University Hospital Würzburg, Institute of Experimental Biomedicine, Würzburg, Germany; **3** University of Würzburg, Rudolf Virchow Center, Würzburg, Germany; **4** University Hospital Würzburg, Department of Pediatrics, Würzburg, Germany

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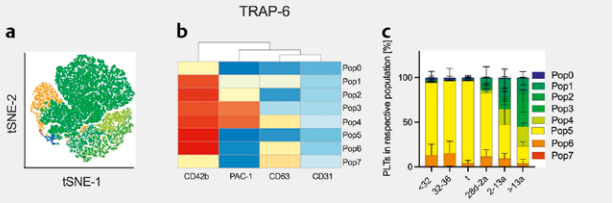
Introduction Erythrocytes undergo an overall well characterized switch from fetal to postnatal circulation, which is reflected by stage-specific expression of hemoglobin chains. Postnatal alterations in thrombopoiesis remain poorly understood. Since prematurity is the major risk factor for postnatal bleeding and recent studies have shown that transfusion of (adult) platelet concentrates might increase mortality, detailed understanding of the ontogeny of platelet phenotype and function is crucial.

Method We recruited 66 individuals, stratified into 6 cohorts: (I) neonates < 32 weeks (wk) gestational age (GA); (II) neonates 32–37 wk GA; (III) term neonates, (IV) infants 28 wk-2 a; (V) 2–12 a; (VI) > 13 a. Neonates were assessed at up to three time points t1: 0–2 d, t2: 3–7 d, and t3: 8–14 d. Platelets were investigated flow cytometrically and subpopulations analyzed using t-SNE and automated clustering algorithms. Integrin GPIIb/IIIa activity was assessed by monitoring spreading on fibrinogen-coated cover slips (▶ **Fig. 1**).



▶ **Fig. 1** Continuous evolution of platelet subpopulations; Platelets in whole blood were stimulated with TRAP-6 [5 µM]. Platelet subpopulations show a contiuous shift from a CD63-/PAC-1- or CD63 + / PAC-1- pattern towards a CD63 + /PAC-1 + predominant pattern in adolescents. (A-C) Representative curve displayed in A. Figure legends displayed in B.

Results Platelets were immunophenotyped in response to selected agonists and compared to the resting condition by a complex multi-color panel. The surface expression of the surface receptors CD9, CD29, CD41a, CD49d/e/f, CD61, GPVI, CLEC-2, and TLR-4 was overall unaltered in respect to a marginally reduced platelet size in preterms. Platelet subpopulations were identified by FlowSom-based clustering. In response to TRAP-6, platelets among all neonatal cohorts (I-III) were found to be in the PAC-1high (Pop3, Pop4) or PAC-1intermediate(Pop2) cluster, in contrast to 51 % (Pop3,4) or 15 % (Pop2) in adolescents. This finding was further corroborated when neonatal platelets were seeded on fibrinogen-coated surfaces. These platelets showed a massive defect in spreading, implying an impaired outside-in activation defect (80 % neonatal platelets in spreading stage 1 + 2 vs. adolescents: 80 % in stage 3 + 4, $p < 0.05$). In contrast, agonist-induced granule release indicated by CD62P or CD63 surface neo-exposition was unaltered or just slightly reduced in neonates suggesting that granule release in preterm/neonatal platelet is uncoupled of integrin

activation. Coupling of agonist-triggered granule release and GPIIb/IIIa activation evolved continuously with age throughout all platelet subpopulations (Fig. 1). In neonates, we found increased platelet-leukocyte aggregates (PLA), especially with neutrophils or monocytes, but not with T- or B-cells. While blockade of PSGL-1 or GPIIb/IIIa reduced the number of PLAs in adults, antagonism of PSGL-1, but not GPIIb/IIIa reduced PLA formation in neonates, suggesting that GPIIb/IIIa function matures until adolescence.

Conclusion Our study shows that platelet function does not just switch from fetal to mature thrombopoiesis, but undergoes a continuous development until adolescence. The separate responsiveness of granule release and integrin activation might reflect specific platelet functions during the fetal and neonatal period compared to the adult platelet.

Conflict of Interest No conflicts of interest to declare.

T-05-03 Agenesis of vena cava inferior (AVCI) and Hypereosinophilic Syndrome (HES): two rare causes of severe thrombosis and its challenging treatment in adolescents

Authors Wieland I, Becker K A

Institute Hannover Medical School, Pediatric hematology and oncology, Hannover, Germany

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Introduction The agenesis of vena cava inferior (AVCI) is a rare vascular abnormality with an estimated prevalence of up to 1 %. The embryological development of VCI is a complex fusion of 3 vein pairs finished in the 8. gestational week. Embryonal, intrauterine or peripartal thrombosis of VCI are discussed to cause AVCI. Patients are usually asymptomatic.

The hypereosinophilic syndrome (HES) is defined as eosinophils > 1.5/nl without parasitic, allergic or other secondary causes. Different organs or organ systems can be involved. The prevalence is unknown.

Method We want to report two rare causes of severe thrombosis and its challenging treatment in adolescents: 3 patients with AVCI and 2 with HES.

Results Patients with AVCI: Three boys, aged 15 years, developed extended deep vein thrombosis of legs and pelvis (pat 1 and 2 both sites extended; 3. extended right site). All three showed an AVCI in diagnostic workup. Except hyperhomocysteinemia in one patient, no hereditary thrombophilia or thrombophilic trigger could be detected. Despite sufficient therapeutic anticoagulation with apixaban, only patient 3 achieved a recanalization. Two patients developed early severe postthrombotic syndrome. **Patients with HES**: A 16 years old girl was admitted with suspected bleeding in the right breast and left eye, after 4 weeks of anticoagulation with rivaroxaban due to a deep vein thrombosis of the right leg. In the initial blood count 28 eosinophils/nl were detected. The assumed hemorrhage was a skin microthrombosis of the breast, resulted in a large necrosis. Anticoagulation was switched to enoxaparin twice daily. During a break for surgery, she developed an additional thrombosis of the V. ophthalmica superior resulting in a Visus Lux. To avoid long breaks, anticoagulation was bridged with unfractionated heparin for the next multiple surgeries without additional thrombotic events.

A 13 years old boy suffered from an eosinophilic granulomatosis with polyangiitis (EGPA; Churg-Strauß-Syndrome) with skin and lung involvement and 6.690 eosinophils/nl (33 %) in the initial blood count, increasing to 45 %. Despite eosinophils < 1.0/nl after treatment with steroids, and thrombopenia of 21 platelets/nl, he developed an extended TVT of left leg. Due to thrombopenia, anticoagulation started with half-therapeutic dose tinzaparin, escalating with increasing platelets to full-dose within days. Unfortunately, thrombosis did not improve much after 4-6 weeks treatment.

Conclusion AVCI and HES are two very rare reasons of severe thrombosis in adolescents with therapeutic challenges.

Especially in young males with extended TVT of right or both sites, AVCI should be excluded. Patients with AVCI have a high risk for treatment failure, early post-thrombotic syndrome and relapse of thrombosis after discontinuation of anticoagulation.

HES is associated with a very high risk for thrombosis. A consequent anticoagulation is necessary despite thrombopenia or surgery. Rivaroxaban seemed not sufficient to prevent microthrombosis.

Conflict of Interest IW: Research funding: CSL Behring; Clinical trials/studies: Boehringer-Ingelheim, Pfizer, Roche/Chugai, Shire, Sobi; Consultings: Bayer, Biotest, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche/Chugai, Shire/Takeda, Sobi. KL: CSL Behring

T-06 | Coagulation Disorders and Malignancy

T-06-01 Expression and release of tumor cell tissue factor triggers recurrent thromboembolism in a patient with endometrial cancer

Authors Beckmann L[1], Lennartz M[2], Poch A[1], Holstein K[1], Bokemeyer C[1], Langer F[1]

Institutes **1** University Cancer Center Hamburg (UCCH), University Medical Center Eppendorf, Department of Hematology and Oncology, Hamburg, Germany; **2** University Medical Center Eppendorf, Institute of Pathology, Hamburg, Germany

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Introduction Although cancer-associated thrombosis (CAT) is a frequent complication in patients with malignancies [1], its treatment remains challenging in daily practice. Here, we report the clinical course of a 51-year-old woman who developed a highly thrombogenic paraneoplastic coagulopathy due to locally advanced endometrial cancer.

Method Routine laboratory work-up, including analysis of tumor markers and coagulation parameters, was carried out using standard in-house techniques. In addition, plasma microvesicles (MVs) were isolated from citrate-anticoagulated whole blood by double high-speed centrifugation and analyzed for tissue factor (TF) procoagulant activity (PCA) by a chromogenic Xa generation assay. Further, tissue sections were analyzed for cellular TF expression by immunohistochemistry using a specific monoclonal antibody.

Results Despite therapeutic anticoagulation with various agents, including rivaroxaban, fondaparinux and low-molecular-weight heparin (LMWH), the patient experienced recurrent venous and arterial thromboembolism with at least seven distinct thromboembolic events within 3 months. Plasma D-dimers were profoundly increased, but extensive laboratory work-up ruled out antithrombin deficiency, overt disseminated intravascular coagulation, antiphospholipid syndrome, paroxysmal nocturnal hemoglobinuria, and autoimmune (or spontaneous) heparin-induced thrombocytopenia. In addition, molecular testing for JAK2V617F, prothrombin G20210A and factor V gene mutation Leiden was negative, while tumor markers CA125, CA15-3, CA19-9, and CEA were markedly elevated. Imaging studies, including computed tomography, magnetic resonance imaging and positron emission tomography, eventually revealed locally advanced uterine cancer FIGO stage IVB. Histopathological analysis was consistent with an endometrioid adenocarcinoma showing strong TF expression. In addition, abundant release of MV-associated TF PCA was measured in patient plasma. Anticoagulation was continued with the direct thrombin inhibitor, argatroban. Initially, unusually high dosages were required to obtain activated partial thromboplastin times within the therapeutic target range, indicating massive intravascular thrombin generation. Multimodal antineoplastic treatment, including neoadjuvant chemotherapy followed by surgery and postoperative radiotherapy, resulted in clinical cancer remission, which was paralleled by normalization of tumor markers, CA125 and CA19-9, as well as plasma levels of D-dimer and TF-bearing MVs. Thus, anticoagulation was continued with the LMWH, enoxaparin. At 15 months of follow-up, the

patient was still in complete remission. No further thrombotic events have occurred.

Conclusion Continuous anticoagulation with argatroban and multimodal anticancer treatment may be necessary to control TF-driven paraneoplastic coagulation activation with recurrent CAT in endometrial cancer.

Conflict of Interest L.B., M.L. and A.P. declare no conflicts of interests relevant to the content of the submitted abstract. K.H. has received honoraria for advisory boards or speaker fees from Bayer, Bristol-Myers Squibb and Pfizer, and unrestricted research grants from Bayer and Pfizer. C.B. has received personal fees for consultancy from Bayer, Bristol-Myers Squibb and Sanofi, and travel support from Bristol-Myers Squibb, Pfizer and Sanofi. F.L. has received personal fees for lectures or consultancy and/or research support from Bayer, Boehringer-Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, LEO Pharma, Pfizer, Sanofi, and Viatris.

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T-06-02 BMS-262084, a FXIa inhibitor, interferes with tumor cell-induced coagulation activation only in tumor cells with low tissue factor (TF) procoagulant activity (PCA)

Authors Mäder J, Rolling C C, Voigtländer M, Schulenkorf A, Regenhardt J, Bokemeyer C, Lehr C, Langer F, Beckmann L

Institute University Cancer Center Hamburg (UCCH), University Medical Center Eppendorf, Department of Hematology and Oncology, Hamburg, Germany

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Introduction Inhibition of coagulation factor XI (FXI) has become a promising new antithrombotic strategy with a presumably low bleeding risk [1]. While it is efficacious and safe in prevention of postoperative venous thromboembolism in orthopedic patients [2–5], its role in treatment and prophylaxis of cancer-associated thrombosis (CAT) is less clear. Here, we provide mechanistic insights into FXI inhibition in tumor cell-induced coagulation activation.

Method FXIa amidolytic activity was assessed by in-house chromogenic assays. Coagulation activation by four distinct cancer cell lines (i.e., BxPC-3, MCF-7, THP-1, and HL-60) was analyzed by single-stage clotting and modified thrombin generation assay in the presence or absence of the FXIa inhibitor, BMS-262084, and peak or trough concentrations of tinzaparin (0.85 and 0.2 IU/mL) and rivaroxaban (270 and 26 ng/mL). Coagulation initiation by recombinant human TF (rhTF) served as control. Further, tumor cell-induced platelet aggregation was recorded by light transmission aggregometry in a similar way.

Results While BMS-262084 potently inhibited FXIa, with concentrations > 0.5 µM showing complete neutralization of its amidolytic activity, it interfered with rhTF-induced fibrin formation only at low rhTF concentrations. Under these conditions, the effect of 10 µM BMS-262084 was comparable to that of FXa inhibition by peak levels of tinzaparin or rivaroxaban. Similarly, the ability of equal BMS-262084 concentrations to mitigate tumor cell-induced fibrin formation, thrombin generation and platelet aggregation inversely correlated with the magnitude of cellular TF expression. Consistently, BMS-262084 had negligible effects on tumor cell-induced fibrin formation and platelet aggregation by highly procoagulant BxPC-3 cells, but potently prevented both thrombogenic effects in the presence of very low TF expressing HL-60 cells. Of note, the most prominent BMS-262084 anticoagulant effect was recorded in the modified thrombin generation assay, particularly on peak and total thrombin generation. In this assay, BMS-262084 even significantly reduced peak and total thrombin generation of highly procoagulant BxPC-3 cells suggesting that BMS-262084 predominantly interfered with the intrinsic amplification loop of coagulation.

Conclusion FXI inhibition predominantly interferes with the propagation phase of TF-triggered coagulation activation by tumor cells. Thus, the anticoagulant potency of FXI inhibitors may critically dependent on levels of TF expression by cancer cells, a finding with potential clinical implications for CAT prophylaxis and treatment.

Conflict of Interest J.M., A.S., J.R., C.L. and L.B. declare no conflicts of interests relevant to the content of the submitted abstract. CC.R. has received travel support from Pfizer. M.V. declares personal fees for lectures from Bristol-Myers Squibb and travel support from Bayer, Bristol-Myers Squibb and LEO Pharma. C.B. has received personal fees for consultancy from Bayer, Bristol-Myers Squibb and Sanofi, and for travel expenses from Bristol-Myers Squibb, Pfizer and Sanofi. F.L. has received personal fees for lectures or consultancy and/or research support from Bayer, Boehringer-Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, LEO Pharma, Pfizer, Sanofi, and Viatris.; The submitted work was supported in part by an Early Career Research Grant from the GTH e.V. to L.B..

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T-06-03 Markers of coagulopathy in multiple myeloma

Authors Chasakova K[1], Slavík L[2], Starostka D[1], Úlehlová J[2], Papajík T[2], Minařík J[2]

Institutes **1** Hospital in Havirov, Department of clinical hemathology, Havířov, Czech Republic; **2** University Hospital and Faculty of Medicine and Dentistry, Palacký University Olomouc, Department of hemato- oncology, Olomouc, Czech Republic

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Introduction Multiple myeloma (MM) is a lymphoproliferative disease characterized by clonal proliferation and accumulation of neoplastic and osteolytic skeletal involvement. Some of hemostasis disorders are attributed to M-Ig interactions with blood clotting factors (acquired von Willebrand's disease, acquired hemophilia A or deficits of other coagulation factors, circulating anticoagulant, hyperviscosity, amyloidosis and lupus anticoagulant) or with platelets (acquired thrombocytopathy), M-Ig-independent effects (thrombocytopenia, other thrombocytopathies, DIC, immobility and hypercalcaemia)
The aim of our work is to detect abnormalities of coagulation in patients in with newly diagnosed MM suitable for intensive chemotherapy - depending on the activity of the disease, which predispose patients to thrombotic resp. bleeding complication in MM. TGT is a global coagulation assay that measures the global capacity of blood plasma to form thrombin. Several clinical studies have shown that increased TG in platelet poor plasma (PPP) predicts an increased risk of (recurrent) VTE.

Method We included 53 patients with newly diagnosed multiple myeloma in this study. Patients with MM were investigated examined by coagulation tests for detecting both bleeding and thrombotic tendency with following coagulation tests: PT, APTT, TT, fibrinogen, antithrombin, D-Dim, levels of coagulation factors (II, V, VII, X, VIII, IX, XI and XII), vWF, lupus anticoagulant, protein C, protein S, resistance to activated protein C and thrombin generation assay modified with activated protein C. We also monitored plasma cell counts and serum paraprotein levels in these patients.

Results All markers were evaluated (average value, standard deviation) to the disease activity defined by the paraprotein level resp. number of plasma cells.

A significant correlation was found between D-Dim and paraprotein p = 0.0031 resp. plasma cells p = 0.0006 and between vWF vs. paraprotein p = 0.0053 not between plasma cells p = 0.42, which is interesting. Correlations of these markers can predict bleeding conditions, however our ambition is to detect markers of thrombotic risk as well. For this purpose, we examined the modified TGT, which can identified thrombotic pathology in eight cases (15 %), while genetically determined thrombophilias were detected in only 3 % of patients.

Conclusion In newly diagnosed patients with MM, were commend increased attention to the level of D-Dim and vWF, especially in patients with higher disease activity in order to estimate possible bleeding or thrombotic complications and modified TGT for thrombotic complication. , for which long term observation is needed.

Conflict of Interest I have no conflict of interest.

T-06-04 Tissue Factor Pathway Inhibitor is associated with risk of venous thromboembolism and all-cause mortality in patients with cancer

Authors Englisch C[1], Moik F[1, 2], Thaler J[1], Koder S[1], Preusser M[3], Pabinger I[1], Ay C[1]

Institutes **1** Medical University of Vienna, Department of Medicine I, Clinical Division of Haematology and Haemostaseology, Vienna, Austria; **2** Medical University of Graz, Department of Internal Medicine, Division of Oncology, Graz, Austria; **3** Medical University of Vienna, Department of Medicine I, Clinical Division of Oncology, Vienna, Austria

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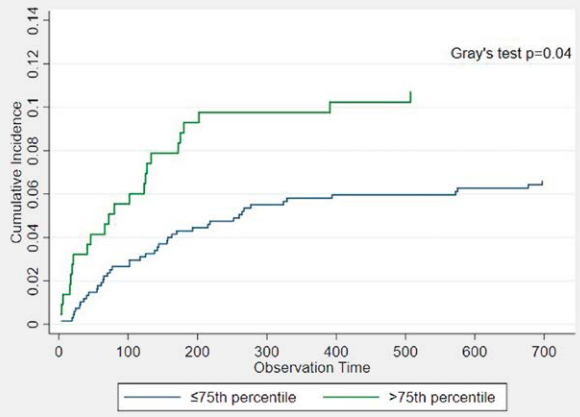
Introduction Venous thromboembolism (VTE) is a common complication in patients with cancer. Several biomarkers have been found to predict risk of VTE in patients with cancer. However, the role of natural inhibitors of haemostasis in cancer-associated VTE is less studied. Tissue factor pathway inhibitor (TFPI) is a natural anticoagulant that inhibits complexes of tissue factor and factor VIIa via its K1 domain and factor Xa via its K2 domain. Conflicting results about its association with VTE risk were reported in the general population, and it has been discussed whether TFPI may act as a surrogate marker for circulating TF levels. In comparison to the non-cancer population, higher TFPI levels were reported in patients with cancer. We aimed to investigate TFPI levels in patients with cancer and explore their association with risk of VTE and all-cause mortality.

Method Total TFPI antigen levels at study inclusion (Imunbind total TFPI ELISA kit, American Diagnostica Inc., USA) were measured in patients included in the Vienna Cancer and Thrombosis Study (CATS), which is a prospective observational cohort study including patients with newly diagnosed or recurrent cancer. Patients were followed for objectively diagnosed, independently adjudicated VTE for up to 2 years. The association between TFPI-levels and VTE-risk was analyzed in competing risk analysis, considering death as competing outcome event. The association of TFPI-levels with all-cause mortality was assessed in Cox regression analysis.

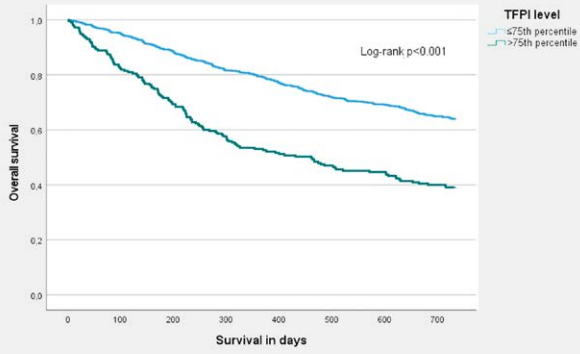
Results TFPI was analyzed in 898 patients (median age 62 years, interquartile range [IQR]: 53-68; 407 (45 %) female). Over a median follow-up time of 22 months (IQR: 7-25), 67 patients were diagnosed with VTE and 387 patients died (6-, 12-, and 24-month cumulative risk: 5.5 %, 6.7 %, 7.5 % and 15.1 %, 27 %, 42.1 %).

Patients had median TFPI levels of 56.4 ng/mL (IQR: 45-70). Distribution of TFPI levels did not significantly differ between cancer types. Patients with metastatic vs non-metastatic disease had higher TFPI levels (median: 60 vs 50.4 ng/mL, p < 0.001).

In multivariable analysis adjusting for age, sex, cancer type and stage, baseline levels of TFPI were associated with risk of VTE (SHR per doubling: 2.01, 95 % confidence interval [CI]: 1.25-3.24; 1-year cumulative incidence >75th vs ≤75th percentile: 9.7 vs 5.8, p = 0.04, ▶ **Fig. 1**). Further, TFPI was independently associated with risk of all-cause mortality (adjusted HR per doubling: 1.86, 95 %CI: 1.47-2.35; 1-year cumulative incidence >75th vs ≤75th percentile: 47.2 vs 21.7, p < 0.001, ▶ **Fig. 2**).



▶ **Fig. 1** Cumulative VTE Incidence of patients (n = 898) with TFPI levels ≤ 75th (n = 680) (≤ 70 ng/mL) versus >75th percentile (n = 218) (>70ng/mL). Patients were divided according to their TFPI level and the group with levels under 70 ng/mL (≤ 75th percentile) was compared to the group with levels over 70 ng/mL (>75th percentile) within a Fine and Gray subdistribution hazard model, p = 0.04



▶ **Fig. 2** Overall-survival of patients (n = 898) with TFPI levels ≤ 75th (n = 680) (≤ 70 ng/mL) versus >75th percentile (n = 218) (>70 ng/mL). Patients were divided according to their TFPI level and the group with levels under 70 ng/mL (≤ 75th percentile) was compared to the group with levels over 70 ng/mL (>75th percentile) within a Kaplan Meier analysis and with a log-rank test p < 0.001

Conclusion TFPI levels are independently associated with risk of VTE and all-cause mortality in patients with cancer. Interestingly, patients with metastatic disease had higher TFPI levels. TFPI levels might represent a surrogate and could be a biomarker for the prediction of VTE risk and mortality in patients with cancer.

Conflict of Interest No conflicts of interest to disclose.

T-06-05 Cardiovascular biomarkers for the prediction of adverse cardiovascular events and mortality in patients with cancer

Authors Moik F[1, 2], Kraler S[3], Montecucco F[4], Liberale L[4], Nopp S[1], Englisch C[1], Lapikova-Bryhinska T[3], Akhmedov A[3], von Eckardstein A[5], Wenzl F[3], Pabinger I[1], Lüscher T[3, 6], Ay C[1]

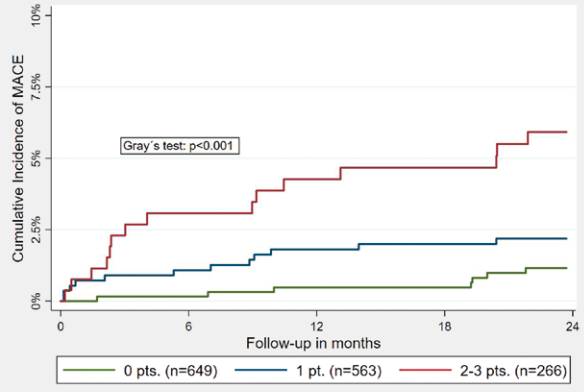
Institutes **1** Medical University of Vienna, Department of Medicine I, Clinical Division of Haematology and Haemostaseology, Vienna, Austria;

2 Medical University of Graz, Department of Internal Medicine, Division of Oncology, Graz, Austria; 3 University of Zürich, Center for Molecular Cardiology, Zürich, Switzerland; 4 First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, Genoa, Italy; IRCCS Ospedale Policlinico San Martino Genoa - Italian Cardiovascular Network, Genoa, Italy; 5 University Hospital of Zurich, Institute of Clinical Chemistry, Zürich, Switzerland; 6 Imperial College and Kings College London, Heart Division, Royal Brompton & Harefield Hospitals, London, UK

DOI 10.1055/s-0042-1760491

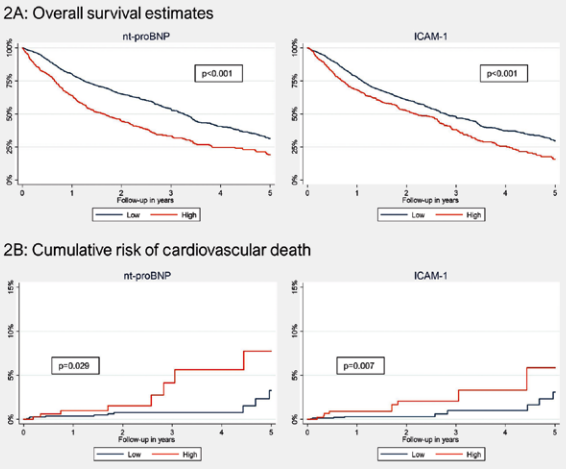
Introduction Patients with cancer are prone to develop thromboembolic and atherothrombotic events. Advances in anti-cancer therapies lead to prolonged cancer-specific survival, putting patients at increasing risk of major adverse cardiovascular events (MACE). Hence, tools to personalize risk stratification to predict and eventually prevent cardiovascular complications in patients with cancer are an unmet medical need. Here, we aimed to assess the predictive utility of cardiovascular biomarkers in a representative population of oncologic patients.

Method In a single-center prospective cohort study (Vienna Cancer & Thrombosis Study - CATS), cardiovascular biomarkers (lipoprotein(a), NT-proBNP, P-selectin, E-selectin, L-selectin, ICAM-1, VCAM-1, sLOX-1) were explored for their predictive utility towards MACE (i.e., myocardial infarction, ischaemic stroke, cardiovascular death), cardiovascular-, and all-cause mortality. Patients were followed for MACE for 2 years, whereas a prolonged follow-up period (5 years) for mortality outcomes was feasible due to data availability from the national death registry. MACE and cardiovascular mortality were analysed by competing-risk regression, accounting for non-cardiovascular death as competing outcome event, adjusting for age, sex and smoking status. All-cause mortality was analysed in Cox regression analysis, adjusting for age, sex, cancer stage and type.



▶ **Fig. 1** Additive effect of biomarkers for the prediction of MACE in patients with cancer.; Patients were assigned + 1 point each for levels of NT-proBNP ≥ 75th percentile, ICAM-1 ≥ 75th percentile, and L-selectin ≤ 25th percentile. Cumulative incidences were obtained in competing risk analysis, accounting for non-cardiovascular mortality as the competing outcome event.

Results In total, 2,192 adult patients with newly diagnosed or recurrent cancer were included (median age: 62 years; 53 % male). Over a median follow-up of 23 months, 50 MACEs occurred (cumulative 1- and 2-year incidence: 1.7 % [95 % confidence interval, CI: 1.2-2.3] & 2.4 % [1.8-3.1]). MACE-risk was independently associated with baseline levels of NT-proBNP (sub-distribution hazard ratio, SHR, per double: 1.28 [95 %CI: 1.06-1.54]), ICAM-1 (SHR: 1.53 [1.06-2.20], and L-selectin (SHR: 0.63 [0.44-0.90]), but not for lipoprotein(a), P-selectin, VCAM-1, or sLOX-1. An additive predictive effect of biomarkers was observed in the derivation of a point-based prediction score (▶ **Fig. 1**). Long-term cardiovascular mortality was independently associated with levels of NT-proBNP (SHR: 1.42 [95 %CI: 1.17-1.72]) and ICAM-1 (1.62 [1.05-2.48]). Accordingly, risk of all-cause mortality independently increased with higher NT-proBNP (HR: 1.16 [95 %CI: 1.10-1.22]) and ICAM-1 (1.15 [1.06-1.25]). No significant association with cardiovascular mortality or all-cause mortality was observed for the remainder of biomarkers (▶ **Fig. 2**).



▶ **Fig. 2** Overall survival estimates (2A) and cumulative risk of cardiovascular death (2B) according to dichotomized biomarker levels. Biomarkers were dichotomized at the 75th percentile of distribution, comparing patients below this cut-off ("low"), to those at or above the cut-off ("high"). Overall survival estimates were obtained in Kaplan-Meier analysis, whereas cumulative risk of cardiovascular death was analysed in a competing-risk framework, accounting for non-cardiovascular-cause mortality as competing outcome event.

Conclusion Cancer patients are at substantial risk of cardiovascular events, with a predictive utility of NT-proBNP, L-selectin, and ICAM-1 for short-term MACE-risk. Further, NT-proBNP and ICAM-1 independently predict long-term risk of cardiovascular- and all-cause mortality. Our findings suggest a possible benefit of incorporating cardiovascular biomarkers in personalized risk prediction models to develop individualized cardiovascular prevention strategies in oncologic patients.

Conflict of Interest No conflicts of interest to disclose.

T-07 | Haemotherapy Concepts in Complex Haemostasis Disorders, Intensive Care and Evidence-Based Platelet Transfusion

T-07-01 Thrombocytopenia in patients with cardiogenic shock treated with extracorporal life support system versus conservative treatment

Authors Huseynov A[1], Hoffmann U[1], Kittel M[2], Baumann S[1], Behnes M[1], Duerschmied D[1], Akin I[1]

Institutes 1 University Medical Center Mannheim, Department of Medicine, Cardiology, Angiology, Hemostasis and Intensive Care, Mannheim, Germany; 2 University Medical Center Mannheim, Institute for Clinical Chemistry, Mannheim, Germany

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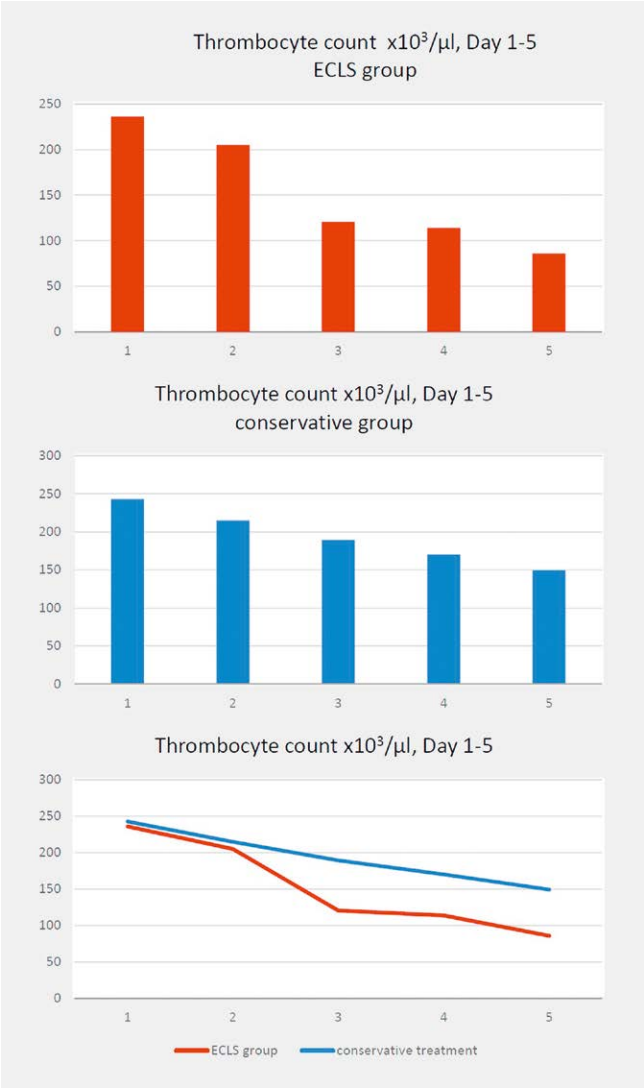
Introduction Cardiogenic shock is a complex, and hemodynamically diverse state of end-organ hypoperfusion that is associated with multiorgan failure. The extracorporal membrane oxygenation is one of the therapeutic interventions which is frequently used to stabilize the patient's condition. Despite increasing experience with this treatment, there is a lack of data on the management of some patient's groups. This study investigated the occurrence of thrombocythopenia in patients with conservative treatment and patients receiving veno-arterial membrane oxygenation (VA ECMO) support in cardiogenic shock.

Method We reviewed the retrospectively collected data of 69 patients admitted to our hospital (2019-2022) with cardiogenic shock. The patients were randomized to conservative and extracorporal life support system (ECLS) treatment groups. Thrombocyte count in day 1 to day 5 and the occurrence of thrombocyte mass transfusion were evaluated during hospitalization (▶ **Fig. 1**).



▶ **Fig. 1** Thrombocyte count in cardiogenic shock; Thrombocyte count Day 1-5 in ECLS group and conservative treatment group.

Results The study population consist of 69 patients with infarct associated cardiogenic shock. Among them 37 (53.6 %) were admitted with ST-elevation myocardial infarction, 32 (46.4 %) with non-ST elevation myocardial infarction or malignant heart rhythm disorders leading to cardiogenic shock. All patients have had coronary artery disease and were treated with urgent percutaneous coronary intervention. About 80 % of patients (55 patients) survived out of hospital cardiac arrest at admission. The 5-day mortality was 52.2 % and the overall in-hospital mortality was 62.3 %. The mean duration of VA ECMO in ECLS patients was 67.35 hours (95 % CI 45.77 to 88.93). The overall mean thrombocyte count at the admission was 242,43 x103/µl (95 % CI 221.22 to 263.64 x103/µl) without any difference in both groups (236.0 vs 234.1 x103/µl, p = 0.95). A drop in platelets count occurred in both groups, but significantly more pronounced in the ECLS group compared to patients without ECLS (at day 3: 120.5 vs 189.5 x103/µl, p = 0.0018 and at day 5 86.0 vs 149.5 x103/µl, p = 0.0014). The rate of transfusion of platelets was generally very low and occurred only in 4 cases (2 in ECLS group (6.2 %) and 2 in conservative group (5.4 %).

Conclusion Thrombocytopenia in cardiogenic shock is a common condition and is more pronounced in patients with ECLS treatment. However, the need for a platelet transfusion rarely occurs regardless of whether the patient is treated with ECLS or only conservatively

Conflict of Interest The Authors declare no conflict of interests.

T-09 | Perioperative Haemostasis

T-09-01 Periinterventional management of edoxaban in major procedures: results from the DRESDEN NOAC REGISTRY

Authors Köhler C, Tittl L, Marten S, Naue C, Beyer-Westendorf J
Institute University Hospital "Carl Gustav Carus", Thrombosis Research Department of Medicine I, Division Haematology, Dresden, Germany
DOI 10.1055/s-0042-1760493

Introduction Edoxaban is a non-vitamin K dependent oral anticoagulant (NOAC) licensed for venous thromboembolism (VTE) treatment or stroke prevention in atrial fibrillation (SPAF). Major surgical procedures are not uncommon in anticoagulated patients but data on perioperative edoxaban management are scarce.

Method Using data from the prospective DRESDEN NOAC REGISTRY we extracted data on major surgical procedures in patients who took edoxaban within the preceding 7 days. Periinterventional edoxaban management patterns and rates of outcome events were evaluated until day 30 after procedure.

Results Between 2011 and 2021, 3448 procedures were identified in edoxaban patients, including 287 (8.3 %) major procedures. Overall, patient characteristics were comparable for major and non-major procedures, but significant differences existed with regard to gender, concomitant antiplatelet therapies and the proportion of patients with a CHA2DS2-VASc score ≥ 2 (▶ **Table 1**).

	All procedures N=3448	Major procedures N=287	Non-major procedures N=3161	p-value
Male, n (%)	2057/3448 (59.7)	144/287 (50.2)	1913/3161 (60.5)	0.0006
Median Age (25-75th percentile), years	74.0 (67.0-79.0)	74.0 (67.0-80.0)	74.0 (67.0-79.0)	0.3989
Median BMI (25-75th percentile), kg/m²	28.1 (25.4-31.0)	28.4 (25.4-31.5)	28.1 (25.4-31.0)	0.4425
Indication for edoxaban				0.3764
– SPAF, n (%)	2816/3448 (81.7)	237/287 (82.6)	2579/3161 (81.6)	
– VTE, n (%)	611/3448 (17.7)	50/287 (17.4)	561/3161 (17.7)	
– off-label indication, n (%)	21/3448 (0.6)	0	21/3161 (0.7)	
Concomitant antiplatelet therapy, n (%)	169/3448 (4.9)	6/287 (2.1)	163/3161 (5.2)	0.0212
Heart failure, n (%)	778/3448 (22.6)	69/287 (24.0)	709/3161 (22.4)	0.5316
Arterial hypertension, n (%)	2864/3448 (83.1)	234/287 (81.5)	2630/3161 (83.2)	0.4706
Diabetes, n (%)	1012/3448 (29.4)	81/287 (28.2)	931/3161 (29.5)	0.6614
Prior TIA, stroke, or systemic embolism, n (%)	342/3448 (9.9)	24/287 (8.4)	318/3161 (10.1)	0.3569
PAD/CAD, n (%)	567/3448 (16.4)	52/287 (18.1)	515/3161 (16.3)	0.4242
Impaired renal function*, n (%)	484/3448 (14.0)	47/287 (16.4)	437/3161 (13.8)	0.2334
$CHADS_2$ ≥2, n (%)	2126/3448 (61.7)	179/287 (62.4)	1947/3161 (61.6)	0.7960
CHA_2DS_2-VASc ≥2, n (%)	3015/3448 (87.4)	262/287 (91.3)	2753/3161 (87.1)	0.0400
CHA_2DS_2-VASc ≥4, n (%)	1508/3448 (43.7)	138/287 (48.1)	1370/3161 (43.3)	0.1209
HAS-BLED score ≥2, n (%)	1824/3448 (52.9)	152/287 (53.0)	1672/3161 (52.9)	0.9826

▶ **Tab. 1** Patient characteristics at baseline of patients with edoxaban undergoing 3448 surgical or I; * Impaired renal function was defined as current or history of GFR <50 ml/min.; BMI = body mass index; PAD/CAD = peripheral arterial occlusive disease/coronary artery disease; SPAF = stroke prevention in atrial fibrillation; TIA = transient ischaemic attack; VTE = venous thromboembolism

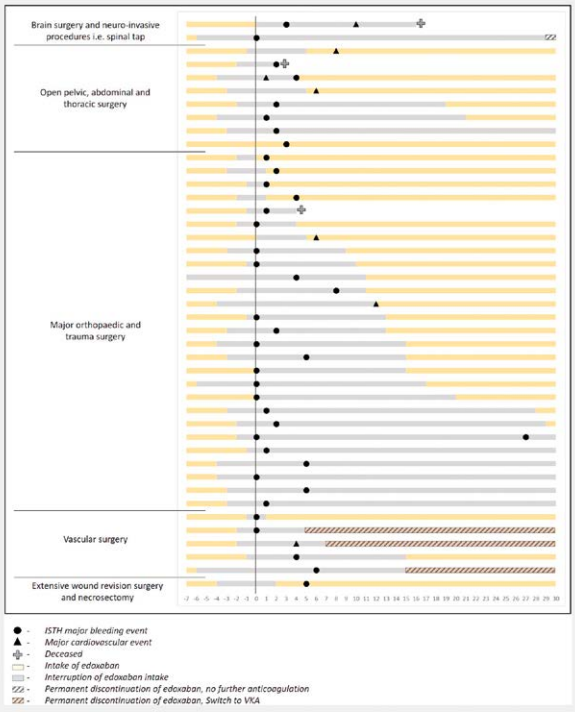
Major procedures consisted of orthopaedic/trauma surgery (44.3 %); open pelvic, abdominal or thoracic surgery (30.4 %), central nervous system surgery and procedures (13.9 %), vascular surgery (9.1 %) and extensive wound revision surgery (2.4 %).

A scheduled interruption of edoxaban was observed in 284/287 major procedures (99 %) with a total median edoxaban interruption time of 11.0 days (25-75th percentile 5.0-18.0 days). Heparin bridging was documented in 183 procedures (46 prophylactic dosages, 111 intermediate and 26 therapeutic dosages).

Overall, 7 (2.4 %; 95 %-CI 1.2 %-4.9 %) major cardiovascular events (5 VTE, 2 arterial thromboembolic events) occurred and 63 bleeding events were observed in 287 major procedures (22.0 %; 95 %-CI 17.6 %-2.71 %), comprising of 38 ISTH major bleeding events (13.2 %; 95 %-CI 9.8 %-17.7 %) and 25 ISTH CRNM bleedings (8.7 %; 95 %-CI 6.0 %-12.5 %).

Rates of major cardiovascular events with or without heparin bridging were comparable (6/183; 3.3 %; 95 %-CI 1.5 %-7.0 % vs. 1/36; 2.8 %; 95 %-CI 0.5 %-14.2 %; p = 0.7173). ISTH major bleeding occurred numerically more frequent in patients receiving heparin bridging (30/183; 16.4 %; 95 %-CI 11.7 %-22.4 %) versus procedures without heparin bridging (2/36; 5.6 %; 95 %-CI 1.5 %-18.1 %; p = 0.1542) (▶ **Fig. 1**). Within 30 days of follow up, 6 patients died (2.1 %; 95 %-CI 1.0 %-4.5 %) with causes of death being a ruptured truncus coeliacus following palliative angioplasty for an infiltrating pancreas cancer (ruled as fatal bleeding), septic organ failure, pneumocystis jirovecii pneumonia, COVID-19-pneumonia, septic complications following clipping of a ruptured cerebrovascular aneurism or terminal malignant disease. No fatal cardiovascular event occurred.

Conclusion Within the limitations of our study design, periprocedural edoxaban management seems effective and safe in routine care. Use of heparin bridging seems to have limited effects on reducing vascular events but may increase bleeding risk.



▶ **Fig. 1** Time-frequency-plot of periprocedural edoxaban management and heparin bridging; Time-frequency-plot of major cardiovascular outcomes, ISTH major bleeding and death in relation to periprocedural edoxaban management and heparin bridging. Of note, the figure depicts only patients who developed clinical outcomes of interest.

Conflict of Interest J.B.-W. has received honoraria and research support from Bayer, Boehringer Ingelheim, Daiichi Sankyo, Pfizer, Alexion, Norgine, DOASENSE and Sanofi. L.T. has received honoraria and travel support from Daiichi Sankyo and Bayer. S.M. has received honoraria from Daiichi Sankyo and Bayer. None of the other authors declared a conflict of interest with regard to the NOAC registry or this manuscript.

T-12 | Laboratory Diagnostics and Poc

T-12-01 A sensitive and selective method for the measurement of activated factor X based on complex formation with tissue factor pathway inhibitor

Authors Engelmaier A[1], Prodinger G[2], Weber A[2]
Institutes 1 Baxalta Innovations GmbH, a Takeda company, Pharm Sci, Vienna, Austria; 2 Baxalta Innovations GmbH, a Takeda company, PDT, R&D, Vienna, Austria
DOI 10.1055/s-0042-1760494

Introduction Low molecular weight chromogenic or fluorogenic substrates allow the sensitive measurement of activated coagulation factor X (FXa) but could show sometimes deficits in selectivity as only the interaction of the protease's active site with the substrate determines this assay characteristic. When measuring closely related proteases often the sum of activity rather than the activity of a single protease is determined. Adding specific inhibitors is a valid approach for increasing selectivity but this also increases the assay's complexity. Here we describe the use of solid phase-bound tissue factor pathway inhibitor (TFPI) to measure FXa by formation of the immobilized inhibitor-protease complex and its immunological detection by an FX-specific antibody.

Method TFPI (R&D Systems), diluted in phosphate-buffered saline (PBS) to a concentration of 10 µg/mL was coated to Maxisorp F96 plates at 4 °C overnight. After a washing step with PBS containing 0.05 % Tween 20 (PBST), plates were blocked with PBST containing 0.5 % skimmed dry milk, 3 mM EDTA and 120 KIE aprotinin: This buffer was used also as dilution buffer for samples and purified FXa (Haematologic Technologies), used as the assay standard. Serial dilution series were incubated with the plate and the TFPI-FXa complex formed was detected with an anti-FX IgG peroxidase conjugate (CoaChrom) after a washing step. Bound peroxidase activity was measured with SureBlue (KPL).

Results An accurate and sensitive six-point log-log calibration curve which ranged from 0.03 to 1.04 ng purified FXa was obtained reproducibly. Addition of the FXa-specific synthetic inhibitor Rivaroxaban caused complete signal inhibition with an 50 % inhibition rate elicited at a concentration of 177 ng/mL. This demonstrated the assay's selectivity for FXa. Furthermore, none of the six purified proteases tested (thrombin, FVIIa, FXIa, FXIIa, kallikrein, and plasmin) showed any significant alteration of the concentration-response curve obtained for purified FXa, when aprotinin was included in the dilution buffer to specifically inhibit plasmin, which is known to degrade TFPI. Analysis of a prothrombin complex concentrate demonstrated that even a 500-fold excess of the FX zymogen did not result in a significant signal. In contrast, despite the complexity of the protein matrix of an activated prothrombin complex concentrate (aPCC) and the presence of significant levels of FX zymogen the concentration-response curves obtained for the aPCCs were highly parallel to that obtained for the purified FXa standard. Inter-run precision determined in six separate measurements for four aPPC lots ranged from 3.7 % to 5.4 %.

Conclusion The TFPI-FXa complex formation assay described enables for a sensitive, robust, and reliable FXa measurement with high selectivity for FXa.

Conflict of Interest All authors are full-time employees of Baxalta Innovations GmbH, part of Takeda.

Hämostaseologie 2023; 43: S1–S106

T-12-02 Sensitive and unambiguous measurement of the activated contact activation factor XII

Authors Engelmaier A[1], Weber A[2]
Institutes **1** Baxalta Innovations GmbH, part of Takeda, Pharm Sci, Vienna, Austria; **2** Baxalta Innovations GmbH, part of Takeda, PDT R&D, Vienna, Austria
DOI 10.1055/s-0042-1760495

Introduction The advent of chromogenic and fluorogenic low molecular weight peptide substrates has revolutionized protease measurements in terms of sensitivity but has not fully resolved issues related to selectivity. When measuring closely related proteases often the sum of their activities rather than the activity of a single protease is determined as the reaction between low molecular weight substrate and protease is focused on the protease's active site only. Adding specific inhibitors to increase the assay's sensitivity has been described as a valid approach but this increases the complexity of the assay. Here we describe the use of solid phase-bound corn trypsin inhibitor (CTI) to selectively measure activated factor XII (FXIIa) after formation of the immobilized CTI-FXIIa complex and its immunological detection by a FXII zymogen-specific antibodies.

Method CTI (Enzyme Research Laboratories) was diluted to 10 µg/mL in phosphate-buffered saline (PBS) and coated at 4 °C overnight to a NUNC Maxisorp F96 plate (100 µL/well). After a washing step with PBS containing 0.05 % Tween 20 (PBST), the plate was blocked by incubation with PBS containing 10 mg/mL human serum albumin and 2 mM benzamidine (200 µL/well, room temperature, 60 min). The washed plate was then incubated with serial dilution series of standard and samples. Purified FXIIa (Enzyme Research Laboratories) was used to establish the assay calibration curve. After a final washing step, the CTI-FXIIa complex formed was detected by an anti-human FXII IgG peroxidase conjugate (The Binding Site). Finally, bound peroxidase activity was measured with SureBlue (KPL).

Results The five-point log-log calibration curve ranged from 0.6 to 10 ng FXIIa/mL. Purified activated factor XI and kallikrein, present at 2-fold and 3-fold excess respectively, demonstrated essentially no influence on the concentration-response curve obtained for FXIIa. Similarly, the presence of a human immunoglobulin G concentration of as high as 2.5 mg/mL did not alter the characteristics of the FXIIa concentration-response curve.

Conclusion The CTI-FXIIa complex formation assay for the measurement of FXIIa showed adequate sensitivity and high human immunoglobulin G levels did not interfere. Mixing experiments demonstrated the high assay selectivity as excesses of the related proteases activated FXI and kallikrein demonstrated no effect on the FXIIa calibration curve. This high selectivity is obtained by the combined effects of the specific binding to CTI followed by the specific binding of the anti-FXII detection antibody, which is used to measure the amount of complex formed bound to the solid phase.

Conflict of Interest Both authors are full-time employees of Baxalta Innovations GmbH, part of Takeda.

T-12-03 Limited concordance of heparin/PF4 antibody assays for the diagnosis of heparin-induced thrombocytopenia: an analysis of the TORADI-HIT study

Authors Hammerer-Lercher A[1], Nilius H[2], Studt J-D[3], Tsakiris D A[4], Greinacher A[5], Mendez A[1], Schmid A[6], Wuillemin W[7], Gerber B[8], Kremer Hovinga J A[9], Vishnu P[10], Graf L[11], Bakchoul T[12], Nagler M[2]
Institutes **1** Kantonsspital Aarau AG, Institute of Laboratory Medicine, Aarau, Switzerland; **2** Inselspital, Bern University Hospital, University of Bern, Department of Clinical Chemistry, Bern, Switzerland; **3** University and University Hospital Zurich, Division of Medical Oncology and Hematology, Zürich, Switzerland; **4** Basel University Hospital, Diagnostic Haematology, Basel, Switzerland; **5** Universitätsmedizin Greifswald, Institut für Immunologie und Transfusionsmedizin, Greifswald, Germany; **6** City Hospital Zurich Triemli, Clinic of Medical Oncology and Hematology, Zürich, Switzerland; **7** Cantonal Hospital of Lucerne and University of Bern, Division of Hematology and Central Hematology Laboratory, Luzern, Switzerland; **8** Oncology Institute of Southern Switzerland, Clinic of Hematology, Bellinzona, Switzerland; **9** Inselspital, Bern University Hospital, University of Bern, Department of Hematology and Central Hematology Laboratory, Bern, Switzerland; **10** CHI Franciscan Medical Group, Division of Hematology, Seattle, USA; **11** Cantonal Hospital of St Gallen, St. Gallen, Switzerland; **12** University Hospital of Tübingen, Centre for Clinical Transfusion Medicine, Tübingen, Germany
DOI 10.1055/s-0042-1760496

Introduction Anecdotal reports suggest that the correlation between heparin/PF4 antibody assays for the diagnosis of heparin-induced thrombocytopenia (HIT) is limited. To address this issue, we investigated the correlation between widely used assays and explored possible factors contributing to the variability.

Method This is a large, prospective cohort study with 10 participating tertiary care hospitals including 1'393 patients with suspected HIT in clinical practice. HIT was defined by a positive heparin induced platelet activation (HIPA) test serving as a reference standard. Three immunoassays that measure heparin/PF4 antibodies (chemiluminescent immunoassay (CLIA; Acustar HIT IgG, Instrumentation Laboratory), enzyme-linked immunosorbent assay (ELISA; Lifecodes PF4 enhanced, Gen-Probe Incorporated), particle gel immunoassay (PaGIA; Diamed ID-PaGIA Heparin/PF4, DiaMed SA)) were used to investigate their comparability. Additionally, possible factors that could have an impact on assay result were examined: sex (male, female), age (< 65 years, ≥ 65 years), unfractionated heparin exposure, thrombosis (no thrombosis, thrombosis), cardiovascular surgery, and intensive care unit. The statistical software "R" was used to calculate correlations, diagnostic odds ratios (DOR) and z-scores.

Results Of 1'393 patients 119 patients (8.5 %) with a positive HIPA result were classified as HIT positive and had a higher median 4Ts score (5; interquartile range (IQR): 4 - 6) compared to those without HIT (3; IQR: 2 - 4). Correlations between immunoassays were weak ($r \leq 0.65$). None of the tested, possible factors contributing to this inconsistency reached a practicable relevance to explain this situation. The clinical performances of all three assays were high (DOR values CLIA: 361 (confidential interval (CI): 142 – 914), ELISA: 322 (CI: 101 – 1029), PaGIA 236 (CI: 58 – 966)). If DOR values were investigated separately for each assay and potential contributing factor no significant difference was observed. This was partly also true for z-scores, that reached higher values in HIPA positive compared to HIPA negative patients. Both, DORs and z-scores revealed markedly overlapping CI concerning the possible influencing factors.

Conclusion The correlation between widely used heparin/PF4 antibody assays was poor, and key patient characteristics could not explain this variability. Even though the clinical performance of these assays is high, standardization of assays is requested to improve comparability. This is particularly important to support future applications in algorithms integrating immunoassay test results.

Conflict of Interest Michael Nagler received fundings from Swiss National Science Foundation (SNSF) and International Society on Thrombosis and Haemostasis (ISTH).

T-12-04 Use of DOAC Dipstick point-of-care testing in patients with acute stroke and transient ischemic attack

Authors Ziegler M[1], Kern R[2], Szabo K[1], Hoyer C[1], Willmann O[2], Mink S[2], Harenberg J[3], Spannagel U[3], Weiss C[4], Hetjens S[4]
Institutes **1** University Medical Center Mannheim, Department of Neurology, Mannheim, Germany; **2** Klinikum Worms gGmbH, Department of Neurology, Worms, Germany; **3** DOASENSE GmbH, Heidelberg, Germany; **4** University Medical Center Mannheim, Department of Biometry and Biomathematics, Mannheim, Germany
DOI 10.1055/s-0042-1760497

Introduction In acute stroke or transient ischemic attack (TIA), rapid information about the plasma concentration of direct oral anticoagulants (DOAC) is important for treatment decisions such as systemic thrombolysis, endovascular therapy, or antidote administration in case of hemorrhagic stroke. As standard coagulation assays are either not sufficiently reliable or have long turnaround times, point-of-care-testing with DOAC dipstick may be of additional value in this setting.

Method About 250 patients with acute stroke within the past 24 hours, aged >18 years, admitted to the emergency care units at two centers will be included into the study after having obtained written informed consent. Biographic data, therapeutic interventions, global and specific chromogenic substrate assays, routine clinical chemistry parameters and DOAC Dipstick tests are documented or measured as soon as possible and within eight hours after admission to hospital. DOAC Dipstick results are obtained from urine samples analyzed immediately after sampling. Colors of the specific factor Xa and thrombin inhibitor pads are analyzed visually and by a reader for presence of DOACs. Data will be analyzed by SAS software for primary outcome and for interfering factors.

Results The ongoing bi-center observational prospective study is the first on patients with acute ischemic stroke, hemorrhagic stroke or TIA under real-life conditions. It intends to retrieve an unequivocal test result as early as possible and within 8 hours after stroke unit admission. Moreover, the comparability of visual and reader data of DOAC Dipstick will be analyzed.

Conclusion Preliminary results of this on-going study will be presented at the conference.

Conflict of Interest MZ, KS, CH, OW, SM, SH, CW, and RK declare no conflicts of interest. US receives honoraria from DOASENSE GmbH. JH is founder and general manager of DOASENSE GmbH. Materials of DOAC Dipstick are provided by DOASENSE GmbH.

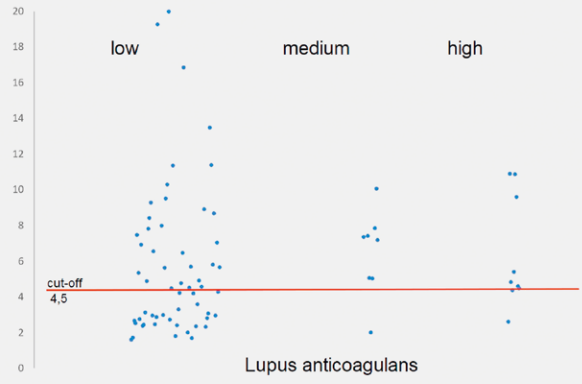
T-12-05 Can APC inhibition by lupus anticoagulants indicate antibody thrombogenicity?

Authors Bradacova P, Slavik L, Úlehlová J, Procházková J, Hluší A
Institute Faculty of Medicine and Dentistry, Palacký University Olomouc; University Hospital Olomouc, Department of Hematology-Oncology, Olomouc, Czech Republic
DOI 10.1055/s-0042-1760498

Introduction Lupus anticoagulans is the most important factor in the detection of antiphospholipid syndrome (APS). APS is a hypercoagulable state accompanied by the presence of heterogeneous antiphospholipid antibodies (aPL) that nonspecifically affect hemostasis. The effect is due to their binding to phospholipid surfaces, thereby prolonging screening coagulation tests such as aPTT, but this does not correlate with the incidence of thrombotic complications in APS. This situation is clarified by the theory that the main target of antibodies is the activated protein C (APC) system, the elimination of which may manifest as thrombotic complications.

Method The aim of this study was to determine the thrombogenicity of lupus anticoagulans antibodies using a modified thrombin generation assay inhibited by protein C (TGA) in a cohort of 90 samples with suspected APS. TGA was measured with/without APC and the ratio of the two measurements (as for APC resistance) was evaluated, with a cut-off of ≤4.5 (90th percentile) calculated using 21 patients with factor V Leiden heterozygous mutation (FV Leiden heterozygote).

Results The cohort was divided according to the prevalence of lupus anticoagulans into low, intermediate and high titers. The results of the modified TGA test showed that the incidence of patients with low TGA ratio did not depend on lupus anticoagulans antibody titer (low titers 35 %, intermediates titers 27 % and high 30 %), but at the same time it correlates very well with the clinical manifestation of APS in these patients (▶ **Fig. 1**).



▶ **Fig. 1** Thrombogenicity lupus anticoagulans antibodies.

Conclusion Performing TGA may help us to identify patients in all groups who are at risk of thrombotic and pregnancy complications. For this reason, it appears beneficial to include thrombogenicity determination by the modified TGA test in all positive lupus anticoagulans antibody detections.

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Conflict of Interest No conflict of interest

T-12-06 Discriminatory potential of platelet function reference ranges in cardiovascular disease

Authors von Ungern-Sternberg S[1,2], ten Cate V[1,2], Panova-Noeva M[1,2], Dahlen B[2], Buch G[2], Rapp S[2], Prochaska JH[1,2], Strauch K[3], Beutel ME[4], Schuster AK[5], Lackner KJ[6,7], Münzel T[6,8], Wild PS[1,2], Jurk K[1,2]
Institutes **1** University Medical Center of the Johannes Gutenberg-University Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **2** University Medical Center of the Johannes Gutenberg-University Mainz, Preventive Cardiology and Preventive Medicine, Department of Cardiology, Mainz, Germany; **3** University Medical Center of the Johannes Gutenberg-University Mainz, Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), Mainz, Germany; **4** University Medical Center of the Johannes Gutenberg-University Mainz, Department of Psychosomatic Medicine and Psychotherapy, Mainz, Germany; **5** University Medical Center of the Johannes Gutenberg-University Mainz, Department of Ophthalmology, Mainz, Germany; **6** German Center for Cardiovascular Research (DZHK), Partner Site Rhine-Main, Mainz, Germany; **7** University Medical Center of the Johannes Gutenberg-University Mainz, Institute for Clinical Chemistry and Laboratory Medicine, Mainz, Germany; **8** University Medical Center of the Johannes Gutenberg-University Mainz, Department of Cardiology, Cardiology I, Mainz, Germany
DOI 10.1055/s-0042-1760499

Introduction Cardiovascular diseases are characterized by altered platelet activity. To distinguish between physiological and pathological platelet activity, it is essential to establish reference ranges of standardized platelet (re)activity parameters in the population, which was a major aim of this study. Furthermore, the discriminative ability of values outside these reference ranges was assessed for various cardiovascular diseases.

Method A reference group (n = 329; 51 % women; age: 35-85 years) was defined in a representative cohort study after exclusion of cardiovascular-related diseases and platelet function-interfering medications. Reference values for platelet function parameters were set at the 5th/95th percentiles. Multivariable linear regression was applied to assess age and sex dependence, and to identify cardiovascular determinants of platelet function parameters measured by flow cytometry, light transmission aggregometry, PFA-200, and calibrated automated thrombinography. In the total cohort with platelet function data

(n = 789), the prevalence ratio of coronary artery disease (CAD), myocardial infarction (MI), history of stroke, atrial fibrillation (AF), chronic heart failure (CHF), and venous thromboembolism (VTE) was estimated in relation to values outside the platelet function reference ranges by robust Poisson regression.

Results The percentage of CD63 + platelets was higher in men (median:2.60 %, IQR:1.20/5.26 %) than women (1.90 %, 0.70/3.60 %), which both decreased with age. Collagen/ADP-induced occlusion time decreased (β [log-transformed DV] = -0.03, p = 0.044) with age and platelet aggregability, spontaneously and in response to collagen, increased with age (β [log-transformed dependent variable, DV] = 0.094, p = 0.0031; β = 1.58, p = 0.029). Tissue factor-triggered endogenous thrombin potential (ETP) decreased (β = -33.8, p = 0.021) and lag time increased (β = 0.348, p = 0.0046) with age. Arterial hypertension, obesity and active smoking were independent determinants of age, sex and other risk factor of in vivo platelet activation as well as platelet aggregation and thrombin generation in vitro. Increased prevalence of CAD, MI, AF, CHF, and VTE was observed for values outside of reference limits for platelet adhesion (von Willebrand factor dependent), aggregation and coagulation parameters. In addition, history of stroke and AF were more prevalent with values exceeding the reference range for CD63 + and CD62P + platelets, respectively.

Conclusion Platelet function test values, which exceed or fall below population-based reference ranges may predict increased prevalence of different cardiovascular diseases.

Conflict of Interest One author has received research funding outside the present study from Boehringer Ingelheim, Sanofi-Aventis, Bayer Healthcare, Daiichi Sankyo Europe, and Novartis, and received outside the present study honoraria for lectures or consulting from Boehringer Ingelheim, Bayer HealthCare, Evonik, AstraZeneca and Sanofi-Aventis.

T-12-07 Monitoring of argatroban in COVID-19 ICU patients: a prospective study comparing aPTT, point-of-care viscoelastic testing with ECA-test and dTT to LC/MS/MS

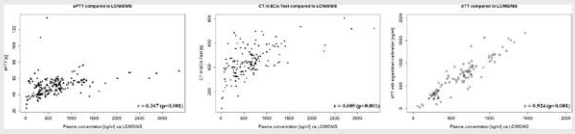
Authors Heubner L[1], Oertel R[2], Tiebel O[3], Güldner A[1], Mehlig-Warnecke N[1], Vicent O[1], Beyer-Westendorf J[4], Renner B[2], Koch T[1], Spieth P[1]

Institutes **1** University Hospital "Carl Gustav Carus", Technische Universität Dresden, Department of Anesthesiology and Intensive Care Medicine, Dresden, Germany; **2** Faculty of Medicine, Technische Universität Dresden, Institute of Clinical Pharmacology, Dresden, Germany; **3** University Hospital "Carl Gustav Carus", Technische Universität Dresden, Institute of Clinical Chemistry, Dresden, Germany; **4** University Hospital "Carl Gustav Carus", Technische Universität Dresden, Division of Hematology and Hemostasis, Department of Medicine I Thrombosis Research, Dresden, Germany

DOI 10.1055/s-0042-1760500

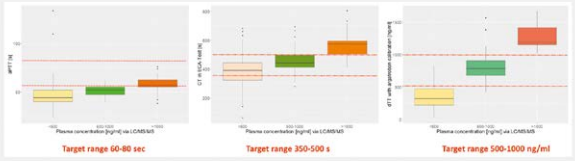
Introduction Argatroban is indicated for treatment of heparin-induced thrombocytopenia, but is also used in critical ill COVID-19 patients presenting with extensive thrombin overload. Direct drug monitoring is not available and argatroban dosing is mainly based on activated partial plasmin time (aPTT), which has limitations in hypercoagulable patients with increased FVIII [1, 2]. The aim of this study was to compare correlation of routine clotting tests (aPTT, ecarin clotting time [ECA-CT] and diluted thrombin time [dTT]) [3] to argatroban plasma levels measured by gold standard mass spectrometry (LC/MS/MS).

Method From 06/2021 to 03/2022, 205 samples from 22 COVID-19 ICU patients were analyzed: aPTT and dTT on STA R Max3-Analyzer (Stago Deutschland GmbH, Germany) using the BIOPHEN DTI Kit with Argatroban-calibration (CoaChrom Diagnostica GmbH, Austria); ECA-CT was measured using ClotPro ecarin assay. LC/MS/MS was performed using an RP column, a solvent gradient and an API4000 mass spectrometer with electrospray. Correlation was analyzed using Pearson correlation coefficient r in R version 3.2.4. This study was approved by the Ethics Committee of the Technical University of Dresden, Germany (BO-EK-64022022) and registered with German Clinical Trials Register DRKS00028689.



▶ **Fig. 1** Scatter plot diagram comparing aPTT, ecarin clotting time (CT ECA-Test) and dTT to argatroban plasma concentrations measured by LC/MS/MS.

Results From 205 samples with LC/MS/MS analysis, 195 were compared to aPTT, 153 to ECA-CT and 105 to dTT. In 40 samples, dTT was not measureable due high bilirubin values. Compared to LC/MS/MS, correlation of dTT was highest (r = 0.924), followed by ECA-CT (r 0.609) and aPTT (r 0.367; p < 0.001; ▶ **Fig. 1**). When recommended cut-offs for argatroban plasma levels (500-1000 ng/ml according to SmPC) were applied, dTT (when measurable) and ECA-CT better identified critical values of argatroban plasma values > 1000ng/ml than aPTT (▶ **Fig. 2**).



▶ **Fig. 2** Box plots comparing aPTT, ccarin clotting time (CT ECA-Test) and dTT to different clinical relevant argatroban plasma concentrations measured by LC/MS/MS.

Conclusion Argatroban in critical ill COVID-19 patients should be monitored using dTT. If dTT is not possible or measurements are highly time-sensitive, point-of-care ClotPro ECA-test should be preferably used instead of aPTT.

Conflict of Interest None to declare.

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T-12-08 Monitoring of unfractionated heparin in critical ill ICU patients: a prospective study comparing aPTT and point-of-care viscoelastic testing with IN /HI ratio to anti-Xa

Authors Heubner L[1], Dietze A[1], Tiebel O[2], Güldner A[1], Vicent O[1], Beyer-Westendorf J[3], Koch T[1], Spieth P[1]

Institutes **1** University Hospital "Carl Gustav Carus", Technische Universität Dresden, Department of Anesthesiology and Intensive Care Medicine, Dresden, Germany; **2** University Hospital "Carl Gustav Carus", Technische Universität Dresden, Institute of Clinical Chemistry, Dresden, Germany; **3** University Hospital "Carl Gustav Carus", Technische Universität Dresden, Division of Hematology and Hemostasis, Department of Medicine I Thrombosis Research, Dresden, Germany

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Introduction Anticoagulation is indicated for the prevention or therapy of thromboembolic events, but remains highly challenging considering the high risk of bleeding events in critically ill patients.Unfractionated heparin (UFH) is widely used as preferred anticoagulation for patients on intensive care units (ICU) due to its beneficial short half time and fast elimination. For monitoring of UFH, activated partial thromboplastin time (aPTT) is mainly used, but aPTT can be misleading in both directions [1]. While high factor VIII plasma values may decrease aPTT, a reduced factor XII under extracorporal circulation may prolong aPTT that no longer correlates to anticoagulation intensity [2]. Anticoagulation monitoring using specific UFH calibrated anti-Xa levels is an established alternative to overcome aPTT limitations but is rarely available 24/7 [3, 4]. Using point-of-care (POC) viscoelastic testing (VET) [5] with a specific ratio between clotting time (CT) in intrinsic test (IN-test) compared to heparinase test (HI-test) – which includes the inactivation of heparin in the probe - might help to determine the UFH effect in critically ill patients [6].

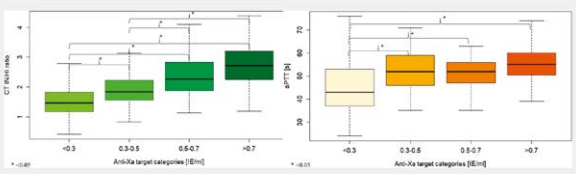
Method From 09/2020 to 07/2022, 467 samples from 120 adult ICU patients receiving UFH therapy were prospectively collected. Samples for aPTT, anti-Xa measurement and POC VET using ClotPro (Haemonetics, Boston, Massachusetts, USA) were simultaneously collected. Measurement for aPTT (C.K. Prest) and anti-Xa (Liquid AntiXa) were performed using STA R Max 3 device (Stago Deutschland GmbH, Düsseldorf). Correlation was analyzed using Kruskal-Wallis test in SPSS version 27 and R version 3.2.4. This study was approved by the local Ethics Committee at the Technische Universität Dresden, Germany (BOEK- 374072021) and registered with the German Clinical Trials Register DRKS00028689.

Results 467 samples under UFH treatment were included in this analysis, the majority of these patients were treated for COVID-19 associated acute distress syndrome. According to our institutional guidelines, anti-Xa target levels for UFH were set at 0.3-0.5 IE/ml for standard high-risk prophylaxis and 0.5-0.7 IE/ml for therapeutic anticoagulation therapy with values <0.3 and > 0.7 being defined as under- or over-treatment. Table 1 presents the median aPTT and CT IN/HI ratio values for patients within these anti-Xa categories. CT IN/HI ratio correlation to anti-Xa levels was considerably better than aPTT correlation (▶ **Tab. 1**). Notably, aPTT could not exactly discriminate between patients receiving UFH dosages correlating to high-risk prophylaxis or therapeutic anticoagulation.

anti-Xa [IE/ml]	<0.3	0.3-0.5	0.5-0.7	>0.7
n= 467	264 (57 %)	86 (12 %)	41 (6%)	76 (16 %)
aPTT [s]	43 (37; 53)	52 (46; 59)	52 (47; 56)	55 (51; 60)
CT IN/HI ratio	1.5 (1.2; 1.8)	1.8 (1.6; 2.2)	2.3 (1.9; 2.8)	2.7 (2.3; 3.2)

▶ **Tab. 1** median test values (1st; 3rd quartile) for each predefined ant-Xa target category

Conclusion Whole blood POC VET using a specific heparinase-approach (IN/HI ratio) is superior to aPTT in detecting patients in or out of targeted anti-Xa levels. POC VET should be made available for ICUs as bedside test and might help to guide anticoagulation management in critical ill patients, being faster and potentially more widely available than lab-based anti-Xa testing (▶ **Fig. 1**).



▶ **Fig. 1** Box plots comparing ClotPro IN/HI ratio and aPTT to doffernt clinical relevant predefined anti-Xa target categories in patients under anticoagulations with UFH.

Conflict of Interest None to declare.

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T-12-09 Biobanking for testing hemostasis: Long-term stability of citrated plasma samples

Authors Nilius H[1], Henny C[2], Giabbani E[3], Sulzer I[4], Kremer Hovinga J A[4], Nagler M[1]

Institutes **1** Inselspital Bern University Hospital, Departement of Clinical Chemistry, Bern, Switzerland; **2** Interregional Blood Transfusion SRC Ltd., Bern, Switzerland; **3** University Hospital Zurich, Deparement of Laboratory Medicine and Pathology, Zurich, Switzerland; **4** Inselspital Bern University Hospital, Departement of Haematology and Central Haematology Laboratory, Bern, Switzerland

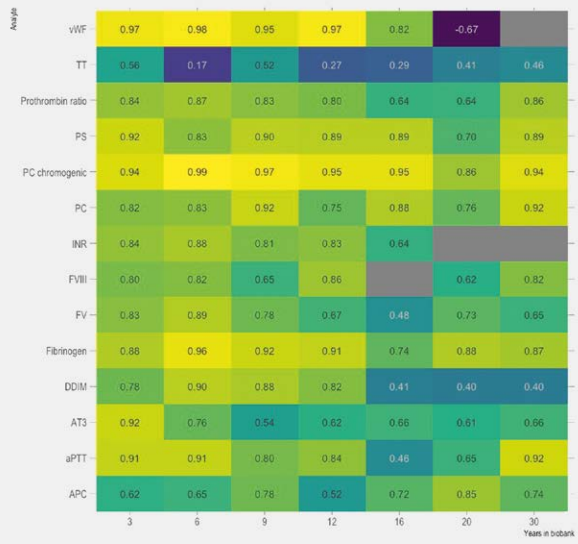
DOI 10.1055/s-0042-1760502

Introduction Biobanking plays an essential role in contemporary translational research. However, the long-term stability of citrated plasma samples for testing hemostasis is virtually unknown. In this study, we aimed to investigate the consistency of plasma samples that were stored for up to 30 years.

Method This evaluation study used 280 citrated plasma samples from a prospective cohort of patients referred for venous thromboembolism risk assessment. We selected forty samples for each group, which were stored at –80 °C for 3, 6, 9, 12, 16, 20 or 30 years. The following hemostasis parameters were measured at the time point of collection (1988-2014) and again recently: Prothrombin ratio, INR, activated partial thrombin time (aPTT), thrombin time (TT), fibrinogen, d-dimers, factor V, factor VIII, von-Willebrand-factor (antigen; vWF), protein s, protein c (coagulometric), protein c (chromogenic; PC), antithrombin, and APC resistance. The analytical principles, devices and reagents have remained similar over the years. Spearman's correlation coefficient was calculated for each time point and a correlation matrix was created. A linear model was fitted to the data to assess if the relative change was associated with storage time.

Results Applying an approximate cut-off of a spearman's correlation coefficient of 0.8, the prothrombin ratio was stable for 12 years, INR for 12 years, aPTT for 12 years, fibrinogen for 12 years, d-dimers for 12 years, factor V for 6 years, factor VIII for 6 years, vWF for 16 years, protein s for 16 years, protein c (coagulometric) for 9 years, protein c (chromogenic) for 30 years, and antithrombin for 3 years. Associations were particularly low regarding TT, and APC. Linear regression confirmed the storage time as a relevant predictor in the majority of parameters (▶ **Fig. 1**).

Conclusion Although the majority of parameters have remained relatively stable over several years, the long-term stability of most hemostasis parameters cannot be predicted with certainty. These results must be confirmed in an independent dataset.



▶ **Fig. 1** Spearman's correlations coefficients for different hemostasis parameters depending on storage time.; vWF - von-Wllebrand-factr (antigen), TT - thrombin time, PS - protein s, PC chromogenic - protein c chromogenic, PC - protein c coagulometric, FVIII - factor VIII, FV - factor V, DDIM - d-dimers, AT3 - antithrombin, aPTT - activated partial thrombin time, APC - APC resistance.

Conflict of Interest All authors report no conflict of interest for the present study.

T-12-10 Therapeutic interventions and use of point of care DOAC Dipstick test in stroke patients: an interim analysis of a prospective cohort study

Authors Eid E[1], Schlachetzki F[1], Spannagel U[2], Harenberg J[2], Hetjens S[3]
Institutes 1 Bezirksklinikum Regensburg, Regensburg, Germany; 2 Doasense GmbH, Heidelberg, Germany; 3 Medical Faculty Mannheim, Medical Statistics and Biomathematics, Mannheim, Germany
DOI 10.1055/s-0042-1760503

Introduction A fast therapeutic decision is important in patients with stroke. A fast and reliable diagnostic point of care (POC) test to determine the anticoagulant effects of direct oral anticoagulants (DOACs) may assist the medical decision-making process in patients with suspected stroke. In the present study, we evaluated the use of the DOAC Dipstick test strip, which analyzes the presence of direct oral thrombin (DTI) or direct oral factor-Xa inhibitors (DXI). We investigated how the DOAC Dipstick test affected treatment interventions and the speed of diagnosis between diagnostic POC and diagnostic imaging and treatment.

Method This prospective, controlled multicentre study will include around 200 participants with acute stroke. Patients underwent cranial computerized tomography or nuclear magnetic resonance tomography. Urine samples were taken for DOAC Dipstick test. The study was approved by the local ethics boards and all patients gave written informed consent. The present analysis included 57 patients recruited in one centre and descriptive data is presented.

Results The indications for DOAC treatment were non-valvular atrial fibrillation (n = 47) other indications (n = 3) or unknown anticoagulation (n = 7). Patients were treated with apixaban (n = 20), edoxaban (n = 17), rivaroxaban (n = 9), and dabigatran (n = 4). Of the 7 patients with unknown anticoagulation, 2 (28 %) were identified as being treated with apixaban or rivaroxaban by a positive DOAC Dipstick result. DOAC Dipstick test results (n = 49 patients) were available within 75 min before the diagnostic imaging in 12 patients, 6 min before the diagnostic imaging in 11 patients, and between 15 and 147 min after diagnostic imaging in 26 patients. Six patients had a positive DOAC Dipstick test result for DXI; 3 of these were treated with local thrombolysis, 2 were treated with mechanical thrombectomy, and 1 was treated with 4-factor prothrombin-complex concentrate (PPSB). Seven patients had a negative DOAC Dipstick test result for DXI and DTI; 4 of these were treated with local thrombolysis, 2 were treated with mechanical thrombectomy, and 1 was treated with PPSB because of major haemorrhage. We also observed that the DOAC Dipstick test was performed later in patients with a low NIHSS score (National Institute of Health Stroke Scale) than in patients with a medium or high NIHSS score. The intervention outcomes were uneventful.

Conclusion In conclusion, the DOAC Dipstick test was rapidly and easily performed in patients with stroke admitted to an emergency care unit of a medium sized hospital. Most tests were performed before or within a short time frame of diagnostic procedures, facilitating medical decisions for therapeutic interventions. Future work should determine whether time to the DOAC Dipstick test affects the severity of NISSS scores.

Conflict of Interest EE, FS, SH none declared, US receives honoraria from Doasense GmbH, JH is founder and managing director of Doasense GmbH

T-13 | Haemophilia

T-13-01 Emicizumab prophylaxis for the treatment of people with moderate or mild Hemophilia A without Factor VIII inhibitors: results from the primary analysis of the HAVEN 6 study

Authors Oldenburg J[1], Hermans C[2], Negrier C[3], Lehle M[4], Chowdary P[5], Catalani O[4], Jiménez-Yuste V[6, 4], Schmitt C[4], Ventriglia G[4], Windyga J[7], Kiialainen A[4], d'Oiron R[8], Moorehead P[9], Koparkar S[10], Teodoro V[4], Shapiro AD[11]
Institutes 1 University of Bonn, Director of the Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany; 2 Cliniques Universitaires Saint-Luc, Brussels, Belgium; 3 University, Lion, France; 4 F. Hoffmann-La Roche Ltd, Basel, Switzerland; 5 Royal Free Hospital, Haemophilia and Thrombosis Centre, London, UK; 6 Hospital Universitario La Paz, Autónoma University, Madrid, Spain; 7 Institute of Hematology and Transfusion Medicine, Warsaw, Poland; 8 Bicêtre Hospital AP-HP, University Paris-Saclay and UMR_S1176 INSERM, Le Kremlin-Bicêtre, France; 9 Memorial University of Newfoundland, St John's, NL, Canada; 10 Genentech, San Francisco, CA, USA; 11 Indiana Hemophilia and Thrombosis Center, Indianapolis, IN, USA
DOI 10.1055/s-0042-1760504

Introduction Emicizumab is a bispecific monoclonal antibody that substitutes for missing activated factor (F)VIII in people with hemophilia A (HA). This primary analysis ofHAVEN 6 (NCT04158648) aims to assess safety and efficacy of emicizumab prophylaxis in people with non-severe HA without FVIII inhibitors.

Method HAVEN 6 is a Phase III, open-label study of emicizumab in people with moderate or mild HA without FVIII inhibitors who warrant prophylaxis as assessed by Investigator. Informed consent and ethics approval were obtained. Participants received subcutaneous emicizumab 3mg/kg weekly for 4 weeks, then 1.5mg/kg weekly, 3mg/kg every 2 weeks, or 6mg/kg every 4 weeks. Safety endpoints include adverse events (AEs), serious AEs (SAEs) and AEs of special interest, including thromboembolic events (TEs) and thrombotic microangiopathies (TMAs). Efficacy endpoints include negative binomial regression model estimates of annualized bleed rates (ABRs) (▶ **Tab. 1**).

AE	Participants (N=72)
Total number of AEs, n	248
Participants with ≥1 AE, n (%)	
Any AE	60 (83.3)
AE with fatal outcome	0 (0)
AE leading to withdrawal from treatment	0 (0)
AE leading to dose modification/interruption	0 (0)
Grade ≥3 AE	4 (5.6)
Treatment-related AE*	15 (20.8)
Local injection-site reaction	12 (16.7)
Total number of SAEs,† n	10
Participants with ≥1 SAE, n (%)	8 (11.1)
AE of special interest, n (%)	
Systemic hypersensitivity/anaphylactic/anaphylactoid reaction	0 (0)
Thromboembolic event‡	1 (1.4)
Thrombotic microangiopathy	0 (0)

▶ **Tab. 1** Efficacy Summary of the HAVEN 6 study.

Results As of 30-Oct-2021, 72 participants (70.8 % [n = 51] moderate; 29.2 % [n = 21] mild; 95.8 % [n = 69] male; 4.2 % [n = 3] female) received emicizumab. Median follow-up was 55.6 weeks. At baseline, 37 participants (51.4 %) were on FVIII prophylaxis; 24 (33.3 %) had target joints. Within 24 weeks prior to study entry, participants had a median (range) of 2.0 (0–96) bleeds and a model-based ABR (95 % CI) of 10.1 (6.93–14.76). Sixty participants (83.3 %) had ≥ 1 AE and 15 (20.8 %) had ≥ 1 emicizumab-related AE; no AEs led to treatment withdrawal/modification/interruption (Table 1). Ten SAEs were reported by eight participants (11.1 %), none emicizumab-related. There were no deaths or TMAs. One participant experienced a Grade 1 thrombosed hemorrhoid unrelated to emicizumab, classified as a TE. Model-based ABRs (95 % CI) were 0.9 (0.55–1.52) for treated bleeds, and 2.3 (1.67–3.12) for all bleeds (Table 2). Forty-eight participants (66.7 %) had zero treated bleeds (▶ **Tab. 2**).

¤	Treated·participants·(N=72)¤				
	Treated·bleeds¤	Treated·joint·bleeds¤	Treated·spontaneous·bleeds¤	Treated·target·joint·bleeds¤	All·bleeds¤
Model·based·mean·ABR·(95%·CI)¤	0.9·(0.55–1.52)¤	0.2·(0.09–0.57)¤	0.2·(0.11–0.33)¤	0.1·(0.03–0.40)¤	2.3·(1.67–3.12)¤
Calculated·mean·ABR·(95%·CI)¤	0.9·(0.02–5.48)¤	0.2·(0.00–4.15)¤	0.3·(0.00–4.23)¤	0.1·(0.00–3.92)¤	2.3·(0.35–7.75)¤
Calculated·median·ABR·(IQR)¤	0.0·(0.00–0.98)¤	0.0·(0.00–0.00)¤	0.0·(0.00–0.00)¤	0.0·(0.00–0.00)¤	1.0·(0.00–3.11)¤
Calculated·ABR·range¤	0.00–7.05¤	0.00–3.63¤	0.00–6.09¤	0.00–3.21¤	0.00–21.04¤
Participants·with·zero·bleeds,·n·(%)‡¤	48·(66.7)¤	64·(88.9)¤	59·(81.9)¤	68·(94.4)¤	24·(33.3)¤

▶ **Tab. 2** Safety Summary of the HAVEN 6 study.

Conclusion These data show continued efficacy and a favorable safety profile of emicizumab in people with non-severe HA without FVIII inhibitors who warrant prophylaxis

Conflict of Interest CH has received research funding from Bayer, Shire/Takeda, Pfizer, Novo Nordisk, CSL Behring, and Sobi; honoraria and speaker's bureau from Bayer, Shire/Takeda, Pfizer, Novo Nordisk, CSL Behring, Octapharma, Sobi, LFB, CAF-DCF, F. Hoffmann-La Roche Ltd., UniQure, and Bio Marin; **CN** has served as a consultant to BioMarin, Novo Nordisk, F. Hoffmann-La Roche Ltd. / Genentech, Inc., Sanofi, Sobi, and Shire/Takeda; has received research funding from Novo Nordisk, F. Hoffmann-La Roche Ltd. / Genentech, Inc., Sanofi, and Sobi; honoraria from Bayer, Novo Nordisk, F. Hoffmann-La Roche Ltd. / Genentech, Inc., Sanofi, Sobi, Spark, and Shire/Takeda; and has been a member of an entity's Board of Directors or advisory committees for Bayer, BioMarin, CSL Behring, Novo Nordisk, Pfizer, F. Hoffmann-La Roche Ltd. / Genentech, Inc., Sanofi, Sobi, Spark, Shire/Takeda, and UniQure; **ML** is an employee and holds stock in F. Hoffmann-La Roche Ltd.; **PC** has served on advisory boards for Bayer, Boehringer Ingelheim, CSL Behring, Chugai, Freeline, NovoNordisk, Pfizer, Roche, Sanofi, Spark, Sobi and Takeda; and has received research funding from Bayer, CSL Behring, Freeline, Novo Nordisk, Pfizer, SOBI and Takeda; **OC** is an employee of F. Hoffmann-La Roche Ltd.; **VJY** received grant/research support from Grifols, Novo Nordisk, F. Hoffmann-La Roche Ltd, Takeda, Bayer, CSL Behring, Pfizer, consultancy fees from Grifols, Novo Nordisk, F. Hoffmann-La Roche Ltd., Takeda, Bayer, CSL Behring, Pfizer, and BioMarin; **BMB** is an employee and stockholder in F. Hoffmann-La Roche Ltd; **CS** is an employee and stockholder in F. Hoffmann-La Roche Ltd., and is co-inventor of a patent related to an anti-FIXa/FX bispecific antibody; **GV** is employee and stockholder in F. Hoffmann-La Roche Ltd.; **JW** is an employee at Institute of Hematology and Transfusion Medicine; received research funding from Baxalta, Novo Nordisk, Rigel Pharmaceuticals, F. Hoffmann-La Roche Ltd, Shire and Takeda; honoraria from Alfasigma, Bayer AG, Baxalta, CSL Behring, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd, Sanofi-Aventis, Shire, Sobi, Swixx BioPharma, Takeda and Werfen; **AK** is an employee of and stockholder in F. Hoffmann-La Roche Ltd.; **RD** received research funding from Shire, Takeda, CSL Behring, LFB, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd, Biomari, Sobi and Sanofi; honoraria: Shire, Takeda, CSL Behring, LFB, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd, Biomarin, Sobi, Sanofi, UniQure and Spark; **PM** holds membership on an entity's Board of Directors or advisory committees for F. Hoffmann-La Roche Ltd Canada and Bayer; **SK** is an employee of Genentech, Inc.; **VT** is employee of F. Hoffmann-La Roche Ltd.; **AS** is an employee of Indiana Hemophilia and Thrombosis Center, Inc.; received consultancy fees from Sangomo Biosciences, Prometic Life Sciences, Sigilon; research funding from Bioverativ, Sanofi, Genentech, Inc., Kedrion Biopharma, Novo Nordisk, Pfizer, Sigilon, Takeda, ProMetic Life Sciences and Freeline; honoraria from Genentech, Inc., Sigilon, Novo Nordisk, Pfizer and Catalyst Biosciences; speaker's bureau from Genentech, Inc., Novo Nordisk; membership on an entity's Board of Directors or advisory committees for Novo Nordisk Hemophilia Foundation, Genentech, Inc., Sigilon, Novo Nordisk and Sanofi; **JO** has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai Pharmaceutical Co., Ltd., CSL Behring, Freeline, Grifols, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda

T-13-02 Endogenous FVIII activity and procedure-related FVIII use and bleeding: post hoc analysis of GENEr8-1

Authors Quon DV[1], Wang J-D[2], Wang M[3], Pepperell D[4], Park YS[5], Klamroth R[6], Kenet G[7], Mahlangu J[8], Khoo T-L[9], Robinson T[10], Chavele K-M[10], Pipe S W[11]

Institutes **1** Orthopedic Hemophilia Treatment Center, Los Angeles, USA; **2** Center for Rare Disease and Hemophilia, Taichung Veterans General Hospital, Taichung, Taiwan; **3** Hemophilia and Thrombosis Center, University of Colorado Anschutz Medical Campus, Aurora, USA; **4** Department of Haematology, Fiona Stanley Hospital, Murdoch, Australia; **5** Kyung Hee University Hospital at Gangdong, Seoul, South Korea; **6** Comprehensive Care Haemophilia Treatment Center, Vivantes Klinikum im Friedrichshain, Berlin, Germany; **7** The National Hemophilia Center, and Amalia Biron Research Institute of Thrombosis and Hemostasis, Sheba Medical Center, Tel Hashomer, Tel Aviv University, Tel Aviv, Israel; **8** Hemophilia Comprehensive Care Center, Charlotte Maxeke Johannesburg Academic Hospital, University of the Witwatersrand and NHLS, Johannesburg, South Africa; **9** Haematology Department, Haemophilia Treatment Center, The Royal Prince Alfred Hospital, Sydney, Australia; **10** BioMarin Pharmaceutical Inc., Novato, USA; **11** Departments of Pediatrics and Pathology, University of Michigan, Ann Arbor, USA

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Introduction GENEr8-1 (270-301; NCT03370913) is an ongoing phase 3 trial of valoctocogene roxaparvovec gene therapy for severe hemophilia A. Primary study outcomes demonstrate that valoctocogene roxaparvovec enables endogenous factor VIII (FVIII) production, reduces bleeding episodes, and reduces FVIII concentrate use versus prior FVIII prophylaxis treatment. Here, we report the procedures performed and associated FVIII use post-gene transfer with valoctocogene roxaparvovec in participants of the 301 trial.

Method The 270-301 trial enrolled male participants ≥ 18 years of age with severe hemophilia A previously on FVIII prophylaxis and negative for anti-AAV5 antibodies. All participants received a single infusion of 6x1013 vg/kg of valoctocogene roxaparvovec. Endogenous FVIII activity was assessed by chromogenic substrate assay (CSA) throughout the study and the closest CSA measurement to the time of a procedure or bleeding event was identified.Invasive procedures were defined as major or minor based on commonly used criteria (Solimeno 2018). Per protocol, exogenous FVIII could be used to control bleeding perioperatively at the discretion of the investigator.

Results The intention-to-treat (ITT) population consisted of 134 participants who received valoctocogene roxaparvovec. As of the 2-year data cutoff, in 77 subjects, a total of 260 procedures were performed. Of 111 invasive procedures, 67 required no exogenous FVIII treatment. Of the 44 procedures performed with FVIII treatment, 11 were major (eg, joint debridement, arthrodesis) and 33 were minor (eg, dental extraction, biopsies).

In the ITT population, the post-infusion mean (SD) endogenous FVIII activity level at week 52 was 42.4 (45.3) IU/dL and at week 104 was 22.7 (32.8) IU/dL. For participants who received FVIII treatment for a procedure, mean (range) endogenous FVIII level for a major or minor procedure was 15.6 (< 3–52.1) IU/dL or 16.2 (< 3–93.2) IU/dL, respectively, and 50.5 (< 3–255.7) IU/dL for participants who did not receive FVIII treatment. All 6 participants who underwent the 11 major procedures received perioperative exogenous FVIII regardless of their endogenous FVIII activity. More units of exogenous FVIII were administered for major procedures (mean [range], 255.4 [102.8–538.2] IU/kg) compared with minor procedures (67.2 [13.7–324.3] IU/kg).

Overall, 14 participants had 18 bleeding episodes related to a procedure (approximately 77 % occurring within 48 hrs). FVIII treatment was used for 13 bleeds; using the closest CSA measurement to the time of the bleed, mean (range) FVIII activity for those requiring or not requiring FVIII treatment was 11.0 (< 3–45.2) IU/dL and 60.4 (14.1–117.9) IU/dL, respectively.

Conclusion In GENEr8-1, post-infusion with valoctocogene roxaparvovec, most invasive procedures did not require FVIII treatment. Minor procedures where perioperative FVIII was administered were associated with lower participant endogenous FVIII activity.

Conflict of Interest **Doris V Quon** reports consulting fees from Roche/Genentech, Novo Nordisk, BioMarin Pharmaceutical Inc., Bayer, Takeda Pharmaceutical Company, Octapharma, and Sanofi; has participated as a clinical trial investigator for BioMarin Pharmaceutical Inc., Bioverativ/Sanofi, Roche/Genentech, Shire/Takeda, and uniQure; and has received speaker honoraria and travel support from Roche/Genentech, Novo Nordisk, Takeda, Sanofi, and BioMarin Pharmaceutical Inc. **Jiaan-Der Wang** reports consulting fees from Bayer, Novo Nordisk, Alnylam, Pfizer, Chugai, and Sanofi; and serves as a clinical trial investigator for BioMarin Pharmaceutical Inc., Pfizer, Sanofi, Bayer, Novo Nordisk and Chugai. **Michael Wang** reports consulting fees from BioMarin Pharmaceutical Inc., Bayer, Bioverativ, CSL Behring, Novo Nordisk, Genentech, Takeda, HEMA Biologics, and uniQure; and participation as a clinical trial investigator for BioMarin Pharmaceutical Inc., Bayer, Bioverativ, CSL Behring, Novo Nordisk, Genentech, Takeda, HEMA Biologics, uniQure, Pfizer/Spark, and Octapharma. **Dominic Pepperell** reports consulting fees from Sanofi and a travel grant from Pfizer. **Young Shil Park** reports research support or participation as a principal investigator from BioMarin Pharmaceutical Inc., CSL Behring, Novo Nordisk, Sanofi, Takeda, Pfizer, and Chugai. **Robert Klamroth** reports consulting payments including advisory boards from Bayer, Biotest, BioMarin, Novo Nordisk, Octapharma, Pfizer, Roche/Cugai, Sanofi, Takeda/Shire, and SOBI; clinical trial investigator for Bayer, Biotest, BioMarin Pharmaceutical Inc., Novo Nordisk Pharma Ltd., Octapharma, Pfizer, Roche/Chugai, Sanofi, Takeda/Shire, and SOBI; and speaker honoraria and travel support from Bayer; Biotest; BioMarin Pharmaceutical Inc.; CSL Behring; Daiichi Sankyo Co., Ltd.; LEO; Novo Nordisk Pharma Ltd.; Octapharma; Pfizer; Roche/Chugai; Sanofi; Takeda/Shire; and SOBI. **Gili Kenet** reports research support from Alnylam, Bayer, Opko Biologics, Pfizer, Shire; and honoraria for consultancy from Alnylam, Bayer, Novo Nordisk, Pfizer, Roche, and Takeda. **Johnny Mahlangu** reports consulting payments from Catalyst BioSciences, CSL Behring, F. Hoffman-La Roche Ltd., Novo Nordisk, Spark Therapeutics, and Takeda; research support and/or participation as a principal investigator from BioMarin Pharmaceutical Inc., CSL Behring, Novo Nordisk, F. Hoffman-La Roche Ltd, SOBI, and uniQure. **Steven W Pipe** reports consulting fees from Apcintex, ASC Therapeutics, Bayer, BioMarin Pharmaceutical Inc., CSL Behring, HEMA Biologics, Freeline Therapeutics, Novo Nordisk, Pfizer, Regeneron/Intellia, Roche/Genentech, Sanofi, Spark Therapeutics, Takeda, and uniQure; and service as a clinical trial investigator for BioMarin Pharmaceutical Inc., Freeline Therapeutics, Genentech/Roche, Sanofi, Spark Therapeutics and uniQure. **Tara M Robinson** and **Konstantia-Maria Chavele** are employees and stockholders of BioMarin Pharmaceutical Inc. **Teh-Liane Khoo** has no conflict of interest to report.

T-13-03 Influenza infections destabilize established immune tolerance in HemA mice

Authors Jurado-Mestre B[1], Kotov V[1], Oldenburg J[2], Albert T[2], Kurts C[1], Becker-Gotot J[1]

Institutes **1** University Clinic of Bonn, Institute of Molecular Medicine and Experimental Immunology, Bonn, Germany; **2** University Clinic of Bonn, Institute for Experimental Hematology and Transfusion Medicine (IHT), Bonn, Germany

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Introduction Hemophilia A (HemA) is the most common inherited bleeding disorder, caused by a deficiency of coagulation factor VIII (FVIII). Severely affected individuals follow a prophylactic treatment with intravenous replacement therapy of recombinant or plasma-derived FVIII. Unfortunately, up to 30 % of patients develop inhibitory antibodies (inhibitors) against this protein. Eradication of these inhibitors can be accomplished via immune tolerance induction (ITI) through frequent high-dose administration of FVIII, but tolerance is only achieved in about 70 % of patients. The molecular mechanisms concerning environmental factors which disrupt this process are mainly unknown.

Method In order to characterize the FVIII-specific B cell response, 2 UI per mouse of human recombinant FVIII were intravenously injected into HemA mice in weekly intervals for 3 weeks. To mimic high-dose injections performed during ITI therapy in clinical routine, one group of HemA mice received two injections of FVIII per week. To study the effect of external factors, we additionally infected ITI-treated mice with influenza. After 22 days splenocytes and blood were harvested and further analyzed by flow cytometry and ELISA, respectively.

Results First, we could show that immune tolerance against FVIII during ITI is initiated by programmed death-inducing ligand 1 (PD-L1)-expressing regulatory T cells (Treg) that ligate PD-1 on FVIII-specific B cells, causing them to undergo apoptosis. In contrast, FVIII-deficient mice injected with human recombinant FVIII in a prophylactic manner lacked such Treg and developed inhibitors. Furthermore, a viral infection like influenza A disrupted the induced tolerance resembled by an abrogated PD-1-expression on FVIII-specific B cells which in consequence enhanced the survival of inhibitor-forming B cells. And finally, the breakdown of tolerance is associated with a decrease of active FVIII in the serum.

Conclusion Here, we propose that ITI re-engages the PD-1-mediated immune tolerance mechanism against FVIII that operates in healthy individuals and in hemophilia A patients without inhibitors. Furthermore, this process is mediated by FVIII-specific Tregs and is abolished upon an infection.

Conflict of Interest The authors have declared that no conflict of interest exists.

T-13-04 Interim 52-Week analysis of immunogenicity to the vector capsid and transgene-expressed human FVIII in GENEr8-1, a phase 3 clinical study of valoctocogene roxaparvovec, an AAV5-mediated gene therapy for hemophilia A

Authors Long B, Robinson T, Day J, Yu H, Lau K, Patton K, de Hart G, Henshaw J, Agarwal S, Vettermann C, Gupta S
Institute BioMarin Pharmaceutical Inc., Novato, USA
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Introduction AAV-mediated gene therapy vectors represent a complex drug design with multiple components that may impact immunogenicity. Clinical trials monitor immunogenicity directed toward both the vector delivery system and the expressed transgene product. Valoctocogene roxaparvovec is an AAV5-mediated gene therapy under investigation for the treatment of hemophilia A and encodes a codon-optimized B-domain deleted human FVIII protein (hFVIII-SQ) under control of a liver-selective promoter. This report describes clinical immunogenicity monitoring data from up to 1 year of follow up from GENEr8-1, a Phase 3, single-arm, open-label study in 134 male participants with severe hemophilia A.

Method Prospective patients were required to test negative for anti-AAV5 total antibody (AAV5 TAb) utilizing a bridging electrochemiluminescent (ECLA) screening assay being developed as a companion diagnostic by ARUP Laboratories (Salt Lake City, USA). Additionally, all participants were required to have had at least 150 exposure days to FVIII replacement products without previous clinically detectable FVIII inhibitor development. Following dose administration, patient plasma was analyzed for AAV5 TAb, and a cell-based AAV5 transduction inhibition (TI) assay was used to further characterize the capsid-neutralizing potential of anti-AAV5 antibodies. Development of FVIII inhibitors was monitored using the Nijmegen-modified Bethesda assay, and FVIII-specific TAb were assessed by bridging ECLA. Additionally, peripheral blood mononuclear cells were collected for analysis in an IFN-g ELISpot assay for detection of capsid-specific and hFVIII-SQ-specific cellular immune responses.

Results No participants developed a clinically meaningful FVIII inhibitor response. All participants seroconverted to a high titer AAV5 TAb response following dose administration. The incidence of AAV5 capsid-specific cellular immune responses peaked at Week 2 following dose administration, declined over time and reverted to negative by Week 26 in the majority of subjects. A weekly positive correlation exists between Acapsid-specific IFNg ELISpot responses and plasma levels of the liver enzyme ALT. There was no association between AAV5-specific cellular immune responses and FVIII activity, and no association between FVIII-specific cellular immune responses and ALT or FVIII activity.

Conclusion Immune responses to valoctocogene roxaparvovec were predominantly directed toward the AAV5 capsid and characterized by the production of anti-AAV5 antibodies as well as transient AAV5 capsid-specific cellular immune responses. Though there was no diffinitive association, AAV5 capsid-specific cellular immune responses may be a contributing factor to transient elevations in ALT in some participants. There was no clinical evidence of inhibitor formation in subjects dosed with valoctocogene roxaparvovec.

Conflict of Interest Brian Long, Tara Robinson, Jonathan Day, Hua Yu, Kelly Lau, Kathryn Patton, Greg de Hart, Josh Henshaw, Suresh Agarwal, Christian Vettermann and Soumi Guptaare employees and stockholders of BioMarin Pharmaceutical Inc.

T-13-05 The effect of prophylactic corticosteroid treatment on adeno-associated virus-mediated gene therapy and potential mechanisms of action

Authors Handyside B, Zhang L, Yates B, Xie L, Sihn C-R, Murphy R, Bouwman T, Baridon B, Su C, Bullens S, Ismail AM, Bunting S, Fong S
Institute BioMarin Pharmaceutical Inc., Novato, USA
DOI 10.1055/s-0042-1760508

Introduction Adeno-associated virus (AAV)-based gene therapy can initiate host-immune responses to the AAV vector components or the transgenic protein product which could reduce AAV-mediated expression. Data from preclinical studies suggest that corticosteroid treatment may limit these immune responses and improve gene therapy outcomes.

Method In an earlier study, prednisolone treatment initiated one week after valoctocogene roxaparvovec (AAV5-hFVIII-SQ) administration had no impact on FVIII expression in mice. In a subsequent study we investigated if corticosteroid treatment initiated before AAV administration could improve transgene expression in mice. Mice were dosed daily for 4 weeks with prednisolone (2 mg/kg) or water starting 1 day or 2 hours before administration of 6x1013 vg/kg of an AAV5-HLP-hA1AT vector expressing human α1-antitrypsin (hA1AT) as a reporter.

Results Prednisolone-treated mice had significantly higher serum hA1AT protein levels (1.5- to 2.2-fold) starting 6 weeks after AAV dosing and through study end at 12 weeks. Furthermore, mice treated with prednisolone had a higher percentage of hepatocytes stained positive for vector DNA as well as higher liver full-length vector genome DNA and transgene RNA compared to the non–prednisolone-treated groups. This demonstrates that corticosteroid treatment before AAV administration can improve liver-directed AAV5 expression in mice.

To better understand potential mechanisms involved in this beneficial effect of prophylactic steroid treatment on AAV transduction we further investigated early events after AAV5 dosing in mice with or without prophylactic corticosteroid treatment. RNAseq analyses of liver samples were performed to evaluate differential expression 2h and 24h following AAV5-HLP-hA1AT administration in mice treated with and without prophylactic prednisolone. These analyses showed that prophylactic steroid treatment reduces acute innate immune responses to AAV. For example, serum IL-1b protein levels were increased 2h and 24h after AAV administration which were suppressed with prednisolone treatment.

Moreover, RNAseq showed an upregulation of the AAV5 cell surface co-receptor, PDGFRa, and downregulation of its competitive ligand PDGFa 2 hours after AAV administration in prednisolone treated mice compared to mice not treated with prednisolone. This suggests that prophylactic corticosteroid treatment may increase AAV5 capsid uptake by upregulation of the receptor and downregulation of the ligand.

Conclusion In summary, prophylactic corticosteroid treatment before AAV5 administration improved transgene expression potentially through multiple mechanisms, including suppression of the innate immune response and increased AAV uptake by cells.

Conflict of Interest Britta Handyside, Lening Zhang, Bridget Yates, Lin Xie, Choong-Ryoul Sihn, Ryan Murphy, Taren Bouwman, Brian Baridon, Cheng Su, Sherry Bullens, Ashrafali M. Ismail, Stuart Bunting, Sylvia Fong are employees and stockholders of BioMarin Pharmaceutical Inc.

T-13-06 Non-clinical pharmacodynamic effects and immunogenicity assessment of prophylactic immune modulation prior to gene therapy dose administration in C57BL/6 mice

Authors Long B, Handyside B, Seitel T, Boyer R, Wilson C, Obrochta K, Van Tuyl A, Arens J, Lau K, Gupta S
Institute BioMarin Pharmaceutical Inc., Novato, USA
DOI 10.1055/s-0042-1760509

Introduction The investigation of AAV vectored gene therapies has increased exponentially over the past decade for the treatment of monogenic disorders. Many clinical programs have adopted the use of corticosteroids or other immune modulatory therapies to prevent or treat inflammatory responses and preserve transgene expression following gene therapy dose administration, however there is no clear consensus across sponsors on the optimal immunosuppressive regimen. In the current study, we evaluated the pharmacodynam-

ic and immunomodulatory effects of seven different immunosuppressive agents administered prophylactically prior to gene therapy dose administration and continued for four weeks after.

Method Prednisolone, Mycophenolate (MMF), Rapamycin, Tacrolimus, Dimethyl Fumarate (DMF), Fingolimod (FTY720), and anti-IL6R antibody were administered to C57BL/6 mice starting either 1 or 3 days prior to gene therapy dosing and continued through Day 29. Mice were treated with an AAV5 vector delivering human a1-antitrypsin transgene driven by a liver-selective promoter (AAV5-HLP-hA1AT) on Day 1 and followed for 12 weeks and were evaluated at regular intervals for plasma protein concentrations of hA1AT, anti-AAV5 total binding antibody (AAV5 TAb), and inflammatory biomarkers. Liver tissue collected at termination was evaluated for vector genome copy number and RNA expression.

Results No plasma inflammatory biomarkers were significantly changed from baseline in the reference group following AAV5-HLP-hA1AT administration, however, prophylactic immune modulation did alter some cytokine and chemokine expression profiles. Overall, prophylactic administration of Prednisolone resulted in a statistically significant increase (~1.5 fold) in hA1AT plasma protein concentrations over the reference control, and IP administration of Rapamycin resulted in a transient reduction (~1 log titer reduction) in AAV5 TAb titer. Though there were trends of increased transgene expression with Tacrolimus, Mycophenolate, and IL-6R antagonist, the rest of the immune modulatory reagents did not show any statistically significant benefit over control with respect to either plasma hA1AT or suppression of AAV5 TAb.

Conclusion This study demonstrates that prophylactic immune modulation prior to AAV administration can improve liver-directed transgene expression in mice.

Conflict of Interest Brian Long, Britta Handyside, Theresa Seitel, Ryan Boyer, Chris Wilson, Kristin Obrochta, Andrea Van Tuyl, Jeremy Arens, Kelly Lau and Soumi Gupta are employees and stockholders of BioMarin Pharmaceutical Inc.

T-13-07 Pain relief with Eptacog beta in haemophilia patients with inhibitors

Authors Nowak-Göttl U[1], Hermans C[2], Mahlangu J[3], Ahuja S[4], Carcao M[5], Castaman G[6], Davis J[7], Khan O[8], Miesbach W[9], Reding M[10], Schved J-F[11], Villareal Martinez L[12], Wang M[13], Windyga J[14], Witkop M[15], Young G[16], Bonzo D[17], Mitchell I[18], Kessler C[19]

Institutes **1** University Medical Center Schleswig-Holstein, Kiel, Germany; **2** Catholic University of Louvain, Hemophilia Center of the Saint-Luc University Hospital, Brussels, Belgium; **3** University of the Witwatersrand, Johannesburg, South Africa; **4** Rainbow Babies & Children's Hospital, Cleveland, USA; **5** The Hospital for Sick Children, Toronto, Canada; **6** Center for Bleeding Disorders and Coagulation, Department of Oncology, Careggi University Hospital, Florence, Italy; **7** Pediatric Hemophilia Treatment Center, University of Miami, Miami, USA; **8** University of Oklahoma Health Sciences Center, Oklahoma City, USA; **9** Goethe University Hospital, Frankfurt, Germany; **10** University of Minnesota Medical Center, Minneapolis, USA; **11** University Hospital Montpellier, Montpellier, France; **12** Dr. José Eleuterio González Monterrey University Hospital, Monterrey, Mexico; **13** University of Colorado Denver, Aurora, USA; **14** Institute of Hematology and Transfusion Medicine, Warsaw, Poland; **15** National Hemophilia Foundation, New York City, USA; **16** Children's Hospital Los Angeles, Los Angeles, USA; **17** LFB-USA, Framingham, USA; **18** HEMA Biologics, Louisville, USA; **19** Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, USA

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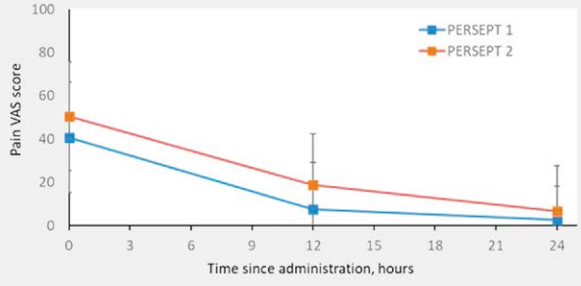
Introduction Bleeding episodes (BEs) into joints and muscles cause acute pain that can be reduced by quick and efficient treatment. Eptacog beta is a new recombinant coagulation factor VIIa approved by the EMA for the treatment of BEs in patients (≥ 12 years of age) with congenital hemophilia A or B with inhibitors (CHABwI) and for the prevention of bleeding in those undergoing surgery or invasive procedures. We herewith describe the impact of eptacog beta treatment on pain relief across two different age groups using data from the pivotal clinical trials.

Method Eptacog beta pain score data originated from two pivotal Phase 3 clinical trials of similar design that evaluated the efficacy and safety of eptacog beta on BE in CHABwI, including one (PERSEPT1) conducted in adults/adolescents and the other one (PERSEPT2) in children. Subjects received initial doses of eptacog beta 75 or 225µg/kg followed by 75µg/kg dose at predefined intervals determined by clinical response. Pain was assessed by the subject, guardian, or caregiver using a visual analog scale (VAS). Successful pain relief was defined by a VAS pain score at 12 hours after eptacog beta administration being lower to that recorded prior to eptacog beta administration. Descriptive statistics were applied for pain analysis.[1–2]

Results Of the 52 subjects evaluated, 27 were ≥ 12 years old with a mean (± SD) age of 31.3 (± 12.35) years and 25 < 12 years old with a mean (± SD) age of 4.9 (± 3.28) years. Majority of the 1017 treated BEs were successfully treated at 12 hours and at 24 hours in both age groups. The proportion of BEs with successful pain relief was approximately 90 % in both groups, irrespective of BE severity. From baseline until 24 hours after eptacog beta administration, the VAS pain score consistently decreased at all time points in both age groups: mean (± SD) VAS pain score of 40.8 (± 25.6) at baseline to 2.8 (± 8.2) at 24 hours in the ≥ 12 year-group and from 50.6 (± 25.1) to 6.7 (± 14.9) in the < 12 year-group, respectively. The median time to pain relief was 3 hours in the older group and above 5 hours in the younger. Concomitant analgesic requirement was less common among older vs younger patients (29.6 % vs 48 %, respectively) (▶ **Fig. 1**).



▶ **Fig. 1** Evolution of pain VAS score by time points.

Conclusion Eptacog beta resulted in clinically relevant pain reduction and bleed resolution across all age groups in CHABwI. A higher analgesic requirement and longer time to pain relief were observed in younger patients, possibly due to difficulty in assessing pain in this younger patient population.

Conflict of Interest Ulrike Nowak-Göttl: Grant/Research support: NovoNordisk; Consultant: LFB, Octapharma, Bayer

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T-13-08 EMIIL - Non-interventional study to investigate the effectiveness of Emicizumab under real-world conditions in pediatric, adolescent, adult and elderly patients with hemophilia A (PwHA) with and without FVIII inhibitors: an interim analysis

Authors Oldenburg J[1], Escuriola-Ettingshausen C[2], Fontana P[3, 4], Wenning S[5], Pfrepper C[6], Heidersdorf B[7], Udvardi P[8], Albisetti M[9]

Institutes **1** University of Bonn, Bonn, Germany; **2** Haemophilia Centre Rhine Main (HZRM), Mörfelden-Walldorf, Germany; **3** University Hospitals of Geneva, Division of Angiology and Haemostasis, Geneva, Switzerland; **4** University of Geneva, Geneva Platelet Group, Faculty of Medicine, Geneva, Switzerland; **5** SRH Kurpfalzkrankenhaus, Heidelberg, Germany; **6** University Hospital Leipzig, Division of Hemostaseology, Leipzig, Germany; **7** Roche Pharma AG, Grenzach, Germany; **8** Roche Pharma Schweiz, Basel, Switzerland; **9** University Children's Hospital, Hematology Department, Zürich, Switzerland

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Introduction Emicizumab is a monoclonal, humanized bispecific antibody binding to coagulation factors IXa and X and thereby taking over the coagulation function of activated factor VIII (FVIII) even in the presence of FVIII inhibitors1. Emicizumab is approved for routine prophylaxis in adult and pediatric patients with hemophilia A (PwHA) with or without FVIII inhibitors. The safety and efficacy of Emicizumab has been described under clinical trial conditions and it has shown a positive benefit/risk profile in phase III trials (HAVEN 1-4)2–5. This prospective non-interventional study (NIS) EMIIL aims to better understand the long-term effectiveness of Emicizumab prophylaxis in PwHA with and without FVIII inhibitors under real-world conditions.

Method EMIIL (ISRCTN58752772) is a single-arm, two cohorts, prospective, multicenter, non-interventional study collecting primary observational data in hemophilia A patients newly treated with Emicizumab. According to the presence of FVIII inhibitors, patients are enrolled in one of the two cohorts (▶ **Tab. 1**):

Efficacy Results	CAP of Cohort A (N=48)
Demographics	
Median Age [range]	22 years [0-75]
Majority age group:	
Adults (18-64 years) [n; n%]	26; 54.2%
Children (0-11 years) [n; n%]	17; 35.4%
Primary Objective	
GLM model-based ABR (95%CI)	0.624 (0.083 - 4.705)
Sensitivity analysis on calculated median ABR	0.00 (0.0 - 3.7)
Secondary Objectives	
Median treatment duration [days]	319.5 (range: 22 - 580)
Patients with zero treated bleeds n (%)	37 (77.1%)
Patients with zero spontaneous bleeds n (%)	43 (89.6%)
Newly developed FVIII inhibitors	none

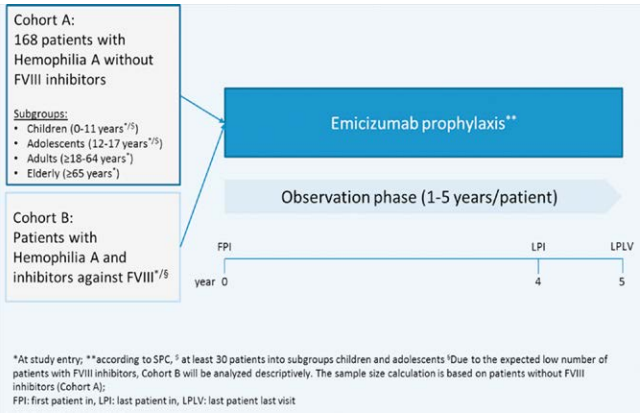
ABR = annualized bleeding rate; CAP = Core Analysis Population; CI = confidence interval; GLM = generalized linear model; n = number

▶ **Tab. 1** Study Design.

- Cohort A: Patients with congenital severe hemophilia A without FVIII inhibitors- Cohort B: Patients with congenital hemophilia A with FVIII inhibitors

Results As of Oct 14th 2022, 106 PwHA have been enrolled. The initial results on the effectiveness of Emicizumab in a real-world setting, particularly the results on GLM model-based annualized bleeding rate (ABR) of treated bleeds and the proportion of patients with zero bleeds appear to be similar to the results of previous clinical trials (Table 1). The interim analysis reveals no new safety signals. Updated, detailed efficacy and safety assessments data will be shared at the GTH 2023 Congress, covering bleed- and safety-related endpoints.

Conclusion The result shown here is based on the first yearly interim analysis. Due to the small number of PwHA included in this interim analysis, no main conclusions can be drawn from the study data. The data reported at GTH 2023 will be based on the second interim analysis and will include updated effectiveness data for Emicizumab under real-world conditions in pediatric, adolescent, adult and elderly patients with hemophilia A (PwHA) with and without FVIII inhibitors from n = 86 study participants.[1–5] (▶ **Fig. 1**).



▶ **Fig. 1** Demographics and Results of the first interim analysis.

Conflict of Interest JO reports having received grants for studies and research from Bayer, Biotest, CSL-Behring, Octapharma, Pfizer, SOBI and Takeda, and travel support as well as personal fees for lectures and advisory board meetings from Bayer, Biogen Idec, Biomarin, Biotest, CSL-Behring, Chugai, Freeline, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sparks, Swedish Orphan Biovitrum and Takeda. CEE has acted as a consultant, and received speaker's fees and/or research funding from Bayer, Biomarin, Biotest, Chugai, CSL Behring, Grifols, Kedrion, LFB, Octapharma, Novo Nordisk, Pfizer, Roche, Sobi, Takeda. PF reports travel grant support from Sobi and NovoNordisk. SW reports consultant and speaker's fees and/or research funding from Bayer, Biotest, Chugai, CSL Behring, Octapharma, Roche, Sobi, Takeda. CP reports grants for studies and research from Chugai/Roche, LeoPharma, Zacros, and Takeda, and personal fees for lectures or consultancy from Bayer, Chugai/Roche, CSL Behring, Novo Nordisk, Pfizer, BMS, SOBI, and Takeda. BH reports Employee, stock owner. PU reports Employee, stock owner. MA received travel and congress financial support from Sobi and Novo Nordisk.

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T-13-09 Low resolution cryo-EM maps and AFM analysis combined with alpha fold model of full-length coagulation Factor VIII sheds light on the conformational positioning of the Factor VIII B domain

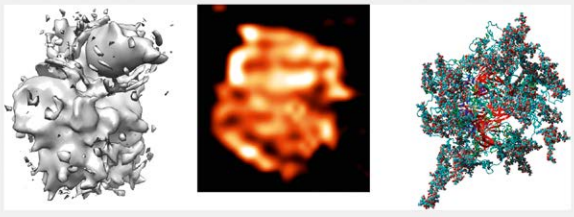
Authors Ramaraje urs SU[1], Ugurlar D[2], Ma B[2], Pellequer J-L[3], Teulon J-M[3], Fenel D[3], Javed H[1], Islam MM[1], Singh S[1], Oldenburg J[1], Biswas A[1]

Institutes 1 Universitätsklinikum Bonn, Institut für Experimentelle Hämatologie und Transfusionsmedizin, Bonn, Germany; 2 Thermo Fisher Scientific, Materials and Structural Analysis Division, GG Eindhoven, Netherlands; 3 Institut de Biologie Structurale, Methods and Electron Microscopy group, Grenoble, France
DOI 10.1055/s-0042-1760512

Introduction The coagulation factor VIII protein (FVIII) is a structurally well-characterized protein with almost 26 crystal structures corresponding to it deposited in the protein structure database. However, in all these structures, the central large glycosylated B domain remains structurally unresolved and therefore structural information on the full length (FL-FVIII) is currently missing. In the current study, we have analyzed the structure of the FL-FVIII using a combination of atomic force microscopy (AFM) and cryo-electron microscopy (cryo-EM) and the recently released alpha fold model.

Method The FL-FVIII was purified from two commercial sources of FL-FVIII, one a recombinant (Kovaltry) and the other one a plasma-derived concentrate (Haemoctin) using gel filtration chromatography. The highly pure protein was subjected to AFM and cryo-EM analysis. Both Kovaltry and Haemoctin were processed on Krios G4 platform. The data processing was performed using the cryoSPARC Software. Air and Liquid AFM was performed for Kovaltry on the AFM platform of IBS, Grenoble, France. Computational studies were performed on the alpha fold model of FL-FVIII. The model was treated in two different settings one where the model was glycosylated, and its heavy and light chains separated at the furin cleavage site and subjected to all atomic molecular dynamic simulation studies while in the other setting the non-glycosylated model subjected to MD simulation studies. The simulation-equilibrated models were analyzed with respect to the cryo-EM and AFM data.[1–3]

Results Cryo-EM and AFM analysis of FL-FVIII showed extensive conformational and particle heterogeneity for the sample purified from Kovaltry unlike Haemoctin which showed relative particle homogenity. Low-resolution maps were obtained from both Kovaltry and Haemoctin samples (6.3 Å and 8.3 Å respectively). Owing to the low resolution of these maps, the B domain was not resolved at an atomic level. However, by rigid fitting of specific domains of FL-FVIII we could identify spatial positioning of parts of the B domain. Computational studies showed that glycosylation has a significant impact in determining the final orientation of the B domain since the non-glycosylated model post equilibration showed an unfolded C terminal while in the glycosylated model, the B domain appeared to stable at a restrictive conformation guided by the formation of select intermediary secondary structures. The conformational variability observed in the cryo-EM and AFM studies was reflected well in the thermal motion observed within the simulation trajectory of the glycosylated alpha fold model of FVIII (▶ **Fig. 1**).



▶ **Fig. 1** Full-length coagulation Factor VIII structure; Side by side images of a 8.3 Å cryo-EM density map, liquid AFM and a simulation equilibrated glycosylated and furin cleaved structure of full-length coagulation FVIII (Left to Right).

Conclusion The FL-FVIII shows a significant amount conformational variability, most of which is attributed to the disordered B domain. Glycosylation plays a key role in the folding and stabilization of the B domain with respect to the rest of the FL-FVIII.

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Conflict of Interest None

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T-13-10 Blood-induced inflammation in hemophilia

Authors Knowles L M[1], Wolter C[1], Menger M D[2], Laschke M W[2], Haegele F[1], Eichler H[1], Pilch J[1]

Institutes 1 University of Saarland Medical Center, Institute of Clinical Hemostaseology and Transfusion Medicine, Homburg, Germany; 2 University of Saarland Medical Center, Institute for Clinical and Experimental Surgery, Homburg, Germany
DOI 10.1055/s-0042-1760513

Introduction The acute-phase response is elevated in hemophilia patients with acute and recent bleeds. Here, we provide evidence that the inflammatory response to injury is deregulated in hemophilia after bleeding.

Method We analyzed blood-induced inflammation and arthrofibrosis in knee joints of transgenic mice with hemophilia A compared to coagulation-competent wildtype mice.

Results To investigate inflammation in response to injury in hemophilia, we punctured the right knee of transgenic mice with hemophilia A with a hypodermic needle and analyzed the subsequent phases of wound healing. While this procedure caused only minor injuries in wildtype mice, we observed the development of large hematomas in the punctured knees of hemophilia mice between days 1 and 7 after puncture that turned irreversibly into arthrofibrosis by day 28. In contrast, blood injected into the knees of wildtype mice, was completely absorbed within 7 days and did not cause fibrosis. To assess inflammation during joint injury, we performed immunohistochemistry for detection of macrophages and neutrophils. In the knees of wildtype mice, we observed infiltrations of neutrophils and macrophages only on day 1 after blood injection while neutrophils in the punctured knees of hemophilia mice persisted beyond day 7. The pro-inflammatory effect in the punctured knees of hemophilia mice was also evident when we probed for macrophages, which in contrast to blood-injected control mice, became first visible after 7 days and persisted until day 56. No relevant blood cell infiltration occurred after saline injection of control mice.

Conclusion We conclude that the presence of free blood in joints causes inflammation and that the pro-inflammatory effect of blood is amplified in hemophilia mice.

Conflict of Interest None

T-13-11 Induction of neonatal tolerance to therapeutic FVIII in hemophilia A

Authors Reyes Ruiz A, Delignant S, Daventure V, Mimoun A, Dimitrov JD, Lacroix-Desmazes S

Institute Centre de Recherche des Cordeliers, INSERM, CNRS, Sorbonne Université, Paris, France
DOI 10.1055/s-0042-1760514

Introduction Injection to pregnant factor VIII (FVIII)-KO mice of the Fc-fused A2 and C2 domains of FVIII leads to their transplacental delivery and induction of partial FVIII-specific immune tolerance in the offspring. We hypothesized that the in utero exposure of the entire FVIII should confer complete FVIII-im-

mune tolerance. Unfortunately, Fc-fused FVIII (rFVIIIFc) crosses the placenta atinsufficient levels (0.03 ± 0.01nM) to foster FVIII-specific tolerance.

Method To evaluate the endocytosis (EEA-1 staining) and lysosomal routing (dextran staining) of Fc-fused molecules we used the human placental cell line BeWo and performed confocal mycroscopy assays. To decode which FVIII domain(s) prevent(s) its placental transfer, we generated different Fc-fused FVIII domains: A1Fc, A3C1Fc, C1Fc, C2Fc and A3C1C2Fc domains. Pregnant FVIII-KO mice were injected at E17.5 with 170picomoles of Fc-fused proteins. Fetuses were collected 4h later and the protein concentration was quantified by ELISA.

Results Using human placental cells, we observed a reduced endocytosis and increased routing towards lysosomes of rFVIIIFc as compared to well transfer protein C2Fc (4.71 ± 1.17nM). Our data show that the Fc-fused light chain of FVIII (A3C1C2Fc) is poorly transferred through the placenta (0.04 ± 0.005nM), suggesting that moieties in the light chain might be implicated in the retention of rFVIIIFc in mother's circulation or placenta. Interestingly, A1Fc, A3C1Fc, C1Fc and C2Fc were all transferred to levels > 1 nM in fetuses' blood.[1]

Conclusion The FVIII C1 and C2 domains are implicated in the interaction with different molecules of the coagulation cascade or catabolic receptors. We hypothesize that the co-engagement of the C1 and C2 domains of rFVIIIFc may explain, at least in part, the reduced transplacental delivery of rFVIIIFc. This research will guide us to optimize the conditions for immune tolerance induction towards FVIII in the offspring.

Conflict of Interest Grant / Research Support: SANOFI Bioverativ #161267A21, SOBI, ANR-18-CE17-0010-02 No. 18181LL, European Union's Horizon 2020 Program No 859974

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T-13-12 HEM-POWR study: Subgroup analysis evaluating the real-world effectiveness and safety of damoctocog alfa pegol in previously treated patients with haemophilia A in Germany

Authors Oldenburg J[1], Wenning S[2], Holstein K[3], von Depka Prondzinski M[4], Filip J[5], Miesbach W[6], Severin K[7], Eichler H[8], Halimeh S[9]

Institutes **1** University Clinic Bonn, Bonn, Germany; **2** SRH-Kurpfalzkrankenhaus Heidelberg, Heidelberg, Germany; **3** Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; **4** Werlhof-Institut, Hannover, Germany; **5** Bayer, Leverkusen, Germany; **6** Universitätsklinikum Frankfurt a.M., Frankfurt am Main, Germany; **7** MV-Zentrum für Onkologie und Hämatologie, Cologne, Germany; **8** Universität und Universitätsklinikum des Saarlandes, Homburg/Saar, Germany; **9** Universitätsklinikum Essen, Essen, Germany

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Introduction Damoctocog alfa pegol (BAY 94-9027) is an extended half-life recombinant factor VIII product approved for treatment of previously treated patients (PTPs) aged ≥ 12 years with haemophilia A [1, 2]. Previous analyses of the ongoing, real-world HEM-POWR study reported data from several clinical sites [3], with most patients recruited from Germany. Here we present effectiveness and safety data from the updated interim analysis of the HEM-POWR study for a subgroup of PTPs from German study sites.

Method HEM-POWR is a prospective, open-label, Phase IV cohort study (NCT03932201), and includes PTPs with mild, moderate or severe haemophilia A receiving damoctocog alfa pegol prophylactically or on demand. Primary and secondary outcomes included annualised bleeding rate (ABR) and safety. The safety analysis set (SAF) comprised PTPs with informed consent and ≥ 1 study dose in the observation period; inclusion in the full analysis set (FAS) required patients to fulfil all inclusion criteria, have a first documented dose of damoctocog alfa pegol in the study and ≥ 1 documented infusion during the observation period. Data were collected in patient diaries and physician records. Ethical approval was obtained for all sites (▶ **Tab. 1**).

Total number of patients in the HEM-POWR study, n	**SAF, n (n=268)**	**FAS, n (n=161)**
Characteristics for subgroup of patients from German study sites	**SAF, n (%) (n=61)**	**FAS, n (%) (n=30)**
Observation period, days, median (Q1, Q3)	262.0 (6.0, 406.0)	336.0 (154.0, 453.0)
Sex, male, n (%)	61 (100.0)	30 (100.0)
Age at enrolment, years, median (Q1, Q3)	35.0 (24.0, 51.0)	35.5 (24.0, 51.0)
Age at enrolment, years, n (%)		
<12	0	0
?12 to <18	6 (9.8)	4 (13.3)
?18 to <65	50 (82.0)	23 (76.7)
?65	5 (8.2)	3 (10.0)
Weight, kg, median (min, max)	80.0 (44.0, 185.0)*	80.0 (44.0, 185.0)†
Severity of haemophilia at initial diagnosis, n (%)		
Mild	1 (1.6)	0
Moderate	10 (16.4)	3 (10.0)
Severe	50 (82.0)	27 (90.0)
Patient history of inhibitors, yes, n (%)	14 (23.0)	8 (26.7)
Prescribed prophylaxis regimen of damoctocog alfa pegol at initial visit, n (%)		
Every day	4 (6.9)	3 (10.0)
Every 2 days	17 (29.3)	12 (40.0)
Every 3–4 days	19 (32.8)	6 (20.0)
Every 5 days	11 (19.0)	6 (20.0)
Every 7 days	7 (12.1)	3 (10.0)
Prophylactic treatment prior to enrolment, yes, n (%)	57 (93.4)	30 (100.0)
Prescribed dose per infusion per kg of damoctocog alfa pegol at baseline during the observation period, IU/kg, median (Q1, Q3)	34.9 (26.3, 44.0)‡	32.3 (27.0, 37.5)§
Patients pretreated with damoctocog alfa pegol	**SAF, n (%) (n=58)[2]**	**FAS, n (%) (n=28)**
Most recent prescribed dosing modality of damoctocog alfa pegol prior to initial visit, n (%)		
Prophylaxis	52 (91.2)	28 (100.0)
Intermittent prophylaxis	0	0
On demand	5 (8.8)	0

*Data missing for 22 patients; †data missing for 11 patients; ‡data missing for 12 patients; §data missing for 5 patients; ?data missing for 1 patient.

▶ **Tab. 1** Patient demographics, baseline characteristics and treatment exposure in the FAS and SAF; Patient demographics and characteristics during baseline and observation period in a subgroup analysis of patients from German clinical study sites. Baseline data include sex, age, weight and disease severity. Exposure in the SAF and FAS includes patients pretreated with damoctocog alfa pegol prior to initial visit, as well as the most recent dosing modality and dose per infusion.

Results At data cut-off (17 August 2022), 61 PTPs were included in the SAF of this sub-population. Most patients presented with severe disease (50/61, 82.0 %; moderate 10/61, 16.4 %; mild 1/61, 1.6 %) and were aged ≥ 18 to < 65 years (50/61, 82.0 %; aged ≥ 12 to < 18 years, 6/61, 9.8 %, Table 1). The median (Q1, Q3) observation period in the SAF was 262.0 (6.0, 406.0) days. Most patients were pretreated with damoctocog alfa pegol (58/61, 95.1 %), with 52/57 (91.2 %; 1 patient missing) having received prophylactic treatment. The median (Q1, Q3) prescribed dose per infusion per kg of damoctocog alfa pegol at baseline during the observation period was 34.9 (26.3, 44.0) IU/kg. A total of 14/61 patients (23.0 %) reported treatment-emergent adverse events, none of which were study drug-related. No inhibitor development or deaths were reported.

A total of 30 patients were included in the FAS and 27/30 (90.0 %) had severe haemophilia A. Total median (Q1, Q3), mean (SD) ABR during the observation period was 0.0 (0.0, 1.1), 1.2 (2.8). Total ABR decreased by 0.0 (-2.0, 0.0), 1.3 (3.0) compared with prior to damoctocog alfa pegol initiation. Data for bleed subtypes are summarised in Table 2. Overall, 22/30 patients (73.3 %) had no bleeds during the observation period, 26/30 (86.7 %) had no spontaneous bleeds and 24/30 (80.0 %) had no joint bleeds. PTPs had a mean (SD) 1.9 (0.9) infusions to control for a bleed, with 48.4 % of bleeds controlled with a single infusion (▶ **Fig. 1**).

Characteristic	FAS, n (%) (n=30)
ABR during the observation period, median (Q1, Q3), mean (SD)	
Total bleeds	0.0 (0.0, 1.1), 1.2 (2.8)
Spontaneous bleeds	0.0 (0.0, 0.0), 0.3 (0.9)
Trauma bleeds	0.0 (0.0, 0.0), 0.7 (2.3)
Joint bleeds	0.0 (0.0, 0.0), 0.4 (1.2)
Spontaneous joint bleeds	0.0 (0.0, 0.0), 0.2 (0.4)
Difference in ABR during the observation period compared with prior to damoctocog alfa pegol initiation, median (Q1, Q3), mean (SD)	
Total bleeds	0.0 (-2.0, 0.0), -1.3 (3.0)
Spontaneous bleeds	0.0 (0.0, 0.0), -0.7 (1.9)
Trauma bleeds	0.0 (-2.0, 0.0), -0.6 (2.1)
Joint bleeds	0.0 (-1.0, 0.0), -1.0 (2.0)
Spontaneous joint bleeds	0.0 (0.0, 0.0), -0.5 (1.4)

▶ **Fig. 1** ABR and difference in ABR throughout the study in the FAS; A summary of ABR during the observation period and ABR compared with prior to damoctocog alfa pegol initiation for total, spontaneous, trauma, joint and spontaneous joint bleeds in a German subgroup analysis in the FAS.

Conclusion These results from the updated interim analysis of the HEM-POWR study provide valuable insights into real-world clinical practice in Germany, inform German stakeholders, and further reinforce the favourable real-world effectiveness and safety of damoctocog alfa pegol in PTPs with mild, moderate or severe haemophilia A.

Conflict of Interest **JO**: reimbursed for attending symposia/congresses and/or received honoraria and/or funds for research from Bayer, Biogen Idec, Biotest, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Swedish Orphan Biovitrum and Takeda. **SW**: reimbursed for attending symposia/congresses and/or received honoraria and/or funds for research from Bayer, Biotest, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Swedish Orphan Biovitrum and Takeda. **KH**: received grants for studies and research from Bayer, Chugai/Roche, CSL Behring, Pfizer and Sobi, and personal fees for lectures or consultancy from Bayer, Biotest, Chugai/Roche, CSL Behring, Novo Nordisk, Pfizer, Sobi, and Takeda. **MvDP**: Received grants from Octapharma and Takeda, and personal fees from Octapharma. **WM**: acted as a paid consultant to Bayer, BioMarin, Biotest, CSL Behring, Chugai Pharma, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Takeda Pharmaceutical/Shire and UniQure, and received funding for research from Bayer, Biotest, CSL Behring, LFB, Novo Nordisk, Octapharma, Pfizer and Takeda/Shire. **KS**: none to disclose. **HE**: reimbursed for attending symposia/congresses and/or received honoraria and/or funds for research from Bayer Vital, BioMarin, Biotest, CSL Behring, Novo Nordisk, Pfizer, Roche and Sobi. **SH**: received grants for studies and research from Bayer, Biotest, CSL Behring, Novo Nordisk, Octapharma, Pfizer Pharma, Swedish Orphan Biovitrum and Takeda, and personal fees for lectures or consultancy Bayer, Biotest, CSL Behring, Chugai, Novo Nordisk, Octapharma, Pfizer, Roche, and Swedish Orphan Biovitrum.

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T-13-13 Updated interim safety analysis of the real-world HEM-POWR study evaluating damoctocog alfa pegol in previously treated patients with haemophilia A

Authors Herbst K[1], Oldenburg J[1], Alvarez Román MT[2], Sanabria M[3], Castaman G[4], Janbain M[5], Matsushita T[6], Meijer K[7], Schmidt K[8], Reding M[9]

Institutes 1 University Clinic Bonn, Bonn, Germany; 2 Hospital Universitario La Paz, Madrid, Spain; 3 Bayer, Basel, Switzerland; 4 Careggi University Hospital, Florence, Italy; 5 Tulane School of Medicine, New Orleans, USA; 6 Nagoya University Hospital, Nagoya, Japan; 7 University Medical Center Groningen, Groningen, Netherlands; 8 Bayer, Berlin, Germany; 9 University of Minnesota Medical Center, Minneapolis, USA

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Introduction Damoctocog alfa pegol (BAY 94-9027) is a PEGylated, extended half-life recombinant factor VIII product, approved for treatment of previously treated patients (PTPs) aged ≥ 12 years with haemophilia A [1, 2]. Real-world effectiveness and safety of damoctocog alfa pegol has been previously reported in earlier analyses of the HEM-POWR study [3]. Here we present the first safety results from the updated interim analysis.

Method HEM-POWR is a prospective, open-label, Phase IV cohort study (NCT03932201) in PTPs with mild, moderate or severe haemophilia A. Eligible patients included PTPs receiving damoctocog alfa pegol as prophylaxis or on-demand. The primary outcome was annualised bleeding rate (ABR) and secondary outcomes included safety. Data were collected in patient e-diaries and physician records. The safety analysis set (SAF) comprised PTPs with informed consent and ≥ 1 study dose in the observation period. Ethical approval was obtained for all sites (▶**Tab. 1**).

Characteristic	SAF, n (%) (n=268)
Observation period, days , median (Q1 , Q3), mean (SD)	233.5 (6.0, 420.0) , 264.7 (237.9)
Country of recruitment, n (%)	
Germany	61 (22.8)
Japan	52 (19.4)
Italy	30 (11.2)
United States of America	29 (10.8)
Canada	19 (7.1)
Taiwan	16 (6.0)
Denmark	15 (5.6)
Greece	13 (4.9)
Other*	33 (12.3)
Sex, male, n (%)	266 (99.3)
Age at enrolment, years, median (Q1 , Q3)	35.0 (23.0 , 48.0)
Age at enrolment, years, n (%)	
<12	1 (0.4)
?12 to <18	28 (10.5)
?18 to <65	224 (83.6)
?65	15 (5.6)
Weight, kg, median (min, max) †	80.0 (44.0, 185.0)
Severity of haemophilia, n (%) ‡	
Mild	8 (3.0)
Moderate	35 (13.1)
Severe	221 (82.5)
Patient history of inhibitors, yes, n (%) §	35 (13.1)
Prescribed prophylaxis regimen of damoctocog alfa pegol at initial visit , n (%)‡	
Every day	6 (2.3)
Every 2 days	32 (12.4)
Every 3–4 days	130 (50.4)
Every 5 days	51 (19.8)
Every 7 days	38 (14.7)
Prophylactic treatment prior to enrolment, yes, n (%)‡	247 (92.2)
Prescribed dose per infusion per kg of damoctocog alfa pegol at baseline during the observation period, IU/kg, median (Q1, Q3)§	37.5 (29.4, 48.4)
Patients pretreated with damoctocog alfa pegol	**SAF, n (%) (n=225)**
Most recent prescribed dosing modality of damoctocog alfa pegol prior to initial visit, n (%)‡	
Prophylaxis	210 (93.3)
Intermittent prophylaxis	2 (0.9)
On demand	10 (4.4)

*Other included Sweden, Spain, Belgium, Colombia, Slovenia and Netherlands (<10 patients each); †Data missing for 106 patients; ‡Data missing for <5 patients; §Data missing for 65 patients. IU, international unit; Q1, first quartile; Q3, third quartile; SAF, safety analysis set; SD, standard deviation .

▶ **Tab. 1** Demographics, baseline characteristics and exposure in the SAF; Demographics and characteristics during baseline and observation period include patients' country of recruitment, sex, age, weight and disease severity. Exposure in the SAF includes patients pretreated with damoctocog alfa pegol prior to initial visit, as well as the most recent prophylaxis regimen and dose per infusion.

Results At data cut-off (17 August 2022), 270 PTPs were enrolled, of which 268 (99.3 %) were included in the SAF. Two enrolled patients (0.7 %) were subsequently excluded from SAF analysis as they did not receive ≥ 1 dose of damoctocog alfa pegol during the observation period (mean [SD] 264.7 [237.9] days). Almost half of patients were from Germany (61/268, 22.8 %) and Japan (52/268, 19.4 %). Most patients presented with severe disease (221/268, 82.5 %) (Table 1). While most patients were aged ≥ 18 to < 65 years (224, 83.6 %), 28 (10.5 %) adolescent (< 18 years) and 15 (5.6 %) older (≥ 65 years) patients were included. Most patients (225/268, 84.0 %) were pretreated with damoctocog alfa pegol within 12 months of enrolment. The most common prophylaxis dosing regimen was every 3–4 days, both prior to enrolment (115/210, 54.8 %) and during the observation period (130/268, 50.4 %). A total of 110/268 patients (41.0 %) had a comorbidity, most commonly hypertension (41/268, 15.3 %), chronic pain (41/268, 15.3 %) and chronic arthropathy (35/268, 13.1 %) (▶ **Tab. 2**).

Characteristic	SAF, n (%) (n=268)
Any TEAE, n (%)	59 (22.0)
Any study drug-related TEAE	0 (0.0)
Any TEAE leading to change of treatment regimen	19 (7.1)
Any TEAE leading to discontinuation of treatment regimen	0 (0.0)
Any TEAE leading to inhibitor development	0 (0.0)
TEAE-related death	0 (0.0)
Any TEAE of special interest	2 (0.8)
Any serious TEAE, n (%)	19 (7.1)
Any study drug-related serious TEAE	0 (0.0)
Any serious TEAE leading to change of treatment regimen*	8 (3.0)
Rotator cuff syndrome	1 (0.4)
Foot fracture	1 (0.4)
Abscess limb	1 (0.4)
Injury (not specified)	1 (0.4)
Gastrointestinal haemorrhage	1 (0.4)
Haematochezia	1 (0.4)
Splenic haemorrhage	1 (0.4)
Cholecystitis	1 (0.4)
Any serious TEAE leading to discontinuation of treatment regimen	0 (0.0)
Any serious TEAE leading to inhibitor development	0 (0.0)
Serious TEAE-related death	0 (0.0)
Any serious TEAE of special interest	1 (0.4)

*Dose increased or interrupted. SAF, safety analysis set; TEAE, treatment-emergent adverse event.

▶ **Tab. 2** Summary of TEAEs in the SAF; A summary of treatment-emergent adverse events (TEAEs) includes any TEAEs or serious TEAEs, those leading to change of treatment regimen, discontinuation, inhibitor development or death.

Overall, 59/268 patients (22.0 %) reported any treatment-emergent adverse event (TEAEs), with 19/268 (7.1 %) reporting serious TEAEs (Table 2). Four adverse events of special interest (AESI) were reported in 2/268 patients (0.8 %); all were hypersensitivity reactions. No drug-related TEAEs, inhibitor development or deaths were reported. The most common TEAEs included various injuries or procedural complications (19/268, 7.1 %), infections or infestations (12/268, 4.5 %) and musculoskeletal or connective tissue disorders (10/268, 3.7 %).

Conclusion These updated interim data continue to support the favourable safety and tolerability profile of damoctocog alfa pegol in PTPs with mild, moderate or severe haemophilia A in a real-world setting.

Conflict of Interest **KH**: none to disclose. **MTR**: receipt of institutional research support from Bayer and BioMarin, member of advisory boards and/or speaker bureaus for Bayer, CSL Behring, Novo Nordisk, Sanofi Genzyme, Takeda and HEMA Biologics. **MTAR**: speaker in advisory boards and sponsored symposia with Novo Nordisk, Bayer, Takeda, Roche, Pfizer, Octapharma, Amgen, Novartis, CSL Behring and Sobi. **MS**: employee of Bayer. **GC**: speaker at satellite symposia during scientific meetings for Bayer, Grifols, LFB, Roche, Sobi, Novo Nordisk, Werfen and Kedrion, member of steering committee of UniQure, participant of advisory boards for Alexion, Bayer, BioMarin, Takeda, CSL Behring, LFB, Novo Nordisk, Pfizer, Roche, Sanofi, Sobi and UniQure, consultant for Roche. **MJ**: member of speaker bureau for Takeda, BioMarin, CSL Behring and Sanofi, consultancy for Takeda, CSL Behring, Sanofi, BioMarin, Genentech and Octapharma, member of Bayer steering committee. **TM**: member of advisory boards for Takeda, Bayer, Novo Nordisk, Chugai and Pfizer, receipt of educational and investigational support from Chugai and Novo Nordisk, received honoraria from Takeda, Bayer, Sanofi, Chugai, CSL Behring, JB Pharma, KMB Pharma, Novo Nordisk, Octapharma and Sysmex. **KM**: receipt of speaker fees from Bayer and Alexion, participation in trial steering committee for Bayer, receipt of consulting fees from UniQure. **KS**: employee of Bayer. **JO**: reimbursed for attending symposia/congresses and/or received honoraria and/or funds for research from Bayer, Biogen Idec, Biotest, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Swedish Orphan Biovitrum and Takeda.

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[3] Reding M, Alvarez Román M, Sanabria M, et al [abstract presented at ISTH 2022] https://abstracts.isth.org/abstract/annualised-bleeding-rate-and-joint-health-second-interim-analysis-of-the-real-world-hem-powr-study-evaluating-the-effectiveness-of-damoctocog-alfa-pegol-in-patients-with-haemophilia-a/. Accessed October 2022

T-13-14 Encore - emicizumab prophylaxis for the treatment of infants with severe hemophilia A without Factor VIII inhibitors: results from the interim analysis of the HAVEN 7 study

Authors Escuriola-Ettingshausen C[1, 2], Pipe S W[3], Collins P[4], Dhalluin C[5], Kenet G[6, 7], Schmitt C[5], Buri M[5], Jimenez Yuste V[8], Peyvandi F[9, 10], Young G[11], Oldenburg J[12], Mancuso ME[13], Kiialainen A[5], Chang T[14], Lehle M[5], Fijnvandraat K[15]

Institutes **1** Haemophilia Centre Rhine Main (HZRM), Mörfelden-Walldorf, Germany; **2** Presenting on behalf of the authors of the original abstract, Mörfelden-Walldorf, Germany; **3** University of Michigan, Ann Arbor, USA; **4** Cardiff University, School of Medicine, Cardiff, UK; **5** F. Hoffmann-La Roche Ltd, Basel, Switzerland; **6** Sheba Medical Center, Ramat Gan, Israel; **7** Tel Aviv University, Tel Aviv, Israel; **8** La Paz University Hospital, Autónoma University, Madrid, Spain; **9** Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia, Milan, Italy; **10** Thrombosis Center and Università degli Studi di Milano, Milan, Italy; **11** Children's Hospital Los Angeles, Los Angeles, USA; **12** University of Bonn, Bonn, Germany; **13** IRCCS Humanitas Research Hospital, Rozzano, Italy; **14** Spark Therapeutics, Inc., San Francisco, USA; **15** University of Amsterdam, Amsterdam, Netherlands

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Introduction Emicizumab (emi) prophylaxis can be started from haemophilia A (HA) diagnosis, reducing bleeding risk in infants with HA (IwHA). This interim analysis (IA) of HAVEN 7 (NCT04431726) evaluates the efficacy, safety and pharmacokinetics (PK) of emi in IwHA1.

Method HAVEN 7 is a Phase 3b, open-label study of emi in IwHA ≤ 12 months (m) without FVIII inhibitors. IwHA receive emi 3mg/kg weekly for 4 weeks (wks), then 3mg/kg every 2 wks for 52 wks; IwHA can then stay on this dose or switch to 1.5mg/kg weekly or 6mg/kg every 4 wks for the 7-year follow-up. Endpoints include: efficacy (treated bleeds; all bleeds; treated spontaneous bleeds; treated joint bleeds); safety; PK; and anti-emi antibodies (ADAs). Annualised bleed rates (ABR) are estimated using a negative binomial regression model.

Results At the IA cut-off (March 31, 2022),54 IwHA (55.6 % ≥ 3– ≤ 12m; 44.4 % < 3m; all male) had ≥ 1 dose of emi; median (range) age: 4.5 (0–11) m at baseline; 16 (1–26) m at cut-off. Minimally treated (≤ 5 exposure days with HA-related treatments (i.e., FVIII, plasma, cryoprecipitate or whole blood products)): 55.6 %; previously untreated: 44.4 %. Median (range) treatment duration: 42.1 (1–60) wks.Overall, 77 bleeds occured in 31 IwHA: 88.3 % traumatic; 5.2 % procedural; 6.5 % spontaneous.One IwHA had 12 bleeds (all traumatic; none joint/muscle; none treated). Overall, 14 treated bleeds, all traumatic, were

reported in 12 IwHA. No IwHA had > 2 treated bleeds. Mean model-based ABR (95 % confidence interval [CI]) for treated bleeds, all bleeds and treated joint bleeds were 0.4 (0.23–0.65), 1.9 (1.35–2.68) and 0.1 (0.01–0.22), respectively. In all, 77.8 % of IwHA had zero treated bleeds, while 42.6 % had no bleeds at all. In total, 92.6 % of IwHA had ≥ 1 adverse event (AE); 16.7 % of AEs were emi-related injection-site reactions. No AEs led to changes in or withdrawal from emi. Eight IwHA reported 12 serious AEs; none were considered emi-related. No deaths/thrombotic events/thrombotic microangiopathies have been reported. None of the 48 IwHA evaluable for immunogenicity tested positive for ADAs. Evaluable PK data in 52 IwHA showed mean trough concentrations of emi increased during loading and were maintained thereafter, slightly above 60µg/mL.[1]

Conclusion This IA indicates that emi is efficacious and well tolerated in IwHA without FVIII inhibitors.

Conflict of Interest CEE has acted as a consultant, and received speaker's fees and/or research funding from Bayer, Biomarin, Biotest, Chugai, CSL Behring, Grifols, Kedrion, LFB, Octapharma, Novo Nordisk, Pfizer, Roche, Sobi, Takeda. PC reported Grant/Research support/Funding statement: Research support from CSL Behring. Consultant: CSL Behring, Roche, Novonordisk. Membership on an entity's board of directors or advisory committees: Member of HAVEN7 steering committee. DC reported Employment: Roche Pharmaceuticals (Basel, Switzerland). KG reported Grant/Research support/Funding statement: BSF, Opko Biologics, Pfizer, Roche, Shire. Consultant: ASC therapeutics, Bayer, Biomarine, Novonordisk, Pfizer, Roche, Sanofi-Genzyme,Sobi, Takeda, Uniquore. Employment: Sheba Medical Center, Tel Hashomer, Israel; Tel Aviv University. Honoraria: Bayer, BioMarin, BPL, CSL, Pfizer,Novonordisk, Roche, Sanofi- Genzyme, Sobi, Spark, Takeda, Uniquore. Membership on an entity's board of directors or advisory committees: PedNet foundation. CS reported Shareholder and Employment F. Hoffmann - La RocheLtd. MB reported Shareholder and Employment F. Hoffmann-La Roche AG. FP reported Honoraria Educational meetings: Grifols, Roche. Membership on an entity's board of directors or advisory committees: Advisory boards: Sanofi, Sobi, Takeda, Roche, Biomarin. GY reported Grant/Research support/Funding statement: Genentech, Grifols, Takeda. Consultant: Bayer, Biomarin, CSL Behring, Genentech/Roche, LFB, Novo Nordisk, Pfizer, Sanofi, Spark, Takeda. Speakers' Bureau: Biomarin, Genentech, Hema Biologics, Sanofi, Spark. Honoraria: Bayer, Biomarin, CSL Behring, Genentech/Roche, Novo Nordisk, Pfizer, Sanofi, Spark, Takeda. Patents/Royalties: Viatris. **JO** has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai Pharmaceutical Co., Ltd., CSL Behring, Freeline, Grifols, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda. MEM acted as paid consultant, speaker and/or advisor for Bayer, Biomarin, CSL Behring, Grifols, Kedrion, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Spark Therapeutics, Sobi, Takeda and UniQure. AK reported Shareholder and Employment F. Hoffmann-La Roche. TC reported Shareholder: F. Hoffmann-La Roche AG. Employment: Spark Therapeutics. ML reported Shareholder and Employment F. Hoffmann-La Roche. CJF reported Grant/Research support/Funding statement: CSL Behring, Novo Nordisk, SOBI. Consultant: SOBI, Sanofi, NovoNordisk, Roche. Membership on an entity's board of directors or advisory committees: EAHAD.

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T-13-15 Safety and efficacy of recombinant Factor IX fusion protein (rIX-FP) in previously untreated patients with haemophilia B

Authors Lemons R[1], Wang M[2], Curtin J[3], Lepatan LM[4], Male C[5], Peyvandi F[6], Von Depka Prondzinski M[7], Wang R[8], McKeand W[8], Seifert W[9], Oldenburg J[10]

Institutes **1** University of Utah, Department of Pediatrics and Primary Children's Hospital, Salt Lake City, USA; **2** University of Colorado School of Medicine, Hemophilia and Thrombosis Center, Colorado, USA; **3** The Children's Hospital at Westmead, New South Wales, Australia; **4** Perpetual Succour Hospital, Cebu, Philippines; **5** Medical University of Vienna, Department of Paediatrics, Vienna, Austria; **6** Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy; **7** Werlhof Institute, Hannover, Germany; **8** CSL Behring, King of Prussia, USA; **9** CSL Behring, Marburg, Germany; **10** University Clinic Bonn, Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany

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Introduction Recombinant fusion protein linking coagulation factor IX (FIX) with albumin (rIX-FP) has been shown to be an effective and well-tolerated treatment for paediatric and adult patients with severe haemophilia B who had been previously treated with factor replacement therapy. This study investigated the safety and efficacy of rIX-FP in previously untreated patients (PUPs) (▶ **Fig. 1**).

Category	Prophylaxis Regimen, N=12	
	PUPs (%)	Events
Any TEAEs	11 (91.7)	109
Intensity:		
Mild	11 (91.7)	86
Moderate	5 (41.7)	21
Severe	2 (16.7)	2
TEAEs related to rIX-FP	2 (16.7)*	2*
Any SAEs	3 (25)†	3

▶ **Fig. 1** Summary of TEAEs in the SAF; A summary of treatment-emergent adverse events (TEAEs) includes any TEAEs or serious TEAEs, those leading to change of treatment regimen, discontinuation, inhibitor development or death.; * The two related TEAEs were recorded as hypersensitivity in the 11-year-old patient who later developed an inhibitor and development of a rash on lower legs and forearms in the second patient. The rash was considered mild in intensity, resolved within four days, and prophylaxis continued in this patient.; †Including the patient who developed an inhibitor (patient had a large gene deletion) and discontinued the study.

Method Patients with moderately severe/severe haemophilia B (≤ 2 % FIX) who had not previously received FIX replacement products received rIX-FP (25–75 IU/kg) via weekly prophylaxis or on-demand treatment over ≥ 50 exposure days (EDs). Primary outcomes were the number of patients who developed FIX inhibitors and incremental recovery. Secondary outcomes included incidence of adverse events and annualised bleeding rates (ABRs) (▶ **Fig. 2**).

Parameter	<6 years N=7	≥6 to ≤12 years N=1	Total N=8
Baseline-corrected PK parameters, mean (SD)			
C_{max} (IU/dL)	63.17 (15.8)	39.90	60.26 (16.8)
IR (IU/dL)/(IU/kg)	1.29 (0.4)	0.80	1.23 (0.4)
Steady-state trough FIX activity on 7-day prophylaxis regimen			
Mean steady-state trough FIX activity, IU/dL (SD)	12.49 (6.1)	-	12.49 (6.1)
Median steady-state trough FIX activity, IU/dL (range)	11.85 (2.7–31.3)	-	11.85 (2.7–31.3)

▶ **Fig. 2** FIX activity and PK parameters after a single rIX-FP dose (50 IU/kg); Cmax, maximum concentration; %CV, percent coefficient of variation; IR, incremental recovery; IU, international unit; N, number of PUPs receiving 50 IU/kg; PK, pharmacokinetic; PUP, previously untreated patient; rIX-FP, recombinant fusion protein linking coagulation factor IX with albumin.

Results In total, 12 paediatric PUPs with a mean age of 1.3 years were treated with rIX-FP with a mean of 68.3 EDs (6/12 prophylaxis; 6/12 on-demand then prophylaxis). Overall, 11/12 patients did not develop FIX inhibitors; one 11-year-old patient who had previously received packed red blood cells developed inhibitors against FIX after 8 EDs and was ultimately withdrawn. Of the 137 treatment-emergent adverse events recorded, only 5 were attributed to rIX-FP. No thromboembolic events or deaths occurred during the study. Across all treatments, 93.8 % of spontaneous bleeding events were controlled with 1–2 rIX-FP infusions. On the prophylaxis regimen, 12 patients had an ABR of 0–3.89, 9 patients had an annualized spontaneous bleeding rate of 0. Seven patients with available data had a mean steady-state FIX activity of 12.5 IU/dL.

Conclusion This Phase 3 extension study provides data to support the safety and efficacy of rIX-FP in paediatric PUPs requiring on-demand or prophylactic treatment for moderately severe/severe haemophilia B, consistent with results in previously treated patients.

Conflict of Interest Advisor, consultancy and/or speaker fees, grants and/or research support from the following pharmaceutical companies were disclosed:; RL; CSL Behring, Novo Nordisk.; MW; Bayer, BioMarin, BioVerativ, CSL Behring, Genetech, Novo Nordisk, Takeda, uniQure.; JC; Bioverativ (paid to the institution), CSL Behring, Shire/Takeda; Bioverativ, Pfizer, Roche, and Sanofi, BioMarin, Freeline (paid to institute).; LL; Pfizer.; CM; Bayer, Takeda, Biotest, Novo Nordisk, Sobi, Biotest, CSL Behring, Grifols, Pfizer, Roche.; FP; Sanofi, Sobi, Takeda and Roche.; MVDP;Baxter, Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Novo Nordisk, Octapharma, Pfizer, Roche and Sobi, Grifols.; RW, WM and WS are employees of CSL Behring.JO; Baxter, Bayer, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma and Pfizer, Biogen Idec, Chugai, Grifols, Roche and Sobi.

T-13-16 Digital joint discovery tool to support hemophilia patient education

Authors Goldmann G, Marquardt N, Oldenburg J
Institute Universitätsklinikum Bonn, Ins. f. Exp. Hämatologie und Trans.-Medizin, Bonn, Germany
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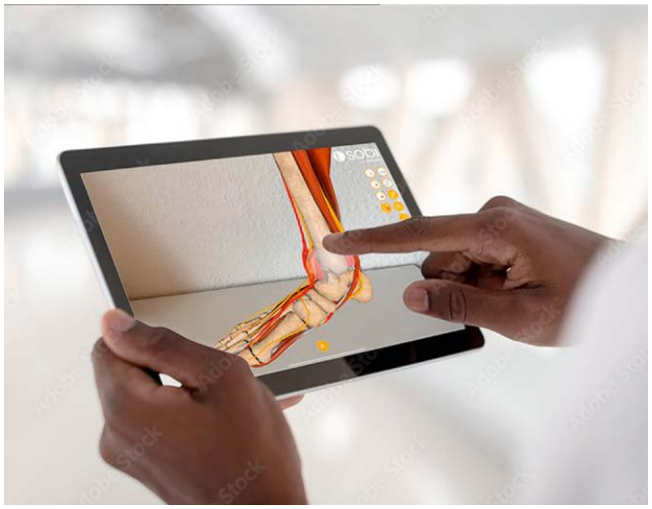
Introduction Recurrent joint bleeding is the most frequent clinical manifestation of severe hemophilia. Unless appropriately managed, even subclinical hemarthrosis can lead to the development of hemophilic arthropathy and chronic pain. The new joint discovery tool shows the anatomical structure of the ancle in different stages of hemophilic arthropathy. The aim of this project is to support HCPs patient information and communication on site (▶ **Fig. 1**).



▶ **Fig. 1** Digital joint discovery tool.

Method Imaging diagnostics of hemophilic patients have been selected such as MRI, ultrasound and X-ray findings and integrated into the development process. This step was followed by a visual transposition as 3D-animation (incl. rotating, tilting, zooming) of the changes in the ancle joint and occurring joint hemorrhages in the course of the disease over time.

Results Defined tissue structures can be visualized such as skin, skeleton, capsule as well as most important muscles, ligaments, fascias, vessels and nerves. Corresponding different stages can be visualized strengthening patient/caregiver education: 1. healthy joint 2. acute joint bleed 3. chronic joint bleed 4. first joint destruction 5. joint destruction. The pathomechanisms underlying the individual clinical picture e.g. changes in the joints such as swelling can be demonstrated. The interactive applications of the 3D-visualization can be sent to the physician via link. App (IOS) and web application are available to support patient communication and education. The joint can also be virtually projected into the room by means of augmented reality.[1]

Conclusion This first joint discovery can contribute to raising patients awareness thus e.g. offering the potential to support adherence to a beneficial prophylactic treatment regime. User surveys have been prepared to accompany the current implementation. The device could be enlarged for further joints e.g. knee and elbow. Potential applications and future developments of this digital tool are currently under evaluation.

Conflict of Interest The joint discovery tool was supported by Swedish Orphan Biovitrum GmbH. The tool can be downloaded from the website pro.sobi.com

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T-13-17 Exploring red blood cells as a novel tolerogenic approach for Factor VIII inhibitors employing immuno-dominant FVIII derived peptides presented on MHC class II

Authors Miranda M[1], Brandsma E[2], Kaijen P[1], Van Alphen F[3], Van Bruggen R[1], Fijnvandraat K[4], Lacroix-Desmazes S[5], Voorberg J[1]
Institutes **1** Sanquin, Department of Molecular Hematology, Amsterdam, Netherlands; **2** Sanquin, Department of Hematopoiesise, Amsterdam, Netherlands; **3** Sanquin, Research Facilities, Amsterdam, Netherlands; **4** University of Amsterdam, Department of Pediatric Hematology, Amsterdam, Netherlands; **5** Centre De Recherche Des Cordeliers, Paris, France
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Introduction The main complication of hemophilia A treatment is the development of neutralizing antibodies (inhibitors) against factor VIII (FVIII). The eradication of anti-FVIII antibodies relies on immune tolerance induction (ITI). Since ITI is efficient in only 60-80 % of cases, within the EDUC8-consortium we are developing innovative methods to reduce the immunogenicity of biotherapeutics. Here we employed bioinformatics and proteomic-based peptide presentation assays to identify promiscuously presented FVIII peptides to use in pioneering immuno-tolerogenic approaches. To this end, we are exploring red blood cells (RBCs) as innovative antigen delivery system to induce tolerance.

Method A database of FVIII peptides presented on different MHC class II alleles was assembled and compared to predicted MHC class II presented epitopes using the 'Immune Epitope Database (IEDB)' website. An additional data-set of naturally processed FVIII peptides was generated by incubating human FVIII with immature monocytes-derived DCs from HLA-typed healthy donors. Specific attention was on the identification of FVIII peptides presented on HLA-DP4, since these alleles are highly prevalent in the Caucasian population.

Results The IEDB website identified a large number of FVIII core peptides presented by multiple MHC class II alleles. To supplement this data-set we successfully developed a mass-spectrometry based protocol to study HLA-DR and HLA-DP antigen presentation utilizing specific monoclonal antibodies. Using this novel approach, approximately 4000 HLA-DR-associated peptides were identified. We detected over 100 HLA-DR and 7 HLA-DP4 presented FVIII

peptides. An HLA-DR presented FVIII peptide was fused to a cell-penetrating peptide and incubated with RBCs. FACS analysis revealed its internalization in a dose-dependent manner. FVIII peptide-treated RBCs phagocytosis by macrophages was assessed using microscopy and MHC class II presentation of FVIII-derived peptides on macrophages was validated. We are currently exploring whether the presentation of immunodominant FVIII peptides on MHC class II results in tolerance induction.

Conclusion Our data provide an inventory of promiscuously presented FVIII-derived peptides which will guide the development of novel tolerogenic approaches for FVIII inhibitors employing RBCs as carrier.

Conflict of Interest There is no conflict of interest.

T-13-18 Emicizumab phamacodynamics assessed by thrombin generation and fibrin clot formation

Authors Bertaggia Calderara D[1], Marchi Cappelletti R[2], Batista Mesquita Sauvage A P[1], Durual S[3], Gomez F J[1], Zermatten M G[1], Aliotta A[1], Casini A[4], Alberio L[1]

Institutes **1** Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Division of Hematology and Central Hematology Laboratory, Lausanne, Switzerland; **2** Faculty of Medicine, University of Geneva, Department of Genetic Medicine and Development, Geneva, Switzerland; **3** University of Geneva, Biomaterials laboratory, University Clinics of Dental Medicine, Geneva, Switzerland; **4** Faculty of Medicine, University of Geneva, Division of Angiology and Hemostasis, University Hospitals of Geneva, Geneva, Switzerland

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Introduction Emicizumab, a bispecific antibody mimicking the activity of FVIII, is used to treat patients with hemophilia A (PwHA) with or without inhibitors. While it is possible to measure its plasma concentration, we cannot assess emicizumab pharmacodynamics.The identification of biological markers reflecting the in vivo hemostatic competence of patients under emicizumab would have a great clinical value.

The aim of this study was to investigate whether global coagulation assays (GCA), measuring thrombin generation (TG) and fibrin clot formation (FCF) could be used for monitoring the biological response to non-factor replacement therapy with emicizumab.

Method We monitored over a period of two months the response of six adult PwHA without inhibitors to emicizumab (Hemlibra F Hoffmann-La Roche, Basel, Switzerland) therapy. PwHA received a weekly dose of emicizumab of 3 mg/kg during weeks (W) 1-4 (loading phase) and 1.5 mg/kg from W5 onwards (maintenance). TG, FCF and fibrin clot structure (FSC) were investigated in platelet poor plasma. Results obtained at emicizumab plateau were compared to patients' baseline, FVIII replacement at peak, and values measured in healthy donors. TG was assessed by Calibrated Automated Thrombogram assay (Stago, France) using low tissue factor; FCF was investigated with Thrombodynamics analyzer (Hemacore, Russia) in a tissue-factor dependent and independent coagulation model and with turbidity assay (polymerization and lysis); FCS was visualized with scanning electron microscopy.

Results

- Steady state plasma concentrations of emicizumab were stable for the individual patients but showed a very large inter-individual variation.
- Parameters of TG and FCF significantly increased compared to patient baseline already during the loading phase, reaching a plateau that lasted until the end of monitoring, but remained at the lower limits of their respective reference values.
- At emicizumab plateau and compared to baseline, fibrin clot network became denser and characterized by thinner fibrin fibers and pores of smaller size close to normal plasma.
- Clot lysis time was significantly increased at emicizumab plateau compared to baseline.
- Of note, patients with similar plasma concentrations of emicizumab displayed very different degrees of individual hemostatic correction.

Conclusion This work suggests the usefulness of GCA to monitor non-factor replacement in PwHA without inhibitors. Our data show that GCA measuring TG and in particular FCF are able to capture emicizumab pharmacodynamics. Noteworthy, individual PwHA achieve variable levels of hemostatic correction despite similar plateau concentrations of emicizumab. These observations indicate that TG and FCF are markers of emicizumab biological activity and that their use might allow personalization of emicizumab treatment. A multi-centric clinical study is needed to evaluate this hypothesis.

Conflict of Interest This study was supported by the 2021 annual research grants of Swiss Hemophilia Network (Switzerland) awarded to Debora Bertaggia Calderara. Lorenzo Alberio is supported by a grant from the Swiss National Science Foundation (SNF 320030-197392), the other authors have nothing to disclose.

T-13-19 PreviouslyuntreatedpatientsinGermany2017 - 2021 – Update 2022

Authors Bidlingmaier C[2], Türknetz M[1], Schultze-Strasser S[1], Escuriola Ettingshausen C[3], Kentouch K[4, 5], Olivieri M[7], Zieger B[6], Königs C[1]

Institutes **1** University Hospital Frankfurt, Goethe University, Department of Paediatrics and Adolescent Medicine, Clinical and Molecular Haemostasis, Frankfurt am Main, Germany; **2** Dr Von Hauner Children's Hospital, University Hospital, Department of Paediatrics and Adolescent Medicine, Paediatric Hemostasis and Thrombosis Unit, Department of Pediatrics, Munich, Germany; **3** HRZM, Moerfelden-Waldorf, Germany; **4** Universitätsklinikum Jena, Klinik für Kinder- und Jugendmedizin, Jena, Germany; **5** Städtisches Klinikum Braunschweig gGmbH, Braunschweig, Germany; **6** Universitätsklinikum Freiburg, Zentrum für Kinder- und Jugendmedizin, Freiburg, Germany; **7** LMU, Pediatric Hemostasis and Thrombosis Unit, Department of Pediatrics, Dr Von Hauner Children's Hospital, University Hospital, Munich, Germany

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Introduction Treatment and outcome data for previously untreated patients (PUPs) with haemophilia A or B (HA or HB) in Germany to date are very limited. In this respect, questions remain about the optimal timing and treatment regimens for PUPs to prevent bleeding and the emergence of neutralising antibodies. The German Paediatric Haemophilia Research Data Base - GEPHARD - was set up as a multicentre observational cohort study to collect and analyse data on PUPs on a nation-wide scale.

Method The GEPHARD study includes all children and adolescents (< 18 years) diagnosed with HA or HB (FVIII or FIX levels < 25 %) since 1st January 2017. This is a prospective study involving most treatment sites in Germany. The registry concentrates on outcomes including development of inhibitors, provides quality assurance as well as serving as a basis for future studies. The first longitudinal analysis was conducted II/2022.

Results The database was implemented in close cooperation with PedNet. Since 1 January 2017, 389 children and adolescents (50-65/y) from 40 participating centres have been reported.Longitudinal data of 82 HA and 46 HB were recorded until 31 January 2021. Of these, 63 and 28 were diagnosed with severe HA and HB, respectively. The median age at diagnosis correlated with severity. Almost all patients with data on treatment regimens were receiving prophylaxis at the time of analysis. By the cut-off date, patients with HA were mainly receiving recombinant extended half-life factors (EHL), followed by non-replacement therapies (NRT) and, recombinant concentrates (SHL).Patients with HB mainly received FIX EHL, followed by SHL. The use of pd concentrates increased, while the use of SHL reduced, depending on the year of diagnosis of the HA patients. The use of EHL increased somewhat, while the use of NRT increased substantially. Among 13 patients with HA and one patient with HB, a current inhibitor was detected.

Conclusion The GEPHARD registry is established and registers an increasing number of participating centres. The GEPHARD community is actively reporting and providing a first data set on the German treatment situation. The longitudinal documentation is quickly increasing and allows further analyses in the nearby future.

Conflict of Interest None Declared

T-13-20 Adults with severe or moderately severe haemophilia B receiving etranacogene dezaparvovec in the HOPE-B phase 3 trial experience a stable increase in mean Factor IX activity levels and durable haemostatic protection after 24 months' follow-up

Authors Pipe S W[1], Leebeek F WG[2], Recht M[3], Key N S[4], Lattimore S[5], Castaman G[6], Coppens M[7], Cooper D[8, 9], Gut R[8, 9], Slawka S[8, 9], Verweij S[8, 9], Dolmetsch R[8, 9], Li Y[10], Monahan P E[10], Miesbach W[11]

Institutes **1** University of Michigan, Ann Arbor, USA; **2** Erasmus MC, University Medical Center, Rotterdam, Netherlands; **3** Yale University, New Haven, USA; **4** University of of North Carolina, Chapel Hill, USA; **5** Oregon Health & Science University, Portland, USA; **6** Center for Bleeding Disorders and Coagulation, Careggi University Hospital, Florence, Italy; **7** Department of Vascular Medicine, Amsterdam Cardiovascular Sciences, Amsterdam UMC, University of Amsterdam, Amsterdam, Netherlands; **8** uniQure BV, Amsterdam, Netherlands; **9** uniQure Inc., Lexington, USA; **10** CSL Behring, King of Prussia, USA; **11** University Hospital Frankfurt, Frankfurt, Germany

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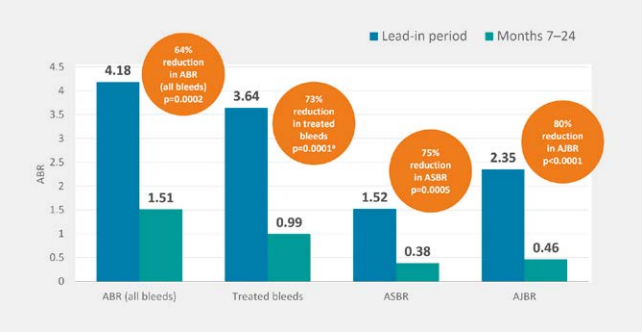
Introduction Etranacogene dezaparvovec (formerly AMT-061) is an investigational gene therapy for haemophilia B comprising an adeno-associated virus serotype 5 (AAV5) vector and a codon-optimised factor IX (FIX) Padua R338L transgene with a liver-specific promoter. The Phase 3 HOPE-B trial (NCT03569891) aims to assess the efficacy and safety of etranacogene dezaparvovec.

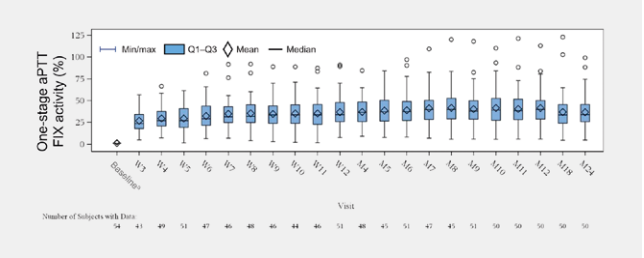
Method In this open-label, single-arm trial, adult males with severe/moderately severe haemophilia B (FIX ≤ 2 %), with/without pre-existing AAV5 neutralising antibodies (NAbs), received etranacogene dezaparvovec (single dose; 2x1013 gc/kg) following a ≥ 6-month lead-in period on FIX prophylaxis. FIX activity, annualised bleed rate (ABR), and FIX infusions were assessed frequently during the lead-in and first 12 months after dosing, then every 6 months during long-term follow-up (Years 2–5). Adverse events (AEs) were recorded.

Results Of the 54 participants, 53 received the full dose and 52 completed 24 months of follow-up. At 464 days (~15 months) post-infusion, one 75-year-old participant died from cardiogenic shock, preceded by a urinary tract infection (unrelated to treatment). Of the 54 participants, 52 (96.3 %) discontinued and remained free of continuous FIX prophylaxis from Day 21 to Months 7–24, including 20 participants with baseline AAV5 NAb titres < 1:700. One participant with a markedly higher AAV5 NAbs titre (1:3212) and one participant who received a partial vector dose (due to an infusion-related reaction) did not express FIX Padua or discontinue FIX prophylaxis. Compared with the lead-in period (mean ABR 4.18), mean ABR (all bleeds) during Months 7–24 post-treatment was significantly reduced by 64 % (mean ABR 1.51; p = 0.0002), sustaining the same bleed reduction that satisfied the primary endpoint of the trial during Months 7–18. Mean ABR for all other bleed types was substantially reduced (▶ **Fig. 1**). Mean FIX activity was 39.0 IU/dL (standard deviation [SD]: ± 18.7; min–max: 8.2–97.1) at Month 6 (n = 51) and 36.7 IU/dL (± 19.0; 4.7–99.2) at Month 24 (n = 50; ▶ **Fig. 2**).

There was an overall 97 % reduction in mean unadjusted annualised FIX consumption from the lead-in period to Months 7–24 (257,339 vs. 8946 IU/year/participant; p < 0.0001). At 24 months post-dose, 38 participants (70.4 %) experienced 93 treatment-related AEs; only one occurred after Month 24 (Day 735 post-dosing). There was an increase in alanine transaminase (with/without increased aspartate transaminase) in 11 participants (20.4 %); 9 participants (16.7 %) received reactive corticosteroids for a mean duration of 79.8 days (SD: 26.6; range: 51–130 days). There were no serious AEs related to study treatment.



▶ **Fig. 1** Improvement in ABR: Months 7-24 post-treatment



▶ **Fig. 2** FIX activity level oder time.

Conclusion After 24 months' follow-up, single-dose etranacogene dezaparvovec resulted in stable FIX Padua expression in participants with AAV NAbs undetected or < 1:700 titer; reduction in ABR remained durable and superior to FIX prophylaxis.

Conflict of Interest SWP has received research funding from Siemens and YewSavin; and consulted for Apcintex, ASC Therapeutics, Bayer, BioMarin, CSL Behring, GeneVentiv, HEMA Biologics, Freeline, LFB, Novo Nordisk, Pfizer, Regeneron/Intellia, Roche/Genentech, Sanofi, Takeda, Spark Therapeutics and uniQure. FWGL has received research support from CSL Behring, Takeda, Sobi and uniQure; consulted for uniQure, Sobi, BioMarin and Takeda; and was a DSMB member for a study by Roche. MR has received research funding from Bayer, BioMarin, CSL Behring, Genentech, Grifols, Hema Biologics, LFB, Novo Nordisk, Octapharma, Pfizer, Sanofi, Spark, Takeda, Spark Therapeutics and uniQure; consulted for Catalyst Biosciences, CSL Behring, Genentech, Hema Biologics, Kendrion, Novo Nordisk, Pfizer, Sanofi, Takeda and uniQure; stood on the board of directors for Foundation for Women and Girls with Blood Disorders Partners in Bleeding Disorders; and was employed by American Thrombosis and Hemostasis Network. NK has acted as the Grants Committee Chair for Novo Nordisk, consulted for CSL Behring and uniQure, and BioMarin. SL participated in an Advisory Board for UniQure. GC consulted for and received honoraria from Bayer, Roche, Sobi, Grifols, Novo Nordisk, Werfen and Kedrion; received research funding from CSL Behring, Pfizer and Sobi; and was a member of the Board of Directors or advisory committees for Ablynx, Alexion, Bayer, Takeda, CSL Behring, Novo Nordisk, Pfizer, Roche, Sanofi, SOBI and uniQure. MC has received financial support for research from Bayer, CSL Behring, Daiichi Sankyo, Portola/Alexion, Roche and uniQure; and consultancy or lecturing fees from Bayer, CSL Behring, Novo Nordisk, Pfizer and Sobi. DC, RG, SS, SV and RD are employed by uniQure. YL and PEM are employed by CSL Behring. WM received funding from Bayer, BioMarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sobi, Takeda/Shire and uniQure.

T-13-21 Durability of bleeding protection and Factor IX activity in individuals with and without adeno-associated virus serotype 5 neutralising antibodies (Titres <1:700) in the phase 3 HOPE-B trial of etranacogene dezaparvovec gene therapy for haemophilia B

Authors Pipe S W[1], Leebeek F WG[2], Recht M[3], Key N S[4], Lattimore S[5], Castaman G[6], Cooper D[7,8], Verweij S[7,8], Dolmetsch R[7,8], Tarrant J[9], Li Y[9], Monahan P E[9], Miesbach W[10]

Institutes **1** University of Michigan, Ann Arbor, USA; **2** Erasmus MC, University Medical Center, Rotterdam, Netherlands; **3** Yale University, New Haven, USA; **4** University of North Carolina, Chapel Hill, USA; **5** Oregon Health & Science University, Portland, USA; **6** Center for Bleeding Disorders and Coagulation, Careggi University Hospital, Florence, Italy; **7** uniQure BV, Amsterdam, Netherlands; **8** uniQure Inc., Lexington, USA; **9** CSL Behring, King of Prussia, USA; **10** University Hospital Frankfurt, Frankfurt, Germany

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Introduction Pre-existing adeno-associated virus (AAV) neutralising antibodies (NAbs) previously limited the efficacy of AAV-based gene therapy. The Phase 3 HOPE-B clinical trial (NCT03569891), which assesses etranacogene dezaparvovec, an AAV5 vector expressing Padua factor IX (FIX), enrolled participants regardless of their baseline AAV5 NAb status. Here we investigate the safety and efficacy of etranacogene dezaparvovec in HOPE-B participants with (NAb+) and without (NAb-) AAV5 NAbs over 18 and 24 months.

Method Adult males with severe/moderately severe haemophilia B (FIX ≤2%; NAb+ or NAb-) received etranacogene dezaparvovec (single infusion; 2x1013 gc/kg) after a ≥6-month lead-in period receiving FIX prophylaxis. FIX activity, annualised bleed rate (ABR) and use of replacement FIX concentrates were assessed regularly during the lead-in and first 12 months post-dose, then every 6 months during long-term follow-up (Years 2–5). Adverse events were recorded. AAV5 NAbs were assessed on the day of dosing (baseline; AAV5 transduction inhibition assay) (▶ **Tab. 1**).

		Baseline	6 months	12 months	18 months	24 months
AAV5 NAb-	n	33	33	32	33	33
	Median (min–max)	1.0 (1.0–2.0)	37.30 (8.4–97.1)	38.65 (5.9–113.0)	35.0 (4.5–122.9)	35.40 (4.7–99.2)
	Mean (SD)	1.15 (0.36)	40.61 (18.64)	44.82 (23.21)	39.87 (24.08)	38.55 (19.19)
AAV5 NAb+	n	21	18	18	17	17
	Median (min–max)	1.0 (1.0–2.0)	35.60 (8.2–90.4)	39.95 (8.5–73.6)	32.00 (10.3–57.9)	33.50 (9.1–88.3)
	Mean (SD)	1.24 (0.44)	35.91 (19.02)	35.54 (17.84)	31.14 (13.75)	32.98 (18.51)

▶ **Tab. 1** Summary of FIX activity (%) by baseline Nab status (FAS)

Results Fifty-four participants were dosed (NAb-, n = 33; NAb+, n = 21 at baseline). Median (Q1–Q3) AAV5 NAb titre in NAb+ participants was 56.9 (23.3–198.9); 20/21 (95%) NAb+ participants had titres of <1:700. One participant (titre 3212 pre-dosing) did not express FIX Padua, and one participant (titre 198.9) received a partial dose. All other participants (52/54) discontinued FIX prophylaxis. At 18 and 24 months post-dose, no correlation between baseline AAV5 NAb titre and FIX activity level was identified, up to titre 1:700 (24-month Pearson's r: -0.29; Spearman's rho: -0.25; R2: 0.086). Sustained FIX activity levels (median [min–max]) were demonstrated at 18 months post-dose in NAb+ <1:700 (32.0% [10.3–57.9]) and NAb- (35.0% [4.5–122.9]) participants and at 24 months post-dose in NAb+ <1:700 (33.5% [9.1–88.3]) and NAb- (35.4% [4.7–99.2]) participants (Table 1). NAb+ <1:700 and NAb- participants had low ABRs during Months 7–18 (1.30 and 0.93) and 7–24 (1.65 and 0.80; Table 2). These ABRs were significantly improved vs lead-in (NAb+ <1:700: 4.29 [vs Months 7–18 p = 0.0005; vs Months 7–24 p = 0.0065]; NAb-: 3.80 [vs Months 7–18 p < 0.0001; vs Months 7–24 p < 0.0001]).

The safety profiles were similar between NAb subgroups. At 24 months, corticosteroid-treated transaminase elevations occurred in 6/33 NAb- (18.2%) and 3/21 NAb+ (14.3%) participants. Infusion-related reactions occurred in 2/33 NAb- (6.1%) and 5/21 NAb+ (23.8%) participants. There was no statistically significant association between infusion-related reactions and NAb status (p = 0.0956) (▶ **Tab. 2**).

Endpoint	≥ 6-month lead-in period	Months 7–18 post-treatment period			Months 7–24 post-treatment period		
	Adjusted ABR (95% CI)[a]	Adjusted ABR (95% CI)[a]	Rate ratio (post-treatment / lead-in) (95% CI)[a,b]	p-value[c]	Adjusted ABR (95% CI)[a]	Rate ratio (post-treatment / lead-in) (95% CI)[a,b]	p-value[c]
All bleeding episodes (FAS; n = 54)	4.38 (3.21–5.44)	1.51 (0.81–2.82)	0.36 (0.20–0.64)	0.0002	1.51 (0.83–2.76)	0.36 (0.21–0.63)	0.0002
All bleeding episodes (FAS; baseline AAV5 NAb <1700) (n = 53)	3.86 (2.89–5.17)	1.07 (0.63–1.81)	0.27 (0.17–0.43)	<0.0001[d]	1.09 (0.67–1.79)	0.28 (0.17–0.46)	<0.0001[d]
All bleeding episodes (baseline AAV5 NAb-) (n = 33)	3.80 (2.56–5.65)	0.93 (0.44–1.98)	0.25 (0.14–0.43)	<0.0001[e]	0.80 (0.39–1.67)	0.21 (0.12–0.37)	<0.0001[e]
All bleeding episodes (baseline AAV5 NAb+ <1700) (FAS; n = 20)	4.29 (3.06–6.01)	1.30 (0.63–2.70)	0.30 (0.15–0.61)	0.0005[f]	1.65 (0.84–3.26)	0.39 (0.18–0.82)	0.0065[g]

▶ **Tab. 2** HOPE-B primary endpoint (ABR) by baseline Nab status

Conclusion Through 24 months of follow-up, etranacogene dezaparvovec provided significant reductions in ABR and an acceptable safety profile, regardless of NAb status. There was no association between baseline NAb status (up to titre <1:700) and long-term durability of FIX expression.

Conflict of Interest SWP has received research funding from Siemens and YewSavin; and consulted for Apcintex, ASC Therapeutics, Bayer, BioMarin, CSL Behring, GeneVentiv, HEMA Biologics, Freeline, LFB, Novo Nordisk, Pfizer, Regeneron/Intellia, Roche/Genentech, Sanofi, Takeda, Spark Therapeutics and uniQure. FWGL has received research support from CSL Behring, Takeda, Sobi and uniQure; consulted for uniQure, Sobi, BioMarin and Takeda; and was a DSMB member for a study by Roche. MR has received research funding from Bayer, BioMarin, CSL Behring, Genentech, Grifols, Hema Biologics, LFB, Novo Nordisk, Octapharma, Pfizer, Sanofi, Spark, Takeda, Spark Therapeutics and uniQure; consulted for Catalyst Biosciences, CSL Behring, Genentech, Hema Biologics, Kendrion, Novo Nordisk, Pfizer, Sanofi, Takeda and uniQure; stood on the board of directors for Foundation for Women and Girls with Blood Disorders Partners in Bleeding Disorders; and was employed by American Thrombosis and Hemostasis Network. NK has acted as the Grants Committee Chair for Novo Nordisk, consulted for CSL Behring and uniQure, and BioMarin. SL participated in an Advisory Board for uniQure. GC consulted for and received honoraria from Bayer, Roche, Sobi, Grifols, Novo Nordisk, Werfen and Kedrion; received research funding from CSL Behring, Pfizer and Sobi; and was a member of the Board of Directors or advisory committees for Ablynx, Alexion, Bayer, Takeda, CSL Behring, Novo Nordisk, Pfizer, Roche, Sanofi, SOBI and uniQure. DC, SV, RD and JT are employed by uniQure. YL and PEM are employed by CSL Behring. WM received funding from Bayer, BioMarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sobi, Takeda/Shire and uniQure.

T-13-22 Improvements in health-related quality of life in adults with severe or moderately severe haemophilia B after receiving etranacogene dezaparvovec gene therapy

Authors Itzler R F[1], Miller J C[2], Monahan P E[1], Pipe S W[3]

Institutes **1** CSL Behring, King of Prussia, USA; **2** Everest Clinical Research, Little Falls, USA; **3** University of Michigan, Pediatrics and Pathology, Ann Arbor, USA

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Introduction Despite current prophylactic therapy for haemophilia B, breakthrough bleeding still occurs, negatively affecting health-related quality of life (HRQoL). The pivotal Phase 3 HOPE-B trial of the investigational adeno-associated virus gene transfer product, etranacogene dezaparvovec, demonstrated sustained factor IX (FIX) activity and bleed protection superior to FIX concentrate prophylaxis. Here we report the impact on HRQoL.

Method Fifty-four adults with haemophilia B (FIX ≤ 2 %) received standard-of-care FIX concentrate prophylaxis in the 6-month lead-in period followed by a single infusion of etranacogene dezaparvovec. HRQoL was assessed with the haemophilia-specific Hem-A-QoL during the lead-in and at 6 and 12 months after etranacogene dezaparvovec. Repeated measures linear mixed models estimated the difference in scores before and after gene therapy. A one-sided p-value ≤ 0.025 for the post-treatment, lead-in period was considered statistically significant. The analyses were not adjusted for multiplicity.

Results Significant model-based mean differences in scores and the percentage improvement compared with the lead-in period were as follows: Total Score (least square [LS] mean -5.50; p < 0.0001; 21.5 %), domains 'Treatment' (LS mean -14.88; p < 0.0001; 59.0 %), 'Feelings' (LS mean -9.42; p < 0.0001; 45.7 %), 'Future' (LS mean -5.02; p = 0.0023; 16.2 %) and 'Work/School' (LS mean -4.99; p = 0.0036; 28.8 %). Results were not significant for the six remaining Hem-A-QoL domains. 'Treatment' reflects how burdened participants are by their haemophilia treatments. 'Feelings' reflects current emotions associated with having haemophilia. 'Future' reflects concerns about how haemophilia will affect participants' life plans. 'Work/School' reflects how well participants think they perform these responsibilities.

Conclusion HRQoL improvements after gene therapy demonstrate that etranacogene dezaparvovec can reduce the burden associated with haemophilia and FIX prophylactic therapy. This may contribute to how people with haemophilia B view their work/school performance as well as providing a sense of optimism for the future.

Conflict of Interest RFI and PEM are employed by CSL Behring. JCM is employed by Everest Clinical Research, contracted by CSL Behring. SWP has received research funding from Siemens and YewSavin; and consulted for Apcintex, ASC Therapeutics, Bayer, BioMarin, CSL Behring, GeneVentiv, HEMA Biologics, Freeline, LFB, Novo Nordisk, Pfizer, Regeneron/Intellia, Roche/Genentech, Sanofi, Takeda, Spark Therapeutics and uniQure.

T-13-23 Durability of Factor IX activity and bleeding rate in people with severe or moderately severe haemophilia B after long-term follow-up in the phase 1/2 Study of AMT-060, and phase 2b and phase 3 studies of etranacogene dezaparvovec (AMT-061)

Authors Miesbach W[1], Recht M[2], Key N[3], Sivamurthy K[4], Monahan P E[4], Pipe S W[5]

Institutes **1** University Hospital Frankfurt, Frankfurt, Germany; **2** Yale University, New Haven, USA; **3** University of North Carolina, Chapel Hill, USA; **4** CSL Behring, King of Prussia, USA; **5** University of Michigan, Ann Arbor, USA

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Introduction Gene therapy for haemophilia B (HB) appears to have a durable response, with data presented at 5 years [1] and 8 years [2] post-dose. We review the durability of AMT-060 and etranacogene dezaparvovec (formerly AMT-061), defined by sustained factor IX (FIX) activity and haemostatic protection. Etranacogene dezaparvovec is similar to AMT-060, differing only in a single amino acid substitution in the F9 gene encoding the highly active, naturally occurring Padua variant. Therefore, durability of response is expected to be similar for AMT-060 and etranacogene dezaparvovec.

Method Three clinical trials are ongoing assessing AMT-060 or etranacogene dezaparvovec in adults with severe/moderately severe HB. Data have been captured at 5 years in the Phase 1/2 AMT-060 study (N = 10; Cohort 1: n = 5, 5x1012 gc/kg; Cohort 2: n = 5, 2x1013 gc/kg; NCT02396342), 3 years in the Phase 2b etranacogene dezaparvovec study (N = 3, 2x1013 gc/kg; NCT03489291) and 2 years in the Phase 3 etranacogene dezaparvovec HOPE-B study (N = 54, 2x1013 gc/kg; NCT03569891).

Results In the Phase 1/2 AMT-060 study, mean FIX activity (one-stage assay) remained stable in both cohorts at 5 years (Cohort 1, 52 weeks: 4.4 %, Year 5: 5.2 %; Cohort 2, 26 weeks: 6.9 %, Year 5: 7.4 %). At 3 and 2 years of follow-up in the Phase 2b and 3 studies, respectively, mean FIX activity remained in the near-normal range (Table). In the Phase 2b study, mean FIX activity increased from Week 3 (23.4 %; n = 3) to Year 3 (36.9 %; n = 2). Mean FIX activity was sustained in the Phase 3 study (Month 6: 38.95 %, n = 51; Year 2: 36.66 %, n = 50). This was observed in the full analysis set (FAS; n = 54) and modified intent-to-treat population (n = 52) that excluded two non-responders (one with an adeno-associated virus 5 neutralising antibody titre of 3212; one that received a partial dose; Table). In the Phase 1/2 AMT-060 study, mean annualised bleeding rates (ABR) were maintained from Year 1–5 (Cohort 1: 7.30–6.40; Cohort 2, 1.58–0.20). In Phase 2b, no bleeding episodes occurred between 2.5 and 3 years of follow-up, and ABR at 3 years was 0.22. Two bleeding episodes occurred in one participant < 18 months post-dose; the participant received FIX product replacement therapy (1700 IU) after each bleed. In the Phase 3 study, ABR (all bleeds) at Months 7–18 and 7–24 post-dose was 1.51 (reduced from 4.18 at baseline; FAS, n = 54; Table). ABR reduced from 4.00 at baseline to 0.95 at Months 7–24 in the modified intent-to-treat population (n = 52). Reductions in all bleed types were maintained at Months 7–24. No new safety signals were identified at 5 and 3 years of follow-up for AMT-060 and etranacogene dezaparvovec, respectively (▶ **Tab. 1**).

		Week		Month					
	Baseline[a]	3	6	6	12	18	24	30	36
Phase 2b									
Mean FIX activity level (%)	n=1 5.10	n=3 23.40	n=3 30.57	-	n=3 40.77	n=2 46.95	n=3 44.20	n=3 50.03	n=2 36.90
Phase 3, full analysis set									
Mean FIX activity level (%)	n=54 1.19	-	-	n=51 38.95	n=50 41.48	n=50 36.90	n=50 36.66	-	-
Mean ABR (all bleeds)	n=54 4.18	-	-	-	0–12 months n=54 1.33	7–18 months n=54 1.51	7–24 months n=54 1.51	-	-
Phase 3, modified intent-to-treat population[b]									
Mean FIX activity level (%)	n=52 1.19	-	-	n=51 38.95	n=50 41.48	n=50 36.90	n=50 36.66	-	-
Mean ABR (all bleeds)	n=52 4.00	-	-	-	-	-	7–24 months n=52 0.95	-	-

▶ **Tab. 1** Mean uncontaminated FIX activity levels and ABR after etranacogene dezaparvovec

Conclusion Gene therapy for HB appears to have a durable response. In the Phase 1/2 AMT-060 study and the Phase 2b and Phase 3 etranacogene dezaparvovec studies, FIX activity is sustained and reductions in bleeding events remain stable.

Conflict of Interest WM received funding from Bayer, BioMarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sobi, Takeda/Shire and uniQure. MR has received research funding from Bayer, BioMarin, CSL Behring, Genentech, Grifols, Hema Biologics, LFB, Novo Nordisk, Octapharma, Pfizer, Sanofi, Spark, Takeda, Spark Therapeutics and uniQure; consulted for Catalyst Biosciences, CSL Behring, Genentech, Hema Biologics, Kendrion, Novo Nordisk, Pfizer, Sanofi, Takeda and uniQure; stood on the board of directors for Foundation for Women and Girls with Blood Disorders Partners in Bleeding Disorders; and was employed by American Thrombosis and Hemostasis Network. NK has acted as the Grants Committee Chair for Novo Nordisk, consulted for CSL Behring and uniQure, and BioMarin. KS and PM are employed by CSL Behring. SWP has received research funding from Siemens and YewSavin; and consulted for Apcintex, ASC Therapeutics, Bayer, BioMarin, CSL Behring, GeneVentiv, HEMA Biologics, Freeline, LFB, Novo Nordisk, Pfizer, Regeneron/Intellia, Roche/Genentech, Sanofi, Takeda, Spark Therapeutics and uniQure.

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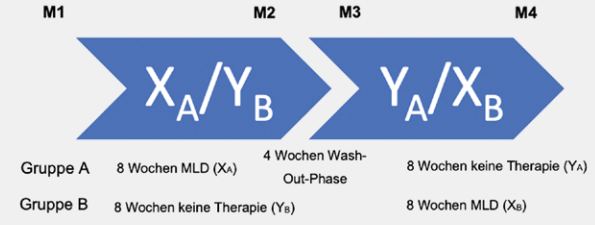
T-13-24 Randomized-controlled cross-over pilot study on the effectiveness of manual lymphatic drainage in patients with hemophilic arthropathy

Authors Dayo J, Miesbach W
Institute Universitätsklinikum Frankfurt a.M., Frankfurt, Germany
DOI 10.1055/s-0042-1760527

Introduction Patients with hereditary hemophilia often clinically present with signs of spontaneous bleeding in joints. If bleeding occurs more than three times in the same joint within six months, it is defined as a target joint. Affected joints can be swollen, painful and restricted in movement. Most seriously patients can develop chronic destructive hemophilic arthropathy.**Manual lymphatic drainage (MLD)** is a special skin displacement technique used in complex decongestion therapy to treat swollen parts of the body and to remove interstitial inflammatory mediators by stimulation of lymphatic vascular system (LVS) (▶ **Fig. 1**).

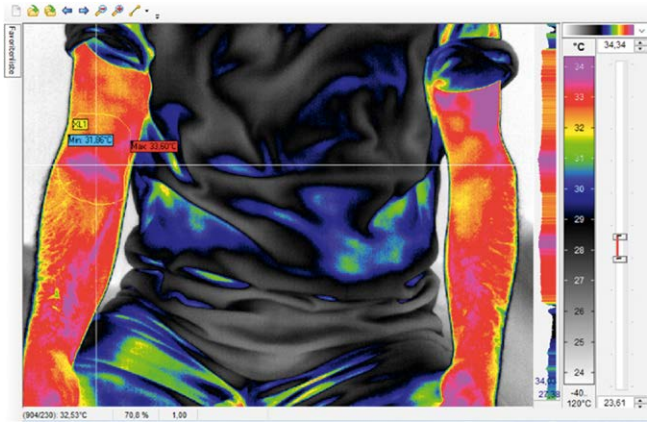


▶ **Fig. 1** Flowchart of cross-over study design. Parameters were measured directly pre and post each period (time points M1-M4).

Method The single-center randomized-controlled, cross-over-pilot study included 14 male hemophilia A o B patients with a target joint to evaluate effects of MLD on their joint condition. Study design (Fig. 1): intervention group A started with an 8-week MLD treatment period (30 min MLD twice a week). This was followed by a wash-out phase of 4 weeks and a non-treatment period of further 8 weeks. Intervention group B was treated in reverse order of periods. A total of four parameters were examined directly pre and post each period:

1. Pain situation (Visual analogue scale, VAS)
2. Joint mobility (Range of motion, ROM)
3. Joint status (Hemophilia Health Joint Score, HJHS)
4. Irritation state of target joint (infrared thermography)

Results VAS revealed a significant effectiveness of MLD treatment on pain situation ($p < 0.003$). A moderate (n = 2) to significant (n = 6) pain reduction was reported by 66.7 % of the patients after treatment period (threshold = -2 points) whereas 33.3 % showed mild (n = 2) to no (n = 1) pain reduction. The overall median pain was reduced by 2.5 ± 1.33 points, with group A (minus 3 points) benefiting more than group B (0 points) as group B showed a slight increase in pain during non-treatment period (median VAS + 1).
In the evaluation of ROM, a slight, but non-significant change in extension (p = 0.202) and flexion (p = 0.010) could be observed. A change in joint mobility of ≥ 5 ° (clinically relevant) could be measured in 5 of 14 patients during MLD treatment period (▶ **Fig. 2**).



▶ **Fig. 2** Example of infrared thermographic examination.

In the Hemophilia Health Joint Score, no significant effect could be detected. Nonetheless a median HJHS score reduction of -3 ± 0.78 points was determined in both groups during MLD period. For item score "joint pain", a post-interventional reduction of at least -1 point was recorded in 58.3 % of the patients (n = 7). Infrared thermographyshowed no significant effect for MLD on joint irritation state. Overall, an average temperature change of + 0.35 °C ± 0.10 occurred. An increase in temperature occurred at n = 8 and a decrease in temperature at n = 4.
Conclusion The pilot study showed that Manual lymph drainage can have a significant pain-relieving effect in patients with hemophilic arthropathy. VAS documentation and HJHS score could prove the pain-relieving effectiveness of this therapy. Multicentric, randomized control studies are required for further evaluation.
Conflict of Interest Institutional budget (budget of sponsor/PI). Prof. Dr. med. W. Miesbach received a Joint Health Research Grant from Bayer Vital GmbH. No further external funding.

T-13-25 Structural alteration and impact on pain perception in patients with haemophilia

Authors Schmidt A[1], Sigl-Kraetzig M[2], Richter H[3], Vogler T[4], Tomschi F[1], Hilberg T[1]
Institutes **1** Bergische Universität Wuppertal, Lehrstuhl für Sportmedizin, Wuppertal, Germany; **2** IPFW - Institut für pädiatrische Forschung und Weiterbildung, Blaubeuren, Germany; **3** Hämophiliezentrum, Münster, Germany; **4** Zentrum für Sportmedizin, Münster, Germany
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Introduction Joint haemorrhage in patients with haemophilia (PwH) leads to changes of the synovium, cartilage, and bone, that can result in chronic pain. To date, it is unclear, which of the affected structures are the determinants of the enhanced pain state in PwH. To identify the distinct sources of pain, the structural joint condition was assessed by ultrasound (US) and pressure pain thresholds (PPT) were determined to gauge the sensitivity of pain.
Method 79 patients with moderate or severe haemophilia A or B (PwH) (severe A = 54, B = 13; moderate A = 11, B = 1) were included. Thus, a total of 459 (n = 459) haemophilia-specific joints (ankle, knee, and elbow joints) of PwH were investigated sonographically and by means of PPT. Structural alterations were detected by applying the Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) system. This US scanning protocol discriminates between activity of the joint by measuring the degree of synovitis and damage of the joint by evaluating the extent of cartilage and/or bone degeneration. Pain status was assessed by measuring PPT at landmarks selected on the basis of US-findings. The lower the PPT values, the higher the pain sensitivity. To identify whether an altered pain perception is associated with increased joint

activity, three subgroups were analysed (Syn0 = none/minimal, Syn1 = mild/moderate, Syn2 = severe). To explore the potential link between joint damage and an altered pain perception, destruction of cartilage and/or bone was clustered in four subgroups (Dam0 = none, Dam1 = mild, Dam2 = moderate, Dam3 = severe).

Results A total of 156 (n) ankle, 149 knee, and 155 elbow joints were analysed. One-way ANOVA revealed a group-specific difference for PPT based on the severity of synovitis (Syn0 (n = 110) = 52.5 ± 28.3, Syn1 (n = 243) = 41.8 ± 21.2, Syn2 (n = 106) = 44.8 ± 20.5; mean ± SD in Newton; p ≤ .001) and joint damage (Dam0 (n = 282) = 48.8 ± 24.9, Dam1 (n = 38) = 43.1 ± 22.5, Dam2 (n = 40) = 37.8 ± 19.9, Dam3 (n = 99) = 38.4 ± 17.6; mean ± SD in Newton; p ≤ .001). Bonferroni-corrected post-hoc analysis revealed significant differences for synovitis between Syn0 vs. Syn1 (p ≤ .001) and Syn0 vs. Syn2 (p = .042) as well as for joint damage between Dam0 vs. Dam2 (p = .027) and Dam0 vs. Dam3 (p = .001). Spearman's correlation analysis suggests an inverse correlation between PPT and the magnitude of joint damage (r = -.199, p ≤ .001), but not for the degree of synovitis (r = -.075, p = .109).

Conclusion Structural alterations in both the synovium and cartilage and/or osseous tissue lead to mechanical hyperalgesia in PwH. In synovitis, even mild changes seem to affect pain perception, with the effect not increasing at higher levels of inflammation. In terms of joint damage, severe degeneration leads to a sensitized pain state most robustly, whereas initial changes do not appear to significantly affect pain perception.

Conflict of Interest All authors stated that they had no interests, which might be perceived as posing a conflict or bias.

T-13-26 HSCT in a newborn suffering from SCID and severe haemophilia A

Authors Schober S[1], Holzer U[1], Lang P[1], Döring M[1], Icheva V[2]
Institutes **1** University Children's Hospital Tuebingen, Department I – General Pediatrics, Haematology/Oncology, Tuebingen, Germany; **2** University Children's Hospital Tuebingen, Department II – Pediatric Cardiology, Intensive Care Medicine and Pulmonology, Tuebingen, Germany
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Introduction SCID (severe combined immunodeficiency) and severe haemophilia A are two very rare congenital diseases (incidence SCID 1:50 000 newborns, haemophilia A 1:5 000 male newborns). The coincidence of these two potentially life-threatening diseases in newborns has not been reported so far. Today, SCID can be cured by HSCT (haematopoietic stem cell transplantation). However, managing a newborn with severe haemophilia A during intensive HSCT treatment with increased bleeding risk is very challenging.

Method We describe a newborn with SCID, genetically verified by a homozygous pathogen missense variant in RAG1 gene at 5 weeks of age. Initially, SCID was suspected by a pathological newborn screening test. In addition, the boy suffered from severe haemophilia A caused by an inversion of intron 22, which was detected postnatally due to familial haemophilia predisposition. The parents were consanguine (cousins). So far there were no known SCID cases in the family. Fortunately, the boy had no severe infections since he was admitted to the HSCT ward at the age of 16 days.

Results As no matched sibling donor could be found, the patient received T-cell depleted peripheral stem cells from a matched unrelated donor after a myeloablative conditioning regimen (fludarabine, thiotepa, treosulfan, ATG) at the age of three months. The first substitution of elevated half-life factor VIII product (Efmoroctocog alfa) was required during implantation of the central venous catheter. During the initial phase of HSCT factor VIII was supplemented twice a week with 250IE. During the aplastic period (d + 2 – d + 16) with a platelet count < 100,000/µl, factor substitution was individually adjusted to achieve factor VIII levels > 50 % (40IE/kg/BW daily to every other day). After haematopoietic reconstitution, with a platelet count > 100,000/µl, factor VIII substitution was reduced to 250IE twice a week, and finally in the absence of bleeding symptoms to once a week (follow up until d + 61). With this individualized management there were no bleeding complications and no inhibitor was detected. There were no relevant side effects during HSCT.

Conclusion This is the first case report of a newborn suffering from SCID and severe haemophilia A. HSCT is feasible in this situation without bleeding complications if an individual substitution regimen is applied depending on the platelet count. So far, the patient did not develop any factor VIII inhibitor although intron 22 inversion is associated with an elevated inhibitor risk of about 25 %. One could assume that the risk for developing inhibitors is lower in a patient with SCID as the immune system is impaired. After HSCT, the inhibitor risk presumably remains decreased due to the fact that the healthy donor cells were exposed to normal factor VIII levels. However, this is speculative and further studies are needed to investigate haemophilia patients undergoing HSCT and haemophilia patients suffering from immunodeficiencies.

Conflict of Interest There are no conflicts of interest.

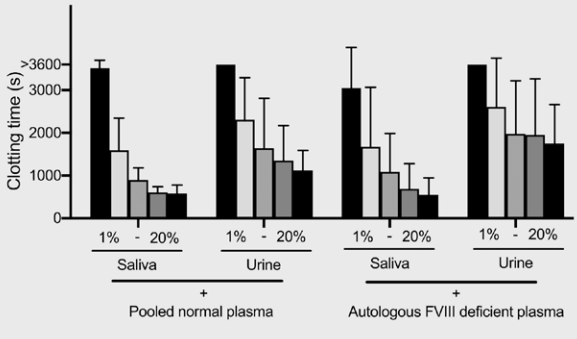
T-13-27 Saliva and urine from persons with hemophilia A trigger coagulation bypassing factor VIII

Authors Thaler J[1], Samadi N[1], Kraemmer D[1], Hu Y[2], Knöbl P[1], Pabinger I[1], Ruf W[3], Nieuwland R[2], Ay C[1]
Institutes **1** Medical University of Vienna, Department of Medicine I, Vienna, Austria; **2** Amsterdam University Medical Center, Laboratory of Experimental Clinical Chemistry, Amsterdam, Netherlands; **3** Johannes Gutenberg-University Mainz, Center for Thrombosis and Hemostasis, Mainz, Germany
DOI 10.1055/s-0042-1760530

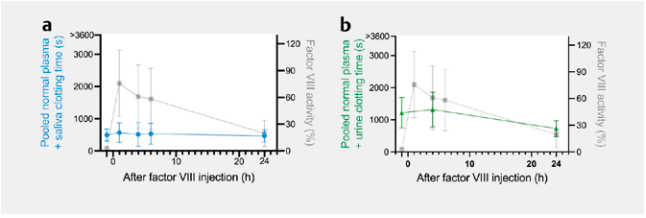
Introduction We previously demonstrated that saliva and urine from healthy individuals trigger coagulation via tissue factor/activated factor VII (TF/FVIIa) complex exposing extracellular vesicles (EVs). In persons with hemophilia A, saliva and urine may provide hemostatic protection via TF/FVIIa complex exposing EVs.

Method Saliva, urine, and plasma were collected from 5 male persons with severe hemophilia A before and after prophylactic administration of FVIII concentrates. For plasma clotting experiments, saliva and urine were mixed with pooled normal plasma, autologous FVIII-deficient plasma, and commercial FVII-deficient plasma.

Results Saliva and urine from persons with hemophilia A triggered clotting of pooled normal plasma and of autologous FVIII deficient plasma (▶ **Fig. 1**). The coagulant potential of saliva and urine did not change after intravenous administration of FVIII concentrates (▶ **Fig. 2a** and ▶ **2b**). Saliva and urine triggered clotting also in FVII-deficient plasma, which lacks TF and FVII, and this coagulant potential was inhibited by antibodies against FVII (clone 3G12) and TF (clone HTF-1) . The bulk of the coagulant potential of saliva and urine was pelleted by differential centrifugation, confirming that the coagulant activity is associated with EVs. Taken together, these findings confirmed the presence of TF/FVIIa complex exposing EVs in saliva and urine from persons with hemophilia A, which are able to trigger clotting in FVIII-deficient plasma.



▶ **Fig. 1**



▶ **Fig. 2**

Conclusion In FVIII-deficient plasma from persons with hemophilia A, saliva and urine act as endogenous FVIII-bypassing agents that trigger clotting via TF/FVIIa complex exposing EVs. Efficient FXa generation by saliva and urine seems to compensate for the lack of intrinsic tenase complexes (i.e. FVIII/FIXa complexes) in persons with hemophilia A. This may explain why persons with hemophilia A rarely develop mucosal bleedings.

Conflict of Interest The authors have no competing conflicts of interest to declare.

T-13-28 Single center experience with emicizumab for primary bleeding prophylaxis in children < 2 years of age with severe hemophilia A

Authors Reschke M, Cuntz F, Ruthenberg J, Holzhauer S, Hagedorn N
Institute Charité, University Berlin, Department of Pediatric Oncology and Hematology, Berlin, Germany
DOI 10.1055/s-0042-1760531

Introduction Primary bleeding prophylaxis with FVIII is still standard of care for patients with severe hemophilia A. Prophylaxis usually starts at the end of the first year of life. Major challenges of prophylaxis are venous access and development of neutralizing antibodies against factor VIII. With emicizumab an effective alternative drug for primary prophylaxis has been introduced. We report the management of primary prophylaxis with emicizumab in children < 2 years of age with hemophilia.

Method Retrospective single center case series of children < 2 years of life at initiation of emicizumab treatment, who were treated at the Charité Hemophilia Comprehensive Care Center in Berlin between 2018 and 2022. We collected data on disease type, treatment strategy, efficacy and safety.

Results We included seven patients who started prophylaxis with emicizumab before the age of two years. Prophylaxis was started at a median age of 13 months (range 6-21 months). Treatment strategies were as follows: (A) Treatment with emicizumab with no prior exposure to factor VIII products, (B) Prior factor VIII exposure > 100 exposure days or (C) Switch to emicizumab within the first 100 exposure days due to poor venous access. Median follow up time since starting emicizumab was 14 months (6-51 months). Dosing interval in 6 of 7 patients was every 28 days. Two patients additionally received factor VIII associated with head-trauma to protect from potential bleeds. No spontaneous or traumatic bleeds occurred in a median observation time of 14 months (range 6-51 months). We measured emicizumab blood levels in 6 of 7 patients; 54 % of the emicizumab levels were below the lower reference value (Table 1). Dose adjustments were made only according to body weight. No relevant side effects to emicizumab have been observed. Caretaker satisfaction is high.

Conclusion In a case series of seven children with severe hemophilia A and emicizumab for primary prophylaxis, emicizumab provided excellent bleeding protection irrespective of dosing interval or blood drug level. No spontaneous or traumatic bleeding occurred in any patient during a median observation period of 14 months. In our experience, emicizumab provides a safe and easy to perform bleeding prophylaxis for young patients (▶ **Fig. 1**).

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age at start of Emicizumab *(month)*	9	6	14	14	21	13	12
Factor VIII activity *(%)*	<1	<1	<1	<1	<1	<1	<1
Exposure days							
Before Emicizumab	0	0	0	>100 <150	>100 <150	>100 <150	4
During Emicizumab	0	0	0	1 ED FVIII	1 ED FVIII	0	0
FVIII-Inhibitor (BE Units)							
Before Emicizumab	-	-	-	Yes, max 1 BE	-	-	-
During Emicizumab	-	-	-	-	-	-	-
Emicizumab							
Dosage interval [days]	28	28	28	28	28→14	28	14
Dosage	6mg/kgBW	6 mg/kgBW	6 mg/kgBW	6mg/kgBW	6 mg/kgBW → 3 mg/kgBW	6mg/kgBW	3 mg/kgBW
Drug level *[µg/ml]* [54,6 +/-14,3 µg/ml]	82,1	Not available	32,3 38,5	44,3	35,7	52,0 52,2 30,0 23,5 26,9	71,6
Bleeding							
Spontaneous	0	0	0	0	0	0	0
Traumatic	0	0	0	0	0	0	0
Surgery	0	0	0	0	0	0	0
Applying person	parents	parents	parents	parents	parents	parents	parents
Side effects	none	none	pain at the injection site	none	none	none	none
Follow up time *[months]*	6	14	51	7	7	32	18

▶ **Fig. 1** Patient characteristics; BW = body weight, ED = Exposure days, kg = kilogram, BE = Bethesda.

Conflict of Interest Madlen Reschke: CSL Behring, Octapharma, Chugai, Takeda, LFB, SOBI, Pfizer, NovoNordisk, Alexion.; Susanne Holzhauer: Chugai, Biomarin Advisory Boards.

T-13-29 Immune tolerance achievement by low dose factor VIII in PUPs and MTPs under prophylaxis with emicizumab: two case reports

Authors Lohse J, Boiti F, Knöfler R
Institute Medical Faculty Carl Gustav Carus of Technical University Dresden, Dept. of Pediatric Haemostaseology, Dresden, Germany
DOI 10.1055/s-0042-1760532

Introduction The major concern of emicizumab monotherapy in previously untreated patients (PUPs) and minimal treated patients (MTPs) with hemophilia A (HA) is a possibly delayed inhibitor development. To avoid this complication, it could be considered to substitute FVIII regularly to achieve immune tolerance.

Method We present two cases where this concept was used successfully so far.

Results Patient 1 was diagnosed to have severe HA shortly after birth because of the known carrier state of his mother. Until the age of 10 months, when emicizumab prophylaxis was started, he received twice plasmatic (pd) factor VIII (FVIII) because of a trauma. No relevant breakthrough bleeds occurred. At the age of 2 years and 3 months we started a weekly application of 250 U pd-FVIII to avoid inhibitor development. Inhibitor formation was investigated every three FVIII applications. After 29 exposure days (ED) we changed to a biweekly schedule until the 50th ED, when the patient received pdFVIII every 4 to a maximum of 6 weeks. The FVIII dose was not increased over time.

Patient 2 was diagnosed at the age of 9 months because of a marked soft tissue bleeding tendency. Prophylaxis with emicizumab was also started at the age of 10 months and after another 20 months we initiated the same regimen as described for patient 1 concerning the accompanying FVIII application.

In both patients no inhibitor formation was detected so far. For the future we plan to guide the parents in i.v. injection technique and to maintain the low dose FVIII substitution every 4 weeks.

Conclusion We present a so far successful regimen for immune tolerance achievement in 2 patients with severe hemophilia A under emicizumab prophylaxis. Because more data are needed to evaluate this concept, both patients were included in the German GEPHARD registry. The GEPHARD cohort includes children with prophylaxis treatment of hemophilia A with either FVIII or emicizumab and also in combination of both as seen in our two cases. Longitudinal data are pending.

Conflict of Interest There is no conflict of interest.

T-13-30 Relation of FVIII activity levels with global assays of hemostasis in persons with hemophilia A

Authors Kraemmer D[1], Rejtő J[1], Quehenberger P[2], Ay C[1], Pabinger I[1], Königsbrügge O[1]

Institutes 1 Medical University of Vienna, Department of Medicine I, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; 2 Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria

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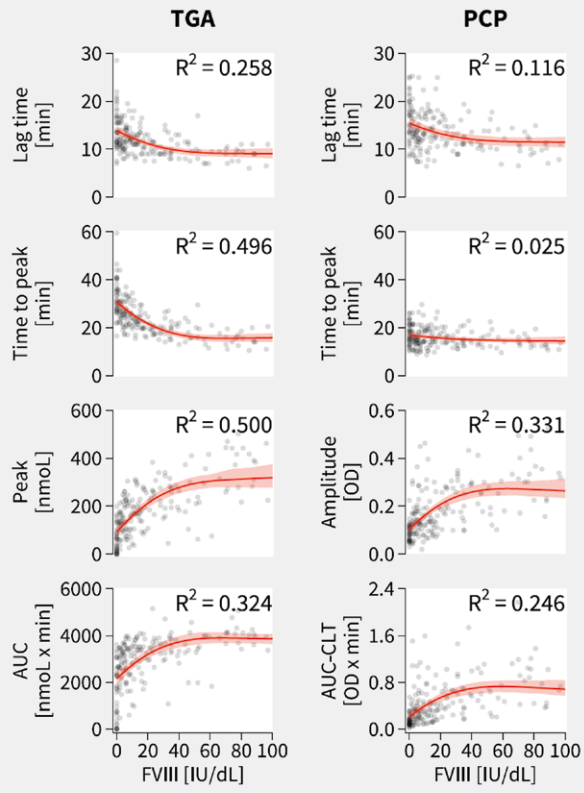
Introduction Global assays of hemostasis provide a more detailed view of thrombin generation and clot formation and may be helpful in identifying persons with hemophilia at a higher risk of bleeding. Here, we investigated the continuous relation between factor VIII (FVIII) activity as measured in routine clinical care and parameters of two global assays of hemostasis.



▶ **Fig. 1** FVIII activity was fitted with restricted cubic splines (3 knots) and regressed on the respective global assay parameters using ordinary least squares regression models. The red lines depict fitted values and the shaded regions 95 % confidence bands. Depicted R^2 values were corrected for overftting via bootstrapping.; Abbreviations: AUC, area under the curve; AUC-CLT, area under the clot-lysis time; FVIII, factor VIII; OD, optical density; PCP, plasma clot properties; R^2, coefficient of determination; TGA, thrombin generation assay.

Method We included samples of subjects with hemophilia of all severities enrolled in our biobank and, additionally, collected samples of patients with severe hemophilia when attending our clinic for pharmacokinetic studies in routine clinical care. We subsequently measured thrombin generation using a commercially available assay (TGA, Technothrombin, Technoclone, Vienna, Austria) and assessed plasma clot formation and lysis (PCP) with a turbidimetric assay, as described previously.[1] FVIII activity was measured by chromogenic substrate assay (CSA, Biophen, Hyphen Biomed, Neuville-sur-Oise, France). We estimated the variance of parameters of global assays explained by FVIII activity (R2) using ordinary least squares regression models with restricted cubic splines, further correcting for overfitting via bootstrapping. As some subjects provided multiple samples at different FVIII activity levels, 95 % confidence intervals (95 % CI) were corrected for cluster sampling by computing bootstrap estimates.

Results In total, we collected 197 samples of 92 participants with hemophilia (severe, n = 35; moderate, n = 17; mild, n = 40; mean age, 40.4 ± 14.6 years). Pearson correlation coefficients of parameters within TGA and PCP ranged from 0.64 to 0.87 and -0.09 to 0.95, respectively, and from 0.30 to 0.60 between both assays. Expectedly, parameters of both global assays saw a steeper initial change associated with increasing FVIII activity until reaching a plateau at factor levels corresponding to the upper bound of mild hemophilia. The proportion of variance accounted for by FVIII activity was, in general, lower in PCP, particularly for time to peak (R2 = 0.025). The curve parameters with the highest proportion of their variance accounted for by FVIII levels were peak thrombin (R2 = 0.500) and optical density amplitude (R2 = 0.331) in TGA and PCP, respectively. Thus, FVIII levels only accounted for a minority of the variance found in parameters of both global assays (▶ **Fig. 1**). Adding age, fibrinogen, and type of factor (endogenous, exogeneous with standard half-life, or exogenous with extended half-life) as predictors did lead to an improvement in model fit and R2 for most parameters, in particular amplitude and area under the clot-lysis time of PCP (Table 1) (▶ **Tab. 1**).

	Univariable Models			Multivariable Models		
Outcome Parameter	**FVIII***	**(95% CI)**	**R^2**	**R^2**	**LR X^2**	***p*-value**
Lag time (TGA), min	-4.14	(-5.75 to -2.81)	0.258	0.279	9.81	0.044
Time to peak (TGA), min	-13.44	(-16.03 to -11.40)	0.496	0.489	0.15	0.997
Peak (TGA), nmoL	184.6	(153.2 to 221.2)	0.500	0.517	8.57	0.073
AUC (TGA), nmoL x min	1563.4	(1168.4 to 1972.1)	0.324	0.360	14.88	0.005
Lag time (PCP), min	-3.32	(-4.64 to -1.94)	0.116	0.122	9.90	0.042
Time to peak (PCP), min	-1.96	(-3.41 to -0.40)	0.025	0.030	9.27	0.055
Amplitude (PCP), OD	0.154	(0.121 to 0.181)	0.331	0.466	46.18	<0.001
AUC-CLT (PCP), OD x min	0.478	(0.347 to 0.580)	0.246	0.429	59.94	<0.001

▶ **Tab. 1** Optimism-corrected R^2 values of univariable (FVIII activity) and multivariable models (FVIII activity, age, fibrinogen, and factor type). Likelihood ratio tests (LR X^2 with p-values) compare goodness-of-fit of multi- to univariable models.; * Change in outcome parameter at FVIII activity of 40 vs < 1 IU/dL; Abbreviations: AUC, area under the curve; AUC-CLT, area under the clot-lysis time; 95 % CI, confidence interval; FVIII, factor VIII; LR X^2 likelihood ratio chi-square; OD, optical density; PCP, plasma clot properties; R^2, coefficient of determination; TGA, thrombin generation assay.

Conclusion In summary, while FVIII activity accounted for a good proportion of the variation in parameters of both global assays, particularly TGA, a substantial fraction remained unexplained even after incorporating age, fibrinogen, and type of factor as additional predictors.

Conflict of Interest No conflicts of interest.

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T-13-31 Efficacy of emicizumab prophylaxis in patients with severe hemophilia A in Germany: Follow-up evaluation of real-life-data documented by smart medication eDiary

Authors Mondorf W[1], Eichler H[2], Fischer R[4], Holstein K[5], Klamroth R[6], Richter H[7], Sachs U[8], Escuriola-Ettingshausen C[3]

Institutes 1 Haemostas, Frankfurt, Germany; 2 Universitätsklinikum des Saarlandes, Institut für Klinische Hämostaseologie und Transfusionsmedizin, Homburg, Germany; 3 Hämophilie-Zentrum Rhein Main, Mörfelden-Walldorf, Germany; 4 SRH Kurpfalzkrankenhaus Heidelberg, Hämophiliezentrum und Gerinnungsambulanz, Heidelberg, Germany; 5 Universitätsklinikum Hamburg-Eppendorf, Gerinnungsambulanz, Hamburg, Germany; 6 Vivantes Klinikum Friedrichhain, Innere Medizin – Angiologie und Hämostaseologie, Berlin, Germany; 7 Hämophiliezentrum Münster, Münster, Germany; 8 Universitätsklinikum Gießen und Marburg, Zentrum für Transfusionsmedizin und Hämotherapie, Gießen, Germany
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Introduction Systematically documented data on real-world use of emicizumab prophylaxis in patients with severe haemophilia A (PwSHA) in Germany are still lacking. We present real-life datat on efficacy of emicizumab in PwSHA across German haemophilia Treatment Centres (HTCs) as of September 2022.

Method Annual bleeding rate (ABR), annual joint bleeding rate (AJBR) and proportion of bleed-free patients were documented using the electronic diary platform smart medication. Data of PwSHA before and after switch of treatment with FVIII concentrates to emicizumab were evaluated. Patients with >24 weeks of electronic documentation after switch were evaluated.

Results 79 PwSHA from HTCs in Germany in which PwSHA are using smart medication could be included. The median age was 33 years (IQR 36); 66 % were 18 years and older. 39 PwSHA started with electronic documentation when switched to emizicumab. In 40 patients complete electronic documentation before and after switch could be evaluated. All PwSHA were on prophylactic treatment at 1 - 5 days intervals. After switch to emicizumab, the mean AJBR was 0.31 and the mean ABR 0.84 in all patients. In the subgroup of 40 PwSHA with documentation before and after switch, the mean AJBR dropped significantly from 1.60 before and 0.39 after switch ($p < 0.01$) and the mean ABR from 3.29 to 1.27. The proportion of bleeding-free patients increased from 73 % before to 93 % after switching to emicizumab. Despite of additional FVIII treatment in 41 % of patients after the switch to emicizumab, only 4 (5 %) needed additional FVIII due to joint bleeds.

Conclusion The real life-data collected with the electronic patient diary smart medication show a statistically significant decrease of bleeding episodes in this increasing cohort of PwSHA after switching from prophylaxis with FVIII concentrates to emicizumab. The preliminary results of this ongoing study confirm the favourable data with emicizumab prophylaxis from clinical trials.

Conflict of Interest none

T-13-32 A snapshot analysis of a prospective, non-interventional study to evaluate real-life prophylactic treatment schedules of factor VIII concentrates

Authors Halimeh S[1], Behnisch W[2], Escuriola C[3], Feddern J[4], Oldenburg J[5], Seeger S[4], Wenning S[6], Klamroth R[7]

Institutes 1 Gerinnungszentrum Rhein-Ruhr, Duisburg, Germany; 2 Universität Heidelberg, Pädiatrische Hämatologie und Onkologie, Heidelberg, Germany; 3 Hämophiliezentrum Rhein-Main, Mörfelden, Germany; 4 Octapharma GmbH, Langenfeld, Germany; 5 Universität Bonn, Institut für Experimentelle Hämatologie und Transfusionsmedizin, Bonn, Germany; 6 Kurpfalzkrankenhaus Heidelberg, Heidelberg, Germany; 7 Vivantes Klinikum Friedrichshain, Berlin, Germany
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Introduction There is still a need to systematically document the prophylactic treatment of Haemophilia A (HA) in real-life clinical practice. Hence dosing and other elements of HA treatment are documented in a non-interventional fashion. The primary objective of this non-interventional study (NIS) is the assessment of the influence of the weekly prophylactic FVIII dose on annualized bleeding rates (ABR). It further aims to analyze all aspects of prophylactic treatment with Simoctocog alfa (Nuwiq) and the plasma-derived FVIII concentrates Wilate and Octanate.

Method Patients with HA of all ages receiving prophylaxis and having a good compliance are eligible to be enrolled after informed consent is given. All details of bleeding episodes and factor VIII administrations are recorded. Optional study elements comprise assessment of joint scores (HJHS) and a pharmacokinetic (PK)-assessment including dosing simulation for potential adaptation of therapy schedules with a population-based PK using WAPPS-Hemo.

Results As of October 2022, 53 patients were included with ages at study entry ranging between 1 and 91 years. 26 patients below age 12 were enrolled. The median observation time was 31.5 months. In total, 447 episodes were documented as bleeds (57.3 % mild, 33.3 % moderate, 6.9 % of severe intensity). 75 % of episodes had a traumatic cause. 97 % of rated episodes received an "excellent/good" assessment of treatment. Mean ABR was 2.94 for all bleeds, 0.7 for spontaneous bleeds and 2.01 for traumatic bleeds. These reflects the high proportion of children in the study with a large inter-patient variability, however. 35 surgeries in 18 patients were performed during the observation time. No inhibitor formation or any other adverse drug reactions were observed (▶ **Tab. 1**).

		No of Patients
All		53
Exposure Days	≥150	46
	1-149	6
	0	1
Severity of Disease	Severe	46
	Moderate	1
	Mild	5 (between 6 and 18% FVIII:C basal activity)
Age	0-11	26
	12-91	27
	>60	3
History of inhibitors	Yes	8
	No	44
	unknown	1
Target joints present	Yes	32
	No	21
	Children aged 0-11 with target joints	11
Medical History of ICH		3

▶ **Tab. 1** Patient Status at Study Entry.

Conclusion This snapshot analysis with a long observation time of a median of 31.5 months delivered important data on the ABR in real-life treatment of haemophilia A. It further confirms the outstanding tolerability and efficacy of the concentrates investigated.

Conflict of Interest Dr. Halimeh, Dr. Behnisch, Dr. Escuriola, Prof. Oldenburg, Dr. Wenning, PD Dr. Klamroth: Investigators of the study; J. Feddern, S. Seeger: employees of Octapharma GmbH

T-13-33 Second- and third-generation FVIII-specific CAR Treg responses to FVIII are functionally affected by the combination of FVIII-specific scFvs and intracellular costimulatory domains

Authors Scatigna S, Warken J, Orlowski A, Schultze-Strasser S, Königs C, Schmidt A

Institute University Hospital Frankfurt Am Main, Department of Paediatrics and Adolescent Medicine, Clinical and Molecular Haemostasis, Frankfurt am Main, Germany
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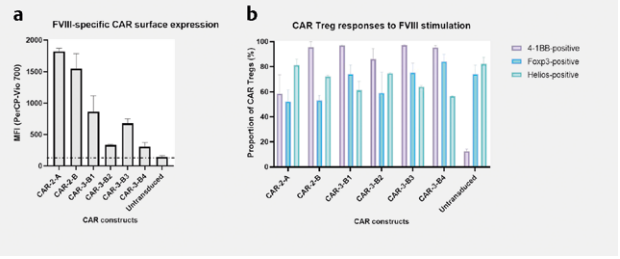
Introduction About one third of severe HA patients treated by protein replacement therapy with factor VIII (FVIII) develop inhibitors. As FVIII-specific chimeric antigen receptor (CAR) regulatory T cells (Tregs) were shown to suppress T and B cell responses to FVIII, we aimed to develop and compare second- and third-generation FVIII-specific CAR Tregs to reverse inhibitor-formation.

Method We designed second-generation FVIII-specific CARs CAR-2-A and CAR-2-B, characterized by two different single-chain variable fragments (scFvs). ScFv affinity for FVIII was measured by affinity ELISA. Combination of second-generation CARs with costimulatory domains such as CD28 and others involved in Treg homeostasis, resulted in third-generation CARs (only CAR-3-B1-4 data shown). CAR construct-containing lentiviral vectors were used to transduce Tregs. CAR Treg responses to FVIII were investigated stimulating rested CAR Tregs with FVIII. CAR Tregs were analyzed by flow cytometry for CAR expression and expression of Treg- and activation-specific protein markers including Foxp3 and Helios. Cytokine analysis was assayed by bead array.

Results Second-generation CARs showed a higher surface expression than third generation CARs (▶ **Fig. 1a**). CAR-3-B2- and CAR-3-B4-Tregs showed the lowest CAR surface expression. All CAR Tregs were able to recognize and respond to FVIII in stimulation experiments, as indicated by the activation marker 4-1BB upregulation (▶ **Fig.1b**). CAR-2-A showed a lower response to FVIII than CAR-2-B. Of note, FVIII affinity of both scFvs was in the low nanomolar range. Following stimulation, the amount of CAR Tregs expressing Foxp3 was compared to untransduced Tregs. Expression was lower for CAR-2-A, CAR-2-B and CAR-3-B2, indicating that these CAR variants do not sustain the Treg phenotype. CAR-3-B4-Tregs showed the highest amount of Foxp3 expression. Interestingly, Helios-expressingCAR Tregs portion was generally lower than for untransduced cells, except for CAR-2-A-Tregs (Fig.2A). Cytokine analysis revealed that CAR-3-B1 and CAR-3-B4 showed the highest secretion of the immunosuppressive cytokine IL-10. On the other hand, CAR-2-B, CAR-3-B1 and CAR-3-B3-Tregs produced the highest amount of TNF-α and INF-γ. These preliminary data suggest that CAR-3-B4 might be the most promising CAR for generation of functional FVIII-specific CAR Tregs.



▶ **Fig. 1** FVIII-specific CAR surface expression and CAR Treg responses to FVIII; **a** FVIII-specific CAR surface expression. Second and third-generation FVIII-specific CAR surface expression was defined by MFI values of PerCP-Vio 700 following FVIII stimulation of CAR Tregs. Second-generation CARs were named as CAR-2-A and CAR-2-B. Third generation CAR variants were named as CAR-3-B1 to CAR-3-B-4. **b** CAR Treg responses to FVIII stimulation. Percentages of 4-1BB-, Helios- and Foxp3-positive CAR Treg populations were obtained gating on CD4+ CD25+ CD127low/- CAR+ Tregs.

Conclusion Costimulatory domain combinations introduced into FVIII-specific CAR variants revealed clear differences in terms of cellular responses. Variable responses among CAR Tregs might be influenced by the scFv affinity for FVIII, CAR surface expression levels and/or signaling pathways induced by different costimulatory domain combinations. Further experiments are ongoing to select the best FVIII-specific CAR variants that in the context of Tregs show to suppress anti-FVIII immune responses.

Conflict of Interest none

T-13-34 Preanalytical quenching of FVIII using FVIII-inhibitors improves the specificity of functional emicizumab testing

Authors Shahidi-Hamedani N, Klein C, Goldmann G, Marquardt N, Rühl H, Pötzsch B, Oldenburg J, Müller J
Institute University Bonn Medical Center, Institute of Experimental Haematologie and Transfusion Medicine, Bonn, Germany
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Introduction While the measurement of FVIII-activity in haemophilia A (HA) patients treated with Emicizumab (Emi) is easily possible using chromogenic FVIII-assays based on bovine factors, functional measurement of plasma Emi is potentially falsified by the presence of FVIII [1]. However, the latter may be of interest in severe HA patients treated with Emi and additional need for FVIII or in Emi prophylaxis in patients with moderate or mild HA [2]. The aim of the present study was to assess different strategies for quantification of plasma Emi levels in the presence of FVIII-activity.

Method Evaluated strategies comprised (1) calculation of actual Emi plasma levels based on measured FVIII-activity and FVIII-affected Emi values, (2) silencing of FVIII-activity by FVIII-inhibitors or (3) heat-inactivation (HI, 56 °C for 40 min) of patient plasma samples in order to extinguish FVIII-activity before Emi analysis. FVIII-deficient plasma was spiked at different ratios with Emi and FVIII to initially evaluate strategies 1 and 2. For FVIII-inhibitor treatment, samples were mixed 1 + 1 with a commercially available FVIII-inhibitor plasma preparation (approx. 15 BU/ml) before analysis. Plasma samples obtained from patients treated with Emi were used for further assessment and to determine the impact of HI on Emi measurements (strategy 3). Emi plasma levels were measured using a modified FVIII one-stage clotting-assay calibrated against Emi. Plasma FVIII-activities were determined using a chromogenic FVIII-assay based on bovine factors. Multiple linear regression (MLR) analysis was used to allow for calculation of actual Emi plasma levels according to strategy 1.

Results Spiking experiments at different Emi concentrations (20 to 80 µg/ml) revealed a linear relationship between increasing plasma FVIII-activity (up to 2 IU/ml) and determined (FVIII-affected) Emi levels. MLR analysis showed an adjusted multiple R-squared value of >0.99 and allowed for calculation of actual Emi levels in patient plasma samples (relative error [RE] <10 %) based on Emi- and FVIII-activity measurements. Pre-analytical mixing of plasma samples with FVIII-inhibitor plasma (1 + 1) demonstrated efficient inhibition of FVIII-activity and yielded good recovery (RE <10 %) of Emi plasma levels as measured in the absence FVIII-activity. In contrast to the successfully applied strategies 1 and 2, HI of patient plasma samples according to strategy 3 resulted in a significant decrease of determined functional Emi levels (mean loss: 48 %, range: 35 % to 58 %).

Conclusion The presence of FVIII activity impairs the specificity of functional Emi-testing in a concentration-dependent manner. Quenching of FVIII activity through addition of FVIII-inhibitors increases the specificity of Emi-testing, allowing accurate measurement of Emi plasma levels. MLR analysis may be used for results correction. HI significantly decreased the sensitivity / accuracy of functional Emi-testing.

Conflict of Interest JM received honoraria from Roche / Chugai, Siemens Healthineers and Stago. JO has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai Pharmaceutical Co., Ltd., CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda. NM has received honoraria for speaking/consulting, funds for research and travel support from Bayer, NovoNordisk, Chugai, CSL Behring, Octapharma, Pfizer, Roche, Shire/Takeda.

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T-13-35 A single-centre approach for a safe and individualized tolerization phase for the treatment of PUPs with severe haemophilia A

Authors Halimeh S[1, 2], Rott H[1], Kappert G[1], Siebert M[1], Reinhardt D[2]
Institutes **1** Gerinnungszentrum rhein ruhr, Duisburg, Germany; **2** Universitätsklinikum Essen, Kinderklinik III, Essen, Germany
DOI 10.1055/s-0042-1760538

Introduction Pediatricians treating newly diagnosed severe haemophilia A patients are confronted with the challenge to protect the children against bleeds that are potentially life-threatening or may have long-term damaging effects on joints. The protection that early prophylaxis with coagulation factor VIII (FVIII) provides, may however put the patients at risk for inhibitor development. We report our experience with an individualized approach to managing PUPs at a single haemophilia treatment center (HTC) in Germany.

Method PUPs with severe hemophilia A (FVIII:C <1 %) treated at the Gerinnungszentrum Rhein-Ruhr (GZRR), Germany, between January 2013 and August 2022 were followed prospectively. Our treatment approach includes early prophylactic physiotherapy and treatment with a human plasma-derived FVIII (pdFVIII/VWF) concentrate for at least the first 50 exposure days (EDs). Prophylaxis was adapted with individualized dose escalations according to bleeding tendency and prolonged on-demand treatment was avoided where possible, by choosing doses up to 80 IU/kg. Close contact between parents and haemophilia center ensures that decisions to treat with factor VIII concentrate can be done on a case-by-case basis. Non-urgent surgical procedures were postponed within the first 100 EDs. Ultrasound was performed every 3–6 months to check for joint bleeds. In addition, patients receive individualized physiotherapy to preserve joint health. In contrast to this patient group, between 2003 and 2012 9 PUPs with severe haemophilia A were treated predominantly with recombinant factor concentrate and with less selectiveness in treating bleeds.
Inhibitor levels were measured using the modified Bethesda assay every 3–4 EDs until ED 100, and every 3 months thereafter for 2 years for all patients.

Results Data from 39 consecutive PUPs were collected, of which 36 had started treatment. At the time of data cut-off 29 patients had received over 50 EDs of FVIII treatment, 8 patients had more than 20 EDs. 27 patients had started prophylaxis within the first 10 EDs. The initial schedule was tailored to each patient and ranged from 21 IU/kg every 10 days up to 40 IU/kg twice per week. One patient was treated on-demand for an intracranial bleed from ED 1–100. Only 3 spontaneous bleeds in 3 patients were treated; 33 patients (92 %) remained free of treated spontaneous bleeds during prophylaxis. 1 of 36 treated patients had developed an inhibitor after the 4th exposure day (2.8 %). Of the 9 patients treated conventionally before 2013, 9 (44 %) patients had developed an inhibitor. Ultrasound and MRI assessment showed no sign of joint modification.

Conclusion The individualized management approach with a close monitoring of the patients during the high-risk phase for development of an inhibitor, choice of FVIII concentrate and its restricted use in on-demand treatment seems to be a safe way to guide the patients to be tolerant to FVIII while protecting from consequences of serious bleeds.

Conflict of Interest None

T-13-36 The effect of DNA methylation on inhibitor development in haemophilia A patients treated with FVIII concentrates

Authors Chand H[1], Hassan S[1, 2], Cairo A[3], Palla R[1], Peyvandi F[1, 3]
Institutes **1** University of Milan, Department of Pathophysiology and Transplantation, Milan, Italy; **2** Leiden University Medical Center, Department of Clinical Epidemiology, Leiden, Netherlands; **3** Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy
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Introduction Haemophilia A (HA) is a hereditary X-linked haemorrhagic bleeding disorder. Following the treatment with exogenous FVIII, roughly one-third of patients with severe HA develop anti-FVIII antibodies (inhibitors), that renders treatment ineffective. Our aim is to detect CpG sites that are differentially methylated (DMCs) in peripheral blood mononuclear cells (PBMCs) of patients with and without an inhibitor, using a genome-wide approach, to better understand the biological pathways that leads to inhibitor development.

Method A case-control study was performed using 37 inhibitor-positive HA patients and 53 inhibitor-negative HA patients from the SIPPET study cohort. Reduced representation bisulfite sequencing was performed on PBMC DNA samples using the Agilent SureSelectXT Human Methyl-Seq kit. Alignment, deduplication and methylation calling was done using Bismark. Differentially methylated CpG sites were identified using logistic regression. Adjustment for multiple testing was done using the Benjamini-Hochberg method. To identify biological pathways associated with inhibitor development, over-representation analysis using the Reactome database was performed on genes associated with differentially methylated CpG sites.

Results Overall, information on 407,601 CpG sites was obtained. Of these, 2,962 CpG sites were significantly differentially methylated (unadjusted P-value < 0.05), with 2,246 sites being hypermethylated and 716 being sites hypomethylated. Of the total DMCs, 48 % were found in the introns, 44 % in intergenic region and 4 % each in exons and promoters. An association with a gene was found for 1,957 out of 2,962 DMCs. Reactome pathway analysis of these 1,957 genes did not identify any significant pathways involved in immune regulation.

Conclusion Differentially methylated CpG sites were identified in PBMC DNA samples from Haemophilia A patients. However, the role of these CpG sites in inhibitor development is unclear and needs further investigation.

Conflict of Interest H.Chand, S.Hassan and A.Cairo declare no conflict of interest. R.Palla : speaker fee for educational meeting organized by Novonordisk. F.Peyvandi : Advisory boards: Sanofi, Sobi, Takeda, Roche, Biomarin. Educational meetings: Grifols, Roche.

T-13-37 Intrinsic difference in cellular response between full-length and B-domain deleted FVIII HEK293 secreting cells: implication for gene therapy

Authors Al Rifai R, Jamil MA, Nüsgen N, Ibrahim M, El Hazzouri S, Oldenburg J, El-Maarri O
Institute Universitätsklinikum Bonn, Institut für experimentelle Hämatologie und Transfusionsmedizin, Bonn, Germany
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Introduction Hemophilia A treatment relied on replacement of FVIII, but recently new treatment approaches aimed at overcoming the shortcomings of factor replacement. AAV-based gene therapy have achieved good results, but are still far from universal applicability as they still face many challenges. A major problem is the lack of sustainability of FVIII levels; we also still have no idea on the status of the transfected cells in the liver. Most of these protocols target hepatocytes and use a BDD-FVIII, implying two non-natural phenomena linked in one way or another to the deterioration of FVIII blood levels over 2-3 years.This gradual decrease in FVIII levels over time strongly demand a thorough investigation of the suspected mechanisms at the cellular level of transfected cells.The latter include abnormal control/levels of expression and abnormal cellular trafficking leading to cytotoxicity in the cells.The phenomenon could be dose-dependent and could be related to different intra-cellular pathways of BDD-FVIII.Understanding the reasons behind this phenomenon is the first step to avoiding its side effects.This will be directly beneficial for the patient, since better optimization of gene therapy protocols could be achieved. The aim of

this study to compare the cellular response to different forms (FL vs BDD) and levels of FVIII in HEK293 cells

Method We established single cell HEK293 clones transfected with FL or BDD-FVIII; each clone has a unique level of expression and secretion.We examined the correlations between FVIII copy number and levels of expression, secretion and storage as well as homeostatic cellular parameters such as residual ATP levels, cell proliferation rate, co-segregation and co-localization of intracellular markers with FVIII.In addition, we performed 3´RNA-Seq for expression profiling of the established clones and analysed the data using bioinformatics tools

Results Our results show clear differences in expression (higher in BDD), intracellular localization, secretion (higher in BDD) and intracellular storage potential (higher in FL) between BDD and FL expressing clones.Cell proliferation rate was higher in FL while residual ATP was higher in BDD clones.Co-localization of cellular markers with FVIII shows a reduced static presence of FVIII-BDD in ER/ERGIC/GABARAP and GAPARAPL1/lysosome, while a higher presence is observed in GABARAPL2- and LC3B-coated vesicles.Bioinformatics analysis confirmed most of the phenotype, as differentially activated pathways and processes include cytoplasmic organization, energy-related, stress-related, and cell proliferation.Finally, expression association with secretion potentials revealed the absence of overlap between BDD and FL expressing clones, indicating a clear divergence in response to overexpression of BDD and FL-FVIII

Conclusion Our data indicate a different cell response towards BDD and FL expression and secretion in HEK293 cells.This is due to the different pathway traversed by BDD FVIII in the absence of its b domain.

Conflict of Interest No conflict.

T-13-38 Establishment and specification analysis of LSEC-like endothelial cells for the detection of endogenous FVIII

Authors Chawla P, Rath M, Oldenburg J, Singer H

Institute University Clinic Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany

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Introduction In the liver vasculature the oxygenated arterial and nutrient rich portal venous blood mix within the sinusoidal space that drains into a hypoxic central vein. These sinusoids are lined with liver sinusoidal endothelial cells (LSEC) which are unique endothelial cells. They have a variety of important functions and one of them is secretion and expression of FVIII. Pluripotent stem cells from healthy donors can be a source of generating organ specific endothelial cells in vitro. However, maintaining the specification and expression of LSEC markers (F8) remains a challenge. Currently, embryoid bodies with a combination of adrenomedullin pathway activation, TGF-ß inhibition and hypoxia are a state of the art to derive LSEC-like EC from IPS. A 2D monolayer-based approach established by our group can not only increase the proliferative potential of these endothelial cells but also help in maintaining their specification.

Method IPS cells were seeded as a single cell suspension and differentiated into mesoderm through GSK3ß suppression and activation of Wnt signaling using small molecules bFGF, BMP4 and CHIR. Mesoderm induced cells were further treated with VEGF-A and GSI that maintain Notch inhibition and increase the venous vascular cell population. Subsequently, angioblasts were isolated with CD34 + MACS positive selection and maintained in medium containing low VEGF-A under hypoxic conditions. Induction of LSEC-like cells was implemented by adding small molecules SB431542 and 8-Br-cAMP. Venous, arterial and LSEC specific markers FCGR2, Stab2 & F8 were analyzed by rtPCR. Specification analyses were conducted by FACS for detection of definitive mesoderm markers (KDR, CD235a/b and CD56) and venous markers for angioblasts and LSEC-LCs (CD31, CD34, CD184 and CD73). A method to enrich intracellular FVIII protein by immunoprecipitation is curently under development, using VEGFR2 specific antibody as positive control and a combination of FVIII-specific antibodies to detect protein-specific peptides by mass spectrometry.[1–9]

Results RT-PCR: Early angioblasts (Day6) show an increase of venous marker COUP-TF and NT5E after four days under low VEGF-A conditions (Day10). During LSEC induction (Day10-16), LSEC markers show clear increase compared to HUVEC: FCGR2b (2fold), Stab2 (37fold) and F8 (7fold). FACS: Day4 mesoderm shows a KDR + CD235a/b + CD56 + phenotype. Day 6 angioblasts show a venous CD31 + CD34 + CD184lowCD73high phenotype which is maintained until Day 15 LSEC-LC

Conclusion This LSEC-like cellular model provides 7-fold higher F8 expression compared to a normal vascular EC in vitro system. FACS analysis shows the right phenotype for modeling iPSC into Liver sinusoidal endothelial cells. The additional presence of LSEC specific receptors enables a more native and precise disease modeling for HA research and therapy

Conflict of Interest No conflict of interest

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T-13-39 Interim analysis in patients from Germany and Switzerland of the A-MORE study: a 48-month, multi-centre, observational study to evaluate long-term effectiveness of rFVIIIFc on joint health

Authors Graf L[1], Tiede A[2], Ahlin H[3], Bystrická L[4], Bauer E[5], Piccand M[6], Holzhauer S[7]

Institutes **1** Center for Laboratory Medicine, Hemophilia and Hemostasis Center, St. Gallen, Switzerland; **2** Hannover Medical School, Department of Hematology, Haemostasis, Oncology and Stem Cell Transplantation, Hannover, Germany; **3** Sobi, Stockholm, Sweden; **4** Sobi, Stockholm, Sweden; **5** Sobi, Martinsried, Germany; **6** Sobi, Martinsried, Germany; **7** Charité University Medicine, Department of Paediatric Haematology and Oncology, Berlin, Germany

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Introduction The A-MORE study primarily aims to evaluate the long-term effectiveness of efmoroctocog alfa (referred to herein as rFVIIIFc) on joint health in patients with haemophilia A (PwHA) in a real world setting.

Method A-MORE (NCT04293523) is an ongoing 48 month prospective, non interventional study across 14 countries in Europe and the Middle East that enrolled PwHA of all ages and severities who received ≥ 1 dose of prior rFVIIIFc

prophylaxis (PPX). Here we present baseline characteristics and interim data of enrolled patients, from Germany and Switzerland, with ≥ 3 months follow-up (FU) at last assessment before data cut off (24 June 2022).

Results At data cut-off, 68 PwHA with ≥ 3 months FU (1 female, 67 males; 58 severe, 9 moderate, 1 mild) were enrolled with median interquartile range (IQR) age of 33.3 years (22.7 – 46.2). 68 PwHA enrolled from Germany and Switzerland represented 30 % of all patients included in the interim analysis (68/223). 3 PwHA were previously untreated patients (PUP). Positive inhibitor history was reported in 30.9 %. At baseline, 30.9 % received primary PPX (27.9 % secondary, 11.8 % tertiary, 29.4 % unknown). Within 12 months pre-study, 65 PwHA had extended half-life (EHL) treatment. During this period, mean/median IQR overall annualized bleeding rates (ABR), spontaneous and joint bleed rates (n = 67) were 1.5 / 0.0 (0.0 – 1.0), 0.9 / 0.0 (0.0 – 0.0) and 0.6 / 0.0 (0.0 – 1.0); 55.2 % of patients had zero bleeds. The presence of impaired joints at enrolment was reported in 44.1 % of patients (30/68). At baseline, median IQR rFVIIIFc dose/week was 91.3 (68.1 – 117.6) IU/kg; median IQR injection frequency/week was 2.3 (2.0 – 3.0). Adherence was rated very good/good in all 97 % of patients (n = 64/6466).

Median IQR on-study FU was 260 days (215 – 339). Median IQR dose/week during the FU was 96.4 (71.3 – 124.5) IU/kg and median IQR injection frequency/week was 2.4 (2.0 – 3.0), with adherence rated very good/good in all patients (n = 17). Mean/median IQR overall ABR, spontaneous and joint bleed rates (n = 68) at FU were 1.1 / 0.0 (0.0 – 1.0), 0.6 / 0.0 (0.0 – 0.0) and 0.7 / 0.0 (0.0 – 1.0); 66.2 % of patients had zero bleeds. No new safety signals were identified at data cut-off; no recurrence of inhibitor has been observed in PwHA with positive inhibitor history; inhibitor development was reported in 1/3 PUP with severe haemophilia.

Conclusion A-MORE regional analysis of patients from Germany and Switzerland showed long-term effectiveness of rFVIIIFc-PPX with sustained effective bleed protection and maintained good adherence. Prospective observation over 48 months will further evaluate the protective effect of rFVIIIFc PPX.

Conflict of Interest LG: Consulting fees from Bayer, CSL Behring, Novo Nordisk, Octapharma, Roche, Sobi. AT: Consulting fees/research grants from Alnylam, Bayer, Biogen Idec, Biotest, Boehringer Ingelheim, Chugai, CSL Behring, Daiichi Sankyo, LEO Pharma, Novo Nordisk, Octapharma, Pfizer, Portola, Roche, Sobi, and Takeda. HA, LB, EB, MP: Sobi employees SH: Consulting fees from Boehringer Ingelheim, Biomarin, BMS, Pfizer.

T-13-40 Survey on the care reality of people with mild hemophilia A and B in Germany - the burden of mild hemophilia

Authors Alesci R S[1], Halimeh S[2], Miesbach W[3], Koenigs C[4], Holstein K[5], Olivieri M[6]

Institutes **1** IMD Gerinnungszentrum Hochtaunus, Bad Homburg, Germany; **2** Gerinnungszentrum Rhein-Ruhr, Duisburg, Germany; **3** Goethe University Hospital, Med. Klinik III; Frankfurt Haemophiliezentrumntre, Frankfurt, Germany; **4** Goethe University Hospital, Klinikum der Kinder- und Jugendmedizin, Haemophiliezentrumntre, Frankfurt, Germany; **5** Universitätsklinikum Hamburg-Eppendorf, Med. Klinik III, Haemophiliezentrum, Hamburg, Germany; **6** Universitätsklinikum München LMU, Kinderklinik, Hämophiliezentrum, München, Germany

DOI 10.1055/s-0042-1760543

Introduction Patients with mild hemophilia (residual factor activity > 5 to < 40 %) represent a very heterogeneous group. Therapeutic approaches are as individual as the bleeding tendency. Patients may feel less perceived by the medical profession than patients with moderate or severe hemophilia. It is likely that the extent of disease and bleeding risk of patients with mild hemophilia are underestimated, resulting in an undersupply of factor concentrate, both during surgical procedures and trauma. So far, not much data exists on both joint problems of patients, nor on the management of surgical procedures. The aim of this patient survey is to gather insights into the treatment situation of patients aged 12 years and older with mild hemophilia A or B and their quality of life

Method Using an anonymous cross-sectional patient survey, we documented online patients 'experience with factor concentrates and other treatments. Patients ≥ 12 years with diagnosed mild hemophilia A or B participated in the survey. We evaluated patients 'satisfaction regarding the therapy situation and the support by physicians, and assessed the impact on Quality of Life (EQ-5D-5L).

Results We could include 44 patients (35 hemophilia A, 5 hemophilia B, 3 unknown) with a median age at survey of 33 years (mean 36.0 years, 12-75 years). The median age at diagnosis was 6.0 years (mean 10.6, 0-37 years). Median factor activity was 14.0 % (mean 18.1; 4-55 %). 84.2 % (n = 32) were treated with factor concentrates in the past. The most frequent reasons for treatment were surgery or joint bleedings (each 65.6 %, n = 21). Approximately half of the patients (n = 20) had complications caused by untreated bleeding due to accidents (4), surgical bleeding (11), dental treatment (10), joint bleeding (10) and spontaneous bleeding (2). Only 4 patients (10.3 %) were prophylactically treated with factor concentrates. Health state indicates slight problems with mobility, slight problems with washing or dressing, slight problems with doing usual activities, moderate pain or discomfort and slight anxiety or depression.

Conclusion Bleeding complications, especially joint bleeding, seem to be highly underestimated in patients with mild hemophilia A and B. Obvious, there is still a lack of awareness. To start an early prophylaxis to avoid joint damage (hemophilia arthropathy) should be discussed even in patients with mild hemophilia.

The QoL is surprisingly reduced, too. More data about joint bleeding is needed.

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T-13-41 Real-world experience on the use of rIX-FP in patients with hemophilia B: interim results from a prospective, non-interventional study in Germany

Authors Oldenburg J[1], Holzhauer S[2], Wenning S[3], Olivieri M[4], Linhoff T[5], Pfrepper C[6]

Institutes **1** University Clinic Bonn, Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany; **2** Charité University Medicine, Department of Pediatric Hematology and Oncology, Berlin, Germany; **3** SRH Kurpfalzkrankenhaus, Heidelberg, Germany; **4** LMU Munich, Paediatric Thrombosis and Haemostasis Unit, Paediatric Haemophilia Centre, München, Germany; **5** CSL Behring, Hattersheim, Germany; **6** University Hospital Leipzig, Division of Hemostaseology, Leipzig, Germany

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Introduction The efficacy and safety of rIX-FP, a long-acting fusion protein, in patients with haemophilia B has been previously demonstrated in clinical trials. rIX-FP provides dosing flexibility allowing selected patients to receive prophylaxis at intervals of 7, 10, 14 or 21 days. Data from routine clinical practice on the use of rIX-FP are required. Therefore, prospective, multicentre studies are ongoing in order to obtain data on the effectiveness and tolerability of rIX-FP when used to treat patients with haemophilia B during routine clinical practice.

Method A non-interventional study was initiated in Germany in March 2018. All patients with haemophilia B were eligible for enrolment. Patients are routinely monitored every 3–12 months and followed-up for 3 years or until 100 exposure days.

Results At the interim data cut-off on 14 October 2022, 73 patients were enrolled across more than 20 German sites. The recruitment was completed, and follow-up is ongoing. Patients had a mean (SD) age of 27.5 (19.6) years (range, 0.6–80) and the majority (90.7 %) had moderate or severe haemophilia B (n = 64). At enrolment in the study, patients had an estimated mean (SD) of

105.5 (75.8) exposure days to rIX-FP (n = 65). Seven patients discontinued the study: two were lost to follow-up, two switched to another factor IX product, one due to physician´s decision (non-related to rIX-FP) and two for other reason. Seventy-seven adverse events (AEs) in 34 patients have been reported, none of which were considered related to rIX-FP. Sixteen AEs were considered serious. To date, no patients have developed inhibitors.

Conclusion Data from this study indicate that rIX-FP prophylaxis is well tolerated in both adults and paediatrics in routine clinical practice. Further data collection is ongoing to assess the long-term effectiveness and tolerability of rIX-FP in the real-world clinical setting.

Conflict of Interest JO received grants/research support from Baxter, Bayer, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma and Pfizer, consultancy fees from Baxter, Bayer, Biogen Idec, Biotest, Chugai, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche and Swedish Orphan Biovitrium, and speaker fees from Baxter, Bayer, Biogen Idec, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer and Swedish Orphan Biovitrium; MO received grants/research support from Bayer, Biotest, Takeda, CSL Behring, Octapharma, Pfizer, Shire, Roche and Swedish Orphan Biovitrium, consultancy and speaker fees from Bayer, Biotest, Novo Nordisk, Takeda, CSL Behring, Pfizer, Roche and Swedish Orphan Biovitrium; CP received speaker honoraria from BMS, CSL Behring, Pfizer, Roche, Shire Takeda, acted as a medical advisor for Bayer HealthCare, Chugai, CSL Behring, Novo Nordisk, Pfizer, Roche, Shire Takeda, received research grants from Fujimori, LeoPharma, Takeda, Roche; SH and SW have no conflicts of interest to declare. TL is CSL Behring employee.

T-13-42 Low bone mineral density in hemophiliacs: cell culture experiments to elucidate the role of coagulation factors in bone metabolism

Authors Bernar A[1], Gebetsberger J[1], Schirmer M[2], Streif W[1]
Institutes **1** Medical University of Innsbruck, Department of Pediatrics I, Innsbruck, Austria; **2** Medical University of Innsbruck, Department of Internal Medicine, Clinic II, Innsbruck, Austria
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Introduction Patients with severe haemophilia A do not only suffer from spontaneous bleeding into joints or soft tissues, but they are also prone to low bone mineral density (BMD) independent of commonly accepted risk factors including smoking and reduced physical activity1,2. Nowadays evidence accumulates that FVIII plays a role outside the coagulation system3. FVIII may directly interact with the main players of bone physiology, including the receptor activator of nuclear factor kappa-B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) axis and/or the wingless related integration site (Wnt)/β-catenin pathway, as well as pro-inflammatory cytokines. Alternatively, the effect on bone metabolism could happen downstream of FVIII and e.g. the missing interaction with von Willebrand factor (vWF) or decreased thrombin production is causative. Previous studies on that topic led to controversial results. Since clinical studies can be affected by a complex interplay of confounding factors (e.g. viral infections, medications, hypovitaminosis D), more experimental studies are urgently needed.

Method We are performing cell culture experiments with both main cell types of bone metabolism – osteoblasts (murine MC3T3-E1, human SaOs2), and osteoclasts (isolated from human peripheral blood) – in order to investigate the effect of several coagulation factors, including FVIII, FIX, FX, vWF, vWF-FVIII complex and thrombin, on cell growth and functioning. In a first attempt, we established and optimized qualitative and quantitative assays. Additional biochemical and molecular biological methods are used to elucidate involved signaling pathways, shedding light on the interactions between coagulation and bone physiology.

Results First results on the optimization of the Alizarin Red S assay showed that calcium is able to significantly improve mineralization of osteoblasts (human and murine) in a dose dependent manner. We expect in further experiments that coagulation factors have an impact on osteoblast and/or osteoclast activity, by either stimulating or inhibiting cell growth and/or functioning.[1–3]

Conclusion Understanding the underlying mechanisms of coagulation factors in bone metabolism will provide additional guidance for the development of effective and safe treatment strategies in order to prevent low BMD in patients with haemophilia A. This is essential, especially now as non-replacement therapies have been introduced for treatment of haemophiliacs of all ages.

Conflict of Interest There are no conflicts of interest.

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T-13-43 Patient-reported symptoms to predict intra-articular location of joint bleeds

Authors Goldmann G[15], Oldenburg J[15], Hukauf M[14], Garcia Frade L J[2], Jiménez Yuste V[3], Bonanad Boix S[4], Canaro Hirnyk M[5], Benítez O[6], Gómez del Castillo MDC[7], Sigl-Krätzig M[8], Halimeh S[9], Sparber-Sauer M[10], Heller C[11], Juranek S[12], Friedrich C[13], Zierk J[16], Tiede A[1]
Institutes **1** Hannover Medical School, Hannover, Germany; **2** Hospital Universitario Rio Hortega, Valladolid, Spain; **3** Hospital Universitario La Paz, Madrid, Spain; **4** Hospital Universitari i Politècnic La Fe, Valencia, Spain; **5** Hospital Universitario Son Espases, Palma de Mallorca, Spain; **6** Hospital Universitario Vall d Hebron, Barcelona, Spain; **7** Complexo Hospitalario Universitario A Coruña, A Coruña, Spain; **8** Blaubeuren Hemostasis Center, Blaubeuren, Germany; **9** Hemostasis Center, Duisburg, Germany; **10** Klinikum der Landeshauptstadt Stuttgart, Stuttgart, Germany; **11** University Hospital Frankfurt, Pediatric Hemostasis Center, Frankfurt, Germany; **12** Pediatric Thrombosis and Hemostasis Unit, Dr. von Hauner Children's Hospital, LMU Munich, München, Germany; **13** University Children's Hospital, Klinikum Oldenburg AöR, Oldenburg, Germany; **14** StatConsult, Magdeburg, Germany; **15** Bonn University Hospital, Bonn, Germany; **16** Erlangen University Hospital, Erlangen, Germany
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Introduction Joint bleeding is still a challenging complication of hemophilia. Distinguishing between intra-articular hemarthrosis and peri-articular soft tissue hematomas appears important but little guidance exist to support self-diagnosis by patients in the home care setting.

Method This study was designed to compare patient-reported symtpoms of intra-articular and peri-articular bleeds. Sixteen centers in Spain and Germany using the Haemoassist 2 electronic diary participated in the study. Patients with severe or moderate hemophilia were included if they had at least one qualifying ankle, knee, or elbow bleed with available self-assessment. The set of patient-reported symptoms included new or worsened restriction of joint mobility (M), swelling (S), pain (P), and tingling or local heat sensation (T). Logistic regression models were obtained from randomly assigned training and validation sets (60:40).

Results Three hundred and ten patients reporting 755 bleeding events were included. Comparing intra-articular and peri-articular bleeds, the symptoms M (68 % vs. 26 %), S (55 % vs. 41 %), P (74 % vs. 55 %), and T (19 % vs. 8 %) were consistently more frequent. The predictive model derived from logistic regression included all patient-reported symptoms and the affected joint. In the training set, 92 % of hemarthroses were correctly identified. This was confirmed in the validation set (sensitivity 93 %, positive predictive value 81 %, negative predictive value 77 %). The area under the receiver-operator curve was 0.84 (95 % confidence interval 0.79-0.89) in the validation set. The data were used to construct a simple self-assessment nomogram to predict joint bleeding.

Conclusion Patient-reported symptoms were different comparing intra-articular hemarthrosis with peri-articular soft tissue bleeds. A simple self-assessment tool provided good diagnostic performance and should be further validated in studies involving imaging or other clinical diagnostic strategies.

Conflict of Interest G. Goldmann Consultant for: Sobi, Takeda, Bayer, Octapha, Novo Nordisk, Biotest, Roche, J. Oldenburg Grant/Research support from: Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Sobi, Takeda, Consultant for: Bayer, Biogen Idec, Biomarin, Biotest, CSL Behring, Chugai, Freeline, Grifols, LFB, Novo Nordisk, Octapha, Pfizer, Roche, Sanofi, Spark Therapeutics, Sobi, Takeda, M. Hukauf Employee of: StatConsult, L. Garcia Frade Consultant for: Sanofi, Pfizer, Octapharma, V. Jiménez Yuste Grant/Research support from: Sobi, Biomarin, Grifols, Bayer, CSL Behring, Pfizer, Roche, Novo Nordisk, Takeda, Consultant for: Sobi, Biomarin, Grifols, CSL Behring, Pfizer, Roche, Novo Nordisk, Takeda, S. Bonanad Boix: None Declared, M. Canaro Hirnyk Consultant for: Bayer, CSL Behring, Novo Nordisk, Octapharma, Roche, Sobi, Takeda, O. Benítez Grant/Research support from: Sobi, Consultant for: CSL Behring, Pfizer, Sobi, Bayer, Novo Nordisk, Takeda, Octapharma, M. D. C. Gómez del Castillo Consultant for: Roche, Bayer, Amgen, Werfen, Sobi, Novo Nordisk, Pfizer, Takeda, M. Sigl-Krätzig: Consultant for: CSL Behring, Biotest. S. Halimeh Grant/Research support from: Bayer, Biotest, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Takeda, Consultant for: Bayer, Biotest, CSL Behring, Novartis, Novo Nordisk, Octapharma, Pfizer, Sobi, Takeda, M. Sparber-Sauer Consultant for: Bayer, Roche, Sobi, C. Heller Grant/Research support from: Bayer, Biotest, CSL Behring, Novo Nordisk, Pfizer, Roche, Sobi, Takeda, Consultant for: Bayer, Biotest, CSL Behring, Novo Nordisk, Pfizer, Roche, Sobi, Takeda, S. Juranek Consultant for: Pfizer, Biotest, C. Friedrich: None Declared, J. Zierk: None Declared, A. Tiede Grant/Research support from: Biotest, Octapharma, Pfizer, Roche, Consultant for: Bayer, Biomarin, Biotest, Chugai, Roche, Takeda, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Sobi.

T-13-44 Switching to extended half-life rFVIIIfc: transition of therapy and pharmacokinetics in pediatric patients with severe hemophilia A

Authors Miebach G, Juranek S, Lieftüchter V, Bidlingmaier C, Olivieri M
Institute Ludwig-Maximilians-Universität München, Pädiatrische Hämostaseologie, Dr. von Haunersches Kinderspital, München, Germany
DOI 10.1055/s-0042-1760547

Introduction To decrease bleeding incidents especially joint bleeding and ensure quality of life for patients with severe haemophilia A prophylactic treatment is standard of care at present. By using an extended half-life (EHL) recombinant factor VIII fc-fusion protein (rFVIIIfc, efmoroctocog alfa) the focus is on fewer injections and higher trough levels in patients to further decrease annual bleeding rates. If the decision is made to switch to a rFVIIIfc product, the therapy change must be well prepared and monitored. The objective of this review is to demonstrate the transition from standard half-life (SHL) products to rFVIIIfc and its outcome.

Method Prophylactic therapy in pediatric patients with severe haemophilia A was switched to a rFVIIIfc product. Dosage and frequency stayed identical to their previous FVIII product. Patients were selected either because they wanted less frequent factor doses or because they did not achieve sufficient trough levels with their previous therapy. Trough levels for each patient before and after switching to a rFVIIIfc were compared. Pharmacokinetic measurements were performed 3 to 4 weeks after switching. FVIII plasma levels were measured before injection of rFVIIIfc (trough level), 1 hour and 6 hours after injection. Factor VIII pharmacokinetics were generated for each patient via Web-Accessible Population Pharmacokinetics Service-Hemophilia (WAPPS-Hemo).

Results 8 patients aged between 3 and 17 years (median 10 years) were switched to the rFVIIIfc product. Prior therapies were rFVIII products (octogoc alpha) or plasma-derived factor VIII concentrates administered at a median dose of 2000 units (1500 - 5000 units) approximately every 2 days (minimum every 3 days, maximum every day). Patients received a median of 52 units per kg body weight per injection (31 - 87 units). The median trough level of prior therapy was 5 % (< 1 % - 14 %). By switching therapy to the EHL concentrate, patients achieved a median trough level of 9,5 % (2 % - 34 %).

Recovery of FVIII after 1 hour showed a median plasma level of 111,5 % (60 % - 172 %). After 6 hours the median plasma level had decreased to 71,5 % (45 % - 174 %). After 3-4 weeks of rFVIIIfc therapy there was no inhibitor development in any patient, no sign of atraumatic or extensive bleeding incidents and most importantly no joint bleeding in patients.

Conclusion Despite lower half-life of FVIII in the pediatric population all patients that switched to the rFVIIIfc product showed higher trough levels than before, no inhibitors were detected so far and no bleeding occurred. Pharmacokinetics showed FVIII activity levels as high as in healthy individuals for at least the first 6 hours after injection. Because of high trough levels fewer factor doses per week could be discussed depending on each patients need. In summary, the therapy transition to the rFVIIIfc product in this small cohort of patients was found to be highly successful and served the patients benefit.

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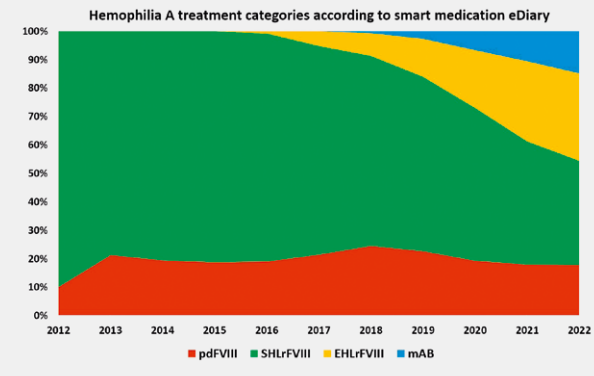
T-13-45 Changes in hemophilia A therapy - an analysis of real-world data from smart medication eDiary over the past 10 years

Authors Roesch A[1], Schmoldt D[1], Mondorf W[2]
Institutes 1 smart medication eHealth Solutions GmbH, Frankfurt, Germany; 2 Haemostas, Frankfurt, Germany
DOI 10.1055/s-0042-1760548

Introduction When the smart medication platform was launched in 2012, prophylaxis and on-demand therapy in hemophilia A was performed with either plasmatic factors (pdFVIII) or recombinantly produced short half-life preparations (SHLrFVIII). In 2016, recombinant extended half-life preparations (EHLrFVIII) and in 2018, monoclonal antibodies (mAB) were added as treatment options. Analysis of real-world data from smart medication eDiary over the past 10 years shows the change in therapy in favor of the more modern treatment options EHLrFVIII and mAB.

Method The smart medication eDiary electronic diary went live in 2012. Since then, more than 1500 patients from more than 45 treatment centers have used the platform to document their prophylaxis and on-demand therapy. Based on the documentation data, the number of patients who used one of the therapy options was evaluated to show the change in therapy over the past 10 years (▶ **Fig. 1**).



▶ **Fig. 1** Hemophilia A treatment categories according to smart medication eDiary.

Results The results show that modern therapy options with 30.8 % EHLrVIII and 14.9 % mAB (in total more than 45 %) have now replaced therapy with SHLrVFII with 36.6 % and will probably continue to replace it in the coming years. The use of plasmatic factors, however, remains at a constant level of just below 20 %.

Conclusion The data analysis in figure 1 shows that the use of recombinant short half-life preparations is gradually being replaced by modern therapeutic options, such as recombinant extended half-life preparations and monoclonal antibodies. However, the use of plasmatic factors remains at an almost constant low level.

Conflict of Interest none

T-13-46 The effects of single and combined knockouts of GABARAPs proteins family on FVIII secretion

Authors Ibrahim M, Nusgen N, Oldenburg J, Elmaarri O
Institute Universitätsklinikum Bonn, Institut für Experimentelle Hämatologie und Transfusionsmedizin (IHT), Bonn, Germany
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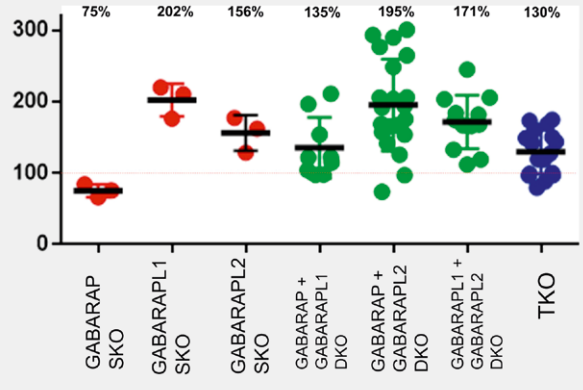
Introduction It is well established that upon synthesis, FVIII enters the ER to be folded and transported to the ERGIC compartment by interacting with chaperones and cargo proteins (ER: CANX and CALR; ERGIC: LMAN1 and MCFD2) which constitute the conventional secretory pathway (1). The highly similar ATG8 homolog proteins, GABARAP, GABARAPL1 and GABARAPL2 are gaining high interest due to the many roles that they play, in autophagy related and autophagy non-related functions (2). Albeit highly similar, these proteins seem to play differential roles that still require much investigation (3). We hypothesize that GABARAPs play a role in the intracellular trafficking of FVIII. However, due to the high similarity between these proteins and possible compensation among them, it is difficult to distinguish individual functions (4).

We aim to study the effect of the absence and/or presence of individual GABARAPs proteins on the secretion level of FVIII. To tackle this problem we created cells knockouts carrying one, two or none of the GABARAPs, in order to study their individual separate role(s) (▶ **Fig. 1**).



▶ **Fig. 1** Dot plot showing the measured FVIII activity in our different KO clones.

Method We performed CRISPR-cas9 mediated single, double and triple knockouts of GABARAPs (GABARAP, GABARAPL1 and GABARAPL2) in HEK293 cells stably expressing FVIII. The study was performed on HEK293 modified to secrete stable FVIII levels. This clone was named HEK28. Our KOs were identified by Sanger sequencing and verified by Western blot analysis using antibodies against GABARAP (anti-GABARAP E1J4E, Cell-Signaling technology), GABARAPL1 (anti-GABARAPL1 D5R9Y, Cell-Signaling technology), and GABARAPL2 (anti-GABARAPL2 D1W9T, Cell-Signaling technology) proteins. To observe the effect of the KO on FVIII, we measured FVIII Activity in the medium using the Chromogenix Coamatic Factor VIII assay.

Results Our results showed a decrease of secreted FVIII in GABARAP SKO (to 75 %) compared to increase in GABARAPL1 (to 201 %) and GABARAPL2 SKO (to 156 %). For DKO, all three combinations showed an increase in FVIII relative to WT (GABARAP-GABARAPL1 to 135 %; GABARAP-GABARAPL2 to 195 %; GABARAPL1-L2 to 171 %). A similar increase was also observed in TKO although it was less than that in DKO (to 130 %).[1–4]

Conclusion Our results show that in the SKO, the GABARAPs play a gene specific but variable role manifested by opposite effects on FVIII secretion (GABARAP SKO vs GABARAPL1 and GABARAPL2 SKO). However, in DKO and TKO, an additive role in the secretion of FVIII was seen. This is shown by the cumulative values of FVIII measurements in DKO and TKO relative to WT.

Conflict of Interest none

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T-13-47 Insights in pain processing of affected versus non-affected structures

Authors Ransmann P, Tomschi F, Schmidt A, Brühl M, Hilberg T
Institute University of Wuppertal, Department of Sports Medicine, Wuppertal, Germany
DOI 10.1055/s-0042-1760550

Introduction Patients with haemophilia (PwH) suffer from a coagulation disorder leading to repetitive bleedings into joints, which can result in painful haemophilic arthropathy. Along with joint arthropathy, higher local pain status in PwH was observed using a hand-held electronic pressure algometer in previous studies [1, 2]. This study aims to explore pain sensitivity in non-affected structures in form of compression pain on the musculus triceps surae. For this, the cuff pressure algometry is used and compared to pain perception of the electronic pressure algometer.

Method 39 PwH and 42 healthy control subjects (Con) enrolled in this study. Joint health status was assessed. pain sensitivity was measured with the hand-held electronic pressure algometer and cuff pressure algometry. Pressure pain thresholds of the algometer (PPT) were measured at both knee and ankle joints as well as the forehead. Subsequently, thresholds of cuff pressure were measured at the left and right calf (CPT). Hereby, mechanical compression pain of deep soft tissue (muscle, fascia) is induced. Noticing that high values indicate less pain sensitivity and vice versa.

Results Results of 36 PwH and 38 control subjects were analyzed. The values of joint health status of PwH were significantly higher (28.9 ± 14.6 (mean ± SD)) compared to Con (5.5 ± 5.7 (mean ± SD)); $p \leq 0.001$, $r = -0.79$). The Mann-Whitney-U-Test revealed that PPT of the knee and ankle joints were lower in PwH (e.g., knee left: 56.6 ± 25.4; ankle left: 46.6 ± 24.2 (mean ± SD)) compared to Con (e.g., knee left: 76.4 ± 29.8, $p = 0.003$, $r = -0.34$; ankle left: 55.8 ± 20.2, $p = 0.023$, $r = -0.25$ (mean ± SD)). Values of the right side displayed similar results. No difference was observed between PwH and Con in PPT values at the forehead (PwH: 39.5 ± 14.9; Con: 37.6 ± 13.9 (mean ± SD), $p = 0.404$, $r = -0.09$). Contrastingly, PwH had significantly higher scores of the CPT compared to Con in both left (PwH: 30.5 ± 16.9; Con: 19.7 ± 9.3 (mean ± SD), $p = 0.010$, $r = -0.29$)

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and right calves (PwH: 30.2 ± 15.6; Con: 22.0 ± 11.5, p = 0.026, r = -0.26 (mean ± SD)).

Conclusion While PPT of the knee and ankle joints are lower in PwH, scores of CPT are significantly higher in PwH compared to Con. This reveals a paradox situation, highlighting that PwH experience local, joint- and synovitis-related pain, whereas pain sensitivity of non-affected soft tissue structures is lower indicated by higher CPT values. This goes along with non-significant values of the PPT in the forehead. The results are different to what has been expected. Though the underlying physiological mechanisms, induced by cuff pressure on the lower leg are not yet entirely clarified. Hence, future investigations focusing on the different types of pain processing and effects of cuff are needed to verify these results.

Conflict of Interest There is no conflict of interest.

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T-13-48 Production of FVIII-specific iTregs using tolerogenic dendritic cells

Authors Schnepper P, Orlowski A, Königs C, Schmidt A

Institute Goethe University Frankfurt, University Hospital, Department of Paediatrics and Adolescent Medicine, Clinical and Molecular Haemostasis, Frankfurt am Main, Germany

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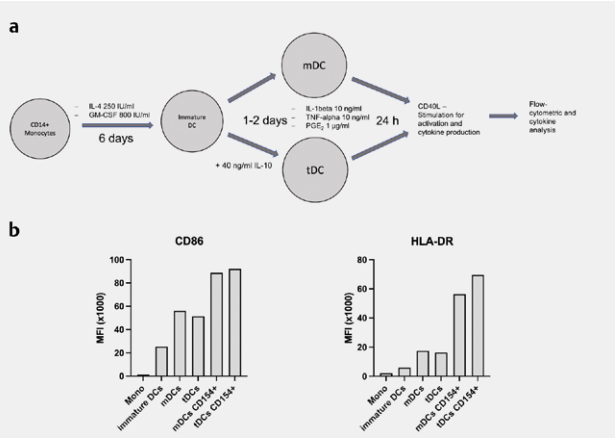
Introduction Inhibitor development during the treatment of haemophilia A patients still represents a major challenge. Despite extremely demanding conventional immune tolerance induction, specific approaches to reverse inhibitor development are still missing. In this study we were aiming to induce FVIII-specific regulatory T cells (iTregs) from naïve CD4 + T cells using tolerogenic dendritic cells (tDCs). iTregs induced from patient T cells might in the future be used to tolerize inhibitor-positive patients towards FVIII.

Method The generation of tDCs was performed from isolated CD14 + monocytes (Fig. 1A) based on a previously described protocol (1). During culture, cells were analysed for surface marker and cytokine expression. To verify further maturation and cytokine production of tDCs and mDCs following stimulation, cells were stimulated with CD4 + T cells expressing CD154 following FVIII stimulation after maturation. Generated tDCs and mDCs were subsequently used for priming of autologous naive CD4 + T cells with FVIII. Additionally, the Fab fragment of an anti-TNF-α antibody was added to a part of the tDCs to enhance their suppressive function (2). Cells were kept in culture for 14 days with the addition of IL-2 on day 7 followed by a resting period in cytokine free medium for 2 days. To determine the regulatory function of potential iTregs, eF450-labelled mDC-primed T effector cells were incubated with autologous mitomycin C-treated PBMCs and different ratios of tDC-primed T cells as iTregs in the presence of FVIII and proliferation of T effector cells was compared after 5 days by flow cytometric analysis.[1–2]

Results Analysis of DC surface markers CD86 and HLA-DR revealed a slightly lower expression in tDCs compared to mDCs followed by a higher increase following stimulation with CD154 + T cells, that were activated by FVIII stimulation (Fig. 1B), as expected (1) and only tDC stimulation resulted in production of IL-10. Looking at T cells primed with mDCs and tDCs in the presence of FVIII, cultures showed different chemokine expression. Additionally, the production of pro-inflammatory TNF-α and IFN-γ was higher in mDC cultures while regulatory IL-10 and TGF-β were more produced in tDC cultures. In the suppression assay, a donor- and dose-dependant suppression of FVIII-specific T effector cell proliferation was observed in the presence of tDC-primed iTregs (▶ **Fig. 1**).



▶ **Fig. 1** Scheme of tDC generation surface marker analysis of DC populations; A) Monocytes were isolated and cultured in the presence of IL-4 and GM-CSF. For tDC induction IL-10 was added 1 h prior to addition of IL-1β, TNF-α, and prostaglandin E2 for further maturation. Matured cells were stimulated with CD154 + T cells to induce IL-10 secretion of tDCs. B) Representative CD86 and HLA-DR surface marker profile of monocytes and DCs during differentiation.

Conclusion We established a protocol for generating mDCs and tDCs that could be used for priming of naïve CD4 + T cells with FVIII, resulting in a regulatory cytokine milieu during priming in the presence of tDCs. So far, potentially induced Tregs were able to partly suppress T effector proliferation. An enrichment of FVIII-specific T cells after priming appears necessary to successfully inhibit FVIII-specific T effector proliferation in suppression assays.

Conflict of Interest The authors declare no conflict of interest.

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T-13-49 Characterization of the influence of heat- and acid-treatment on the FVIII-antibody interaction in order to improve inhibitor diagnostics

Authors Schmidt A[1], Stichel D[1], Salzmann-Manrique E[2], Königs C[1]

Institutes **1** Goethe University Frankfurt, University Hospital, Department of Paediatrics and Adolescent Medicine, Clinical and Molecular Haemostasis, Frankfurt am Main, Germany; **2** Goethe University Frankfurt, University Hospital, Department for Pediatric Stem Cell Transplantation and Immunology, Frankfurt am Main, Germany

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Introduction We have previously reported, that acid-treatment of FVIII-containing plasma samples is advantageous over heat-treatment to detect FVIII-specific antibodies in treated patients, as heat-treatment often leads to false-positive results. However, the number of samples in which we could detect FVIII-specific antibodies using acid-treatment was low. Therefore, here we aimed to further characterize the impact of heat- and acid-treatment on the binding properties of FVIII-specific antibodies.

Method A total of 23 murine FVIII-specific monoclonal antibodies were analysed in two different ELISAs: first, binding of antibodies to immobilized FVIII in the presence of soluble FVIII with or without acid-treatment was analysed,

to identify if binding to immobilized FVIII could be competed with FVIII and if the competition could be reversed by acid treatment of the soluble FVIII-antibody mixture. Second, we tested binding of heat- and acid-treated antibodies to native, as well as heat- and acid-treated immobilized FVIII, to further quantify the susceptibility of FVIII and antibodies to these treatments.

Results Analysis of the influence of heat- and acid-treatment on antibodies and FVIII, revealed that antibody-binding to FVIII was more influenced by heat- than acid-treatmtent. Interestingly, if FVIII was also treated with heat or acid, only heat-treatment led to a further decrease of binding, pointing out that a high proportion of antibodies might still bind to acid-treated FVIII. However, in the competition ELISA for 17 of 23 antibodies, binding to immobilized FVIII could be competed by soluble FVIII and restored by acid-treatment. Competition resulted in a mean of 66,18 % (± 13,29 %) reduction of the ELISA signal and acid-treatment resulted in a mean restoration of 88,26 % (± 18,01 %) of the initial ELISA signal. Remaining 6 antibodies either could not be competed by soluble FVIII (2) or could be competed with soluble FVIII but binding was not restored by acid-treatment (4), like previously shown for the human antibody BO2C11.

Conclusion Our data indicate, that FVIII as well as antibodies are more affected by heat-treatment than by acid-treatment. Treatment of FVIII in complex with antibodies should ideally ensue the denaturation of FVIII while still being mild for the antibodies. When comparing binding of acid-treated antibodies to native and acid-treated FVIII, binding was not significantly reduced by additional acid-treatment of FVIII, indicating, that antibodies might still bind to acid-denatured FVIII in the plasma sample. However, analysis of 23 monoclonal murine antibodies revealed, that most antibodies bound again to FVIII after acid-treatment of the antibody and competing FVIII and binding was only reduced by a mean of 12 % compared to binding without competition and acid treatment. Thus acid-treatment of FVIII-containing plasma samples appears to be a beneficial approach to detect masked immune responses in treated haemophilia patients.

Conflict of Interest The authors declare no relevant conflict of interest

T-13-50 Establishment of a flow-cytometry panel to analyse binding of FVIII to lymphocyte populations

Authors Albers S, Schmidt A, Orlowski A, Schultze-Strasser S, Königs C
Institute Goethe University Frankfurt, University Hospital, Department of Paediatrics and Adolescent Medicine, Clinical and Molecular Haemostasis, Frankfurt am Main, Germany
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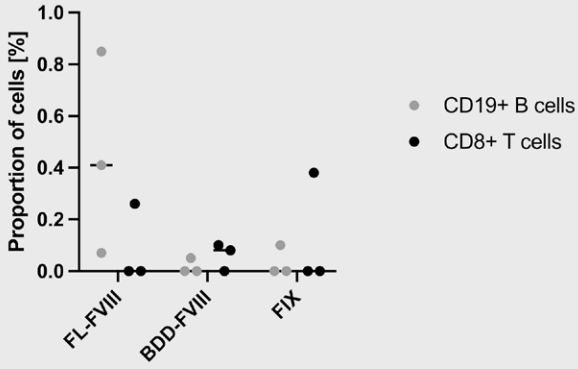
Introduction In haemophilia A, specific neutralising antibodies, also known as inhibitors, occur in about 30 % of patients, target FVIII and therefore inhibit the procoagulant effect of coagulation factor VIII (FVIII). The use of CAR-transduced regulatory T cells (CAR-Tregs) is considered a promising approach to eliminate inhibitors and induce tolerance. FVIII-specific Tregs have been shown to modify and suppress the immune reaction against FVIII and thus inhibit the proliferation of FVIII-specific T effector cells and reduce FVIII-specific antibodies. The mode of action for this suppression is unknown for the soluble antigen FVIII. Furthermore, it is unclear whether FVIII immobilises on cells and is therefore presented differently to the immune system.

Method A flow cytometric panel was established with lymphocytes of three healthy donors to analyse the main lymphocyte populations: CD3 + CD4 + T cells, CD3 + CD8 + T cells, CD19 + B cells and CD56 + NK cells. To analyse the ability of these populations to bind FVIII, we labelled full-length FVIII (FLFVIII), B-domain deleted FVIII (BDDFVIII) and FIX as control with AF647 and quantified the labelling level by absorption measurements. We used FVIII in physiological concentrations and equimolar amounts of FIX for good comparison. Citrated blood samples were freshly obtained from healthy volunteers and stained with the described panel after lysis of erythrocytes.

Results After testing several labelled proteins for the staining, we decided to use proteins only labelled about once per molecule to be able to compare signal intensities within flow-cytometric samples. Using the designed panel, T cells, B cells and NK cells in fresh blood samples were quantified in the expected ranges. With our current protocol, the proportion of FVIII-AF647-stained cells was below one percent and positive cell populations could only be detected for CD19 + B cells and CD3 + CD8 + T cells (▶ **Fig. 1**). CD3 + CD8 + T cells of one healthy volunteer showed higher binding to FIX than FVIII. interestingly, for our analysed volunteers, CD19 + B cells bound better to FLFVIII than BDDFVIII.



▶ **Fig. 1** Binding of labelled FL-FVIII, BDD-FVIII and FIX to CD19 + B cells and CD8 + T cells.; Binding of AF647-labelled proteins to CD19 + B cells and CD8 + T cells was donor-dependant. B cells showed higher binding to FL-FVIII compared to BDD-FVIII and FIX.

Conclusion FVIII but also FIX bound to lymphocytes could be detected in healthy volunteers. Endogenous FVIII of healthy individuals might already occupy a big proportion of binding sites. The impact of endogeneous FVIII on our results is currently being analysed. Likewise, the same analyses are in progress in haemophilia A patients without endogenous FVIII. Our results might offer an explanation for the induction of FVIII-specific CAR-Tregs and further have the potential to explain open questions in the procoagulant activity of transfused FVIII.

Conflict of Interest The authors declare no conflict of interest

T-13-51 The beauty of real-world data. EHL factor concentrates contribute to declining annual bleed rates

Authors Wüst F[1], Pekrul I[1, 2], Ganslmeier M[1, 2], Spannagl M[2], Möhnle P[2]
Institutes **1** Ludwig-Maximilians University, University Hospital, Dept. for Anaesthesiology, Munich, Germany; **2** Ludwig-Maximilians University, University Hospital, Dept. for Transfusion Medicine, Cell Therapy and Haemostaseology, Munich, Germany
DOI 10.1055/s-0042-1760554

Introduction Optimized haemophilia care with its zero-bleed strategy is critical for patient outcomes. Various therapeutic options are available for treatment individualization, offering higher trough factor levels and a reduction in therapeutic burden. However, does switching from conventional to novel factor concentrates (CFC, FC) with extended half-life (EHL) improve the overall number of bleeds (NBs) in a real-world patient cohort?

Method We performed a retrospective data analysis (01.01.2014 to 31.12.2020) in patients with severe Haemophilia A (HA) in our centre to evaluate the association between different FCs with FC consumption, spontaneous bleeding rates and adherence to the prescribed therapeutic regimen.

Results See conclusion.

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Conclusion Data from 69 patients with complete documentation qualified for analysis. With the introduction of EHLs, the proportion of plasma and recombinant CFC steadily declined, while the number of EHL-FCs sharply increased, accounting for 49 % of FC used for HA treatment in 2020. NBs were more than halved in 2020 compared to 2018 (124 vs. 58; Fig.1). This observation are the result of various measures: The drop in bleed rates may be associated 1) with the increasing use of EHL-FCs, 2) with therapy optimization based on factor VIII measurements (CFCs, EHLs), 3) a closer monitoring (e.g. increased number of visits, eDiary) and 4) increased efforts in patient education. Some of the implemented measures were aimed at improving adherence, but no impact is evident in our data. However, the reduction in NBs may be associated with switching to EHL-FCs. However, there is still a long way to go when targeting zero-bleeds (▶ **Fig. 1**).

▶ **Fig. 1** The beauty of real-world data. EHL factor concentrates contribute to declining annual bleed rates; Figure 1 Therapeutic regimes based on CFC-P or –R decreased over time. Switching to EHL-FCs may be associated with a clinical significant decrease in bleeding rates. The total consumption of FCs increased over time (CFC and EHL-Fcs). (Abbr. CFC-P, -R conventional factor concentrates plasma, -recombinant, EHL extended half-life factor concentrate, NB number of bleeds, FC factor concentrate, IE/kg lean BW).

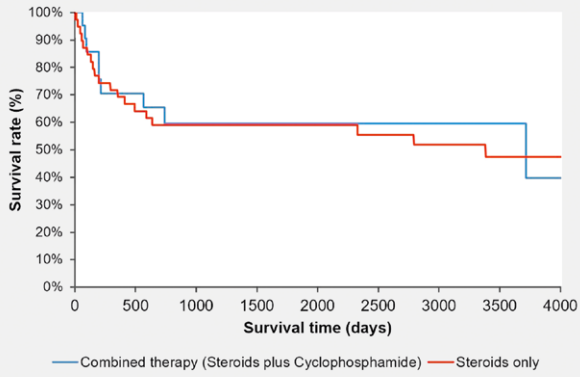
Conflict of Interest The authors declare no relevant conflict of interest.

T-14 | Acquired Haemophilia

T-14-01 Long term observation of therapy and outcome in acquired hemophilia A

Author Dreier L
Institute Johann Wolfgang Goethe-Universität Frankfurt am Main, Medizinische Klinik 2/Hämostaseologie/Universitätsklinikum Frankfurt am Main, Frankfurt am Main, Germany
DOI 10.1055/s-0042-1760555

Introduction Aquired hemophila A (AHA) is a rare autoimmune disease (incidence 1/1.000.000 per year), which is caused by autoantibodies against factor VIII (FVIII) and can lead to potential life-threatening bleeding events.
Primary therapy goals are prevention and control of bleeding events, inhibitor eradication with immunosuppression and therapy of primary disease. Today's therapy recommendations suggest a combination of hemostatic and immunosuppressive treatment (IST).
The aim of the study was to examine different therapy strategies used over the last 30 years on treatment effects, mortality and patient's outcome. Adverse events (AEs) while and after treatment, AHA-relapses and many other secondary outcome parameters were also monitored. Primary purpose was to provide a more precise prognosis and better aftercare for future patients.
Method The study was a monocentric retrospective and in parts prospective observational study of 79 AHA-patients, treated between 1995 and 01/2021 at the hemophilia center of Universitätsklinikum Frankfurt a. M.. The data collection was based on existing patient files and telephone interviews to record and confirm AEs that occurred after therapy. Research protocol was approved by the ethics committee.
Results To explore the impact of different IST strategies (steroids alone vs. steroids plus cyclophosphamide) on patients' survival, a Kaplan Meier survival analysis was performed. The analysis showed no significant difference in patients survival regardless which IST was used, $\chi^2(1) = 0.257$, $p = .612$ (▶ **Fig. 1**).



▶ **Fig. 1** Kaplan Meier survival analysis comparing IST.

The effect of susoctocog alfa regarding remission was also investigated. With susoctocog alfa therapy, complete remission was achieved in 10 patients (71.4 %), partial remission in one patient (7.1 %) and no remission after the initial AHA episode in 2 patients (14.3 %). In one patient (7.1 %), the remission status remained unclear.
In comparison, in the group of patients who did not initially receive susoctocog alfa or another FVIII preparation, complete remission was achieved in 33 patients (55.9 %), partial remission in 9 patients (15.3 %) and no remission in 10 patients (16.9 %). In this group, the remission status remained unclear in 7 patients (11.9 %) (▶ **Table 1**).
Conclusion The choice of IST was based on FVIII-activity (%) and antibody-titer (BE/ml). Patients with low FVIII-activity and higher antibody-titer usually received stronger IST in form of combined therapy. The fact that the Kaplan Meier analysis showed no significant differences in survival between both IST-groups speaks in favor of the therapy consideration that was made.
The susoctocog alfa therapy of AHA shows promising results regarding the achievement of a complete remission. However, due to the relatively small sample size, more data needs to be collected.

	Remission after initial therapy				
	Complete Remission (CR) [n (%)]	Partial Remission (PR) [n (%)]	No Remission [n (%)]	Unclear Remission-status [n (%)]	Total
Susoctocog alfa	10 (71.4)	1 (7.1)	2 (14.3)	1 (7.1)	14
No Susoctocog alfa	33 (55.9)	9 (15.3)	10 (16.9)	7 (11.9)	59

▶ **Tab. 1** Susoctocog alfa therapy and remission.

Due to the rarity of AHA and a lack of RCTs, retrospective studies like this make a major contribution to provide more precise prognosis and better aftercare for future patients.
Conflict of Interest Institutional budget, no extrernal funding (budget of sponsor/PI).

T-14-02 Monitoring of emicizumab in acquired hemophilia A

Authors Türkantoz H, Heubisch M CL, Bokies J, Tiede A, Werwitzke S
Institute Hannover Medical School, Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover, Germany
DOI 10.1055/s-0042-1760556

Introduction Emicizumab is a humanized bispecific antibody mimicking the cofactor activity of factor VIII (FVIII) and is currently licensed for congenital hemophilia A. It is also increasingly used in patients with acquired hemophilia A (AHA). Monitoring of emicizumab in AHA can be complicated by concomitant medication with recombinant porcine FVIII (susoctocog alfa, rpFVIII) or recovering FVIII activity in the context of remission. Vice versa, emicizumab may interfere with the monitoring of residual FVIII activity and complicate the detection of remission.

Method We developed a comprehensive monitoring program for patients with AHA using emicizumab. Assays to monitor emicizumab activity included a chromogenic FVIII activity assay based on human components (Emi-CSA), and a modified clot-based assay (Emi-CBA). FVIII interference was eliminated by the addition of specific anti-FVIII antibodies or heat inactivation. To eliminate the interference of emicizumab in activated partial thromboplastin time (APTT) and APTT-based assays, anti-emicizumab Fab fragments were used.

Results Emi-CSA and Emi-CBA were both suitable for measuring emicizumab in clinical and spiked samples. The lower limits of detection and quantification were slightly lower for Emi-CSA compared to Emi-CBA. The intra- and inter-assay variations were within acceptable ranges (< 15 %) for both assays. Recombinant human or porcine FVIII interfered with emicizumab activity in both assays. Interference of rpFVIII and residual human FVIII was prevented by adding anti-FVIII antibodies or heat inactivation. Emicizumab was completely neutralized by Fab fragments to enable APTT-based assays, including FVIII activity and inhibitor titer. Emicizumab monitoring informed about pharmacokinetics and drug concentration in all patients examined and indicated the development of neutralizing anti-drug antibodies (ADA) in one patient with AHA in the context of severe recurrent bleeding.

Conclusion Modification of coagulation methods allows monitoring of emicizumab, residual FVIII and inhibitor titer in patients with AHA. The monitoring program established here will be helpful for assessing the use of emicizumab in clinical trials and during real-world use.

Conflict of Interest H.T. has nothing to disclose. M.C.L.H. has nothing to disclose. J.B. has nothing to disclose. A.T. received grants for research and honoraria for lectures or consultancy from Bayer, Biotest, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, and Takeda. S.W. received grants for research and honoraria for lectures or consultancy from Biotest, Octapharma, and Stago.

T-15 | Von Willebrand Syndrome

T-15-01 Impaired hemostatic capacity in patients with low von Willebrand factor

Authors Mehic D[1], Hofer S[1], Quehenberger P[2], Sunder-Plaßmann R[2], Haslacher H[2], Ay C[1], Pabinger I[1], Gebhart J[1]
Institutes 1 Medical University of Vienna, Department of Medicine I, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; 2 Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria
DOI 10.1055/s-0042-1760557

Introduction In the most recent guidelines on the diagnosis of von Willebrand disease (VWD),1 not only patients with von Willebrand Factor (VWF) < 30 IU/dL, but also those with a bleeding tendency and VWF levels 30-5IU/dl were defined as having VWD. However, data on the hemostatic capacity are scare in patients with VWD and no data on differences between patients with < 30 IU/dL and those with 30-5IU/dL are available. Recently, we could show an impairment of the hemostatic potential in patients with bleeding disorder of unknown cause (BDUC),2 who share a similar phenotype to patients with low VWF.3

	VWF 30-50 IU/dl (n=38)	VWF< 30 IU/dl (n=10)	p*	BDUC (n=383)	p*	Healthy controls (n=100)	p*
	n (%)	*n (%)*		*n (%)*		*n (%)*	
Sex, female	33 (86.8)	8 (80)	0.628	330 (86.2)	0.908	80 (80.0)	0.351
Caucasian ethnicity	38 (100)	10 (100)	-	379 (99.0)	-	99 (99.0)	-
Blood Group type O, n (%)	31 (81.6)	8 (80)	0.909	177 (46.3)	**<.001**	37 (37.0)	**<.001**
	median (IQR)	*median (IQR)*		*median (IQR)*		*median (IQR)*	
Age, years	31.5 (22.0-24.3)	37.0 (30.3-39.3)	0.601	42.0 (29.0-54.0)	0.005	40.5 (29.0-50.0)	**0.035**
BMI, kg/m²	22.8 (20.1-26.1)	25.2 (21.6-28.7)	0.250	23.3 (21.0-26.5)	0.228	22.2 (20.5-25.2)	0.807
VWF:Ag, IU/dl	55.5 (50.0-60.2)	35.5 (31.3-39.8)	**<.001**	100 (82.0-126.0)	<.001	104.0 (88.8-134.0)	**<.001**
VWF:RCo, IU/dl	46.0 (41.0-49.0)	26.0 (20.5-28.0)	**<.001**	87.0 (69.0-127.0)	<.001	91.0 (71.0-127.0	**<.001**
FVIII IU/dL	80.5 (69.8-97.3)	62.0 (53.8-79.3)	0.154	128.0 (103.0-163.0)	<.001	128.0 (105.0-156.5)	**<.001**
APTT, sec	36.9 (35.4-41.9)	37.6 (36.2-42.9)	0.374	35.5 (33.4-37.7)	<.001	35.1 (33.3-36.6)	**<.001**
PT, %	92.0 (85.0-97.3)	92.5 (89.0-98.0)	0.542	95.0 (88.0-104.0)	0.015	99.0 (90.3-108.5)	**<.001**
Fibrinogen, g/dl	277.0 (242.0-315.8)	293.5 (266.8-324.3)	0.852	317.0 (276.0-365.0)	<.001	290.5 (244.3-340.3)	0.254
Hemoglobin, g/dl	13.3 (12.6-14.4)	13.7 (12.4-15.4)	0.926	13.5 (12.8-14.2)	0.919	13.7 (13.0-14.4)	0.264
Platelet count, G/L	247.5 (213.0-321.0)	225.0 (188.5-345.8)	0.378	246.0 (213.0-284.0)	0.161	257.0 (220.3-295.5)	0.704
Bleeding phenotype							
Number of patients above the Vicenza score cut-off	36 (94.7)	9 (90)	0.512	357 (93.2)	1.000	0 (0)	**<.001**
Vicenza bleeding Score, median (IQR)	5.0 (3.0-7.0)	6.5 (2.8-10.3)	0.482	5.0 (3.0-8.0)	0.819	0 (0-0)	**<.001**
ISTH-BAT§	4.0 (4.0-7.0)	7.5 (3.0-9.8)	0.172	6.0 (4.0-8.5)	0.123	na	na
Number of bleeding manifestations	3.0 (2.0-6.0)	3.0 (2.0-5.0)	0.658	3.0 (2.0-40)	0.373	na	na

▶ **Tab. 1** Clinical characteristics and bleeding phenotype in all patient groups and healthy controls.

Method We analyzed the hemostatic capacity and compared clinical data of 38 patients with low VWF levels (30-50 IU/dL) to 10 patients with very low VWF levels (< 30 IU/dL), 383 patients with BDUC and 100 healthy controls (HC). Patients had been consecutively included into the Vienna bleeding biobank (VIBB), a prospective cohort-study (EC No 603/2009).2 The hemostatic capacity was assessed by thrombin generation (TG, Technothrombin, Technoclone, Vienna, Austria) and plasma clot formation and lysis (PCF, method by Wolberg et al, according to SCC recommendations of the ISTH4,5).

Results Patients' characteristics and bleeding phenotype are summarized in Table 1. Blood group O was more common in VWD patients compared to BDUC and HC. Patients with low VWF levels did not differ significantly in bleeding severity (measured by the Vicenza and ISTH bleeding score and the number of bleeding manifestations) in comparison to patients very low VWF levels (< 30 IU/dL) or BDUC, albeit a tendency towards increased bleeding severity in patients with very low VWF was seen (▶ **Tab. 1**).

TG and PCF, but not clot lysis, were equally impaired in patients with low- and very low VWF compared to HC (Table 2). Compared to BDUC, TG was also significantly decreased in low VWF patients, whereas there were no relevant differences in PCF. No strong correlations of the hemostatic capacity with the bleeding phenotype (Vicenza and ISTH-BAT scores, number of bleeding manifestations) was seen (data not shown). Age ($\beta = -0.149$, $p < .001$), sex ($\beta = 0.206$, $p < .001$) and fibrinogen levels ($\beta = 0.330$, $p < .001$) affected TG (AUC) in multivariate analysis (linear regression, $R2 = 0.207$) of all patients, whereas there was no significant impact of VWF- or FVIII levels, or blood group O. PCF ($R2 = 0.360$) was determined by factor VIII- ($\beta = 0.251$, $p < .001$) and fibrinogen levels ($\beta = 0.534$, $p < .001$) but also not by VWF or blood type [1–5].

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Conclusion In our study, we have observed a predominance of female sex and blood group O in patients with low VWF, in line to published data. We found a significantly decreased hemostatic capacity in patients with low VWF levels, which was to the same extend impaired as in patients with very low VWF. Our data support the diagnosis of VWD in bleeding patients with low VWF (▶ **Tab. 2**).

	VWF 30-50 IU/dl	VWF <30 IU/dl	p*	BDUC	p*	HC	p*
Thrombin generation							
N (%)	35 (92.1)	10 (100)		364 (95.0)		100 (100)	
	median (IQR)	median (IQR)		median (IQR)		median (IQR)	
Lag time, min	11.6 (9.6-13.1)	10.3 (9.0-12.7)	0.151	10.6 (9.1-12.6)	0.087	9.1 (8.1-11.1)	0.064
Velocity index, nmol/l/min	16.5 (8.1-33.8)	19.6 (13.0-37.7)	0.539	33.8 (18.2-55.9)	**0.015**	65.8 (32.3-115.4)	**<.001****
Peak thrombin, nmol/L	155.9 (99.7-220.2)	184.65 (149.1-264.3)	0.314	239.5 (161.5-239.5)	**0.008****	362.5 (251.2-475.4)	**<.001****
TTP, min	20.6 (17.1-25.1)	20.1 (17.2-22.6)	0.359	18.1 (15.1-21.6)	**0.010**	15.1 (12.1-18.6)	**<.001****
AUC, nmol/l x min	2789.7 (2236.8-3505.0)	3169.0 (2759.3-3565.0)	0.239	3274.5 (2855.2-3746.0)	**0.004****	3784.9 (3302.9-4067.1)	**<.001****
Plasma clot formation and lysis							
N (%)	38 (100)	10 (100)		383 (100)		100 (100)	
Lag time, min, median (IQR)	10.4 (8.3-16.3)	14.8 (11.3-19.5)	0.130	10.4 (7.4-14.0)	0.275	9.3 (7.7-12.2)	**0.036**
Vmax, OD/min, mean (SD)	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.533	0.1 (0.1-0.2)	**0.029**	0.2 (0.1-0.2)	**<.001****
ΔAbs, OD 405nm, mean (SD)	0.6 (0.5-0.8)	0.7 (0.6-0.8)	0.996	0.7 (0.6-0.8)	0.497	0.5 (0.4-0.6)	**<.001****
TTP, min	22.2 (15.6-25.2)	23.5 (18.4-29.2)	0.214	19.5 (14.4-23.5)	0.383	9.2 (7.7-12.2)	**<.001****
CLT, min, median (IQR)	16.5 (12.4-18.4)	14.2 (13.0-16.9)	0.233	16.0 (13.5-19.7)	0.964	16.0 (13.8-19.8)	0.190

▶ **Tab. 2** Thrombin generation and plasma clot formation/lysis in patients with low VWF in comparison to very low VWF, BDUC and healthy controls.

Conflict of Interest The Vienna bleeding biobank was supported by an unrestricted grant of CSL Behring, the Medical-scientific fund of the Mayor of the federal capital Vienna (Nr. 20023) and the Anniversary Fund of the Austrian National Bank (Nr. 18500).; D.M. received honoraria from CSL Behring for advisory board meetings.; C.A. received honoraria from Bayer, CSL Behring, NovoNordisk, Pfizer, Roche, Sobi and Takeda for lectures and participation in advisory board meetings.; I.P. received honoraria from CSL Behring, Novartis, Amgen and Sobi for lectures and advisory board meetings.; J.G. received honoraria for lectures and advisory board meetings and research funding for the Medical University of Vienna from CSL Behring, Novartis, Amgen and Sobi.

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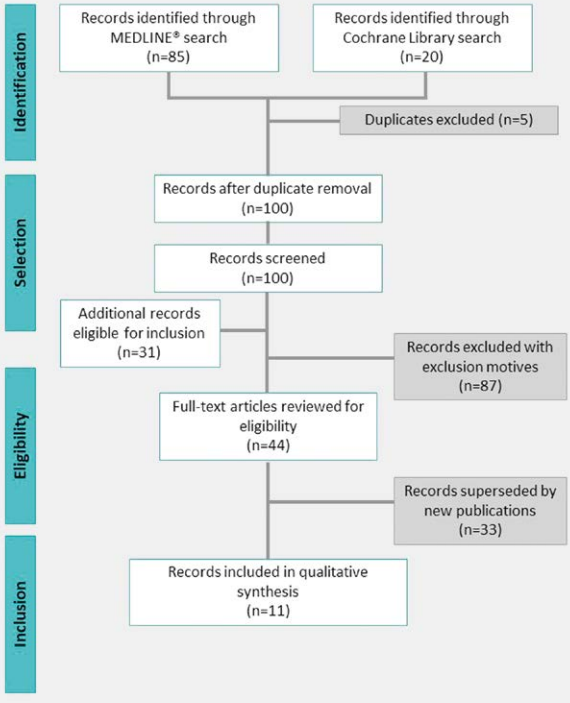
T-15-02 Efficacy, safety and consumption of plasma-derived von Willebrand factor (VWF)/Factor VIII (FVIII) concentrate with 2.4:1 VWF:FVIII ratio for the treatment of von Willebrand Disease: a systematic review

Authors Rugeri L[1], Thomas W[2], Schirner K[3], Heyder L[3], Auerswald G[4]
Institutes **1** Hospices Civils de Lyon - Unite d'Hemostase Clinique, Hôpital Cardiologique Louis Pradel, Lyon, France; **2** Cambridge University Hospitals NHS Foundation Trust, Cambridge Biomedical Campus, Cambridge, UK; **3** CSL Behring Innovation GmbH, Marburg, Germany; **4** Coagulation Centre, Bremen Central Clinic, GeNo Ltd, Parent-Child-Centre Prof. Hess, Bremen, Germany
DOI 10.1055/s-0042-1760558

Introduction The therapeutic goal in the management of von Willebrand disease (VWD) is to treat or prevent bleeding events by correcting the deficiency of von Willebrand factor (VWF) and coagulation factor VIII (FVIII). Plasma-derived human coagulation FVIII/human VWF (pdVWF/FVIII; Voncento®/Biostate®) is indicated in all age groups for prophylaxis and treatment of haemorrhage or surgical bleeding in patients with VWD when desmopressin is ineffective or contraindicated. A systematic literature review was conducted to evaluate the efficacy, safety and consumption of pdVWF/FVIII in the treatment of patients of all ages with mild, moderate and severe inherited VWD.

Method TheMEDLINE and Cochrane Library databases were searched for publications presenting results of studies of pdVWF/FVIII in patients with inherited VWD. All retrieved articles were assessed against predefined inclusion/exclusion criteria following Cochrane group recommendations. Pharmacovigilance data were also collected.



▶ **Fig. 1** PRISMA flowchart of the systematic literature review.

Results Eleven publications from eight study cohorts were identified for inclusion (▶ **Fig. 1**). All were from multicentre studies and included both paediatric and adult patients. Eight publications included evaluations of the efficacy of

pdVWF/FVIII for on-demand treatment, eight included long-term prophylactic treatment, and eight described surgical prophylaxis. Treatment protocols and pdVWF/FVIII administration methods differed between studies, as did safety evaluations. The clinical response was rated as excellent/good in 67–100 % of non-surgical bleeds treated on-demand, 89–100 % in the treatment of breakthrough bleeds occurring during long-term prophylaxis, while haemostatic efficacy in surgical procedures was 75–100 %. Pharmacovigilance data showed low incidence rates for adverse events including FVIII/VWF inhibitors, thromboembolic events, hypersensitivity reactions and transmission of infectious agents.

Conclusion This review provides a comprehensive summary of studies that evaluated the use of pdVWF/FVIII in VWD demonstrating the long-term effectiveness and safety of this pdVWF/FVIII across all ages, types of VWD and treatment settings.

Conflict of Interest LR: consultant for CSL Behring and Octapharma, honoraria from LFB. GA: honoraria from CSL Behring. WT: speakers' fees from Takeda, Bayer, Sobi, Pfizer, NovoNordisk, CSL Behring, Alexion, Portola, Sanofi and participated in advisory boards for Pfizer, Takeda, Ablynx, Sanofi, Daiichi Sankyo, LFB, Grifols and Novo Nordisk. KS and LH are employees of CSL Behring.

T-15-03 Investigation Of endometriosis symptoms and tumor marker CA 125 in patients With hereditary Von Willebrand disease

Authors Finger J[1], Eladly F[2], Roth S[1], Rauchfuß S[1], Tahmasbi Rad M[3], Miesbach W[1]

Institutes **1** University Hospital Frankfurt, Goethe University, Department of Haemostaseology and Hemophilia Center, Medical Clinic 2, Institute of Transfusion Medicine, Frankfurt am Main, Germany; **2** University Hospital Frankfurt, Goethe University, Department of Medicine, Hematology/Oncology, Frankfurt am Main, Germany; **3** University Hospital Frankfurt, Goethe University, Department of Obstetrics and Gynecology, Frankfurt am Main, Germany

DOI 10.1055/s-0042-1760559

Introduction Women with hereditary Von Willebrand Disease (VWD) show prolonged and heavy menstrual bleeding as common symptoms. According to Sampson's theory retrograde menstruation can be a cause of endometriosis, a condition defined by the presence of endometrial tissue in extrauterine locations. Therefore, our hypothesis is that women with stronger retrograde menstruation due to VWD have a higher chance of developing endometriosis. The aim of this non-interventional single-center study is to investigate a possible association between the two diseases and to evaluate the use of tumor marker CA125 determination and a gynecological questionnaire as a screening tool for endometriosis.

Method Female adult patients prior to climacteric with hereditary VWD of all types and all degrees of severity are recruited during routine visits. As primary endpoint the frequency of patients with elevated serum levels of CA 125 is determined. CA 125 is often elevated in patients with endometriosis. Secondary, every patient receives a questionnaire about the gynecological history including previous gynecological surgeries as well as symptoms known to be associated with endometriosis. Additional data is collected about age, known cancer disease(s), current infections, ISTH BAT Bleeding Score, VWD type, actual VWD parameters and therapy. Data will be analyzed using descriptive statistics. Correlations between endometriosis symptoms, levels of CA 125, blood coagulation parameters and ISTH BAT Score will be assessed.

Results To date 32 patients were included in the study. Their characteristics are displayed in Table 1. The mean concentration of CA 125 was 20,84 U/ml (range: 4,4 – 112, SD 23,98). Out of 4 patients (12,9 %) who showed elevated levels of CA 125, 1 patient was later diagnosed with endometriosis. 5 patients (15,6 %) underwent surgery for (suspected) endometriosis in the past resulting in 3 confirmed diagnoses. In total 4 patients (12,5 %) in the study group had confirmed endometriosis. The evaluation of the questionnaire showed the following: 26 patients (83,9 %) were suffering from painful menstruation. 15 patients (50 %) reported either occasional or frequent painful intercourse. 12 patients (37,5 %) reported painful defecation. 5 patients (16,1 %) reported painful urination (▶ **Tab. 1**).

Characteristic	n = 32
Mean age (range, SD)	37,28 (21-54, 8,69)
Type of VWD	
Type 1	26 (81,3%)
Type 2N	3 (9,4%)
Type 2A	2 (6,2%)
Type 3	1 (3,1%)
Mean ISTH BAT Score[1] (range, SD)	4,52 (1-19, 4,34)
Surgery for endometriosis	5 (15,6%)

▶ **Tab. 1** Characteristics of Patients.

Conclusion Though the rate of confirmed endometriosis lies within the range of the general population there seems to be a high prevalence of symptoms associated with endometriosis among this group of women with VWD. However, the still very small sample size of 32 patients does not yet allow for any definitive statements regarding the association between VWD and endometriosis. If an association can be shown, awareness of endometriosis potentially being a frequent disease among women with VWD could lead to earlier detection and initiation of therapy.

Conflict of Interest All authors declare that they have no conflicts of interest.

T-15-04 Treatment of a patient with von Willebrand disease type 2B and severe thrombocytopenia due to mutation p.V1316M with thrombopoietin receptor agonist Avatrombopag

Authors Mondorf W[1], Geisen C[2], Mondorf C[1]

Institutes **1** Haemostas-Frankfurt, Haemostas, Frankfurt am Main, Germany; **2** Blutspendedienst, DRK, Frankfurt am Main, Germany

DOI 10.1055/s-0042-1760560

Introduction A 34-year old woman presented with a history of severe thrombocytopenia. Immunthrombocytopenia was diagnosed according to typical bleeding patterns (recurrent nose bleeds, petechia) and exclusion of common thrombocytopenia associated disease. Episodes of steroid treatments were repeatedly terminated due to side effects, so that splenectomy was considered. She was transferred prior to ovariectomy because of cystic bleedings.

Method Laboratory finding confirmed severe thrombocytopenia. As other family members also had thrombocytopenia further laboratory assessments were done.

Results Because of ongoing bleeding and thrombocyte count of 13.000/µl Avatrombopag was initiated. A dose of 20 mg every second day showed a rapid increase of thrombocyte count to 233.000/µl. Further assessment showed von Willebrand activity of 14 % and activity of 63 %. Multimer analysis revealed a Type 2B von Willebrand Disease and genetic assessment p.V1316M mutation. As Von Willebrand activity decreased further with increasing thrombocyte count treatment with TPRA was reduced to 20 mg once weekly with thrombocyte counts between 36.000 und 48.000/µl. Ovariectomy could be avoided. No petechial bleeding was observed since then.

Conclusion Von Willebrand disease type 2B due to p.V1316M mutation should be considered in severe thrombocytopenia especially with a positive family history. As case records showed adverse effects after thrombocyte transfusion,

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probably due to enhanced thrombocyte aggregation, splenectomy may therefore be contraindicated. Treatment with low dose TPRA leading to a moderate increase of thrombocyte count may be effective as well as treatment with von Willebrand concentrates in case of surgery.

Conflict of Interest None

T-15-05 Correlation between von Willebrand Factor and the PI3K/Akt signalling pathway in the control of angiogenesis

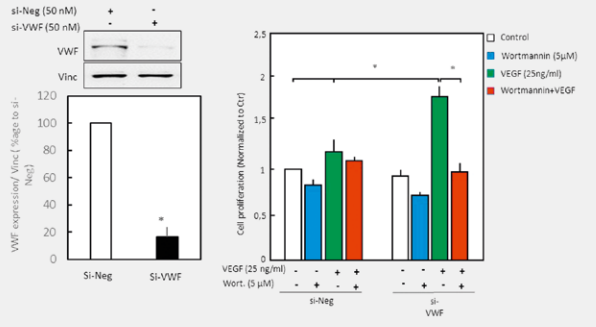
Authors Arshad M, Krüggeler J M, Schröder M, Brehm M A

Institute University of Siegen, Department of Digital Health Sciences and Biomedicine, Siegen, Germany

DOI 10.1055/s-0042-1760561

Introduction Angiodysplasia is the most common vascular abnormality in the gastrointestinal tract and a hallmark of pathological angiogenesis associated with von Willebrand disease (VWD). VWD arises from mutations in the von Willebrand factor (VWF) gene, leading to qualitative or quantitative defects in the VWF protein. VWF is a plasma glycoprotein mainly known for its function in hemostasis, but recently also a role in angiogenesis was described. VEGF and Ang-2 signaling seem to be involved in increased angiogenesis in VWF knock-down cells, however, the mechanism by which VWF modulates angiogenesis is incompletely understood. Thus, we strive to identify signaling pathways downstream of VEGF and Ang-2, which are affected by VWF. Here, we focused on PI3K/Akt signaling; a downstream pathway of the Ang-2 receptor Tie-2.

Method The study was conducted in cultured human umbilical vein endothelial cells (HUVECs), in which a VWD phenotype was mimicked by siRNA knock-down of VWF. Cell proliferation was determined by the WST-1 assay, cell migration by a scratch wound-healing assay, tube formation by a Matrigel assay, and protein expression by Western blotting (WB). The PI3K/Akt pathway was investigated by incubation with wortmannin (5 µM for 24 h), an inhibitor of PI3K. Akt phosphorylation was determined by WB employing an antibody specific for phosphorylated Ser473 in Akt.



▶ **Fig. 1** Enhanced proliferation of VWF knock-down cells can be reversed by PI3K inhibition.; (A) Western blotting (upper panel) and densitometric analysis of VWF expression (lower panel) shows that siRNA-VWF (50 nM) reduced VWF expression 30 h after transfection by 83 %. (B) After siRNA transfection, HUVECs were exposed to Wortmannin (5 µM) (blue bars), VEGF (25 ng/ml) (green bars) or VEGF plus Wortmannin (red bars) for 24 h, n = 3.

Results We confirmed that HUVECs, in which VWF was downregulated (84 ± 6.89 %) by siRNA (▶ **Fig.1a**, si-VWF), react to treatment with vascular endothelial growth factor (VEGF; 25 ng/ml) by showing enhanced cell proliferation (1.79 ± 0.086 fold vs. 1.3 ± 0.164 fold) (Fig.1B, si-VWF, green bar) and cell migration (1.54 ± 0.084 fold) compared to controls. Moreover, Matrigel assay data revealed that downregulation of VWF enhanced tube formation of ECs in a VEGF-independent manner. In response to treatment with wortmannin, cell proliferation (▶ **Fig.1b**, si-VWF, red bar) and tube formation were reduced to normal levels in si-VWF cells and migration was inhibited to the same extent in control and si-VWF cells. Therefore, PI3K inhibition abolished both the VEGF dependent and independent effects of VWF knock-down. Interestingly, reduced VWF expression was further accompanied by an upregulation of Akt phosphorylation, a downstream target of PI3K.

Conclusion The data of the present study indicate the PI3K/Akt pathway to be involved in VWF-regulated angiogenesis. Targeting the PI3K/Akt axis may offer a new therapeutic maneuver for the treatment of angiodysplasia.

Conflict of Interest No

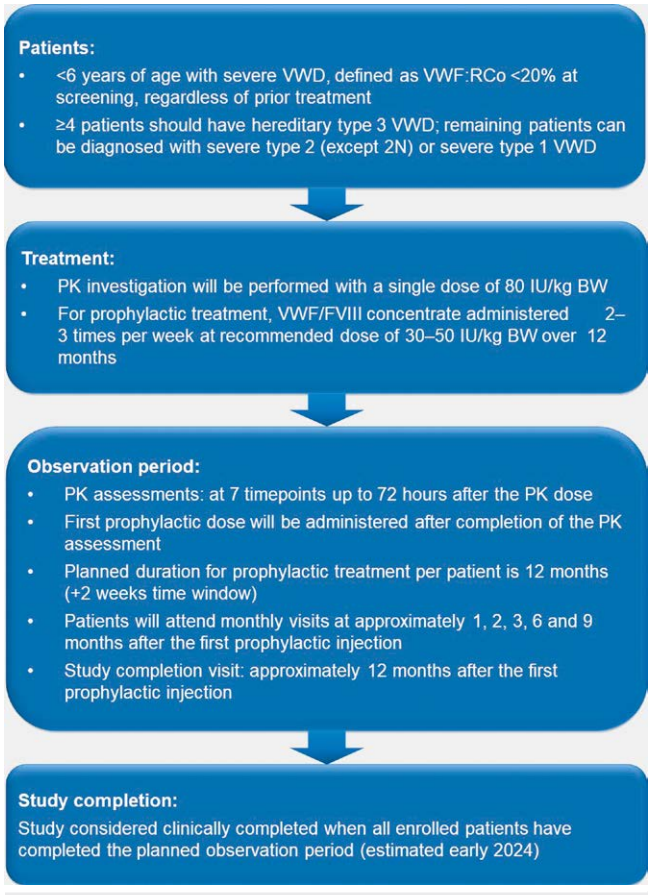
T-15-06 Phase 3 study of the efficacy, pharmacokinetics, immunogenicity and safety of von Willebrand Factor/Factor VIII concentrate in patients with severe von Willebrand Disease under 6 years of age

Authors Jain A[1], Weisz T-E[2], Solomon C[3]

Institutes 1 Loma Linda University School of Medicine, Loma Linda, USA;
2 Octapharma Pharmazeutika Produktionsges.m.b.H, Vienna, Austria;
3 Octapharma AG, Lachen, Switzerland

DOI 10.1055/s-0042-1760562

Introduction Von Willebrand disease (VWD) is a common congenital haemorrhagic disorder affecting around 1 in every 100 individuals. Prophylactic von Willebrand factor (VWF) replacement therapy in patients with severe VWD, including paediatric patients, is associated with reduced mucosal and joint bleeding rates, decreased median annual bleeding rate (ABR), a reduced incidence of major bleeding events (BEs) and good tolerability. WIL-33 will investigate the efficacy, pharmacokinetics (PK) and safety of a plasma-derived, stable, highly purified, double virus inactivated VWF/factor VIII (FVIII) concentrate (Octapharma) in paediatric patients with severe VWD.



▶ **Fig. 1** WIL-33 Study Outline.

Method WIL-33 is anopen-label, prospective, non-controlled, international, multicentre Phase 3 study of VWF/FVIII concentrate efficacy as prophylactic treatment of patients aged < 6 years with severe VWD (defined as screening von Willebrand factor ristocetin cofactor activity [VWF:RCo] < 20 %). WIL-33 plans to enrol 12 patients regardless of prior treatment, of which eight should be evaluable for the primary endpoint; at least four should have hereditary type 3 VWD. Remaining patients can be diagnosed with severe type 2 (except 2N) or severe type 1 VWD (▶ **Fig. 1**).

The primary endpoint is ABR under prophylaxis over the 12-month prophylactic treatment period. Secondary objectives include determination of the VWF/FVIII concentrate PK for VWF activity (VWF:RCo) and FVIII:C (one-stage assay) and incremental in vivo recovery (IVR); data on VWF/FVIII concentrate consumption for prophylaxis, on-demand treatment and surgeries; immunogenic potential; and safety/tolerability. WIL-33 will also examine VWF multimer composition in paediatric patients with type 3 VWD with ≥ 14.5 kg bodyweight (BW). PK will be determined following a single 80 IU/kg/BW dose. Blood samples will be taken pre-dose (baseline), and 15 minutes, 3, 9, 24, 48 and 72 hours after VWF/FVIII concentrate administration. For prophylactic treatment, VWF/FVIII concentrate will be administered 2–3 times per week at 30–50 IU/kg/BW over 12 months. Patients will record BEs in a diary, which will be used to calculate ABR. Treatment for BEs or surgeries will be performed according to pre-established dosing guidelines.

Results To date, 5 patients have been recruited at 5 study sites in 4 countries. Clinical end is expected early 2024, with results anticipated by end of 2024.

Conclusion Prophylactic treatment in other congenital bleeding disorders is widely accepted as standard of care to prevent bleeding in patients, but to date this treatment in patients with VWD is not well characterised. WIL-33 will provide data on the efficacy of prophylactic treatment in paediatric patients with severe VWD below 6 years of age.

Conflict of Interest AJ has received consultancy fees from CSL Behring, Octapharma, Takeda, GBT, Blue Bird Bio, Sanofi, and Novo Nordisk, and has been involved with speaker bureaus for Takeda and Bayer.T-EW and CS are employees of Octapharma.

T-15-07 Von Willebrand-disease_ a patient with a variant of uncertain significance: phenotype like type 1, genotype like type 3

Authors Alesci R S, Hecking C
Institute IMD Gerinnungszentrum Hochtaunus, Bad Homburg, Germany
DOI 10.1055/s-0042-1760563

Introduction We report about a 32 year old women presenting first in 3/2019 due to severe menstrual bleeding, easy bruising, prolonged wound healing.
Testing of DDVAP showed good effectiveness and tranexamic acid was started for the severe menstrual bleeding.
In August 2019 presented in hospital due to vaginal bleedings during pragnancy caused by a retroplancental haematoma. An caesarian section was necessary due bleedings (39. gestational week), postpartal she required transfusion..

Method Von Willebrand-factor activity, -antigen und CBA were measured on ACL AcuStar, Werfen, Germany (HaemoIL).
FVIIIc activity on BCS, Siemens, Germany (fviii deficient plasma).
Multimer analysis was performed by Synlab, Regensburg.
Genetic testing via NGS (illumina; NextSeg)

Results One year later the patient presented with severe menstrual bleeding, neither DDVAP nor tranexamic acid helped..
For the first time the patient recognised pain in her right knee- due to joint bleeds.
We started an on-demand treatment with von Willebrand-factor concentrate during the period of the severe menstrual bleeding (homecare service.
Because the bleeding severity did not match to the vWF levels/multimer analysis we did a genetic testing.
Genetic testing: variant of uncertain significance ACMG class 3:
NM_000552.4:c.5170 10C>T.Intron 29 and NM_000552.4:c.6652C>T. Exon38 p.(Arg2218Trp).
heterozygous (dbSNP;rs61750601.HGMD:CS002689

Conclusion Variant c.5170, 10C > T has been described several times in the literature, but there is no matching evaluation regarding clinical relevance.
Borras et al (1) detected this mutation in patients with Von Willebrand type 3 in a compound-heterozygous form. In a study by Corrales et al (2), however, no visible effect on splicing site could be shown.

▶ **Table 1** (lab results).

Date	FVIIIc (%)	VWF:Ag (%)	VWF:act (%)	VWF:CBA (%)	Ratio	Multimers
19,03,2019	97	47	43	68	0,9	Not poss to evaluate
13.05.2019	337	175	197	2013	1,1	nd
After DDVAP						
16.03.2022	222	151	172	191	1,2	nd
End of pregnancy						
13.52020 (5 weeks after birth)	111	55	54	77	1.1	Not poss to evaluate

The ClinVar database currently regards the variant as conflicting, because there are assessments as „likely benign“ and „variant with uncertain significance“. Baroncino et al (3), associated it with a von Willebrand type 3 in the ISTH SCC VWF database.[1–3]
Further, variant c6652C > T. p.Arg2218Trp in heterozygous form could be detected in exon 38 of the VWF gene.
This mutation is listed only 6 times in the gnomAD database. However, so far it is not listed in the databases NCBI ClinVaar, LOVD, HGMD and ISTH-SSC VWF (▶ **Table 1**).
The REVEL score (Rare Exome Variant Ensemble Lerner), which relies on several predictive programs, is 0.542 in the variant, with a score of more than 0.5 indicating a presumed cause of disease of a variant.
Due to this data situation, the variant must be classified as a variant with uncertain significance. On demand treatment with vWF-concentrate is a way for the patient to prevent bleeding.

Conflict of Interest no conflict of interesst

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T-15-08 Genetic background of von Willebrand disease

Authors Körber S[1], Fleck E[1], Miesbach W[2], Panagiota V[3], Zimmerman R[4], Achenbach S[4], Seifreid E[1], Geisen C[1]
Institutes **1** DRK blood donor service Frankfurt, Molecular Haemostaseology, 60528, Germany; **2** University of medicine Frankfurt, Haemostaseology, 60528, Germany; **3** Medical University Hannover, Haemostaseology,

30625, Germany; 4 Medical University of Erlangen, Haemostaseology, 91054, Germany
DOI 10.1055/s-0042-1760564

Introduction Von Willebrand disease (VWD) is an autosomal hereditary bleeding disorder and in rare situation is also acquired. Its prevalence in the population is very high with about 1 %. There are known quantitative deficiencies (type 1 and type 3) and qualitative deficiencies (type 2). The type 2 VWD contains several possible subtypes, which are often difficult to diagnose with common laboratory methods. The von Willebrand gene consists of 52 exons and is spans about 180 kb of the genome. Our aim was to analyse the molecular defect underlying VWD in a cohort of 72 patients in order to facilitate the classification of the disease.

Method In 72 patients, VWF gene was analysed by Sanger Sequencing. In cases without mutation, MLPA (Multiplex Ligation dependent Probe Amplification) 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.

Results We detected 30 different mutations in the VWF gene. Therefrom, 27 variants were previously described in the Human Genome Mutation database (HGMD) as disease causing mutations. Of these, we identified 23 Missense Mutations, one nonsense-mutation, one frameshift mutation (small deletion) and one splice-site mutation. The most frequent phenotype described for the known mutations was type 1, followed by type 2A and 2B. Two mutations were causative for type 2 N and two mutations for type 2M. Three mutations causative for VWD type 3 were identified. The type 3 mutations were one splice-site mutation, one frameshift mutation (small deletion) and one large deletion of the whole VWF gene. Three variants were novel and not described in the Polymorphism databank of the National Center for Biotechnology Information (dbSNP, NCBI) as Polymorphism. The novel variants were all missense mutations and further analysed to classify the potential impact for disease. The three novel mutations were predicted as potential missense mutations with pathogenic effect. Further functional analysis and phenotype studies will be necessary to determine the type of VWD caused by this mutations.

Conclusion Genetic anlaysis of the VWF gene can help to identify the subtype of VWD by providing insight in the localisation of the mutation in certain domains of the protein or by identifiying already known mutations. In silico methods are useful tools to predict the effect of novel genetic variants on the protein function or structure of the VWF. Nevertheless, these methods cannot replace in vitro analysis by laboratory experiments and common diagnostic tests, multimer analysis, phenotypic studies of patients and co-segregation analysis within families to classify the type of VWD.

Conflict of Interest No conflicts of interest

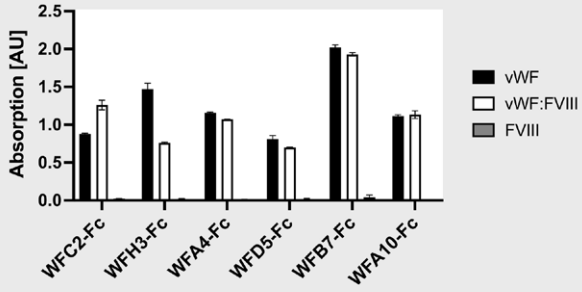
T-15-09 Isolation of vWF-specific antibody fragments from phage-displayed scFv libraries

Authors Schmidt A, Becker-Peters K, Orlowski A, Königs C
Institute Goethe University Frankfurt, University Hospital, Department of Paediatrics and Adolescent Medicine, Clinical and Molecular Haemostasis, Frankfurt am Main, Germany
DOI 10.1055/s-0042-1760565

Introduction Von-Willebrand-Factor (vWF) is a large glycoprotein, circulating as a multimer in the blood stream and serving as carrier protein for coagulation factor VIII (FVIII). While monoclonal antibodies specific for different domains of FVIII are available and can be used to help map the domain specificity of antibodies in plasma, vWf-specific monoclonal antibodies are rare. We therefore aimed to isolate vWF-specific scFvs for generation of vWF-specific antibodies.

Method The HuScL-7 library (Creative Biolabs) was used for selection of vWF-specific single chain variable fragments (scFvs). After three rounds of selection, individual phage clones were analysed for binding to vWF by ELISA. Binding was analysed against vWF alone, vWF in complex with FVIII (vWF/FVIII), as well as FVIII alone and BSA as negative controls. Promising scFvs were cloned into the scFv-Fc format and produced in HEK293T cells to verify binding by ELISA.

Results From 96 phage clones analysed, 32 showed specific binding to vWF when compared to BSA as control. After further analysis of the scFv sequences 12 phage clones were chosen for further analysis and binding of these clones could be verified. So far, eight of these scFv-Fcs were cloned into the scFv-Fc format and six scFv-Fc variants could be successfully expressed in HEK293T cells. Binding for those six variants to vWF could be verified by ELISA (▶ **Fig. 1**). Interestingly, WFH3-Fc showed stronger binding to vWF alone compared to vWF/FVIII, pointing out that scFvs specific for different vWF domains or conformations could be isolated from the phage displayed library.



▶ **Fig. 1** Analysis of scFv-Fc specificity by ELISA.; After cloning vWF-specific scFv clones into the scFv-Fc format binding specificity of scFv-Fcs successfully expressed in HEK293T was analysed by ELISA.

Conclusion We successfully isolated vWF-specific scFvs from a phage-displayed library that could be cloned into the scFv-Fc format and mostly also expressed in HEK293T cells. First analysis revealed differences in binding to vWF and vWF/FVIII for WFH3-Fc. This antibody might be useful for the analysis of vWF/FVIII complexes in plasma samples. Further analysis will be necessary to identify the binding affinity of selected scFv-Fcs as well as mapping their epitopes. ScFv-Fcs might be useful for a more detailed mapping of vWF-specific antibodies in plasma samples.

Conflict of Interest The authors declare no relevant conflict of interest.

T-16 | Gene Therapy of Congenital Bleeding Disorders

T-16-01 Global seroprevalence of neutralizing antibodies against adeno-associated virus (AAV) serotypes of relevance to gene therapy

Authors Rasko J[1,2], Bashirians G[3], Chhabra A[4], Petropoulos C J[5], Wrin T[5], Paliwal Y[6], Henstock P V[7], Somanathan S[3], da Fonseca Pereira C[8], Winburn I[9]
Institutes 1 The University of Sydney, Gene and Stem Cell Therapy Program Centenary Institute and Faculty of Medicine and Health, Sydney, Australia; 2 Royal Prince Alfred Hospital, Cell & Molecular Therapies, Sydney, Australia; 3 Pfizer Inc., Cambridge, USA; 4 Pfizer Inc., New York, USA; 5 Labcorp, South San Francisco, USA; 6 Pfizer Inc., Collegeville, USA; 7 Pfizer Inc., Andover, USA; 8 Pfizer Inc., Sydney, Australia; 9 Pfizer Inc., Walton on the Hill, UK
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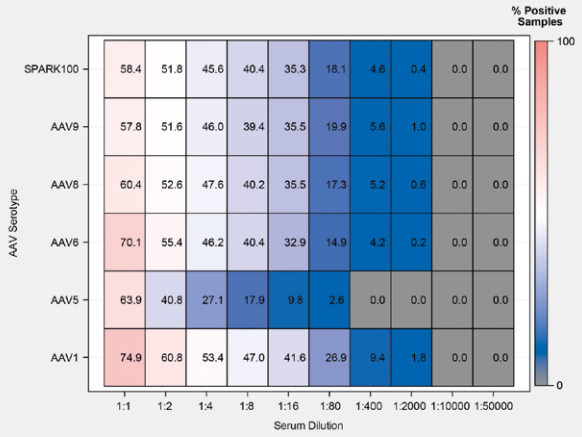
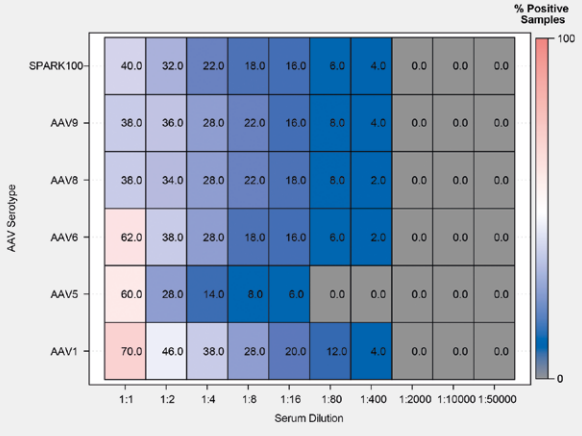
Introduction Recombinant adeno-associated virus (rAAV) vectors have shown encouraging results as candidates for in vivo gene therapy. However, pre-existing neutralizing antibodies (NAbs) against AAV serotypes can interfere with effective target cell transduction. Although there are limited data on the

prevalence of NAbs, previous studies have described geographic variability. Therefore, we performed a multi-country, observational, retrospective, cross-sectional study to assess the prevalence and titer levels of 9 serotypes of NAbs in adults (≥ 16 years) and children (< 16 years) and by various subgroups (geographic region, biological sex, ethnicity). This summary includes results for 6 serotypes (AAV1, AAV5, AAV6, AAV8, AAV9, and AAVRh74var).

Method Participants from 10 countries (Australia, Canada, France, Germany, Italy, Japan, South Korea, Spain, UK, and US) were enrolled from previous non-gene therapy clinical studies (conducted 2015–2019). Serum samples were obtained from the Pfizer Biobank, with data on demographics and clinical characteristics obtained from the clinical trial database. Serum samples (at dilutions 1:1 to 1:50,000) were analyzed by a central laboratory to determine NAb titer.



▶ **Fig. 1** Heat map of prevalence of NAb positivity at different serum dilutions against AAV serotypes in the overall adult population (n=502).



▶ **Fig. 2** Heat map of prevalence of NAb positivity at different serum dilutions against AAV serotypes in the overall pediatric population (n=50).

Results A total of 552 samples (59 % male; < 16 years, n = 50; 16–40 years, n = 95; 41–60 years, n = 407) were analyzed. The primary analysis of seroprevalence in adults showed that the most prevalent NAb at a dilution of 1:1 was AAV1 (74.9 %), followed by AAV6 (70.1 %) and AAV5 (63.9 %; ▶ **Fig. 1**). At 1:2 and subsequent dilutions, AAV5 had the lowest seroprevalence. The primary analysis in children revealed that NAbs against AAV1 were the most prevalent and NAbs against AAV5 (starting at 1:2 dilution) were the least prevalent (▶ **Fig. 2**). Overall, samples in children exhibited lower seroprevalence than samples in adults, consistent with environmental exposure to natural infections during or following childhood. Co-prevalence of NAb positivity (adults and children combined) was most frequently observed for AAV1 and AAV6 across serum dilutions. Co-prevalence of AAV5 was less likely to be observed as the serum dilution level increased. At 1:4 dilution, AAV1/AAV6 co-prevalence was 44.2 %, AAV1/AAV5 was 25.5 %, and AAV5/AAV6 was 25.2 %. Seroprevalence in adults varied by country, with South Korea exhibiting the highest, and Japan, Australia, and the US exhibiting the lowest. The data for adults suggested an association between NAb prevalence and age, biological sex, and ethnicity. The prevalence of NAbs in adults was higher in females, Asians, and older individuals.

Conclusion This large, global study of adult and pediatric participants from 10 countries provides new insights into the prevalence of NAbs against a range of clinically relevant AAV serotypes. Our results highlight variations in subgroups by geographic region, biological sex, ethnicity, age, and clinical disease.

Conflict of Interest JR declares Supply of Material (MTA), consultancy, or honoraria with Bluebird Bio, Cynata, Gilead, Novartis, Pfizer, RareCyte, Roche, and SPARK therapeutics; equity ownership in Genea; and shareholdings with RareCyte and Woke. GB, YP, PVH, SS, MC, CdFP, IW, and AC are employees of and hold stock/options in Pfizer. CJP and TW are employees of and hold equity in Labcorp-Monogram.

T-16-02 Indirect treatment comparisons of the gene therapy etranacogene dezaparvovec (CSL222) vs. extended half-life Factor IX therapies for severe or moderately severe hemophilia B

Authors Klamroth R[1], Bonner A[2], Yan S[3], Monahan P[3], Szafranski K[2], Herepath M[4], Zhang X[3], Gomez K[5]

Institutes **1** Vivantes Klinikum im Friedrichshain, Department for Internal Medicine, Haemophilia Treatment Centre, Berlin, Germany; **2** EVERSANA, Ontario, Canada; **3** CSL Behring, King of Prussia, USA; **4** Optimal Access Life Science Consulting Limited, Swansea, UK; **5** Haemophilia Centre and Thrombosis Unit, Royal Free London NHS Foundation Trust, London, UK

DOI 10.1055/s-0042-1760567

Introduction Etranacogene dezaparvovec (CSL222) gene therapy for haemophilia B delivers Factor IX (FIX) Padua via an AAV5 vector and demonstrated superior efficacy at 24 months in reducing bleeds vs. a 6-month lead-in period of prophylaxis with FIX products in the HOPE-B clinical trial.In the absence of head-to-head comparisons of CSL222 vs. FIX products, indirect treatment comparisons (ITC) can be used. This study aims to compare the efficacy of CSL222 vs. prophylaxis with rIX-FP, rFIXFc and N9-GP using ITC, and support HOPE-B results.

Method Phase 3 pivotal trials were used: HOPE-B for CSL222 (24-month data), PROLONG-9FP for rIX-FP, B-LONG for rFIXFc, Paradigm 2 for N9-GP. Outcomes of interest were annualised bleeding rates (ABR), spontaneous (AsBR) and joint (AjBR) bleeding rates, percentage of patients with no bleeds and FIX consumption. Unanchored inverse probability of treatment weighting method with individual patient data (IPD) from HOPE-B and PROLONG-9FP was used to compare CSL222 vs. rIX-FP. Unanchored matching-adjusted indirect comparison with IPD from HOPE-B was used to compare vs. rFIXFc and N9-GP. Factor ranking was conducted with haematology experts to prioritise baseline variables to be adjusted in ITCs.

Results CSL222 demonstrated statistically significantly lower ABR, AsBR and AjBR vs. rIX-FP; rate ratio (RR) [95 % confidence interval of the RR] for each outcome: 0.19 [0.09, 0.41], 0.08 [0.03, 0.23] and 0.09 [0.03, 0.25], respectively. Similar results were observed vs. rFIXFc and N9-GP. RRs [95 % CI] for ABR, AsBR and AjBR for CSL222 vs. rFIXFc were 0.14 [0.08, 0.25], 0.13 [0.03, 0.59] and 0.15 [0.03, 0.65], respectively. RRs [95 % CI] for ABR and AsBR for CSL222 vs. N9-GP were 0.24 [0.07, 0.82] and 0.13 [0.03, 0.57], respectively. N9-GP did not report AjBR. CSL222 demonstrated significantly higher % of patients with

no bleeds vs. rIX-FP and rFIXFc; odds ratios [95 % CI]: 17.60 [4.77, 64.88] and 5.65 [1.75, 18.21], respectively). CSL222 had significantly lower FIX consumption than all comparators.

Conclusion CSL222 offers patients with severe or moderately severe haemophilia B a single dose treatment option that can significantly reduce bleeding rates and eliminate routine infusions associated with FIX therapies. ITCs supported results from HOPE-B trial.

Conflict of Interest RK has received grant/research support, consultancy fees and/or served on speakers' bureaus for Bayer, BioMarin, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Shire, Biotest, Grifols, Roche and Sobi. SY, PM, and XZ are employees of CSL Behring. AB and KS (as part of EVERSANA) received funding from CSL Behring for this project. MH is a consultant for CSL Behring. KG has received grant/research support, consultancy fees and/or served on speakers' bureaus for Bayer, BioMarin, BPL, Chugai, CSL Behring, Pfizer, Roche, Sobi and Takeda

T-16-03 At the forefront of the introduction of gene therapy in hemophilia A and B: Design and implementation of an innovative software platform ("smart medication Gene") for gene therapy

Authors Roesch A, Schmoldt D, De Witte L
Institute smart medication eHealth Solutions GmbH, Frankfurt, Germany
DOI 10.1055/s-0042-1760568

Introduction In the treatment of rare diseases, especially in hemophilia A and B, gene therapies will strongly increase in importance in the coming years. The complex process of qualification of treatment centers, preparation of patients for therapy, implementation of treatment and subsequent follow-up, including registry reporting over many years can only be implemented with suitable software tools.

Method The aim is to record all gene therapy treatment data in a structured manner in a single software tool and to document it in a specialist patient file. Furthermore, in addition to the documentation requirements of physicians and treatment centers (HCPs, CCCs), such as the patient's suitability for gene therapy, the requirements of the regulatory authorities (EMA, GBA) are also to be recorded. The software tool named "Gene" also supports the collaboration of centers according to the so-called "hub & spoke model". In the hub & spoke model, there is a formalized division of tasks between the centers, i.e. the various necessary activities are assigned to either a "hub" or a "spoke" center, thus organizing and supporting collaboration between the centers. Core elements and functionality of Gene include in particular:

- collaboration and communication functionality
- information sharing between referring center and dosing center along patient's journey from preparation to aftercare
- hub & spoke model according to recommendations of medical societies
- relevant manufacturer information
- compliance documentation
- patient's treatment history in a shared electronic patient file
- requirements of official authorities (i.e. GBA in Germany)
- data acquisition for clinical registries and non-interventional studies
- interfaces to electronic diaries

Results Gene covers all HCP and regulatory (EMA, GBA) requirements related to the recently approved gene therapy in hemophilia A. Gene is currently being validated in selected hemophilia centers in Germany (both dosing and referral centers).

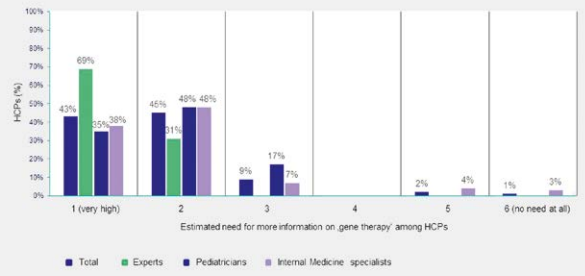
Conclusion The complex treatment process in gene therapy with the high demands on collaboration between treatment centers, documentation and compliance requires an integrated software tool. The focus should be on a targeted collaboration between the service providers to ensure optimal preparation, therapy implementation and follow-up of patients in gene therapy.

Conflict of Interest none

T-16-04 Healthcare professionals (HCPs) in Austria are positive about gene therapy, but expressed a need for a more topic related education

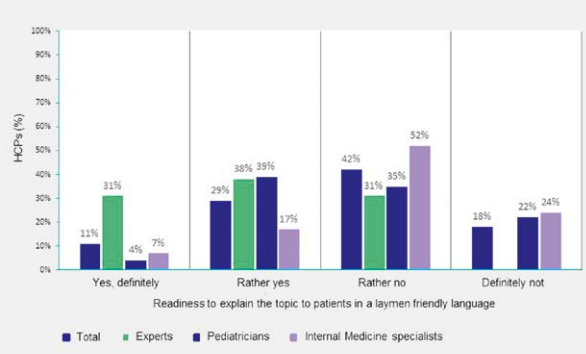
Authors Rupp V M, Windisch M
Institute Pfizer Corporation Austria Ges.m.b.H, Business Unit for Rare Diseases, Vienna, Austria
DOI 10.1055/s-0042-1760569

Introduction Gene therapy development is fast-paced with currently 1480 gene therapies being tested in pre-clinical trials, over 500 active clinical trials and 16 Advanced Therapy Medicinal Products (ATMPs) being approved for use in the EU.1 To better understand the knowledge and perception among health care professionals in Austria about gene therapy, we conducted a survey between April and June of 2021.



▶ **Fig. 1** Do you feel well enough informed to explain the topic to patients in a laymen friendly language?; Figure 2: Readiness to discuss gene therapy with patients. While more than two-thirds of experts feels ready enough to discuss gene therapy related topics with patients, the majority of internal medicine specialists (76 %) and pediatricians (57 %) does not.

Method A questionnaire containing 25 questions on gene therapy perception, knowledge and educational preferences was completed by 65 hospital physicians. This included 23 pediatricians, 29 internal medicine specialists and 13 experts in areas for which gene therapies have already been approved or launches were expected in the near future at the date the survey was conducted.



▶ **Fig. 2** Estimated need for more information on ‚gene therapy' among HCPs; Figure 1: Estimated educational need on 'gene therapy' among HCPs in general. The vast majority of all participants and of the individual groups of experts & specialists rated the need as very high or high. 1 = very high, 6 = no need at all.

Results In general, the need for gene therapy education was rated as high or very high by 88 % of the participants (▶ **Fig. 1**). More detailed questions revealed a lack of understanding of the individual gene therapeutic asset

production steps and the post-infusion monitoring. A total of 68 % of HCPs did not feel informed enough to explain the principles of gene therapies to another colleague, and 60 % expressed insecurities explaining gene therapy related questions from patients in a laymen friendly language (▶ **Fig. 2**). However, 72 % were in general open to discuss gene therapies as a therapeutic option with eligible patients. As a prerequisite they mentioned positive study results, no alternative therapy options, a positive risk-benefit ratio as well as a good safety profile. Nevertheless, 54 % of the participants had a very to relatively positive attitude towards gene therapy, but expressed a need for more detailed information on the technology itself and disease-specific gene therapies.[1]

Conclusion This survey of Austrian healthcare professionals revealed a gap in their gene therapy knowledge, but a general desire for further education.

Conflict of Interest Rupp V.M. and Windisch M. are employees of Pfizer Austria

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T-16-05 Development of an in-house ELISA method for determining the anti-capsid AAV IgG titerin blood donors and hemophilic patients

Authors Milanov P[1], Roth S[1], Rauchfuß S[1], Seifried E[2], Schwäble J[2], Miesbach W[1]

Institutes **1** University Hospital Frankfurt, Department of Haemostaseology and Hemophilia Center, Frankfurt, Germany; **2** German Red Cross Blood Donor Service, Institute for Transfusion Medicine and Immunohematology, Frankfurt, Germany

DOI 10.1055/s-0042-1760570

Introduction The high prevalence of circulating neutralizing antibodies (NAbs) against AAV capsid structures limit gene therapies mainly to patients without preexisting anti-capsid antibodies.Also, determination of anti-AAV antibody titers in patient samples has been so far poorly standardized which hampers accurate comparison of antibody levels in reported studies. In view of these limitations and the necessity to elucidate the impact of the AAV immunity in AAV gene therapy trials, we are aiming at development of a semi-quantitative in-house ELISA method for determining the anti-capsid IgG titer for various AAV serotypes in plasma and serum samples. The project is also aiming at the investigation of cross reactivity of detected antibodies between different AAV serotypes.

Method First, we screened for suitable anti-human IgG HRP conjugated antibodies and optimal blocking conditions for the capsid coated ELISA plates. Then, we evaluated the performance of the anti-AAV capsid ELISA by determining the relative anti-AAV8 capsid human IgG titers in 50 blood donor serum samples on plates coated with a commercial AAV8 vector preparation at 1e9 vg/well. SDS-PAGE analysis was performed to evaluate the purity and the relative capsid amount in various AAV8 and AAV5 vector preparations, whereas absolute capsid amount was measured with an AAV titration ELISA kit. To ensure consistent assessment of IgG titers derived from different measurements, we included an IgG-FC fragment standard that is detected by the same antibody as used for the detection of the anti-capsid directed human IgG molecules from the samples.

Results Our preliminary results concerning the ELISA measurements of the anti-AAV8 IgG titers in 50 serum blood donors' samples show a considerable prevalence for preexisting antibodies in 36 % of the samples. The antibodies in a part of these samples were also confirmed to be cross-reactive with AAV5 capsids. High variance in the full/empty capsid ratio in different AAV8 and AAV5 vector preparations was confirmed by qPCR and SDS-PAGE analysis. Consequently, coating of the plates will be performed according to the viral capsid titer rather than to the vector genome titer. In this regard, we also determined the range of the AAV5 coated capsid (1.10e9 to 1.75e10 capsids/well) at which positive samples behave direct proportional to the amount of the coated capsid.

Conclusion To sum up, we are developing a cost-effective in-house ELISA method that is able to deliver consistent and comparable results by applying capsids from diverse vector preparations and different AAV serotypes for determination of anti-AAV antibodies in patient samples collected over longer time-periods. Due to the high costs linked to AAV vector production, final optimizations will comprise assessment of the minimal capsid amount required to ensure the best classification of the anti-AAV IgG titer in tested samples at different dilutions. Following completing the final optimizations, the in-house ELISA method will be validated.

Conflict of Interest There is no conflict of interest.

T-17 | Diagnosis and Therapy of Haemorrhagic Diathesis

T-17-01 Conclusion from 50 years of reports on prekallikrein or high-molecular-weight kininogen deficiency

Authors Adenaeuer A[1, 2], Barco S[2, 3], Trinchero A[4], Ezigbo E D[5], Falter T[1], Lackner KJ[1], Lämmle B[2, 6], Rossmann H[1]

Institutes **1** University Medical Center Mainz, Johannes Gutenberg University, Institute for Clinical Chemistry and Laboratory Medicine, Mainz, Germany; **2** University Medical Center Mainz, Johannes Gutenberg University, Center for Thrombosis and Hemostasis, Mainz, Germany; **3** University Hospital Zurich, Department of Angiology, Zurich, Switzerland; **4** University Hospital Zurich, Department of Medical Oncology and Hematology, Zurich, Switzerland; **5** University of Nigeria, Department of Medical Laboratory Science, Nsukka, Nigeria; **6** Inselspital, Bern University Hospital, University of Bern, Department of Hematology and Central Hematology Laboratory, Bern, Switzerland

DOI 10.1055/s-0042-1760571

Introduction [if supportFields] > < span style = 'mso-no-proof:yes' > < span style = 'mso-element:field-end' > < /span > < /span > < ![endif]Deficiencies of prekallikrein (PK) and high-molecular-weight kininogen (HK) were discovered in the 1960s and 70s.1,2 Due to their apparent involvement in intrinsic coagulation, scientific interest was focused on hemostasis and possible bleeding during surgeries, as well as a general increase in coagulation activity. Later, knock-out studies in animals suggested protection against pathological clot formation.3,4 Meanwhile, it is very likely that PK and HK do not severely alter hemostasis, as their deficiencies do not cause bleeding diatheses and probably play only a minor role, if any, in thrombotic processes. Aside from coagulation, PK and HK are involved in pathological immunological processes as confirmed in animal models and in individual diseases (e.g. Alzheimer's disease, angioedema, diabetes, asthma).5

Method Systematic evaluation of the literature on PK and HK deficiency and the addition of novel cases, to derive prevalence estimates and clinical conclusions.

Results A total of 111 cases of PK deficiency and 48 cases of HK deficiency were identified and comparatively evaluated.6,7 The first ever conducted prevalence estimates revealed an overall frequency of HK deficiency of ~1/8 mio. and of PK deficiency of ~1/150 000, with a high accumulation among Africans (~1/7000).6,7,8 The clinical data evaluation confirmed previous assumptions but had to be limited to a simple description due to evident data gaps and publication bias. For example, the two most informative clinical records covered bleeding and thromboembolic events (missing in 13-19 %), but event descriptions were often vague or lacked an assessment of risk factors or family history (missing in 34-72 %). Further 44-58 % contained no detailed information on clinical pictures except for bleeding/thrombosis, and in 6-9 %, even basic details like age or sex were absent [1–8].

Conclusion Even current reports on cases of PK/HK deficiency almost exclusively adress parameters and medical histories directly related to hemostasis, despite PK's and HK's many other functions. The impact of rare defects can be revealed by case reports, but their potential to assess the pathophysiology of PK/HK deficiency is limited. This arises from the constraints of brevity and novelty required for publication, causing data gaps and bias, as well as from our evolving understanding of PK/HK. Therefore, encouraged by our prevalence estimates, we started to establish an ISTH-sponsored international registry on PK and HK deficiency to facilitate consistent and comprehensive reporting with prospective follow-up, which is currently lacking. In addition, future case reports should diversify and detail their clinical reporting to improve data quality.

Conflict of Interest None of the authors reports any conflicts of interest with the present work.

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T-17-02 Correlation between coagulation factor residual activities and detection rate of disease-causing variants in Rare Bleeding Disorders

Authors Preisler B, Pezeshkpoor B, Pavlova A, Oldenburg J

Institute University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany

DOI 10.1055/s-0042-1760572

Introduction Deficiency in Rare Bleeding Disorders (RBDs) of coagulant factors FII, FV, FVII, FX, FXI, FXIII and Fibrinogen is diagnosed by testing in the context of bleeding events, family history of factor deficiency or presurgical management. Residual activity of coagulant factors provide important information for the subsequent molecular analysis since they correlate with the detection rate of pathogenic variants/VUS (DRpVV).

Method Over 15 years 2630 unrelated patients (IPs) with RBD factor deficiency were screened for causative variants/VUS. Sequencing by Sanger or NGS, MLPA and CNV analysis were performed.

Results The overall DRpVV shows a large variability between the different coagulant factors. The highest DRpVV was found in F10 and F11 (~80 %) and thelowestwas observed in F13 (27 %). DRpVV was correlated to the residual clotting factor activity.In the severe forms of all factor deficiency except FV, pathogenic variants/VUS were identified in more than 90 % of IPs. In all coagulant factors the DRpVV decreases with the increase of factor residual activity. The DRpVV falls distinctively between the moderate and mild forms of factor deficiencies. In the IPs with residual activity close to normal, the DRpVV for F13 tends towards zero, while most of the factors show values around 20 %.

Conclusion DRpVV correlates with the extent of factor reduction. Therefore, residual factor activity represents an informative tool for the prediction of a genetic variant in the corresponding gene.

Conflict of Interest This study is funded by CSL Behring.

T-17-03 Utility of ACMG classification to support interpretation of molecular genetic test results in patients with FVII deficiency

Authors Alesci R S[1], Hecking C[1], Rackè B[2], Janssen D[3], Dempfle C E[4]

Institutes **1** IMD Gerinnungszentrum Hochtaunus, Bad Homburg, Germany; **2** Institut für Immungenetik Kaiserslautern, Labor Genetik, Kaiserslautern, Germany; **3** Med-i-scene Concept GmbH, Weisendorf, Germany; **4** IMD Gerinnungspraxis Mannheim, Mannheim, Germany

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Introduction An internationally shared framework for variant classification in genetic disorders has been established by the American College of Medical Genetics and Genomics–Association for Molecular Pathology (ACMG–AMP). The EAHAD F7 variant database has been prepared to list the pathogenicity of variants based on the ACMG classification. However, there is a lack of data on the utility of ACMG classification for the interpretation of molecular genetic test results in patients with FVII deficiency (1).

Method Records of patients (pts) with FVII deficiency examined at the Coagulation Center Hochtaunus, Bad Homburg and Coagulation Center Mannheim, Germany, between 08/2012 and 12/2021 where retrospectively reviewed. Molecular genetic analyses were performed at the Institute for Immunology/Genetics, Kaiserslautern, Germany. The ISTH-SSC bleeding assessment tool was used to generate a bleeding score (BS). Cut offs for an abnormal ISTH-BS were ≥ 3 for children, ≥ 4 for males, ≥ 6 for females. In addition, treatment for substitution of FVII deficiency was documented. The cohort was divided into five groups according to genotype and pathogenicity of F7 gene variants: (1) wild-type, (2) ACMG 1 (benign) or ACMG 2 (likely benign), only, (3) ACMG 3 (uncertain significance), only, or not classified, (4) single heterozygous ACMG 4 (likely pathogenic) or ACMG 5 (pathogenic) ± single heterozygous ACMG 1–3, (5) homozygous ACMG 4–5 or ≥ 2 heterozygous ACMG 4–5 or ≥ 1 heterozygous ACMG 4–5 plus either homozygous ACMG 1 c.1238G > A modifying variant or ≥ 2 ACMG 1–3.

Results In total, 111 pts. (74 females, 37 males, median age 32.5 years, range 0–84 years) with FVII deficiency (mean FVII:C 41.2 % ± 15.5 %) were included. F7 gene variants were detected in 89 pts. (number of variants per patient: median 2, maximum 10) with 53 different variants listed: 13 ACMG 1 or 2 variants (c.1238G > A in 68 pts.), 10 ACMG 3 variants, 2 variants remained unclassified, and 28 ACMG 4 or 5 variants (c.1061C > T in 18 pts.). Mean FVII:C in group 5 was 29.5 ± 13.6 % versus 48.6 ± 14.0 % in group 1, 45.7 ± 14.5 % in group 2, 44.1 ± 11.0 % in group 3, and 43.4 ± 13.6 % in group 4. 11 of 31 pts. (35.5 %) in group 5, had abnormal ISTH-BS (n = 7) and/or history of on demand substitution with recombinant factor VIIa (n = 5) versus 4 of 80 pts. (5.0 %, n = 1 abnormal ISTH-BS, n = 3 substitution) in groups 1 (n = 2/22), 2 (n = 1/29), 3 (n = 0/9) and 4 (n = 1/20) [1–2].

Conclusion ACMG classification is a promising tool to improve interpretation of genetic test results in pts. with FVII deficiency. The results of this retrospective analysis suggest phenotype differences between pts. with a homogenous ACMG 4–5 F7 gene variant or specific combinations of heterogenous ACMG 4–5 ± ACMG 1–3 variants, on one side, and pts. with F7 gene wildtype, ACMG 1–3 variants, only, or single heterozygous ACMG 4–5 ± single heterozygous ACMG 1–3 variant, on the other side. This may serve as a basis to develop a genotype/phenotype prediction model in future studies (1,2).

Conflict of Interest DV declares that his company received an unrestricted grant from Novo Nordisk Pharma GmbH, Mainz. RSA had a contract with NovoNordisk for a publication, the other authors declare no conflict of interest.

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T-18 | Autoimmunological Diseases with Thrombocytopenia, Such As Itp, Ttp, Hus

T-18-01 Diagnostic challenges of Thrombotic Thrombocytopenic Purpura: Results from a cohort of 3,100 individual patients in a time period of 21 months

Authors Wilhelm J, Kowalski J, Jarosch C, Budde U, Schneppenheim S

Institute MVZ Medilys Laborgesellschaft mbH, Hämostaseologie, Hamburg, Germany

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Introduction Thrombotic Thrombocytopenic Purpura (TTP) is a life threatening microangiopathy, due to severe deficiency of the von Willebrand factor (VWF) cleaving protease ADAMTS13, which can either be caused by a genetic defect of the ADAMTS13 gene or much more often, by autoantibodies against the protease. TTP is diagnosed by characteristic clinical symptoms of microangiopathy, the presence of schistocytes in the blood smear, thrombocytopenia, elevated LDH and finally, deficiency of ADAMTS13.

Method From Jan-2021 until Sep-2022, 6.323 samples of 3,100 individual patients with a suspected diagnosis of TTP were referred to our laboratory. ADAMTS13 activity was measured by the Technozym ELISA or by the Ceveron s100 automatic assay. ADAMTS13 Ag and ADAMTS13 inhibitor were determined by the Technozym ELISA (all tests from Technoclone). Additionally, we used a variant „Bethesda" method, (Vendramin et al. 2018).

Results 171 patients were diagnosed with severe ADAMTS13 deficiency. Autoantibodies against ADAMTS13 could be detected in 133 of them. However, in 38 TTP patients no particular antibody was identified by the inhibitor ELISA. By means of the Bethesda method we could detect an ADAMTS13 antibody in nine out of 14 patients, and two patients had an Upshaw-Schulman-Syndrome due to an inborn deficiency of ADAMTS13 [1].

Conclusion In roughly 22 % of patients with severely reduced ADAMTS13 activity, the commonly used methods did not detect a significant ADAMTS13 inhibitor. In such cases with clear signs indicative of TTP, a modified Bethesda assay would be helpful, to correctly diagnose acquired TTP and to allow fast, sound and targeted therapeutic decisions. Although hereditary cases, like Upshaw Schulman Syndrome are rare, their diagnosis is of equally high relevance for adequate therapy.

Conflict of Interest The authors declare no conflicts of interest

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T-18-02 Vitamin D deficiency in adult patients with primary immune thrombocytopenia (ITP) from the Vienna ITP Biobank

Authors Schramm T[1], Fillitz M[2], Dixer B[2], Quehenberger P[3], Mehic D[1], Ay C[1], Pabinger I[1], Gebhart J[1]

Institutes 1 Medical University of Vienna, Department of Medicine I, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; 2 Hanusch Hospital, Department of Internal Medicine, Vienna, Austria; 3 Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria

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Introduction Primary immune thrombocytopenia (ITP) is an autoimmune disease, characterized by low platelet counts and heterogeneous bleeding phenotypes. 25-(OH) vitamin D (VD) has been reported to have immunomodulatory properties. Vitamin D deficiency (VDD) has been associated to more severe courses of ITP. In this study, we investigated VD levels in a large and well-characterized adult ITP patient cohort to analyze their association with disease duration, bleeding severity and platelet counts.

Method Levels of VD were investigated in 119 adult primary ITP patients (28 acute, 12 persistent, 79 chronic) and 20 age- and sex- matched healthy controls (HC) included in the Vienna ITP Biobank (EC 1843/2016). Recommended serum VD levels are between 75-250 nmol/L, levels below 75 nmol/L were categorized as VDD. Bleeding severity was recorded by an ITP-specific bleeding assessment tool (SMOG Score). To investigate the influence of VD levels on bleeding severity and platelet counts and selected clinical and laboratory parameters, a linear regression analysis was performed.

Results Table 1 depicts clinical and laboratory data of all ITP patients, ITP patients with VDD, normal VD, and HC. VDD patients in comparison to patients with normal VD levels had a lower rate of women (59.6 % vs 95.0 % vs, p = 0.002) and a higher median BMI than those with sufficient VD (26.3 kg/m2 vs 23.4 kg/m2, p = 0.025). No relevant differences in age, disease duration, bleeding severity, or platelet counts were observed between ITP patients with VDD or normal VD levels

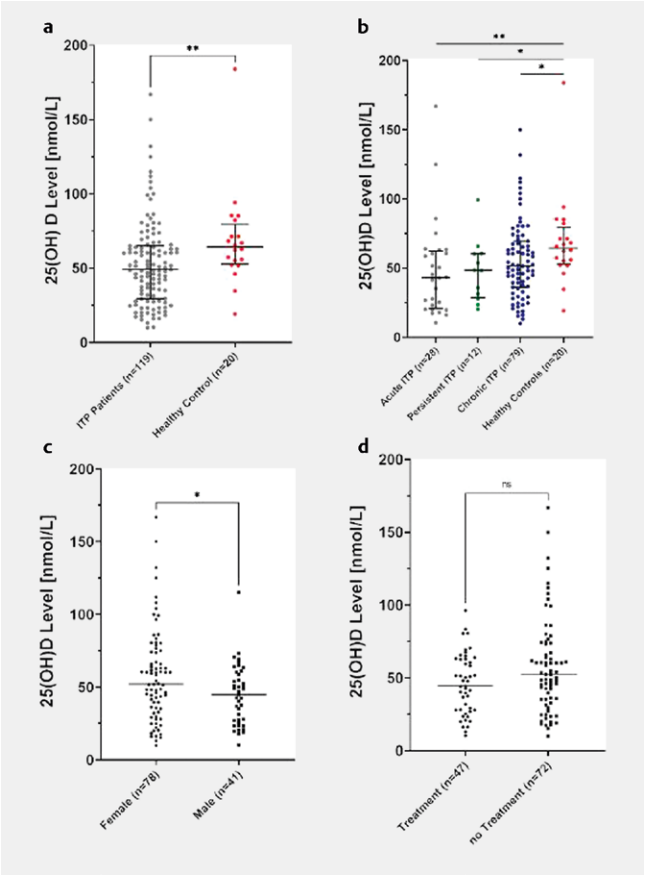
	All ITP patients	*VDD*	*Normal VD levels*	*HC*
Female	78 (65.5 %)	59 (59.6%)	19 (95.0%)	12 (60.0%)
Age, years	43.0 (31.0-56.0)	43.0 (31.0-56.0)	42.0 (28.0-51.5)	52.0 (39.0-57.8)
BMI, kg/m²	25.9 (23.1-29.8)	26.3 (23.2-30.3)	23.4 (21.2-26.8)	25.5 (24.2-28.3)
Disease duration, months	46.0 (4.0-119.5)	40.0 (3.0-117.0)	58.0 (12.5-215.5)	-
Acute ITP)	*28 (23.5 %)*	*24 (24.2%)*	*4 (20%)*	-
Persistent ITP	*12 (10.2 %)*	*11 (11.1%)*	*1 (5%)*	-
Chronic ITP	*79 (66.4 %)*	*64 (64.6%)*	*15 (75%)*	-
Current ITP therapy	47 *(39.5%)*	43 (43.4%)	4 (20%)	-
SMOG Score	2.0 (0.0-4.0)	2.0 (0.0-4.0)	3.0 (1.0-6.8)	0.0 (0.0-0.8)
Skin Score	*1.0 (0.0-3.0)*	*1.0 (0.0-2.0)*	*1.5 (0.3-4.0)*	*0.0 (0.0-0.0)*
Mucosa Score	*0.0 (0.0-1.0)*	*0.0 (0.0-1.0)*	*0.0 (0.0-1.8)*	*0.0 (0.0-0.0)*
Organ Score	*0.0 (0.0-0.0)*	*0.0 (0.0-0.0)*	*0.0 (0.0-2.0)*	*0.0 (0.0-0.0)*
Platelet count, G/L	56.0 (27.0-102.0)	61.0 (29.0-101.0)	45.5 (16.5-104.5)	241.0 (225.5-270.8)
25-(OH) VD, nmol/L	49.1 (29.5-65.1)	44.8 (27.4-60.1)	97.8 (80.6-113.5)	64.3 (52.9-79.5)
<30 nmol/L	*30 (25.2%)*	*30 (30.3%)*	-	*1 (5%)*
<50 nmol/L	*60 (50.4%)*	*60 (60.6%)*	-	*3 (15%)*
Calcium, mmol/L	2.28 (2.21-2.33)	2.19 (1.90-2.50)	2.30 (2.09-2.57)	2.30 (2.23-2.38)

▶ **Fig. 1** Clinical and laboratory data of all patients with primary ITP (n=119), ITP patients with vitamin D deficiency (VDD, n=99), normal vitamin D levels (n=20) and healthy controls (HC)(n=20).
Notes: Values are given either as numbers and percentage or as median (25 th-75 th perecentile). SMOG, skin-mucosa-organ-gradation; 25-(OH) VD, 25-Hydroxy Vitamin D; VDD, Vitamin D deficiency; VD, 25-(OH) VD. SMOG Score was recorded using consensus-based ITP-specific bleeding assessment tool, according to the recommendations of the International Working Group (IWG) for ITP.

ITP patients had lower median (IQR) VD levels than HC (49.1 (29.5-65.1) nmol/L and 64.3 (52.9-79.5) nmol/L, p < 0.01), ▶ **Fig. 1a**.The rate of VDD was high in both groups, even slightly higher in ITP patients than in HC with 99/119 ITP

patients (83.2 %) and 14/20 HC (70.0 %), p = 0.162. Acute, persistent and chronic ITP cases did not differ significantly in VD levels, and HC had significantly higher VD than all three subgroups, ▶ **Fig. 1b**.In ITP patients, VD levels were not associated with bleeding severity (R2 = 0.002, β = 0.045, p = 0.624), platelet counts (R2 = 0.004, β = 0.065, p = 0.485), age, disease duration, BMI, or Ca levels (not shown). However, a dependence of VD by sex (R2 = 0.045, β = 0.213, p = 0.020) and current treatment (R2 = 0.040, β = 0.201, p = 0.029) was observed in the total ITP cohort. Women had higher median (IQR) VD than men (52.2 (26.9-58.7) nmol/L vs 44.8 (35.1-74.3) nmol/L, p = 0.043), treated patients had a tendency for lower VD (▶ **Fig. 1c, d**).

Conclusion In this study, VD levels were lower in ITP patients than in HCs. VD levels were independent of age, disease duration, BMI, Ca levels. No association of VDD with increased bleeding severity was found in our cohort of adult ITP patients. In line with previous data, VD levels did not correlate with platelet counts (▶ **Fig. 2**).



▶ **Fig. 2** A. Significant difference in 25-(OH) D in ITP patients (n=119) versus healthy controls (n=20) (p=0.009) B. Significant differences in 25-(OH) D between acute ITP vs healthy controls (p=0.003), persistent ITP vs healthy controls (p=0.038) and chronic ITP vs healthy Controls (p=0.004). C. Female ITP patients show significantly lower25-(OH) D than male patients (p=0.043) D. No significant difference in 25-(OH) D between treated and untreared ITP patients. ** p<0.05; p<0.01.

Conflict of Interest Ap. Prof. Priv.-Doz. Dr. Johanna Gebhart, PhD: Speakers honoraria and honoraria for advisory boards from Novartis, Amgen, SOBI. Research funding Novartis, Amgen, SOBI. The ITP biobank was supported by Novartis, Swedish Orphan Biovitrum (SOBI), MedMedia Verlag und Mediaservice GmbH and AMGEN GmbH.

T-18-03 Sequential combinations of rapid immunoassays for quick recognition of heparin-induced thrombocytopenia

Authors Steinauer T[1, 2], Rittener-Ruff L[1, 2], Marchetti M[1], Grandoni F[1], Matthey-Guirao E[1], Gomez F J[1], Alberio L[1, 2]
Institutes **1** Lausanne University Hospital (CHUV), Division of Haematology and Central Haematology Laboratory, Lausanne, Switzerland; **2** University of Lausanne (UNIL), Lausanne, Switzerland
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Introduction Heparin-induced thrombocytopenia (HIT) is an adverse drug reaction occurring in <0.2 % to 3 % of patients exposed to heparin that causes a prothrombotic state, increasing the risk of thrombosis. Rapid diagnosis is therefore important. In 2022, Rittener-Ruff et al. (J Thromb Haemost 2022;20:2407) assessed multiple HIT's diagnostic algorithms using rapid immunoassays (IA). Here, we present the interim data of the ongoing internal validation of these algorithms. The goal is to establish their diagnostic accuracy in clinical routine.

Method Three algorithms based on the combination of 4T score with rapid IAs were compared. For each case of our cohort of prospectively enrolled consecutive patients (338 cases with suspicion of HIT for which rapid IAs were performed from 10.2021 to 10.2022), chemiluminescent IA (CLIA) and latex immune-turbidimetric assay (LIA) were performed. The algorithm using CLIA first and LIA for unsolved cases is compared with the one that uses LIA first and CLIA for unsolved cases, and with the "Hamilton algorithm" which employs a scoring system based on CLIA and LIA performed simultaneously (J Thromb Haemost 2020;18:1435). Using heparin-induced platelet activation (HIPA) results as gold standard and PF4-enhanced HIPA (PIPA) for inconclusive cases, we established to what extent the algorithms can correctly exclude or predict HIT.

Results Performing CLIA first and then LIA for unsolved cases correctly excluded HIT in 97,7 % (303/310) and predicted HIT in 89.3 % (25/28). 2.4 % (8/338) of cases were undetermined and 2 mismatches with HIPA were observed. Performing LIA first and then for unsolved cases correctly excluded HIT in 98.1 % (304/310) and predicted HIT in 92.9 % (26/28). 1.8 % (6/338) of cases were undetermined and 2 mismatches with HIPA were observed. "Hamilton algorithm" correctly excluded HIT in 95.2 % (295/310) and predicted HIT in 28.6 % (8/28). 10.4 % (35/238) of cases were undetermined and no mismatches with HIPA were observed. Of note, while the "Hamilton algorithm" requires both IA to be performed, their sequential use allows to reach a diagnosis employing only the first IA in 73.4 % (248/338) of cases with the CLIA/LIA algorithm and in 83.1 % (281/388) of cases with the LIA/CLIA one.

Conclusion A Bayesian approach sequentially employing two rapid IA for anti-PF4/heparin antibodies is effective for an accurate diagnosis of HIT. Performing CLIA and LIA simultaneously according to the "Hamilton algorithm" is less accurate. Based on our retrospective data, the sequential algorithm using LIA first was the best candidate for a prospective validation (J Thromb Haemost 2022;20:2407). The present interim analysis of the ongoing internal validation supports this approach. HIT exclusion or recognition can be achieved in over 98 % of cases with a laboratory turnaround time of less than 1 hour.

Conflict of Interest The authors have no potential conflict of interest.

T-18-04 The Von Willebrand factor multimer ratio and inflammatory markers in autoimmune thrombotic thrombocytopenic purpura

Authors Falter T[1], Rossmann H[1], de Waele L[6], Dekimpe C[6], Roose E[6], von Auer C[5, 2], Müller-Calleja N[1], Häuser F[1], Degreif A[1], Marandiuc D[4], Messmer X[2], Sprinzl M[3], Lackner KJ[1], de Meyer S[6], Jurk K[2], Vanhoorelbeke K[6], Lämmle B[2, 7]

Institutes 1 University Medical Center of the Johannes Gutenberg University, Institute of Clinical Chemistry and Laobratory Medicine, 55131, Germany; 2 University Medical Center of the Johannes Gutenberg University, Center for Thrombosis and Hemostasis (CTH), 55131, Germany; 3 University Medical Center of the Johannes Gutenberg University, Medical Department I, 55131, Germany; 4 University Medical Center of the Johannes Gutenberg University, Transfusion Center, 55131, Germany; 5 University Medical Center of the Johannes Gutenberg University, Department of Hematology, Oncology and Pneumology, 55131, Germany; 6 KU Leuven Campus Kortrijk, Laboratory for Thrombosis Research, 8500, Belgium; 7 Inselspital, Bern University Hospital, University of Bern, University Clinic of Hematology & Central Hematology Laboratory, 3010, Switzerland
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Introduction The protease ADAMTS13 cleaves the high molecular weight and prothrombotic von Willebrand factor (VWF) multimers (MM) secreted by endothelial cells into smaller and less thrombotic active multimers. Severe deficiency of ADAMTS13, caused by autoantibodies, leads autoimmune thrombotic thrombocytopenic purpura (iTTP). VWF seems to be the only relevant substrate of ADAMTS13 and the interaction of both is determining whether an acute iTTP episode develops or not and in particular, infections and inflammations seem to be important factors.

Method In a prospective, two-year study, we continuously enrolled and followed 83 iTTP patients for VWF MM ratio (High-molecular weight/low-molecular weight VWF MM based on SDS-agarose gel electrophoresis (Sebia®)), ADAMTS13 as well as CRP and interleukin 6 (IL-6). Sixteen patients experienced 22 acute episodes during this period and 67 were in continuous remission, with 13 having ADAMTS13 activities < 10 % and 54 ≥ 10 %.

Results iTTP patients in continuous remission had higher VWF MM ratio than controls. However, VWF MM ratios were significantly higher in remission patients with ADAMTS13 < 10 % compared to those with ≥ 10 % (p = 0.0023). Fourteen samples obtained 39 days (IQR 10-45) before an acute iTTP bout (ADAMTS13 < 10 % in 9, 10-26 % in 5) had significantly higher VWF MM ratios than those from 13 patients in sustained remission with ADAMTS13 < 10 % (p = 0.0020). We analyzed CRP and IL-6 to determine whether an inflammatory event was responsible for the increased VWF MM ratios before an acute iTTP relapse. However, neither CRP nor IL-6, which is thought to inhibit cleavage of VWF, were elevated before the acute episode.

Conclusion We demonstrated that the VWF MM ratio is not exclusively dependent on ADAMTS13 activity. The very high VWF MM ratio preceding acute iTTP recurrence with ADAMTS13 between < 10 % and 26 % suggests that VWF processing is hampered more than in similarly ADAMTS13 deficient patients remaining in remission. However, we could not show that an inflammatory process or reduced cleavage due to IL-6 was causative.

Conflict of Interest Prof. Bernhard Lämmle is chairman of the data safety monitoring committees for the Baxalta 281102 and the TAK-755-3002 study (both investigating recombinant ADAMTS13 in he-reditary TTP) and for the Takeda SHP655-201 study (recombinant ADAMTS13 in immune-mediated TTP), all three now run by Takeda. He is a member of the advisory board of Ablynx, now part of Sanofi, for the development of caplacizumab; and received congress travel support and/or lecture fees from Baxter, Ablynx, Alexion, Siemens, Bayer, Roche, and Sanofi.

T-18-05 Utility of D-dimers in the diagnostic work-up of heparin-induced thrombocytopenia (HIT)

Authors Steinauer T[1, 2], Gomez F J[1], Matthey-Guirao E[1], Goodyer M[3], Marchetti M[1], Barelli S[1], Alberio L[1, 2]

Institutes 1 Lausanne University Hospital (CHUV), Division of Haematology and Central Haematology Laboratory, Lausanne, Switzerland; 2 University of Lausanne (UNIL), Lausanne, Switzerland; 3 Hôpital du Valais, Service of Haematology, Sion, Switzerland
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Introduction The most widely used approach for diagnostic of HIT is based on the combination of the 4T score and immunoassays (IA) detecting anti-PF4/heparin antibodies. In our center, we employ a Bayesian diagnostic algorithm incorporating the 4T score and the magnitude of two sequential rapid IAs. Because HIT is characterized by an activation of the coagulation system, and based on our previous experience, we assessed whether the quantitative result of D-dimers could improve the diagnostic work-up.

Method We are currently conducting a prospective validation of our in-house diagnostic algorithm (01.2017 – 10.2022). A confirmatory functional HIPA (heparin-induced platelet activation) test is performed for either a high 4T score and/or any not negative IA result(s). Among this cohort (n = 282), a D-dimer analysis was performed using INNOVANCE D-Dimer of the CS-5100 System (Siemens). The diagnostic performance of D-dimers was evaluated by ROC analysis, allowing us to determine the AUC, the optimal cut-off value, likelihood ratios (LR) of result intervals, and the 100 % negative predictive value (NPV).

Results Among the 282 analysed samples for which HIPA results were available, HIT could be proven by a positive HIPA in 142 cases (50.4 %). The AUC of the ROC curve was 0.711 and the optimal cut-off was identified at a D-dimer value of 3746 ng/ml (sensitivity 82.4 %, specificity 50.7 %). The 100 % negative predictive cut-off value (NPV) for a positive HIPA was at 1'000 ng/ml. Of note, 14/140 (10 %) of non-HIT samples were below the 100 % NPV cut-off value. The D-dimer interval from 1'000 ng/ml to 3746 ng/ml had a LR of 0.432; the one from 3746 ng/ml to 5'000 ng/ml a LR of 1.808; from 5'000 ng/ml to 30'000 ng/ml of 1.121; and the D-dimer interval > 30'000 had a LR of 6.080. The 100 % positive predictive cut-off value (PPV) could not be determined due to the lack of specificity of D-dimers.

Conclusion According to our preliminary date, a D-dimer value below 1'000 ng/ml could be used to exclude HIT. LR of higher result intervals could be useful to modify pretest probability for HIT. However, high D-dimers do not predict a positive HIPA and cannot be used to predict HIT.

Conflict of Interest The authors have no potential conflict of interest.

T-18-06 A Survey on anticoagulation in patient with ITP

Authors Alesci R S[1], Meyer O[2], Matzdorff A[3]

Institutes 1 IMD Blood coagulation centre Hochtaunus, Bad Homburg, Germany; 2 German Red Cross Blood Service NSTOP, Springe, Germany; 3 Asklepios Clinic Uckermark, Dpt. of Internal Medicine II, Schwedt, Germany
DOI 10.1055/s-0042-1760579

Introduction The number of patients who require anticoagulation e.g., for atrial fibrillation is increasing and sometimes also affects patients with immune thrombocytopenia (ITP). Detailed studies on anticoagulation in thrombocytopenia are. In studies on ITP, patients with anticoagulation are usually excluded and on the other hand, patients with thrombocytopenia are often excluded from anticoagulation studies. The goal of this survey was to find out which factors influence the decision making on anticoagulation and ITP-therapy in patients in daily practice.

Method We performed a questionnaire/survey to investigate the preferred management of anticoagulation in ITP patients. The survey described hypothetical patient scenarios and asked potentially influencing factors on the recommendation whether to initiate an anticoagulation or not.

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▶ **Table 1** how does platelet count and bleeding tendency affect the decision to start new ITP-treatment or – if already treated – to change current treatment.

Potentially influencing factor	important	somehow important	undecided	somehow less important	unimportant	Do not know
Severe thrombozytopenia (< 25 x 109/l)	123	36	24	12	4	4
Moderate thrombozytopenia (25-50 x 109/L)	17	31	60	43	48	4
Mild thrombocytopenia >50 x 109/L	10	5	13	28	143	4
Severe bleedings (WHO °3-4)	195	3	0	0	0	5
Moderate bleedings (WHO 2)	62	91	31	8	6	4
Mild Bleedings (WHO 1)	18	39	56	39	46	4

Results We surveyed 229 physicians in Germany, Austria and Switzerland. 210 were specialised in haematology, 13 had subspecialty training in haemostaseology. 100 of them stated, they see 5-10 ITP patients per month. Overall, recommended platelet thresholds for antithrombotic therapy were similar between ITP specialists. Most of the colleagues recommended a minimum platelet count of 50 × 109/L for anticoagulation therapy in most scenarios, see table (further tables and results will be presented at the meeting) (▶ **Table 1**).
Conclusion Anticoagulation in patients with ITP is still a challenge. Obviously, a platelet count above 50 × 109/L is generally regarded as safe. If patients have a lower platelet count, other influencing factors like bleeding tendency, comorbidities and psycho-social status become relevant. Guidelines are very helpful.
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Conflict of Interest no conflict of interest

T-19 | Coagulation and Covid-19 Infection, Vitt, Long-Covid

T-19-01 Possible immune reaction to COVID-19 vaccination - a case report

Authors Malakeh J, Detering C, Gutensohn K
Institute Werlhof-Institut MVZ GmbH, Hannover, Germany
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Introduction Side effects may occur after vaccination against COVID-19. Temporary reactions such as redness, swelling and pain at the injection site, high temperature, fever, tiredness, etc. may be signs of the body's response to the vaccine. Such reactions usually develop within two days after vaccination and last for a few days. With the growing number of vaccinations against SARS-CoV-2 a rising number of reports also showed serious side effects. In some of the most severe cases, life-threatening thrombotic events may develop. We present a case that shows further symptoms that may be due to an immune reaction to the vaccine.
Method In this case report a 67 male smoker presented to our outpatient clinic in April 2022. A few days after vaccination against SARS-CoV-2 with an mRNA vaccine the patient developed pain at all finger tips. The clinical examination showed cool and livid discoloration of all fingers to different degrees; toes were not involved. The symptoms developed progressively over the following weeks into a severe form with progressive fingertip skin necrosis.
Results The blood test showed a CRP of 9.18 mg/l (reference range: 0-3 mg/l) as well as an increased fibrinogen and factor VIII activity. D-dimers were only slightly increased to 290 ng/ml (reference range: < 230 ng/ml) during initial examination. Cold agglutinins, cryoglobulin and cryofibrinogen were tested negative. Angiologic examination revealed small multiple thrombi in the ulnar and digital arteries. Furthermore, the resting ECG showed no dilated ventricles and no indication of a hemodynamically relevant defect. The assessment revealed a good cardiac function overall with no evidence of embolism. Therapy was started with Nifidipine (gold standard in Raynaud's disease), Eliquis 5 mg 1-0-1, and diclofenac following hospital admission. In the further course, the therapy regimen was changed to Ilomedin IV for 4 days once a month. After two weeks, symptoms significantly improved and the signs of necrosis at the fingers disappeared.
Conclusion In summary, a circulatory perfusion disorder associated with microthrombotic events may be a possible side effect of SARS CoV-2 vaccination. A combination of Nifidipine, DOAC and pain therapy has been shown to be an effective treatment of "COVID-fingers" in this case report.
Conflict of Interest There are no relevant conflicts of interest to disclose.

T-19-02 Subsequent vaccinations in VITT patients other than SARS-CoV2 vaccination

Authors Seck S E, Schönborn L, Greinacher A
Institute Universitätsmedizin Greifswald, Institut für Transfusionsmedizin, Greifswald, Germany
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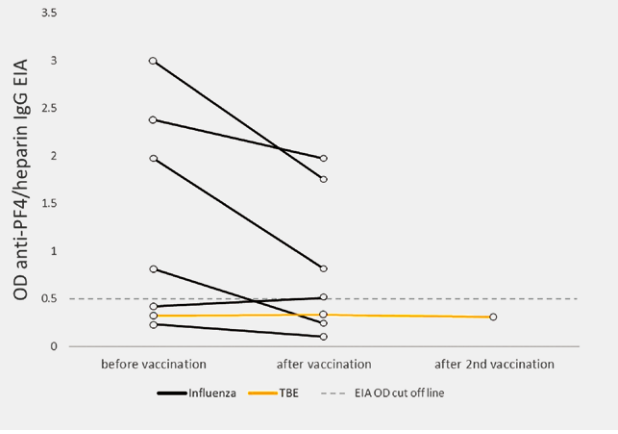
Introduction Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare, but severe side effect after vaccination with adenovirus vector-based vaccines (ChAdOx1 nCoV-19, AstraZeneca and Ad26.COV2.S, Johnson & Johnson/Janssen) in which platelet activating anti-platelet factor 4 (PF4) antibodies cause thrombocytopenia and thrombosis at unusual sites.
Patients and treating physicians are concerned about whether other vaccinations can also trigger thrombosis in patients with a history of VITT. We showed that VITT patients can safely receive their second and third vaccination against Covid-19 with an mRNA-based vaccine. [1] However, there is limited information on whether other vaccines than against Covid-19 could booster platelet activating anti-PF4 antibodies. Uncertainty increased after a report of VITT caused by human papilloma vaccination. [2]
Method In our follow-up study of patients with laboratory confirmed VITT (EUPAS45098), an anti-PF4/heparin IgG enzyme immune assay (EIA) and a PF4-dependent platelet activation assay (PIPA) were performed at regular intervals and after each vaccination reported to us.
Results Seventy-one VITT patients (43 female, median age at VITT diagnosis 48, range 18-80) were followed for a median of 56 weeks (range: 13-77 weeks). During the follow-up period, eight vaccinations other than against Covid-19 were reported: six vaccinations against influenza (three Influvac, two Vaxigrip Tetra, one Influsplit Tetra) and two consecutive vaccinations against tick-borne encephalitis (TBE) in one patient. In six patients who received vaccination against influenza, all patients showed decreasing or stable EIA optical density (OD) levels. None of them showed a reactivation of platelet-activating anti-PF4-antibodies in the PIPA. The patient who was vaccinated against TBE twice showed stable EIA OD levels and remained negative in the PIPA throughout. No new thrombosis or recurrent thrombocytopenia were observed after any vac-

cination. Five out of six patients still received therapeutic anticoagulation, one patient did not receive any anticoagulative drug (▶ **Fig. 1**).



▶ **Fig. 1** Optical density in an anti-PF4/heparin IgG EIA before/after subsequent vaccinations in VITT patients; Figure 1: Optical density (OD) in the in-house anti-PF4/heparin IgG EIA in patients with a history of VITT before and after vaccination against influenza (black) or tick-borne encephalitis (yellow). The two or three samples of each patient are connected by a continuous line. The dotted line is the cut-off EIA at OD 0.5.

Conclusion Similar to observations after consecutive mRNA-vaccinations against Covid-19 in VITT patients, vaccinations against influenza and TBE very unlikely reactivate platelet-activating anti-PF4-antibodies. Further follow up of the VITT patient cohort is performed to detect any new safety signal related to recurrence of VITT.

Conflict of Interest The study has been funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) grants: 374031971 - TRR240; Part of the results reported have been obtained in a study conducted by Universitätsmedizin Greifswald under service contract No. EMA/2021/17/TDA. The views expressed are the personal views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of the European Medicines Agency or one of its Committees or Working Parties.

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T-19-03 The predictive value of coagulation parameters for the course of disease in COVID-19 patients

Authors Cibis C L, Miesbach W, Adam E
Institute Johann Wolfgang Goethe University, Frankfurt, Germany
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Introduction COVID-19 is a systemic disease associated with a high incidence of thrombotic complications. In this study we aimed to identify coagulation parameters as predictors of mortality in hospitalized patients with severe COVID-19 infection.

Method We conducted a non-interventional, national, monocentric observational study of patients treated for COVID infection at the ICU at Frankfurt University Hospital. A total of 410 patients were enrolled in the study between April 1, 2020 and December 31, 2021. Patients had to be 18 years or older and the diagnosis was confirmed by COVID real-time PCR. Coagulation parameters were analysed once on admission to the clinic and 5 to 8 days later.
Variables studied included thromboplastin time, aPTT, fibrinogen, D-dimers, antithrombin, hs-troponin, all coagulation factors and vWF antigen, protein C and protein S. Data was also collected on age, sex, comorbidities, medication, and invasive ventilation, ECMO therapy and dialysis. In order to compare patients regarding their general disease status, the SAPS-II and the Horovitz index were determined at the beginning and end of the observation period.
Univariate and multivariate logistic regression models were then used to screen coagulation parameters for association with mortality in critically ill COVID patients.

Results The arithmetic mean age of patients was 60.9 (± 14.7) years, with 76.1 % being male. Of 410 patients, 259 (63.2 %) received invasive ventilation, 95 (23.2 %) received ECMO therapy and 105 (25.6 %) received renal replacement therapy. The median inpatient length of stay was 16 (IQR: 10-29) days and ICU length of stay was 12 (IQR: 6-25) days. 176 patients (43 %) died because of their COVID disease, 234 (57 %) were discharged home or to other facilities for further treatment.
In univariate logistic regression, increased age (OR = 1,029, 95 %-CI [1,013-1,1,044]), higher SAPS-II (OR = 1,031, 95 %-CI [1,018-1,045]), fibrinogen (OR = 1,002, 95 %-CI [1,001-1,003]), FVIII (OR = 1,004, 95 %-CI [1,001-1,007]) and vWF antigen (OR = 1,005, 95 %-CI [1,003-1,007]) as well as lower antithrombin (OR = 0,981, 95 %-CI [0,971-0,991]), FII (OR = 0,983, 95 %-CI [0,972-0,993]), FXIII (OR = 0,992, 95 %-CI [0,986-0,999]), Horovitz index at admission (OR = 0,994, 95 %-CI [0,990-0,997]) and decreased protein C activity (OR = 0,989, 95 %-CI [0,982-0,996]) were associated with increased mortality.
In the final multivariate regression analysis with backward elimination, low antithrombin activity (OR = 0.987, 95 %-CI [0.974-1.000]), high vWF antigen levels (OR = 1.004, 95 %-CI [1.002-1.007]) and a low Horovitz index (OR = 0.993, 95 %-CI [0.989-0.997]) were identified as independent predictive factors for increased mortality.

Conclusion In the study of 410 COVID patients requiring intensive care, the Horovitz index, antithrombin activity and vWF antigen on hospital admission were identified as independent predictors of mortality.

Conflict of Interest All authors declare that they have no conflict of interest.

T-19-04 Anti-PF4 antibodies after SARS-CoV-2 vaccines: long term follow-up

Authors Uzun G[1], Althaus K[1, 2], Luz B[3], Wolf M[4], Henkes H[5], Bakchoul T[1, 2]
Institutes **1** Center for Clinical Transfusion Medicine, Tübingen, Germany; **2** University Hospital of Tübingen, Institute for Clinical and Experimental Transfusion Medicine, Tübingen, Germany; **3** Klinikum Stuttgart, Institute of Transfusion Medicine, Stuttgart, Germany; **4** Klinikum Stuttgart, Department of Neurology, Stuttgart, Germany; **5** Klinikum Stuttgart, Department of Neuroradiology, Stuttgart, Germany
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Introduction A subgroup of anti-platelet factor 4 (PF4) antibodies can activate platelets via Fcgamma RIIA and cause thrombotic and thrombocytopenic diseases such as heparin-induced thrombocytopenia and vaccine-induced immune thrombotic thrombocytopenia (VITT). Nonpathological anti-PF4 antibodies are detected in 1-7 % of healthy blood donors and in 2-8 % of SARS-CoV-2 vaccinated individuals. In this study, we investigated the long-term course of anti-PF4 antibodies detected after the first SARS-CoV-2 vaccination in healthy subjects and in patients with VITT.

Method Five healthy subjects (all female, median age (range): 40 years (29-62)) who had anti-PF4 antibodies after the first vaccination with ChadOx1 nCov19 (Vaxzevria, AstraZeneca-Oxford) were included. None of the subjects developed VITT. Blood samples were collected as part of a longitudinal study (TüSeRe:exact) evaluating the immune response to SARS-CoV-2 vaccines among employees of an University Hospital. In addition, data from 4 patients with VITT (3 female, median age (range): 44 years (22 -62 years)) were included for long-term follow-up of anti-PF4 antibodies. Anti-PF4/heparin antibodies were measured using a commercially available ELISA assay (Zymutest HIA IgG, Hyphen BioMed, France). Platelet activation was tested with a modified heparin-induced platelet aggregation assay (HIPA).

Results In the non-VITT group, the median (range) OD for IgG anti-PF4/heparin antibodies was 0.69 (0.60-1.83) after the first vaccination. Blood samples were available up to 16 months after the first vaccination (range: 5-16 months). Anti-PF4 antibody levels decreased in all subjects despite further vaccination. However, antibody levels returned to pre-vaccination levels in only one subject. In one subject who had received two doses of ChadOx1 nCov19, anti-PF4 antibodies remained above OD 1.0 at the last follow-up. All samples were negative in the modified HIPA assay. Patients with VITT received mRNA-based vaccine as second vaccination against SARS CoV2. No significant drop in platelet count or new thromboembolic complication was observed.

Conclusion Nonpathological anti-PF4 antibodies can be detected even several months after the first vaccination. The clinical significance of these antibodies in case of subsequent exposure to a vector vaccine or heparin is not yet clear. Furthermore, subsequent vaccination seems safe in VITT patietns.

Conflict of Interest Tamam Bakchoul has received research funding from CoaChrom Diagnostica GmbH, DFG, Robert Bosch GmbH, Stiftung Transfusionsmedizin und Immunhämatologie e.V.: Ergomed, DRK Blutspendedienst, Deutsche Herzstiftung, Ministerium fuer Wissenschaft, Forschung und Kunst Baden-Wuerttemberg, has received lecture honoraria from Aspen Germany GmbH, Bayer Vital GmbH, Bristol-Myers Squibb GmbH & Co., Doctrina Med AG, Meet The Experts Academy UG, Schoechl medical education GmbH, Stago GmbH, Mitsubishi Tanabe Pharma GmbH, Novo Nordisk Pharma GmbH, Leo Pharma GmbH, Swedish Orphan Biovitrum GmbH, has provided consulting services to: Terumo, has provided expert witness testimony relating to heparin induced thrombocytopenia (HIT) and non‐HIT thrombocytopenic and coagulopathic disorders. All of these are outside the current work. Other authors declare no competing financial interests.

T-19-05 Developing an assay to distinguish between HIT and VITT antibodies

Authors Schönborn L[1], Wesche J[1], Fuhrmann J[1], Esteban O[2], Dobosz P[2], Broto M[2], Serra J[2], Llevadot R[2], Greinacher A[1]

Institutes **1** Universitätsmedizin Greifswald, Institute of Transfusion Medicine, Greifswald, Germany; **2** Werfen, Lliçà d'Amunt, Barcelona, Spain

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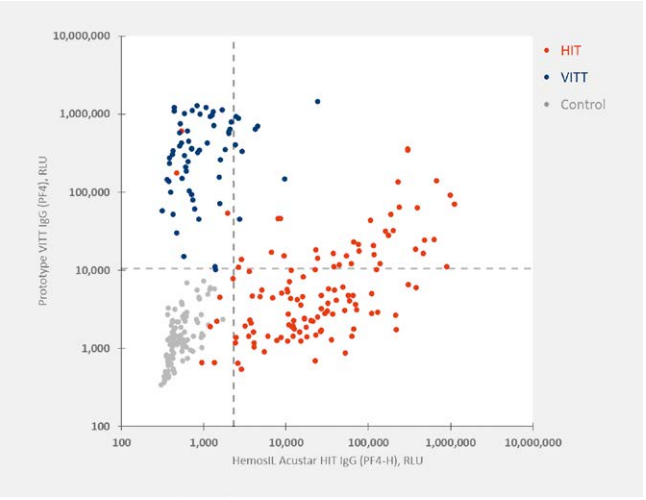
Introduction Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare, but severe side effect after Covid-19 and other vaccinations. First cases of VITT-mimicking antibodies in unvaccinated patients with recurrent thrombosis have been described. Differentiation between heparin-induced thrombocytopenia (HIT) and VITT is difficult in some patients. Widely used enzyme-linked immunoassays (EIA) cannot differentiate between the two, some of them even fail to detect VITT antibodies. So far, differentiation between HIT-like and VITT-like anti-PF4 antibodies can only be performed in specialized laboratories by functional tests using the heparin-induced platelet activation (HIPA) or PF4-induced platelet activation (PIPA) test. We have developed an assay, which can distinguish between HIT and VITT antibodies and can be used in any hospital laboratory.

Method Confirming platelet-activation assays (HIPA and PIPA) were performed as described.[1] We defined 3 cohorts: 1) Negative controls (n = 112, including 35 healthy donors from before 2020, 46 clinical patients suspected for HIT but with negative EIA and HIPA and 31 non-thrombotic patients); 2) classical HIT-patients with positive EIA and HIPA (n = 121); 3) typical VITT patients (n = 63; presenting after vaccination with adenoviral vector-based Covid-19 vaccine and positive EIA and PIPA). Samples were analyzed by an automated coagulation analyzer ACL AcuStar (Werfen / IL Inc., Bedford, MA, USA) using HemosIL AcuStar HIT-IgG(PF4-H) and a prototype of VITT-IgG(PF4) assay according to the manufacturer's protocol. For both assays, raw data was analyzed as relative light units (RLU).

Results All VITT samples were positive in the prototype VITT-assay (▶ **Fig. 1**); only a few (n = 9; 14.3 %) also showed weakly positive results in the HIT-assay. On the other hand, most of the HIT samples showed positive results in the HIT-assay (113; 93.4 %), 34 of them (30.1 %) also reacted positive in the prototype VITT-assay (12 of them strongly; 10.6 %), and three demonstrated an antibody pattern like autoimmune VITT. Negative control samples where all non-reactive in the HIT-assay and served to adjust the cutoff for the prototype VITT-assay.



▶ **Fig. 1** Results of the HemosIL AcuStar HIT-IgG(PF4-H) and of the prototype VITT assay; Results of the HemosIL AcuStar HIT-IgG(PF4-H) are given on the x-axis (cutoff: dashed vertical line). Results of the prototype VITT assay are given on the y-axis (cutoff: dashed horizontal line). HIT sera are shown in red, VITT sera in blue, and controls in grey symbols.

Conclusion The different reaction pattern of samples of HIT and VITT patients using HemosIL AcuStar HIT-IgG(PF4-H) and a VITT prototype assay was able to distinguish between the two antibody entities for the first time. The combination of assays can facilitate a rapid decision whether heparin may be used for treatment and also identify patients with autoimmune-VITT as a cause of recurrent thrombosis.

Conflict of Interest Andreas Greinacher holds a patent for VITT-antibody testing and performed consultant work for Werfen.; Part of the results reported have been obtained in a study conducted by Universitätsmedizin Greifswald under service contract No. EMA/2021/17/TDA. The views expressed are the personal views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of the European Medicines Agency or one of its Committees or Working Parties.

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T-19-06 Long-term outcome of patients with vaccine-induced immune thrombotic thrombocytopenia and cerebral venous sinus thrombosis

Authors Kehr S, Berg P, Müller S, Fiedler S A, Meyer B, Ruppert-Seipp G, Keller-Stanislawski B, Funk M B

Institute Paul-Ehrlich-Institut (Federal Institute for Vaccines and Biomedicines), Division Safety of Medicinal Products and Medical Devices, Langen, Germany

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Introduction In early 2021, unanticipated thromboses, including cerebral venous sinus thrombosis (CVST) with thrombocytopenia, emerged as an adverse reaction (ADR) in patients who had been vaccinated with the AstraZeneca ChAdOx1 nCoV-19 vaccine. This ADR was termed vaccine-induced immune thrombotic thrombocytopenia (VITT) or thrombosis with thrombocytopenia syndrome (TTS). Although sporadic in nature, VITT can result in severe disease

in the individual vaccinee. We followed up on the outcomes and status of neurological recovery of 49 cases of VITT with CVST that were reported to PEI.

Method Assessment of the Extended Glasgow Outcome Scale (GOS-E) was performed within 3–6 months after the initial hospital admissions. Individual Glasgow Coma Scale (GCS) scores were reported by phone or electronically via a questionnaire or medical report by the treating physician of the hospital to which the patient was initially admitted. If a GCS score was not reported, an expert determined a score based on the patient's medical report. For most patients, follow-up was pursued about 3–6 months after hospital admission. The reported outcomes describe the patients' neurological status at 5–38 weeks (mean 20 weeks) after hospital admission. Outcomes were identified in 44 of the original 49 cases.

Results Patient outcomes ranged from good recovery (13 patients, 29.6 %) to moderate disability (11 patients, 25.0 %) and severe disability or vegetative state (6 patients, 13.6 %). Fatal outcomes were reported in 14 patients (31.8 %). As anticipated, initial low GCS scores were associated with poor outcomes. By contrast, GCS scores >10 were typically associated with improved neurological outcomes. Moreover, platelet count nadirs were correlated with patient outcomes. Low platelet counts were observed in fatal cases (GOS-E 1) with a mean count of 17,000 platelets/µL). Likewise, patients with better neurological outcomes (GOS-E scores of 5–6 and 7–8) presented with mean counts of 61,000 thrombocytes/µL. However, the course of the disease was not always predictable and showed significant individual variability.

Conclusion We provide data on the outcome of VITT cases with CVST upon vaccination with the AstraZeneca adenoviral vector ChAdOx1 nCoV-19 COVID-19 vaccine and found that the recovery of patients from CVST was very heterogeneous. While some patients exhibited good recoveries, others developed severe disabilities and major long-term complications. Collectively, our findings highlight the importance of paying attention to early signs of increased intracranial pressure and the onset of thrombocytopenia in patients with a recent history of vaccination with the AstraZeneca adenoviral vector ChAdOx1 nCoV-19 COVID-19 vaccine.

Conflict of Interest The authors declare no competing interests.

T-19-07 Evaluation of the protein C pathway in critically ill patients with severe COVID-19 as compared to bacterial sepsis

Authors McRae H L[1], Bode C[2], Rühl H[1], Müller J[1], Becher T[3], Loßnitzer D[3], Oldenburg J[1], Putensen C[2], Pötzsch B[1]

Institutes **1** University Hospital Bonn, Institute for Experimental Hematology and Transfusion Medicine, Bonn, Germany; **2** University Hospital Bonn, Department of Anesthesiology and Intensive Care Medicine, Bonn, Germany; **3** University Medical Centre Mannheim (UMM), University of Heidelberg, First Department of Medicine, Faculty of Medicine Mannheim, Mannheim, Germany

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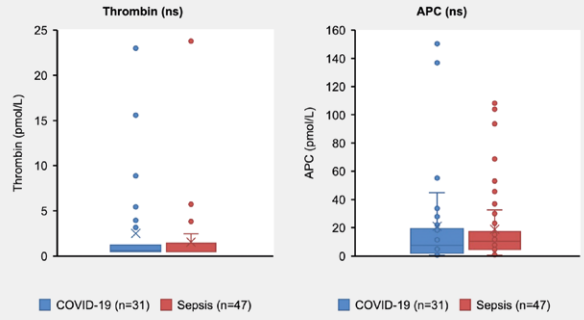
Introduction Endothelial dysfunction has been shown to play a role in severe COVID-19, the pathophysiology of which may be attributed to a myriad of factors including unmitigated immune and inflammatory response, viral-induced injury to the endothelium, end-stage organ failure, and coagulopathy. In addition, severe COVID-19 is most often seen in patients with multiple comorbidities, which themselves are often associated with endothelial dysfunction (such as myocardial and renal failure, as well as thrombotic disorders). However, the literature is still emerging on this topic and there appears to be no consensus on the extent to which endothelial dysfunction plays a role in severe COVID-19.

Method The aim of this study was to assess the functionality of the endothelial protein C pathway in hospitalized patients >18 years of age with severe COVID-19 as compared to those hospitalized with bacterial sepsis. COVID-19 (n = 31) and sepsis (n = 47) patients who were admitted to the ICU were assessed for rates of thrombin and activated protein C (APC) generation. Indirect markers of thrombin formation, including thrombin-antithrombin (TAT) complex, prothrombin fragment 1 + 2 (F1 + 2), as well as D-dimer, and protein C (PC) were measured additionally. Statistical analysis was performed via the Mann-Whitney test and a p value of <0.05 was considered statistically significant.



▶ **Fig. 1** Thrombin and APC formation rates in patients with severe COVID-19 and bacterial sepsis.

Results Plasma levels of free thrombin in COVID-19 and sepsis patients did not differ significantly, with (median, IQR) 0.59 (0.46-1.21) vs 0.57 (0.46-1.10) pmol/L, respectively. TAT was also increased at similar extent in both cohorts (192; 111-325 pmol/L in COVID-19 patients, 148; 73-213 pmol/L in sepsis patients), whereas F1 + 2 was higher in COVID-19 than in sepsis patients, with 850 (440-1940) vs 380 (130-620) pmol/L (p = 1.3 x 10-5). Interestingly, rates of APC formation did not significantly differ between the two groups, with 7.47 (1.99-19.14) vs 9.87 (2.08-16.87) pmol/L (▶ **Fig. 1**). D-dimer and protein C were significantly higher in the COVID-19 patients than in those with sepsis (14.3 vs 8.1 mg/L, p = 0.01, and 92.9 % vs 58.5 %, p = 3x10-8, respectively).

Conclusion We hypothesized that APC formation rates in response to thrombin formation would be significantly lower in patients with severe COVID-19 as compared to those with bacterial sepsis due to the well-known association between severe COVID-19 disease burden and endothelial dysfunction due to the downregulation of thrombomodulin expression. However, our results indicate that this may not be universally true in this patient population, as our observations suggest a largely intact functionality of the protein C pathway. Further studies are warranted to investigate the pathophysiology of severe COVID-19.

Conflict of Interest JO has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai Pharmaceutical Co., Ltd., CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda. All other authors report no conflict of interest.

T-20 | Effects on The Patient's Well-Being and Quality of Life

T-20-01 Pain in patients with hereditary bleeding disorders: evaluation of a survey among people affected in Germany

Authors Goldmann G[1], Halimeh S[2], Oldenburg J[1], Rosenthal M[2], Schulze Schleithoff E[1], Stracke D[3], Becker T[4], Schepperle C[4]

Institutes **1** University Clinic Bonn, Bonn, Germany; **2** Coagulation Centre Rhein-Ruhr, Duisburg, Germany; **3** MediosApotheke an der Charité, Berlin,

Germany; 4 Interessengemeinschaft Hämophiler e.V. (IGH), Rottenburg, Germany
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Introduction Patients with hereditary coagulation disorders often suffer from pain, which usually is a consequence of joint damage. This is a well-known phenomenon. The purpose of the survey was to shed light on how patients are affected and how they perceive pain management in general.

Method In May 2022, the IGH (Interessengemeinschaft Hämophiler e.V.) conducted an online-based anonymous survey. It comprised 20 questions and could be accessed online via the IGH website (https://www.igh.info/) and newsletter (https://ogy.de/schmerz2022), via Facebook (IGH, haemophilia in Germany), Instagram, and Twitter. In addition, the survey was also referred by two haemophilia centres. The IGH survey was intended to clarify whether and how pain is perceived in congenital bleeding disorders and what strategies affected persons or relatives have developed to alleviate the pain. Data collection and analysis were carried out using the online survey tool LamaPoll (https://www.lamapoll.de). Only data of participants who completed the survey were evaluated.

Results Data of 105 participants (49 male, 56 female) were analysed. 36 participants reported haemophilia A, 5 haemophilia B, 53 VWD, and 11 other bleeding disorders. The relative majority of 38 % classified their bleeding disorder as severe, 13 % as moderate, and 30 % as mild. 20 participants (19 %) answered "unknown" or "other". 63 (61 %) of the 105 participants indicated to suffer from pain related to their bleeding disorder, with 10 (16 %) suffering from chronic pain, 10 (16 %) from very frequent pain, and 14 (22 %) from frequent pain. Knee and ankle joints are particularly affected. 29 of the 63 participants (46 %) said that the pain interfered with their everyday lives on an almost daily or weekly basis, resulting in frequent days off work, school or social activities. About 75 % of participants talk about their pain, most of them with their physicians at the coagulation centres. Medication is often taken for pain management, but beyond that, 60 % say they do not seek further support for pain management. If further support is used, it is often physiotherapy. 26 participants (42 %) reported that pain subsided after clotting factor injections. More than half of the participants affected by pain wish to receive better support in pain management.

Conclusion The results of this survey demonstrate that pain is a common phenomenon in persons with haemophilia or von Willebrand's disease. Pain experienced by the patients and its management should be addressed more often during consultation. In addition, better information and holistic care by specialists could improve pain management.

Conflict of Interest In this context no conflict of interest to disclose.

T-20-02 The Post-VTE Functional Status Scale for assessment of functional limitations in patients with venous thromboembolism: construct validity and responsiveness in a prospective cohort study

Authors Steiner D[1], Nopp S[1], Weber B[2], Schlager O[3], Königsbrügge O[1], Klok F A[4], Pabinger I[1], Ay C[1]

Institutes 1 Medical University of Vienna, Department of Medicine I, Clinical Division of Haematology and Haemostaseology, Vienna, Austria; 2 Medical University of Vienna, Department of Dermatology, Vienna, Austria; 3 Medical University of Vienna, Department of Medicine II, Division of Angiology, Vienna, Austria; 4 Leiden University Medical Center, Department of Medicine - Thrombosis and Hemostasis, Leiden, Netherlands
DOI 10.1055/s-0042-1760588

Introduction Venous thromboembolism (VTE) is a common disease with various long-term sequelae such as impaired quality of life and psychosocial consequences. Additionally, a large proportion of patients experience functional limitations after an acute episode of VTE. Recently, the post-VTE functional status (PVFS) scale was proposed to capture these limitations. [1] We performed a prospective cohort study to validate this scale.

Method The PVFS scale, PROMIS physical function short form 10a, EQ-5D-5L, and disease-specific quality of life (VEINES-QOL/Sym, PEmb-QoL) were assessed within three weeks of VTE diagnosis and after a median (IQR) follow-up of 13.4 (12.7-15.9) weeks. To evaluate construct validity of the PVFS scale, we determined correlations of PVFS scale with the other health measurements and investigated differences in patients above and below 70 years of age. Responsiveness was evaluated with a linear regression model, predicting change in PROMIS with change in PVFS scale.

Results We included 211 patients (median (IQR) age: 55.1 (44.1-67.6) years, 40 % women). Pulmonary embolism was diagnosed in 105 (49.8 %) patients and 62.6 % of events were unprovoked (Table 1). The PVFS scale correlated with PROMIS physical function (Spearman's rho (r): -0.67 and -0.63, p<0.001; ▶ **Tab. 1**) and EQ-5D-5L index (r = -0.61 and -0.61, p < 0.001) at baseline and follow-up. Furthermore, PVFS correlated moderately to strongly with disease-specific quality of life. Patients above 70 years of age had significantly higher PVFS grades at follow-up (median (IQR): 2 (0-3) vs. 1 (0-2), p = 0.010). Changes in PVFS scale over time were significantly associated with changes in PROMIS physical function, and for every unit decrease in PFVS scale grade, the PROMIS T-score increased by approximately half a standard deviation. This association remained significant after adjusting for age, sex, type of event, and comorbidities (▶ **Fig. 1**).

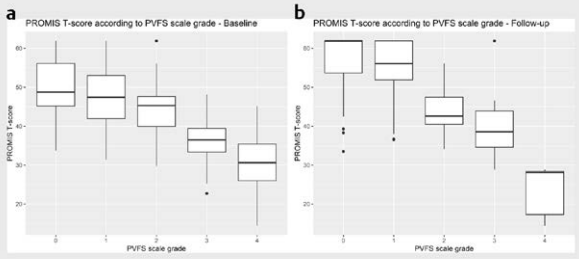
Baseline characteristics (n=211)	
Median age, years (IQR)	55.1 (44.1-67.6)
Female, n (%)	84 (39.8)
Median body mass index, kg/m² (IQR)	27.9 (24.7-31.5)
Type of event, n (%)	
Pulmonary embolism (with or without deep vein thrombosis)	105 (49.8)
Deep vein thrombosis	106 (50.2)
Admitted to hospital, n (%)	79 (37.4)
Unprovoked event, n (%)	132 (62.6)
Provoked event, n (%)*	79 (37.4)
Major persisting risk factor	14 (6.6)
Major transient risk factor	39 (18.5)
Minor transient risk factor	47 (22.3)
History of venous thromboembolism, n (%)	56 (26.5)
Family history of venous thromboembolism, n (%)	66 (31.3)
Comorbidities, n (%)	
Arterial hypertension	81 (38.4)
Cardiovascular disease	31 (14.7)
Respiratory disease	30 (14.2)
Hypothyroidism	16 (7.6)
History of cancer	11 (5.2)
Diabetes mellitus type 2	8 (3.8)
Smoking, n (%)**	
Current	55 (26.1)
Former	46 (21.8)
Never	104 (49.3)

▶ **Tab. 1** PROMIS physical function T-score according to PVFS scale grade at baseline and follow-up.; Legend to Figure 1: Grid A depicts baseline values, grid B follow-up values. Higher PVFS scale grade values indicate more functional limitations, lower PROMIS T-scores indicate worse physical function. Bold line represents median, upper and lower hinge represent third and first quartile, respectively, and outliers (distance to hinge > 1.5x interquartile range) are plotted individually. PROMIS, Patient Reported Outcome Measurement Information System; PVFS, post-VTE functional status.

Conclusion The PVFS scale showed adequate construct validity and responsiveness in a prospective cohort study of patients with VTE. These results suggest that it can be incorporated as additional health measurement and outcome parameter in research and clinical practice.

Conflict of Interest DS, SN, and BW have no potential conflicts of interest do declare. OS received personal fees from Abbott, BARD/BD, Bayer, Biotronik, and Optimed, outside the submitted work. OK received personal fees for lectures and/or participation in advisory boards from BMS. FAK received research

support from Bayer, BMS, BSCI, MSD, Leo Pharma, Actelion, The Netherlands Organisation for Health Research and Development, The Dutch Thrombosis Association, The Dutch Heart Foundation, and the Horizon Europe Program, all paid to his institution and outside the current work. IP received personal fees for lectures and/or participation in advisory boards from Bayer, BMS, Pfizer, and Sanofi. CA received personal fees for lectures and/or participation in advisory boards from Bayer, BMS, Daiichi-Sankyo, Pfizer, and Sanofi.



▶ **Fig. 1** Baseline characteristics; Legend to Table 1: * Some patients had more than one risk factor. * * No data available for 6 patients.

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T-20-03 Assessment of health status, quality of life and physiCAL functIoninG of refUgee hemophiLia patients from UkrAine (CALIGULA Study)

Authors von Mackensen S[1], Hafer L[2], Siebert M[3], Halimeh S[3, 4]
Institutes 1 University Medical Centre Hamburg-Eppendorf, Department of Medical Psychology, Hamburg, Germany; 2 Vilnius University, Vilnius, Lithuania; 3 Coagulation treatment centre rhein-ruhr, Duisburg, Germany; 4 Universitätsklinikum Essen, Hämostaseologische Ambulanz, Kinder Klinik III, Essen, Germany
DOI 10.1055/s-0042-1760589

Introduction Since the beginning of the Russian-Ukrainian war, around 14 million people have fled from the Ukraine; of them around 1 million have come to Germany (as of 19.10.2022). Among them are also peole with haemophilia (PWH) who urgently need medical care. These patients have often previously experienced a different therapeutic standard of treatment in their home countries. This initial situation creates a particular relevance to evaluate the current health status (HS), orthopaedic joint status (OJS) and disease-specific quality of life (HRQoL) as well as the subjective physical functioning (PF) of these PWH. In this context, it is important to record what additional influence haemophilia care has on the refugee PWH, as this can well be modified by therapy options that are part of the standard therapy in Germany.

Method All Ukrainian PWH who have fled Ukraine and are currently treated at German haemophilia treatment centres (HTCs) should be enrolled in the CALIGULA Study. For this purpose, an appeal was launched via the German Society of Thrombosis and Haemostasis Research (GTH) for patient recruitment by the respective HTCs. If not available, patient-reported outcome measures (PROs) were linguistically validated into Ukrainian. Haemophilia-specific PROs included instruments for the assessment of subjective physical functioning (HEP-Test-Q) and for the assessment of HRQoL (Haem-A-QoL, Haemo-QoL). In addition, a short ad-hoc questionnaire on the clinical care of the patients in the home country and on specific escape factors was developed. A validated refugee-specific questionnaire (PMLD: Postmigration living difficulties Questionnaire) to assess the stress experience of the refugee patients was also included. After obtaining the respective informed consent, PWH are asked to complete all Ukrainian PROs. Health care professionals (HCP) assess the OJS by means of the Haemophilia Joint Health Score (HJHS) and document the HS (e.g., form and severity of haemophilia, bleeding frequency and therapy) in a clinical report form. The collected data are analysed, interpreted, and compared with available data from German haemophilia patients.[1–10]

Results So far, 20 PWH (8 Kids, 19 adults) from the HTC in Duisburg were enrolled; 15 further PWH were identified by 7 other HTCs in Germany. Data from these centres are not yet available. Results from all centres will be presented at the GTH congress.

Conclusion The collection of data from refugee haemophilia patients on PROs, joint status and the general refugee situation is intended on the one hand to represent the current state of health of this special population group and on the other hand to enable a comparison with a German comparison group. In view of the current social situation characterised by humanitarian willingness to help, these data are indispensable in order to adapt the therapy to the special and individual needs of the patients in the further course and to enable appropriate treatment planning.

Conflict of Interest None

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T-22 | Fibrinolysis

T-22-01 An improved understanding of native coagulation factor XIII complex structure using cryo-EM

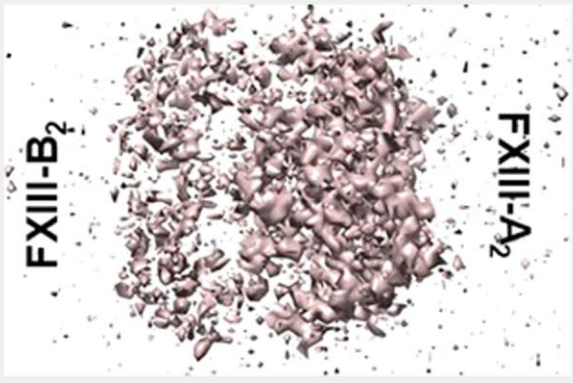
Authors Singh S[1], Uruglar D[3], Hagelukean G[2], Ramaraje Urs SU[1], Islam MM[1], Javed H[1], Geyer M[2], Oldenburg J[1], Biswas A[1]
Institutes 1 University Hospital of Bonn, Institute for Experimental Hematology and Transfusion Medicine, Bonn, Germany; 2 University of Bonn, Institute for Structural Biology, Bonn, Germany; 3 ThermoFischer Scientific, Cryo-EM Unit, Eindhoven, Netherlands
DOI 10.1055/s-0042-1760590

Hämostaseologie 2023; 43: S1–S106

Introduction Coagulation factor XIII (FXIII) exists in plasma as a pro-transglutaminase heterotetrameric complex, FXIII-A2B2.The catalytic FXIII-A subunit is structurally characterized both in its zymogenic as well as activated forms [1, 2]. In recent years, attempts were made to generate a structural model of this stoichiometrically symmetrical FXIII-A2B2 complex based on cross-linking MS data [3]. The present work describes for the first time a high-resolution complex structure of FXIII in its native complexed form using cryo-EM.

Method FXIII complex was isolated and purified from commercially available plasma FXIII concentrate using gel filtration chromatography (Superdex 200 Inc 10/300 column), until a single homogenous, mono-dispersed purified peak corresponding to FXIII. Purified, and the concentrated sample was subjected to negative staining as the first step to ensure the quality. Cryo-grids were prepared using quantifoils. Grids were prepared in a VitroBot Mark IV. Data were collected on a Thermo Scientific KriosG4 Cryo-TEM equipped with an E-CFEG energy Filter, and a Falcon 4 Detector operated in Electron-Event Representation (EER) mode. Data processing, motion correction, and model building were performed using Cryosparc, Coot, and Chimera platforms (▶ **Fig. 1**).

Results We could successfully purify FXIII complex, purified protein complex was stable at higher concentrations of up to 5mg/ml. Of the 409,767 cleaned-up particles picked, almost a 30 % showed FXIII-like behavior which was further refined. After two rounds of refinement, the final map yielded 2.84 Å resolution. When C2 symmetry was applied to the final map, the resolution reached 2.68 Å. The density map clearly depicts with a high resolution a direct interaction of FXIII subunits via sushi domains 1 & 2, as indicated by earlier reports (see figure) [4]. The S1 and S4 domains seem likely to be responsible for the homodimerization of the B subunit in the native FXIII-A2B2 complex.



▶ **Fig. 1** A high-resolution density map of FXIII complex.

Conclusion The high-resolution atomic structure of FXIII complex describes that the complex bears a cyclic symmetry where the regulatory FXIII-B subunit dimer interacts with FXIII-A subunit dimer from one side, via its N- terminal sushi domains which also ascertains them of being responsible for its dimerization.

Conflict of Interest None

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T-22-02 Analyzing secretion-based patterns of the heterozygous species of coagulation Factor XIII B cysteine mutations reported from mild FXIII deficiency using confocal microscopy

Authors Javed H, Singh S, Ramaraje Urs SU, Islam MM, Oldenburg J, Biswas A

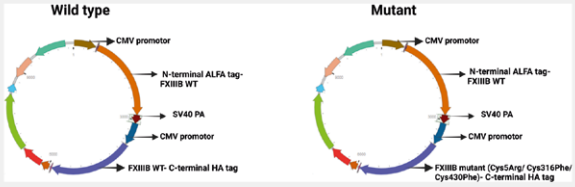
Institute Institute of Experimental Hematology & Transfusions medicine, University Hospital Bonn, Bonn, Germany

DOI 10.1055/s-0042-1760591

Introduction Severe inherited FXIII deficiency is caused by homozygous defects mainly in the F13A1 gene, rarely in the F13B gene. However, in mild FXIII deficiency, which is a milder and mostly asymptomatic form of FXIII deficiency, heterozygous defects in both F13A1 and F13B gene are found to be proportionate in number1. Previous reports have shown that the mutations in F13B gene can lead to secretory deficits2,3. These reports however do not explain the exact functional/secretory status of the heterozygous variant species. Hereby, using a combination of bi-cistronic, bi-tagged mammalian expression vectors and confocal microscopy we explore the secretory patterns of the F13B gene heterozygous mutations and ask the question if these mutations exercise an intracellular dominant negative effect.

Method We used dual promoter bi-cistronic heterozygous co-expression vectors with FXIII-B wild type (WT) cDNA in the first ORF, fused with an N-terminal ALFA tag and the respective FXIII-B variants (Cys5Arg, Cys316Phe, Cys430Phe) in the second ORF fused with a C-terminal HA tag. Both ORFs are regulated by an independent CMV promoter. Additionally for a comparative wild type control we created a bic-cistronic vector in which the wild type FXIII-B cDNA was cloned into both ORFs but with different N and C terminal tags similar to the variant bi-cistronic vectors. HepG2 cells were transiently transfected with the above mentioned vectors using Lipofectamine 3000 transfection reagent. After 24 h, transiently transfected HepG2 cells were fixed in 4 % PFA for 7 min at RT. Cells were then blocked and permeabilized. Constructs expressed were co-stained with anti-ALFA 488 diluted 1:500, anti-HA 555, anti-GM130 647 and anti-Calreticulin 647 diluted 1:250, in PBS containing 1 % BSA and 0.1 % Triton-X 100. The cells were incubated in this staining solution overnight at 4 ° C and washed 3 times for 5 min with PBS. Coverslips were mounted on glass slides using the mounting medium, dried at 37 °C, and visualized. Co-localization values were calculated on ImageJ.[1–3]

Results The signal for variant heterozygous species that was determined primarily by the overlap of AlFA (wild type) and HA (mutant) tag directed antibodies, showed comparable levels for all three Cysteine variants (i.e. Cys5Arg, Cys316Phe and Cys430Phe) when compared with the pure wild type expression. The co-localization values for the wild type FXIII-B tag (i.e. AlFA tag) with the ER signal was also comparable for all the three Cysteine variants when compared with the pure wild type expression (i.e. both cistrons bearing the FXIII-B wild type cDNA). The co-localization values for the wild type FXIII-B tag (i.e. AlFA tag) and also the mutant type FXIII-B (i.e. HA tag) with both the ER and Golgi signal were comparable when compared with the pure wild type expression (▶ **Fig. 1**).



▶ **Fig. 1** Dual promoter bi-cistronic heterozygous mammalian co-expression vectors.

Conclusion From the present analysis no intracellular dominant negative effect of any of the three Cysteine variants can be demonstrated on the secretion of FXIII-B.

Conflict of Interest No conflict of interests

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T-22-03 A novel solid phase assay for the detection of fibrinolytic activities

Authors Quinn P, Kiessig S T

Institute PreviPharma Consulting GmbH, R&D, Mannheim, Germany

DOI 10.1055/s-0042-1760592

Introduction The fibrinolytic system, based on the activation of plasminogen (PLG) into plasmin (PM), targets the dissolution of fibrin clots. An unmet medical need in severe acute thrombotic disorders remains high. Therefore, administration of PLG instead of tPA (PLG activator) or heparin, is under investigation. Human Glu-PLG, as a new candidate, has already been tested in in vivo models.

Method This study engaged itself in establishing an in vitro thrombolysis model (Fibrin Degradation Assay [FiDA]) and adequately validating such a solid phase potency assay satisfying the demands of official guidelines. Biotinylated fibrin was bound to microtiter plates and exposed to Streptavidin-HRP, for subsequent detectability. The addition of PM/activated PLG led to cleavage of the labeled fibrin (permutations with various fibrinolytic components possible). The amount of fibrin-degradation-product-streptavidin-HRP-conjugate in the supernatant was measured on a separate microtiter plate by adding a chromogenic substrate (TMB). The OD450 indirectly represented the PM activity and were plotted against the used output concentrations of PM/PLG to create a plasmin activity plot visualizing the potency of PM/activated PLG.

Results The FiDA showed a direct concentration dependency to PM/PLG, tPA and PAI-1. It can show differences in the fibrinolytic performance depending on the used fibrinolytic components. For human tPA an EC50 of 1.07 nmol/L was found. For human PAI-1 an IC50 of 61.97 nmol/L. The FiDA accuracy was found with an activated purified PLG against an activated Glu-PLG reference standard yielding a recovery of 98 %-100 % across five different PLG concentrations. The testing for precision yielded an intra assay coefficient of variation CVintra ≤ 5 % and inter assay coefficient of variation CVinter < 6.3 % against an activated Glu-PLG reference standard. Within-laboratories variations displayed an intermediate precision with CV < 5 % (plasmin) and CV < 3.5 % (activated PLG). A lower limit of detection for the FiDA was found to be 4.84 nmol/L for plasmin or 10.05 nmol/L for PLG (tPA activated). The effective measurement range was between 20 nmol/L and 1411 nmol/L. The quantification performance of samples with unknown PLG content via FiDA was compared to two commercial ELISA (Glu- & total PLG) systems and an amidolytic assay (STAGO®). The results of the two enzymometric systems were comparable, but overall, slightly lower than the PLG content found by the immunoassays, due to their specificity to only active PLG (see Table 1 below) (▶ **Fig. 1**).

Sample	STA®-Stachrom® PLG [nmol/L]	FiDA PLG [nmol/L]	Glu-PLG ELISA [nmol/L]	Total PLG ELISA [nmol/L]
1	1,783	1,245 ± 24	2,810 ± 488	3,264 ± 43
2	2,261	1,719 ± 1	3,387 ± 483	4,113 ± 286
3	1,543	1,933 ± 9	2,490 ± 164	3,187 ± 396
4	2,130	2,275 ± 26	3,907 ± 654	4,679 ± 320
5	2,870	3,598 ± 22	5,179 ± 738	6,679 ± 1,530

▶ **Fig. 1** Summary of detected PLG contents [nmol/L] of unknown samples through different detection systems; STA analyzer read out gives no information about SD.

Conclusion In conclusion a quantifiable plasmin (or tPA activated PLG) potency assay was established, performing with an adequate accuracy, precision and close to in vivo function. The assay is applicable for qualification of interactions between fibrinolytic system proteins.

Conflict of Interest None for this abstract.

T-23 | Various topics

T-23-01 The impact of enhanced platelet turnover on platelet reactivity in healthy humans

Authors Schwarz S L[1], Wolff M[1], Greinacher A[1], Handtke S[1], Thiele T[2]

Institutes 1 Universitätsmedizin Greifswald, Institut für Transfusionsmedizin, Greifswald, Germany; 2 Universitätsmedizin Rostock, Institut für Transfusionsmedizin, Rostock, Germany

DOI 10.1055/s-0042-1760593

Introduction Platelet turnover describes the process of platelet generation and clearance. We recently showed that a routine double platelet apheresis can enhance platelet turnover. The increase of platelet count after apheresis is associated with a transient increase of young, reticulated platelets in the circulation. Therefore, we aimed to investigate, how the increase of young platelets influences platelet function.

Method Blood from healthy platelet apheresis donors (n ≥ 15) was drawn before apheresis (day 0), immediately after apheresis and on day 3 and 10 after apheresis. Intensity of platelet turnover was stratified by the extent of platelet count recovery at day 3 post apheresis. Platelet reactivity was determined after stimulation with TRAP-6 (thrombin receptor-activating peptide) and CRP (collagen-related peptide) by assessment of CD62P-expression (alpha granule release) and PAC-1 binding (integrin αIIbβ3 activation). Mean fluorescence intensity (MFI) was assessed by flow cytometry.

Results Platelet count recovery from day 0 after apheresis to day 3 was compared between platelet donors. Five out of 15 individuals showed a platelet count recovery above 20 % of platelet count after platelet depletion (rapid turnover). Whereas the other platelet donors showed a slower platelet count recovery until day 3, followed by a stronger platelet count increase after three days (slow turnover). Response to agonists was significantly increased in rapid turnover donors: In comparision to day 0 (baseline) the PAC-1-binding was significantly increased on day 3 after stimulation with TRAP-6 (MFI 4,172 vs 5,951, p = 0.016) and CRP (MFI 5,420 vs 7,769, p = 0.0125). Comparable results

have been obtained for CD62P expression after stimulation with CRP (MFI 28,444 vs 58,135, p = 0.0274). Platelet reactivity returned to baseline values at day 10. No change of platelet reactivity could be detected in donors with slow turnover.

Conclusion The increase of platelet turnover after apheresis is donor dependent. Subjects with a rapid turnover show a transient increase of platelet reactivity whereas no dynamic in platelet function is present in subjects with slow turnover. These findings implicate that platelet turnover influences platelet function in healthy individuals. This may have an impact on diseases associated with increased platelet turnover.

Conflict of Interest No conflict of interest to disclose.

T-23-02 Analysis of single nucleotide polymorphisms in the C4 domain of vWF reveals the novel gain-of-function variant p.Ser2564Arg

Authors Mojzisch A[1, 2], Schneider S W[1], Brehm M A[2]
Institutes 1 University Medical Center Hamburg Eppendorf, Institute for Dermatology and Venerology, Hamburg, Germany; 2 University of Siegen, School of Life Sciences, Department of Digital Health Sciences and Biomedicine, Siegen, Germany
DOI 10.1055/s-0042-1760594

Introduction Von Willebrand Factor (vWF) is a 2813 amino acid large multidomain protein that assembles into multimers. It is exclusively expressed by endothelial cells, megakaryocytes and platelets and is secreted into the peripheral circulation. There it can sense vascular damage and recruit platelets, thereby initiating primary hemostasis. While, on the one hand, dysfunction and loss of vWF cause bleeding symptoms, on the other hand, hyperactivity and elevated plasma levels of vWF can promote thromboembolic events. Recently, two single nucleotide polymorphism (SNP) have been identified that specifically enhance the coagulative potency of vWF by increasing its function. These gain-of-function (GOF) variants are both located in the C4 domain of vWF and are characterized by either higher shear sensitivity, as shown for p.Phe2561Tyr or increased aggregate formation as observed for p.Pro2555Arg.

Method Using the published NMR structure of the vWF-C4 domain as well as the SNP database of Ensembl several SNPs from humans were selected and investigated for their impact on vWF function. The vWF with the SNP of interest was obtained by site-directed mutagenesis of a vWF plasmid, transfection and homozygous expression of vWF in HEK293F cells. These variants were analyzed for common parameters such as secretion, multimer pattern, binding to common interaction partner collagen, GPIbα and GPIIb/IIIa by ELISA, light transmission aggregometry, and platelet capture under flow using a microfluidic system.

Results 11 of 14 analyzed vWF variants showed normal expression and a normal multimer pattern when recombinantly expressed as full-length protein in HEK293F cells. Two variants were not secreted probably due to defects in intracellular trafficking. Among all expressed variants, six showed a mildly increased binding to collagen and platelet receptors GPIbα. Among them, the variant p.Ser2564Arg displayed an increased platelet agglutination in light transmission aggregometry (LTA) and led to accelerated platelet capture under flow in a microfluidic system. These data indicate that the variant p.Ser2564Arg is more sensitive for mechanical forces as it forms platelet aggregation already at lower shear rates.

Conclusion Prothrombotic variants of vWF have only been known for three years and display different phenotypes in vitro. Less is known about the exact mechanism of how these variants alter the vWF structure and function and why they vary among each other. Our study characterizes 14 different variants in the C4 domain of vWF that occur in humans, providing more data that help to identify a pattern and to understand the underlying mechanism for prothrombotic variants in the C4 domain. In addition, our screening revealed the novel GOF SNP p.Ser2564Arg which specifically increases the shear-dependent vWF function in vitro.

Conflict of Interest None

T-23-03 Leveraging medical-AI to speed up Cold Agglutinin Disease detection

Authors D'Avino N, Gray K, Bommer M
Institute Ada Health, Berlin, Germany
DOI 10.1055/s-0042-1760595

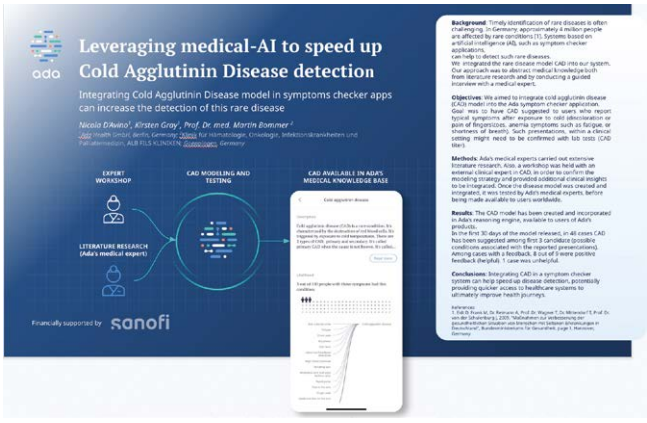
Introduction Timely identification of rare diseases is often challenging. In Germany, approximately 4 million people are affected by rare conditions [1]. Systems based on artificial intelligence (AI), such as symptom checker applications, can help to detect such rare diseases.

We integrated the rare disease model CAD into our system. Our approach was to abstract medical knowledge both from literature research and by conducting a guided interview with a medical expert.

Method Ada's medical experts carried out extensive literature research. Also, a workshop was held with an external clinical expert in CAD, in order to confirm the modeling strategy and provided additional clinical insights to be integrated. Once the disease model was created and integrated, it was tested by Ada's medical experts, before being made available to users worldwide (▶ **Fig. 1**).

Results The CAD model has been created and incorporated in Ada's reasoning engine, available to users of Ada's products.

In the first 30 days of the model released, in 48 cases CAD has been suggested among first 3 candidate (possible conditions associated with the reported presentations). Among cases with a feedback, 8 out of 9 were positive feedback (helpful). 1 case was unhelpful.



▶ **Fig. 1** Integration of CAD model into a symptoms checker app.

Conclusion Integrating CAD in a symptom checker system can help speed up disease detection, potentially providing quicker access to healthcare systems to ultimately improve health journeys.

Conflict of Interest Financially supported by Sanofi-Aventis Deutschland GmbH.

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T-23-04 Developing an assay to determine the individual osteoclastogenic potential of patients with coagulation disorders in-vitro: challenges and pitfalls

Authors Strini T[1], Preinstorfer S[1], Haidl H[1], Tokic S[1], Gallistl S[1], Zieger B[2], Schlagenhauf A[1]
Institutes 1 Medical University of Graz, Department of Pediatrics and Adolescent Medicine, Graz, Austria; 2 Medical Center - University of

Freiburg, Faculty of Medicine, University of Freiburg, Department of Pediatrics and Adolescent Medicine, Freiburg, Germany
DOI 10.1055/s-0042-1760596

Introduction Osteoclasts originating from macrophages are involved in bone resorption, thus, excessive osteoclastogenesis can result in osteoporosis. Generation of osteoclasts is mediated by RANKL (receptor activator of nuclear factor kB ligand) secreted by osteoblasts and inhibited by osteoprotegerin (OPG), a soluble decoy receptor for RANKL. Recent findings suggest a regulatory role of several coagulation proteins (e.g. von Willebrand factor (vWF), factor VIII, thrombin) in this finely tuned RANKL/OPG balance. Absence or dysfunction of these proteins in patients with coagulation disorders may result in a higher degree of osteoclast differentiation leading to chronically elevated bone resorption that increases the risk of long-term bone defects. Furthermore, macrophage polarization has been shown to be altered in patients with hemophilia which would also influence osteoclastogenesis. However, not much is known about the individual osteoclastogenic potential of these patients. Thus, we developed a methodology to evaluate coagulation proteins impact on osteoclastogenesis, and to determine the individual osteoclastogenic potential in-vitro.

Method We isolated peripheral blood mononuclear cells (PBMCs) from the patients' whole blood followed by positive magnetic sorting for CD14 + monocytes. Monocytes were expanded to macrophages with 50 ng/ml recombinant macrophage colony-stimulating factor (rhM-CSF), and osteoclast differentiation was induced by adding 50 ng/ml RANKL in presence/absence of 250 ng/ml OPG. We determined the dose-dependent influence of exogenous vWF, factor VIII, factor X, thrombin, and thrombin receptor activator peptide (TRAP-6). Furthermore, we evaluated the effect of autologous plasma (10 %) in the medium. Cells were fixed, stained for TRAP (tartrate-resistant acid phosphatase), and counterstained with hematoxylin. TRAP in the conditioned medium (TRAcP) was quantified with a colorimetric assay. Additionally, cells were seeded on bovine cortical bone slices for osteoclast resorption analyzed by SEM (scanning electron microscope) and ß-CrossLaps in the supernatant.

Results Addition of RANKL resulted in extensive formation of large multinucleated cells with osteoclast-like morphology, increased TRAcP activity, ß-Crosslaps, and bone resorption which was significantly inhibited by OPG (90,2 ± 3,8 %). Osteoclastogenesis was reduced or delayed by addition of vWF (73,2 ± 3,5 %) and TRAP-6 (62,2 ± 0,9 %), and completely abrogated by thrombin, while factor VIII and factor X showed no effect. Addition of heparinized/citrated autologous plasma showed highly variable osteoclastogenesis while addition of hirudinized plasma resulted in stable response depending on plasma constituents.

Conclusion We developed an assay to evaluate the individual osteoclastogenic potential in-vitro based on the differentiation behavior of patients' cells and the impact of autologous plasma constituents. The next step will be determining the osteoclastogenic potential of patients with von Willebrand disease.

Conflict of Interest The authors declare no conflicts of interest.

T-23-05 Plasma-derived extracellular vesicles: a look at the preanalytics

Authors Wolny M[1], Tertel T[2], Stambouli O[2], Flieder T[1], von Bargen K[1], Knabbe C[1], Giebel B[2], Birschmann I[1]

Institutes **1** Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany; **2** University Hospital Essen, University of Duisburg-Essen, Institute for Transfusion Medicine, Essen, Germany
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Introduction Extracellular vesicles (EVs) are biological nanoparticles produced by virtually all cells. Depending on the cell of origin, these vesicles can carry different proteins on their surface. This makes EVs a promising biomarker for health and disease, especially if they can be obtained from plasma samples. However, the preanalytics of EVs is crucial. The anticoagulants used for blood collection and the processing steps required to prepare plasma for EV analysis could have a significant impact on the diagnostic usefulness of EVs. Another critical aspect is the detection of EVs, as this is a technical challenge due to the small size of EVs. To this end, we have recently qualified imaging flow cytometry (IFCM) as a valid method for phenotypic characterization of antibody-labelled EVs at the single-EV level. In contrast to many other novel single EV characterization methods, this requires almost no additional preparation besides the processing of the EVs described above.

Method We used our optimized IFCM method and a panel of 20 different antibodies covering a cross-section of EV origin to investigate the preanalytics of EV preparation from whole blood. First, we investigated different centrifugation methods (2000xg, 2x: 2500xg, 3800xg, Ficoll gradient) with EDTA-anticoagulated blood. In a further step, eight different anticoagulants as well as serum were considered and the detectability and composition of different EV subpopulations in plasma/serum of healthy donors were studied.

Results Regarding the centrifugation step, we found few differences between the various speeds or gradient centrifugation. In particular, CD9 + and PS + EVs exhibited marginal concentration differences. The choice of anticoagulation during blood collection, in contrast, has a considerable influence on the composition of the EV population. For EVs derived from myeloid cells, e.g. CD16 + or CD71 + EVs, little difference was observed between the different anticoagulants. The EV populations carrying platelet markers, such as CD41 + , CD42a + and CD61 + EVs, showed a clear dependence on the anticoagulation used during blood collection.

Conclusion Our analyses demonstrate that preanalytics have a decisive influence on the composition of the EV population in plasma/serum. In particular, the choice of anticoagulation during blood collection is crucial. We show that anticoagulants have a considerable effect on the concentration and composition of certain EV types, especially platelet-derived EVs, whereas varying centrifugation steps have a rather minor effect.

Conflict of Interest Ingvild Birschmann received speaker's honoraria from Bristol-Myers Squibb/Pfizer, CSL Behring, LFB biomedicaments, Octapharma AG and Siemens Healthcare and performed contract research for Siemens Healthcare and Behnk Elektronik GmbH & Co. Ingvild Birschmann is supported by means of medical writing from CSL Behring and Portola Pharmaceuticals and is a member of the advisory board/expert testimony of LFB biomedicaments, Portola Pharmaceuticals, Siemens Healthcare and CSL Behring. Bernd Giebel is a scientific advisory board member of Innovex Therapeutics SL and Mursla Ltd and a founding director of Exosla Ltd. The other authors state that they have no conflicts of interest to declare.

T-23-06 Identification of key regulators of procoagulant COAT platelet generation by quantitative phosphoproteomic analysis and phosphoflow

Authors Veuthey L[1], Aliotta A[1], Bertaggia Calderara D[1], Pereira Portela C[1], Shehwar D[2, 1], Alberio L[1]

Institutes **1** Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Division of Hematology and Central Hematology Laboratory, Lausanne, Switzerland; **2** Quaid-i-Azam University, Department of Biochemistry, Islamabad, Pakistan
DOI 10.1055/s-0042-1760598

Introduction At the site of vascular injury, by combined activation of COllagen And Thrombin (COAT), a fraction of platelets lose their aggregating properties and become procoagulant enhancing local thrombin generation and fibrin deposition. Decreased or enhanced procoagulant platelet generation lead to bleeding or thrombotic events, respectively. The intracellular signalling underlying this dichotomous activation is only partially described. Here, we investigated whether time-lapse phosphoproteomic analysis could identify key regulators of the procoagulant response. Moreover, a flow cytometry based

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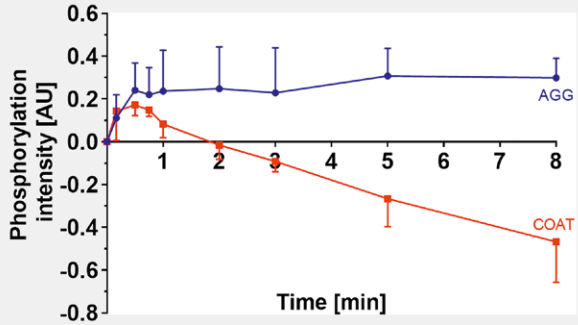
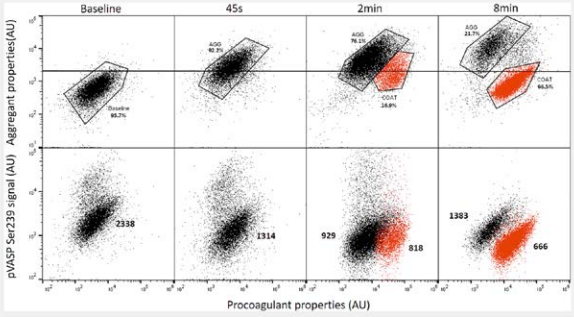
intracellular staining technique (phosphoflow) was developed to confirm and expand on the phosphoproteomic data.

Method Platelets from healthy donors were activated at RT with convulxin and thrombin in presence or absence of calcium, which generated procoagulant or aggregating phenotypes, respectively. Platelets were sampled at baseline and different time points up to 8 min after activation. The phosphoproteomes of unstimulated, aggregating, and procoagulant COAT platelets were analysed by Tandem Mass Tag and quantitative Mass Spectrometry. For the phosphoflow, both phenotypes were generated in the same tube by COAT stimulation in presence of calcium at RT. The aggregant, procoagulant and phosphorylation status were sequentially monitored over 8 min.



▶ **Fig. 1** Phosphorylation status of aggregant and procoagulant platelets by phosphoproteomics; Median phosphorylation intensity (2409 phosphosites) during aggregant (AGG) and procoagulant (COAT) platelets generation compared to baseline. Results are shown in arbitrary unit (AU) of intensity as mean ± SD of 3 healthy donors.



▶ **Fig. 2** Phosphorylation status of pVASP Ser239 during COAT stimulation by phosphoflow; Timelapse of procoagulant convulxin and thrombin (COAT) platelet generation (top panels) and phosphorylation status (bottom panels) of vasodilator-stimulated phosphoprotein (VASP) at Serine 239 in aggregant (AGG, black) versus procoagulant (COAT, red) platelets. (Top panels) the fractions of each subpopulation are indicated next to the gates. (Bottom panels) the median fluorescence of the phosphorylation signal of pVASP Ser239 is indicated for each subpopulation (bottom) in arbitrary unit (AU).

Results We identified over 7200 different phosphorylation sites (phosphosites) corresponding to 1886 unique proteins, out of which 1643 (87 %) showed significant regulation upon stimulation. Our data indicate that during the procoagulant response proteins are gradually dephosphorylated (and hyper-phosphorylated during aggregation) compared to baseline (▶ **Fig. 1**). We identified 65 antithetically regulated phosphosites in the two activation end-points at 8 min: 29 phosphosites were down-regulated in procoagulant and 36 in aggregating platelets. Among these, we observed an antithetical phosphorylation status of sodium-calcium-exchanger (NCX) at Serine381 and Serine382 in procoagulant versus aggregating platelets. This observation is in line with our previous data showing a critical functional role of NCX for the dichotomous activation leading to procoagulant platelets (Thromb Haemost 2021;121:309). Additionally, a differential phosphorylation status was also observed at Serine 239 of the vasodilator stimulated phosphoprotein (VASP). We selected this phosphosite for the development and validation of the phosphoflow. The down-regulation of this phosphosite in procoagulant platelets by phosphoflow confirmed the results from the phosphoproteomic analysis (▶ **Fig. 2**).

Conclusion The present study highlights and confirms the utility of both phosphoproteomic and phosphoflow analysis to detect and observe time-dependent changes of critical molecular regulators of the dichotomous response leading to the generation of procoagulant platelets besides aggregating ones at the site of vascular injury.

Conflict of Interest No conflict of interest to disclose.

T-23-07 The role of Mitochondrial Calcium Uniporter in platelet signalling

Authors Shehwar D[1, 2], Aliotta A[2], Barki S[1], Calderara D B[2], Veuthey L[2], Portela C P[2], Alam M R[1], Alberio L[2]

Institutes 1 Quaid-i-Azam University, Biochemistry, Islamabad, Pakistan; 2 Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Division of Hematology and Central Hematology Laboratory, Lausanne, Switzerland

DOI 10.1055/s-0042-1760599

Introduction Platelet signaling is induced by several agonists such as thrombin, collagen, ADP and adrenaline, by binding to their respective receptors. The resulting elevation of cytosolic calcium, which is induced by several signaling pathways, contributes to platelet activation. Mitochondria are also known to uptake a part of the cytosolic calcium through a specialized influx channel called Mitochondrial Calcium Uniporter (MCU). Mitochondrial calcium has been reported to facilitate the generation of procoagulant platelets upon dual stimulation with thrombin and convulxin. However, the participation of MCU-mediated calcium transport in single agonist-induced signaling and platelet responses remains incompletely understood. Thus, this study aimed to explore the role of mitochondrial calcium in different platelet signaling pathways by blocking MCU with the recently identified inhibitor mitoxantrone (MTX).

Method Expression of MCU was confirmed in platelets at mRNA level by using conventional PCR. Lactate dehydrogenase assay, trypan blue dye exclusion assay and annexin-V binding were performed to investigate the effect of MTX on platelet integrity. Platelet activation by MTX alone was assessed by immunostaining of P-selectin and PAC-1 using flow cytometry. Platelet aggregation was performed on microplate-based spectrophotometer. Agonist-evoked platelet activation was assessed by P-selectin, PAC-1, and externalized phosphatidylserine (PS) using flow cytometry.

Results We demonstrated that MTX did not induce platelet cytotoxicity as indicated by intact plasma membrane and lack of PS externalization. Moreover, platelet surface activation markers (P-selectin and PAC-1) were also not altered upon MTX treatment. Interestingly, our results revealed that MTX significantly decreased platelet aggregation in response to ADP. Likewise, MTX dose-dependently reduced PAC-1 binding and P-selectin expression with ADP. In addition to this, MTX also diminished PAC-1 binding and P-selectin expression induced by thrombin or convulxin. Remarkably, our data also unveiled an inhibitory impact of MTX on Annexin-V positive platelets upon stimulation with either thrombin or convulxin.

Conclusion Altogether, the current study revealed for the first time an activating role of MCU-dependent mitochondrial calcium transport in ADP, thrombin and convulxin stimulated platelet responses.

Conflict of Interest No conflict of interest to disclose

T-23-08 Tumour derived prothrombin interacts with tumour PAR1 receptors

Authors Mouhish Y, Nourse J, Khan E, Danckwardt S
Institute Universitätsmedizin Mainz, CTH, Mainz, Germany
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Introduction Thrombin is a liver-derived serine protease involved in hemostasis, acting through catalytic activation of soluble substrates (fibrinogen) and circulating cells (platelets). In addition, thrombin has a host of actions on cells with functions in development, angiogenesis, wound healing, inflammation, atherosclerosis, brain disorders, and tumour biology through activation of membrane-bound G-protein coupled protease-activated receptors (PARs). Previously, we uncovered extrahepatic prothrombin expression in emerging fibrosarcoma tumours, which drives tumour proliferation and invasiveness (Nourse et al.,bioxiv 2021). Here we investigated the interaction between endogenous tumour-derived (pro)thrombin and PAR1 on the surface of these cells by using bioluminescence resonance energy transfer (BRET).

Method To establish the BRET reporter assay system, we produced a prothrombin-luciferase (emission max 535 nm) and a PAR1-turbo fluorescent protein (emission at 635 nm) fusion construct, which were then used in transient transfection experiments of HEK cells. A bioluminogenic substrate (coelenterazine) was added to the co-transfected cells to elicit the energy transfer between the prothrombin-luciferase and the PAR1 turbo FP, resulting in a specific red fluorescent signal upon the interaction of (pro)thrombin with PAR1. Subsequently, we transfected endogenously prothrombin-luciferase expressing fibrosarcoma cells (obtained from a newly generated transgenic reporter mouse model after chemical tumor induction with methylcholanthrene (Nourse et al.,bioxiv 2021)) with the PAR1 turbo FP construct to study the interaction between endogenous (pro)thrombin and PAR1 using the established BRET assay system.

Results First, we confirmed the production of a functional prothrombin-luciferase fusion protein and the presence of PAR1-turbo FP on the membrane of the cells. After establishing optimal BRET assay conditions, we were able to observe red-shifted signal in transfected cells, which could be modulated with ecarin (increase in red signal) and hirudin (reduced red signal), respectively. These findings confirm a successful function of the established BRET assay principle. Furthermore, we could show an emission stroke shift in endogenously prothrombin expressing cancer cells, suggesting that prothrombin binds to PAR1 receptors located on the membrane of these cells.

Conclusion We successfully established a BRET reporter assay system to monitor (pro)thrombin-PAR1 interaction. We demonstrate that tumor-derived-prothrombin binds to PAR1 receptors expressed on the membrane of the tumor cells. Regarding the wide-spread clinical use of thrombin-targeting by direct oral anticoagulants, determination of the role and underlying mechanisms of thrombin in tumour growth may reveal previously unidentified benefits of selective therapeutic targeting of the hemostatic system in cancer.

Conflict of Interest There is no conflict of interest

T-23-09 Alternative polyadenylation regulates VEGF-coreceptor NRP1

Authors Hartleb S[1, 2, 3], Nourse J[1, 2, 3], Khan E[1, 2, 3], Danckwardt S[1, 2, 3]
Institutes **1** University Medical Centre Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **2** University Medical Centre Mainz, Posttranscriptional Gene Regulation, Cancer Research and Experimental Hemostasis, Mainz, Germany; **3** University Medical Centre Mainz, Institute for Clinical Chemistry and Laboratory Medicine, Mainz, Germany
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Introduction Neuropilin-1 (NRP1) has been identified as Coreceptor to various tyrosine kinase receptors such as VEGFR, PDGFR and TGFβR increasing ligand binding affinity and the receptors downstream signaling. Apart from membrane bound NRP1 a truncated isoform lacking the transmembrane domain exists, which acts as functional antagonist to full-length NRP1. Recently, diversification of the transcriptome at the 3'end by alternative polyadenylation (APA) has emerged as a pervasive and evolutionarily conserved layer of gene regulation. APA is, for example, involved in the IgM heavy chain class switch in activated B-cells resulting in the conversion from a membrane bound to a soluble IgM. Here we set out to explore if and how APA affects the expression of soluble and membrane bound NRP1.

Method The existence and location of several poly-A-sites in the human NRP1 gene was confirmed by 3' RACE PCR. To measure the influence of different APA factors on the expression of NRP1, we transfected BE2C cells with silencing RNAs to knockdown central components that regulate APA (PCF11, CPSF6 and NUDT21). The knockdown efficiency was controlled by western blot. The transfected cells were harvested to isolate RNA and proteins. RT-qPCR was performed to measure expression changes of NRP1 isoforms on mRNA level.[1–2]

Results We confirmed different poly-A-site usage in the human NRP1 gene, which leads to the expression of different NRP1 RNA isoforms. We also demonstrate that CPSF6, a key determinant regulating APA, controls poly-A-site usage, with knockdown of CPSF6 resulting in upregulation of the soluble NRP1 isoform.

Conclusion We show that APA regulates the expression of NRP1, which acts as coreceptor in VEGF, PDGF and TGF-β signaling, modulating angiogenesis. Downregulation of CPSF6 results in the expression of a truncated NRP1 mRNA isoform, encoding a soluble NRP1 protein that lacks the transmembrane domain. Truncated NRP1 thereby functionally competes with full-length membrane bound NRP1 and acts as a soluble decoy receptor. Based on these findings is tempting to speculate that APA evolved as a regulatory mechanism controlling angiogenesis in cancer and vascular diseases such as atherosclerosis and retinopathies.

Conflict of Interest The authors declare no conflict of interest.

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T-23-10 Establishment of an ex vivo assay to investigate thrombus formation using platelet concentrates

Authors Kirschall J[1], Marini I[1, 2], Pelzl L[1, 2], Witzemann A[1], Bakchoul T[1, 2]
Institutes **1** University Hospital of Tuebingen, Institute for Clinical and Experimental Transfusion Medicine (IKET), Tuebingen, Germany; **2** University Hospital of Tuebingen, Center for Clinical Transfusion Medicine GmbH, Tuebingen, Germany
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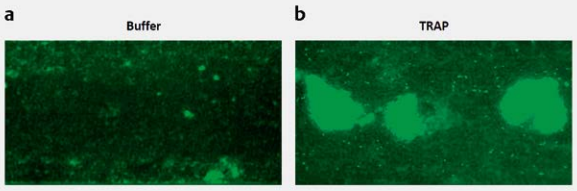
Introduction Platelet concentrates are routinely used to prevent bleeding in patients with impaired platelet function or after injury. Hemostatic function of platelet concentrates has been investigated intensively under steady state settings but until now no standard and robust protocols are available for platelet function testing under shear stress. Our aim is to establish an ex vivo model to evaluate the contribution of platelet concentrates in thrombus formation.

Method First, microfluid channels were coated with collagen (0.1 mg/mL) overnight at 4 °C and the next day blocked with HSA (human serum albumin, 1 %, 1h, room temperature). Next, platelet concentrate and platelet rich plasma (PRP) freshly isolated from healthy donors were incubated with Calcein (4 µM, 15min, room temperature) and recalcified with CaCl (75 mM) and MgCl (37.5 mM). TRAP (thrombin receptor-activating peptide, 2.5 µM) was added to initiate thrombus formation. Finally, platelet-depleted WB (whole blood) samples from healthy donors were spiked-in with platelet concentrate and immunofluorescence pictures were taken randomly.

Results Platelets isolated from two different platelet concentrate bags were tested after 24 hours of storage at room temperature and cells from an healthy donor were used as control. Platelets from both platelet concentrates, after reconstitution, showed stable thrombus formed upon incubation with TRAP, already after 1 minute of perfusion. On the contrary, cells incubated with buffer did not formed any thrombus during the entire period of perfusion (5 minutes, ▶ **Fig. 1**).



▶ **Fig. 1** Representative immunofluorescence pictures of thrombus formed using platelet concentrates upon (**a**) Buffer or (**b**) TRAP (thrombin receptor-activating peptide) stimulation, after 5 minutes of perfusion. Green signal: Calcein.

Conclusion The results indicate that our ex vivo assay, which simulates the platelet concentrate transfusion in thrombocytopenic patients, is suitable to test the hemostatic functions of platelet concentrate under physiological flow conditions.

Conflict of Interest No conflict of interest

T-23-11 Inhibition of cold-induced apoptosis of platelet concentrates improves platelet functionality and half-life

Authors Marini I[1,2], Tamamushi Y[1], Maettler C T[1], Pelzl L[1,2], Althaus K[1,2], Nowak-Harnau S[2], Bakchoul T[1,2]

Institutes **1** University Hospital of Tuebingen, Institute for Clinical and Experimental Transfusion Medicine (IKET), Tuebingen, Germany; **2** Center for Clinical Transfusion Medicine GmbH, Tuebingen, Germany

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Introduction Transfusion of platelet concentrates (PCs) is an essential medical strategy to treat bleeding or to prevent it. However, the standard storage at room temperature enhances the risk of bacterial contamination. We reported that cold-stored PCs had better functionality but reduced survival due to cold-induced apoptosis. In this study, we investigated the impact of apoptosis inhibition on platelet functions and half-life during cold storage.

Method PCs collected from healthy volunteers were stored for 1, 4, 7 and 10 days at 4 °C with or without the apoptosis inhibitor G04 (RhoA GTPase inhibitor). The apoptotic signal was analysed by flow cytometry detecting phosphatidylserine (PS) and mitochondrial membrane potential (MMP). The functionality was assessed: by flow cytometer, testing CD63 and CD62 upon TRAP; by aggregometry and performing an adhesion assay (on fibrinogen coated surfaces). The thrombus formation ability was analysed by thromboelastography. Platelet survival was investigated using the NOD/SCID mouse model.

Results We found that inhibition of RhoA GTPase significantly reduced the percentage of apoptotic cells exposing PS, starting from day 7 in comparison to untreated cells (day 7, $p = 0.030$; day 10, $p = 0.012$). Accordingly, MMP was better conserved in cells stored with G04 (day 7, $p = 0.009$; day 10, $p = 0.008$). Interestingly, upon TRAP stimulation the CD63 levels were significantly higher in the presence of G04 (CD63: day 7, $p = 0.035$; day 10, $p = 0.049$) and CD62 expression was comparable to cells stored in buffer. Similarly, a significantly higher aggregation ability in response to both TRAP ($p = 0.038$) and ristocetin ($p = 0.042$) was measured after incubation with G04 on day 10, compared to untreated cells. Next, we observed that the presence of G04 better maintained the adhesion capability of cold-stored platelets after 4 days, in comparison to buffer ($p = 0.0493$). Next, the thrombus formation ability was not affected by the apoptosis inhibitor. More importantly, a higher percentage of circulating human platelets, after 7 days of cold storage, was detected in the mouse bloodstream upon incubation with G04 compared to untreated cells (2h post injection, $p = 0.0387$, 5h post injection, $p = 0.0355$).

Conclusion Our results show that the inhibition of cold-induced apoptosis significantly reduces the clearance of cold-stored platelets without affecting the haemostatic functionality of the cells. Therefore, the use of apoptosis inhibitor/s may be a promising strategy to prolong the storage time, improve the platelet survival and reduce the risk of bacterial infection post transfusion.

Conflict of Interest Nothing to declare

T-23-12 Role of Neuraminidase A of S. pneumoniae on platelet-bacteria-interaction

Authors Krüger L[1], Handtke S[1], Jahn K[2], Kohler T P[2], Wesche J[1], Hammerschmidt S[2], Greinacher A[1]

Institutes **1** Universitätsmedizin Greifswald, Institut für Transfusionsmedizin, Greifswald, Germany; **2** Universität Greifswald, Interfakultäres Institut für Genetik und Funktionelle Genomforschung, Abteilung Molekulare Genetik und Infektionsbiologie, Greifswald, Germany

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Introduction Streptococcus pneumoniae as an opportunistic human pathogen is a major causative agent of severe community-acquired pneumonia. Pneumococci express pneumolysin (Ply), a cholesterol dependent cytolysin, which forms pores in membranes of eukaryotic cells including platelets. We have shown that pore formation results in diminished platelet function, representing a new pathomechanism during pneumococcal pneumonia. Furthermore, we demonstrated efficient neutralization of Ply with pharmaceutical human IgG (IVIG, Privigen). Besides Ply, S. pneumoniae expresses neuraminidase A (NanA), an enzyme that cleaves specifically sialic acid residues from eukaryotic glycoproteins. This increases binding of Ply to desialylated surface-associated platelet glycoproteins. Here, we aim to decipher how NanA activity affects platelet damage caused by Ply expressing and Ply-deficient strains and the inhibition of these effects by IVIG.

Method Blood was obtained from healthy human donors and isolated platelets were incubated with S. pneumoniae D39 and TIGR4 wild-type strains and their isogenic mutants ΔnanA, Δply, Δcps for 2h and analysed by flow cytometry. Desialylation was evaluated by Erythrina Cristagalli Lectin staining. Pneumolysin binding and membrane permeability was determined by staining with monoclonal anti-ply antibody and -anti-human β-Tubulin antibody. In neutralization experiments, samples were supplemented with human polyvalent immunoglobulin preparations (IVIG; IgG-enriched Privigen®) [5 mg/mL].

Results Compared to wild-type D39 and TIGR4, desialylation of the platelet surface was reduced after incubation with D39ΔnanA and TIGR4ΔnanA (MOI (ratio bacteria to platelets) 0.1; D39: 101,147 vs. 70,202, $p = 0.0078$; TIGR4: 214,786 vs. 69,943 $p < 0.0001$). Ply binding (1,159 vs. 357, $p = 0.0062$) and platelet damage (β-Tubulin expression: 1,989 vs. 470, $p = 0.0054$) were nominally reduced when platelets were incubated with TIGR4ΔnanA mutants compared to wild-types. Similar trends were observed with the D39 equivalents, but failed to reach significance (Ply binding: 369 vs. 259, $p = 0.0648$; β-Tubulin expression: 655 vs. 410, $p = 0.0641$). Desialylation of platelets was decreased when IVIG was added for D39 (44,567 vs. 20,892, $p < 0.0001$) and TIGR4 (95,089 vs. 20,951, $p = 0.0002$). Additionally, IVIG reduced accessibility of β-Tubulin even after infection at MOI 1 (D39: 24,548 vs. 632, $p = 0.0078$; TIGR4: 49,132 vs. 1,237, $p = 0.0078$).

Conclusion NanA and Ply are important virulence factors of S. pneumoniae and profoundly influence the interplay between platelets and this pathogen. The deficiency of NanA in S. pneumoniae reduces Ply binding to the platelet surface, hence also weakening its destructive effect on platelets. Therapeutic doses of human polyvalent immunoglobulin preparations can prevent the effects of both, NanA and Ply. Therefore, human IgG preparations represent

promising candidates for therapeutical intervention during severe S. pneumoniae pneumonia to prevent complications like ARDS.

Conflict of Interest No conflict of interest to be disclosed.

T-23-13 Immune modulation by the endothelial protein C receptor (EPCR) in cancer progression

Authors Pott J, Pagel S, Wilgenbus P, Ruf W, Graf C

Institute University Medical Center Mainz, Center for Thrombosis and Hemostasis (CTH), MAinz, Germany

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Introduction The coagulation system is central to innate immune defense and wound repair and exploited by tumor cells for immune evasion and tumor progression. Insofar coagulation proteases and their receptors contribute to metastasis but also to immunosuppressive cell signaling. The endothelial protein C receptor (EPCR) is expressed by endothelial cells, tumor-associated macrophages (TAM) and CD8 + dendritic cells (DC), and marks cancer stem cells contributing to breast cancer progression. EPCR forms complexes with protein C, FVII and FX that cleave PARs and therefore modulate endothelial barrier function, immune responses, and TLR signaling. We recently demonstrated that macrophage-derived factor Xa as part of the ternary complex EPCR-TF-FVIIa-FXa cleaves protease activated receptor (PAR)2 thereby promoting tumor growth. We aimed to delineate the contribution of EPCR expressed by macrophages (MΦ) and dendritic cells in tumor progression and tumor immune surveillance.

Method We studied tumor progression in the spontaneous breast cancer model PyMT and the transplantable tumor models T241 and MC38. To this end, we used immunocompetent mice with efficient cell type specific deletions of EPCR in MΦ (EPCRfloxLysMcre) or in DCs (EPCRfloxCD11c-cre). We analyzed phenotypes of MΦ and DCs in the tumor and tumor-draining lymph nodes (dLN) by multicolor flow cytometry and mRNA expression profiling.

Results Efficient EPCR deletion in MΦ attenuated tumor growth in the spontaneous breast cancer model PyMT as well as in the transplantable fibrosarcoma model T241. Moreover, EPCR deletion in LysMcre expressing cells, correlated with a reduction in immunosuppressive TAMs in both tumor models. CD103 + DCs that are relevant for tumor-antigen transport from the TME to the dLN were slightly increased to unaltered in the TME and did not differ in dLN of PyMT-EPCRfloxLysM-cre mice. Nevertheless, we detected a significant reduction of progenitor and terminally exhausted T cells in these mice. In the T241 tumor model, we additionally demonstrated a significant reduction in regulatory T cells and an increase in CD8 + cytotoxic T cells in the TME of EPCRfloxLysM-cre mice. In contrast, EPCR deletion in DCs led to lower abundance of CD103 + DCs. In addition, these cells produced less IL-12 when reaching the dLN of PyMT-EPCRfloxCD11c-cre mice. Consistently, in vitro generated DCs from EPCR-recycling deficient mice also showed reduced IL12 production.

Conclusion Our data indicate a role for macrophage expressed EPCR in the local control of cytotoxic T cells in the TME, whereas EPCR on DCs regulates IL12 production and T-cell priming. Thus, coagulation signaling plays diverse roles in the development of anti-tumor immunity and tumor elimination.

Conflict of Interest None

T-23-14 Platelets are pre-activated during thrombocytopenia in a subset of early malaria infections

Authors Witzemann A[1], Pelzl L[1], Marini I[1], Singh A[1], Kreidenweiss A[3], Kremsner PG[3], Bakchoul T[1, 2]

Institutes **1** University Hospital of Tuebingen, Institute for Clinical and Experimental Transfuerimesion Medicine (IKET), Tuebingen, Germany; **2** Center for Clinical Transfusion Medicine GmbH, Tuebingen, Germany; **3** University of Tuebingen, Institute of Tropical Medicine, Tuebingen, Germany

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Introduction Human malaria infections with Plasmodium falciparum are commonly accompanied by a reduction of platelet counts and thrombocytopenia. Usually, platelet counts drop within 10 to 15 days after the initial infection and do not fully recover up until the malaria infection is cleared. Previously, a variety of mechanisms for thrombocytopenia in Malaria have been proposed, including the removal of activated platelets from circulation or anti-Platelet Factor 4 (PF4) antibody-mediated platelet clearance. However, these mechanisms have commonly been studied in natural malaria infection and thus the observation periods are restricted to the symptomatic phase of the disease. Here, we report platelet phenotypes of a cohort of P. falciparum infected vaccine trial participants, covering the initial phase of infection and thrombocytopenia up to two weeks after challenge with malaria parasites.

Method Blood parameters were recruited from malaria-infected and control volunteers enrolled in an anti-Plasmodium falciparum vaccine clinical trial. Participants were challenged with fully-infectious P. falciparum on day one of the observation period. The observation period covered the first two weeks of malaria infection, until parasitemia was detected in blood smear or by PCR. Platelet counts and mean platelet volume were followed daily by peripheral blood counts Platelet activation status was determined by flow cytometry, measuring the expression of CD62P as α-granule, or CD63 as a δ-granule marker. The platelet responsiveness to agonists was quantified after incubation with adenosine triphosphate (ADP) and thrombin receptor-activating peptide (TRAP).

Results In our study cohorts, the malaria infected participants experienced a significant reduction of platelet count between day 11 and 13 post-infection. Platelet count nadir (PLT mean: 104.100/ µL, range: 55.800-138.000/ µL) was accompanied by a statistically significant increase of mean platelet volume (MPV mean: 9.5 fL, range: 7.2-15.9 fL). We found that a subgroup of participants shows pre-activated platelets, as indicated by higher expression of CD62P before stimulation with agonists. Pre-activation did not correlate with platelet count reduction and thrombocytopenia was observed independently of pre-activation in some individuals. Upon challenge with the platelet agonists ADP and TRAP-6, both disease-free and infected participant platelets displayed similar increases of CD62P and CD63 expression.

Conclusion Challenge with malaria parasites could cause a remarkable drop in platelet counts. Our data suggests that pre-activation of platelets co-occurs with thrombocytopenia in a subset of malaria infections. The causal association between circulating activated platelets and thrombocytopenia needs further investigation.

Conflict of Interest The author does not report any conflict of interest.

T-23-15 Endothelial protein C receptor signaling regulates myeloid-biased hematopoiesis under stress and in aging

Authors Nguyen TS[1], Graf C[1], Gur-Cohen S[2], Grunz K[1], Wolz R[1], Royce J[3], Griffin J H[5], Esmon N[4], Esmon C T[4], Ruf W[1, 3]

Institutes **1** University Medical Center Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **2** University of California San Diego, Division of Regenerative Medicine, La Jolla, USA; **3** Scripps Research, Department of Immunology and Microbiology, La Jolla, USA; **4** Oklahoma Medical Research Foundations, Departments of Biochemistry & Molecular Biology and Pathology, Oklahoma City, USA; **5** Scripps Research, Department of Molecular Medicine, La Jolla, USA

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Introduction Hematopoietic stem and progenitor cells (HSPC) replenish all mature blood cells to meet the host's adaptive demand. We showed an important role for the endothelial protein C receptor (EPCR) in maintaining HSC quiescence via integrin α4β1 (VLA4), the small GTPase Cdc42 and PAR (protease-activated receptor) 1 in the bone marrow (BM). To discriminate functions of EPCR in anticoagulation versus signaling, we studied EPCRC/S mice carrying a single intracellular point mutation abolishing normal trafficking of EPCR

through endo-lysosomal compartments. We assessed the contributions of EPCR signaling to stem cell maintenance by analyzing HSPC during mobilization, myeloablation and aging. Moreover, we compared the phenotype of EPCRC/S and PAR1 mutants with selective protease resistance, namely PAR1R41Q and PAR1R46Q mice.

Method We evaluated the quantity of HSPC in the bone marrow (BM) and spleen by multicolor flow cytometry. Mutant EPCRC/S, PAR1R41Q, PAR146Q, and strain matched wild type control mice were analyzed in steady state and after granulocyte colony stimulating factor (G-CSF) mobilization. Additionally, EPCRC/S mice were characterized during fluorouracil (5-FU)-induced myeloablation and aging.

Results At steady state, mutant EPCRC/S mice compared to EPCRWT mice showed increased circulating HSC in the peripheral blood, reduced HSC VLA-4 affinity, and accelerated EPCR clearance from the cell surface of HSC in suspension, linking EPCR signaling to HSC adhesion. Consistently, EPCRC/S showed increased HSC mobilization and extramedullary hematopoiesis upon G-CSF treatment compared to wild type control mice. The changes in of HSPC populations following G-CSF treatment were neither recapitulated by procoagulant protease resistant PAR1R41Q mice nor activated protein C-resistant PAR1R46Q mice, suggesting signaling roles of EPCR independent of proteolytic PAR1 cleavage. Myeloid-biased HSPC of EPCRC/S mice showed higher cell cycle activity compared to stain-matched control in both steady state and G-CSF-induced stress hematopoiesis. Consistent with increased proliferation of myeloid progenitors, EPCRC/S mice were more prone to 5-FU-induced myeloablation. In addition, myeloid biased hematopoiesis typically observed in aging occurred more prominently at earlier age in mice transplanted with EPCRC/S relative to wild type bone marrow.

Conclusion EPCR signaling but not anticoagulant function directly regulates integrin affinity, HSPC activity and mobilization in stress hematopoiesis, myeloablation and aging.

Conflict of Interest None

T-24 | Platelet disorders

T-24-01 Biallelic GNE variants in patients with congenital thrombocytopenia

Authors Boeckelmann D[1], Georgiev H[1], Wasmer S[1], Kornemann E-M[1], Matzner F J[1], Leipold A[2], Erlacher M[1], Hoffmeister K[3, 4], Zieger B[1]

Institutes **1** Medical Center, Faculty of Medicine, University of Freiburg, Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Freiburg, Germany; **2** Children´s Hospital, Division of Pediatric Hematology and Oncology, Karlsruhe, Germany; **3** Translational Glycomics Center, Versiti Blood Research Institute, Milwaukee, USA; **4** Medical College of Wisconsin, Department of Biochemistry and Medicine, Milwaukee, USA

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Introduction Congenital thrombocytopenia can be associated not only with a reduced production, but also with an accelerated degradation of platelets. Sialic acid binds to platelet surface glycoproteins and is known to protect platelets from degradation via the Ashwell-Morell receptor. The GNE gene encodes an enzyme that initiates and regulates the biosynthesis of N-acetylneuraminic acid, a precursor of sialic acids. GNE mutations are autosomal recessive associated with adult-onset progressive GNE myopathy (with or without thrombocytopenia) and autosomal dominant with sialuria. Interestingly, so far only a few children (n < 10) with biallelic GNE variants leading to isolated thrombocytopenia have been described. Recently, we identified compound heterozygous GNE variants in a young girl suffering from severe congenital thrombocytopenia. We showed decreased α2,3 sialic acid and increased terminal galactose and α2,6 sialic acid moieties of the girl´s platelets [1]. In the current study, we aimed to characterize the platelet defect in a family with two children (P1 and P2) affected by congenital thrombocytopenia. P1 is a newborn boy with thrombocytopenia since birth (lowest platelet count 6 x109/L) who needs weekly platelet transfusions. His 16-year-old sister (P2) presented with lifelong thrombocytopenia and suspected chronic immune thrombocytopenia. She received therapy with romiplostim which resulted in stable platelet counts (> 50 x109/L). The parents and two other siblings were clinically not affected.

Method Comprehensive analyses for inherited thrombocytopenia: blood smear, platelet flow cytometry and analysis of platelet sialic acid by lectin (MAA, RCA120) binding. Molecular analysis by next generation sequencing (NGS) panel. Family genotyping using direct sequencing.

Results Flow cytometry (FC) performed for the patients showed severely decreased thrombin-induced CD62-P and CD63 platelet expression hinting to an impaired α- and δ-granule secretion. NGS identified a homozygous GNE variant (NM_001128227.3:c.1727G>C, p.Gly576Ala) with damaging prediction to be present in both siblings. The parents and two healthy siblings are heterozygous carrier of this variant. The alteration is located in the N-acetylmannosamin (ManNAc) kinase domain of GNE. The nucleotide change c.1726G>C leading to Gly576Arg has been described compound heterozygous in a patient with GNE myopathy [2]. For P2, lectin binding analysis showed decreased α-2,3 sialylation (MAA) and increased terminal galactose (RCA120) expression on platelets consistent with loss of sialic acid synthesis and indicative of rapid platelet clearance.

Conclusion We identified a novel biallelic GNE variant located in the ManNAc kinase domain of the bifunctional enzyme that is most likely associated with this case of familial congenital thrombocytopenia. The patients have to be monitored for the development of GNE myopathy later on in life.

Conflict of Interest This research project was partially funded by CSL Behring (ZVT Nr.: ZVS-2019092402).

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T-24-02 Impaired signaling pathways in Glanzmann thrombasthenia platelets

Authors von Bargen K[1], Wolny M[1], Flieder T[1], Hohbein A[1], Dittrich M[2], Kappert G[3], Halimeh S[3], Knabbe C[1], Birschmann I[1]

Institutes **1** Herz- und Diabeteszentrum NRW, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany; **2** Universität Würzburg, Institut für Humangenetik und Bioinformatik, Würzburg, Germany; **3** Gerinnungszentrum Rhein-Ruhr, Duisburg, Germany

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Introduction Activation of platelets during primary hemostasis can induce various processes. Among others, reorganization of the actin cytoskeleton leads to platelet shape change. Preliminary work has shown that the GPIIb/IIIa complex plays a crucial role in this process. Platelets from Glanzmann thrombasthenia (GT) patients with a quantitative or qualitative defect of the GPIIb/IIIa complex were examined. It was found that the GT platelets persisted in an early form of spreading (large number of long pseudopodia) and showed a defect in lamellipodia formation. Therefore, the aim of this study was to investigate the signaling pathways of GT platelets with regard to cytoskeleton reorganization. Since platelets lack a nucleus, signaling pathways are often regulated by post-translational modifications. Here, phosphorylations in particular play a crucial role. Based on this, different signaling molecules downstream of the GPIIb/IIIa complex were investigated to identify possible pathway changes leading to the altered shape change. For this aim, a protein-protein network

was first constructed in silico using PlateletWeb to identify important proteins of the GPIIb/IIIa signaling pathways, which were further investigated.

Method For the study, healthy platelets, GT platelets and also in vitro GT platelets (healthy platelets incubated with a GPIIb/IIIa inhibitor) were examined. Washed platelets (WP) were activated with various agonists (ADP, TRAP with/without CaCl2) for the analysis of different signaling molecules. Subsequently, platelets were lysed and proteins were analyzed by Western blot.

Results Western blot analysis of activated platelets demonstrated that the active form of Src (initial signal effector protein of the GPIIb/IIIa complex, pSrc416) was reduced in the in vitro GT platelets and GT patient platelets compared with healthy donors. This suggests that Src activation via GPIIb/IIIa has an impact on platelet shape change. In addition, increased levels of pVASP239, a marker of platelet inhibition, were detected in resting in vitro GT platelets and GT patient platelets. This indicate that GT platelets are more inhibited than healthy platelets in the resting state.

Conclusion Overall, altered activity of Src and VASP was observed in GT platelets and in vitro GT platelets compared with healthy platelets. This suggests that certain signaling pathways are altered in GT platelets. Further studies, should be performed in the future to identify other impaired signaling molecules.

Conflict of Interest Ingvild Birschmann received speaker's honoraria from Bristol-Myers Squibb/Pfizer, CSL Behring, LFB biomedicaments, Octapharma AG and Siemens Healthcare and performed contract research for Siemens Healthcare and Behnk Elektronik GmbH & Co. Ingvild Birschmann is supported by means of medical writing from CSL Behring and Portola Pharmaceuticals and is a member of the advisory board/expert testimony of LFB biomedicaments, Portola Pharmaceuticals, Siemens Healthcare and CSL Behring. Günther Kappert received support for attending meetings and/or travel from Pfizer, Octapharma, NovoNordisk and Biotest. All other authors have no competing interests.

T-24-03 Distinct functional defects in platelet subpopulations after hematopoietic stem cell transplantation

Authors Mott K[1], Weiß L J[2, 1], Schulze H[1]

Institutes **1** University Hospital Würzburg, Institute for Experimental Biomedicine, Würzburg, Germany; **2** University Hospital Würzburg, Department of Internal Medicine I, Würzburg, Germany

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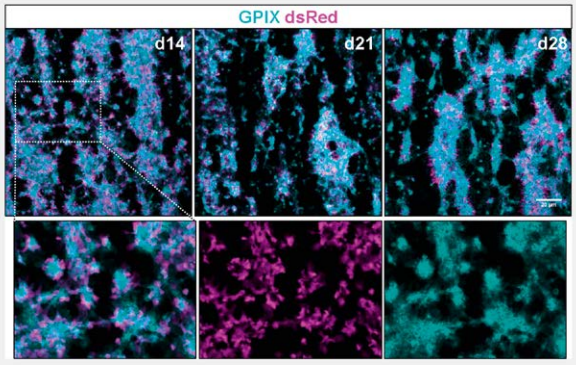
Introduction Total body irradiation (TBI) prior to hematopoietic stem cell transplantation (HSCT) is often the last therapeutic option for hemato-oncological diseases. Complications include bleeding due to prolonged thrombocytopenia but also thrombosis, such as veno-occlusive disease. The role of platelets in HSCT-related complications is yet ill defined.

Method Studies were conducted using C57B6/J wildtype mice subjected to lethal TBI followed by HSCT with bone marrow from ubiquitously dsRed-expressing reporter mice. Platelet surface receptor expression and function was assessed by flow cytometry (FC) and aggregometry. In vitro thrombus formation was analyzed using a microfluidic collagen-coated flow chamber (▶ **Fig. 1**).

Results Platelet receptor expression of GPIb/IX/V, CD9, GPIIb/IIIa, CLEC-2, GPVI, and integrin α2β1 was reduced between day d1 and d14 after HSCT, but returned to control values by d28. Platelet activation in response to high dose thrombin (Thr), collagen-related peptide (CRP-XL) or convulxin (Cvx) was severely impaired even on d28. Receptor expression correlated with platelet size of donor- (dsRed +) or recipient-derived (dsRed-) platelets. On d14, dsRed + platelets were larger, while their receptor expression was comparable to control platelets, indicative of an overall decreased receptor density. dsRed- platelets were smaller with an even higher reduction of receptor expression. By d28, size and receptor levels of dsRed + platelets normalized back to control values, while dsRed- platelets were still significantly smaller with markedly reduced expression of numerous receptors. Assessment of platelet function upon stimulation with Thr, CRP-XL or Cvx revealed hyporeactivity of dsRed- platelets, while dsRed + platelets were unexpectedly hyperreactive compared to controls. In line with our FC data, platelet aggregation was blunted after Thr activation and virtually absent when activated with collagen or CRP-XL, even on d28. When we analyzed in vitro thrombus formation in a whole blood collagen flow chamber total surface coverage was mildly increased, although the thrombus volume remained comparable to control levels. This finding is in contrast to our FC and aggregometry results and suggests a yet unidentified involvement of plasmatic coagulation factors. Intriguingly, we found that dsRed + platelets remained preferentially attached to the edge of the growing thrombi, implying that functionally distinct platelet subpopulations after HSCT could affect thrombus initiation and growth.



▶ **Fig. 1** In vitro thrombus formation on collagen after HSCT; Whole blood from transplanted mice was perfused over a collagen-coated surface on d14, d21 and d28 after HSCT. While the total surface coverage was slightly increased, the thrombus volume was comparable to untreated controls. Donor-derived (dsRed + , magenta) platelets perferentially adhere to the thrombus edge. Platelets were counterstained with an anti-GPIX Dylight488 antibody (cyan).

Conclusion Our platelet subpopulation analysis demonstrated that activity of recipient-derived platelets was massively and sustainably impaired after HSCT, while donor-derived platelets are hyperreactive, which is masked when the entire population is analyzed. Virtually unaltered in vitro thrombus formation implies an unrecognized involvement of the coagulation cascade after HSCT. The identification of a hyperreactive donor-derived platelet subpopulation provides a possible explanation for post-HSCT thrombosis.

Conflict of Interest The authors declare no conflict of interest.

T-24-04 Platelet GPIIbIIIa enriched membrane protrusions (Tether) in healthy and diseased individuals

Authors Wolff M[1], Vater L[1], Zaninetti C[1], Wesche J[1], Heidel F[2], Greinacher A[1]

Institutes **1** Universitätsmedizin Greifswald, Institut für Transfusionsmedizin, Greifswald, Germany; **2** Universitätsmedizin Greifswald, Klinik für Innere Medizin, Hämatologie und Onkologie, Greifswald, Germany

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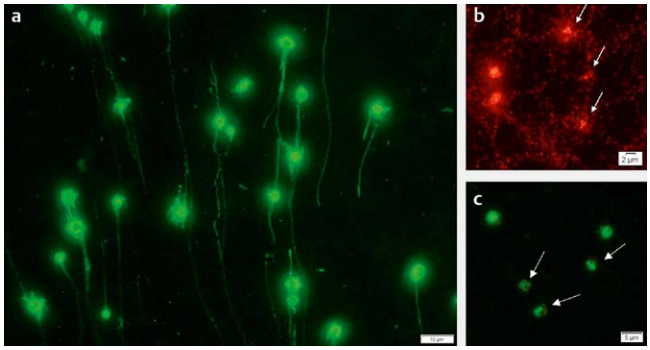
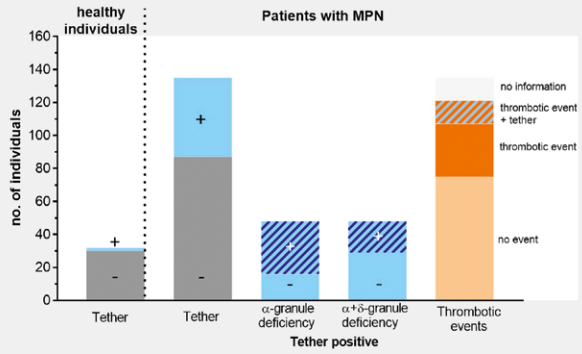
Introduction Platelets mediate adhesion and aggregation via membrane glycoproteins. The glycoprotein (GP)IIbIIIa binds to fibrinogen and other matrix proteins. Recently we observed platelets, forming long, thin membrane protrusions (tethers) that were enriched with GPIIbIIIa in some patients (▶ **Fig.1**). These tethers are very similar to Platelet-derived Integrin & Tetraspanin-rich Tethers (PITTs) described in mouse models [1]. The clinical implications of these tethers remain unclear. We assessed the frequency of this finding in patients

with myeloproliferative neoplasms (MPN). MPN are associated with an increased risk for thrombotic complications.



▶ **Fig. 1** Pattern of the index-patient; The patient presented with intense formation of GPIIbIIIa-enriched membrane protrusions (A) and reduced alpha granule content as represented by staining of vWf(B) and P-selectin (C).



▶ **Fig. 2** Screening of healthy individuals and patients with MPN; **a** cohort of healthy individuals (n = 32) and a cohort of patients with MPN (n = 135) have been assessed for platelet tethers. Tethers were more commonly found in patients with MPN and often associated with alterations of platelet granule content. Thrombotic events occurred in 34 % of the patients; 30 % of the subpopulation with thrombotic events displayed tethers.

Method We analyzed blood smears of patients with confirmed MPN (n = 135); healthy blood donors (n = 32) served as control. Blood smears were stained with 13 primary antibodies targeting platelet structures, e.g. anti CD41 (IIbIIIa), IbIX, CD62P, CD63, von Willebrand factor (vWf). After labeling with fluorescent secondary antibodies, platelets were assessed by immunofluorescence microscopy. At least 5 fields of views have been assessed for each blood smear. Parameters such as length and frequency of tethers were documented.

Results Tethers were observed in 48/135 (36 %) MPN patients. Tethers stained only for GPIIbIIIa, but not for GPIbIX [2]. In about half of tether-positive patients (24/48), tethers were short and present in less than 30 % of the assessed platelet population. Only six patients presented long tethers in a majority of the assessed platelets. The remaining 18 patients displayed moderate tether formation. Furthermore, 32/48 (67 %) tether-positive patients had diminished content of α-granules and 19 of them had a combined reduction of α- and δ-granules. Within the cohort of all 135 MPN patients, 46 (34 %) presented with an arterial or venous thrombotic event in their history; for 14 patients this clinical information was not available. Of the 46 MPN patients with thrombotic events 14 (30 %) formed tethers compared to 30 (39 %) of the subgroup without thrombotic events. In our healthy control cohort 2/32 (6 %) individuals presented with short tethers in less than 10 % of platelets (▶ **Fig. 2**). Both individuals were female, under the age of 35 years and without any documented chronic disease or medication intake.

Conclusion Platelet tethers expressing GPIIbIIIa are present in a subgroup of MPN patients but without strong association to thrombosis. Long thin membrane protrusions enriched with GPIIbIIIa are usually not found in the normal population, while short pseudopodia-like protrusions are also seen in about 5-10 % of healthy subjects. Besides the formation of tether, we noticed further alterations of platelet morphology such as reduced α-and δ-granule content in the patient cohort. This suggests an in-vivo activation of the platelets. Further patient populations with prothrombotic disorders may need to be assessed to better understand the biological role of tethers/PITTs in humans.

Conflict of Interest This project was funded by Early Career Research Grant 2022 of the GTH. M. Wolff receives financial support for travel and accommodation by the company LFB.

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T-24-05 Severe bleeding in a young patient with mild thrombocytopenia due to a novel mutation in growth factor inhibitor 1B

Authors Hammer S[1, 2], Marini I[1, 2], Uzun G[2], Althaus K[1, 2], Bakchoul T[1, 2]

Institutes 1 University Hospital of Tuebingen, Institute for Clinical and Experimental Transfusion Medicine, Tuebingen, Germany; 2 University Hospital of Tuebingen, Center for Clinical Transfusion Medicine, Tuebingen, Germany

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Introduction Inherited thrombocytopenia related to distinctive and clinically relevant bleeding disorder are rare diseases. Special diagnostic tools and intensive medical care are needed for these patients. Chronic thrombocytopenia is a disorder defined by low platelet count (< 150.000 platelets/µl) caused by autoimmune platelet destructions or reduced platelet production by megakaryocytes. Growth factor inhibitor 1B (GFI1B) mutations have been reported to be related to thrombocytopenia and distinctive bleeding tendencies of patients.1,2 In mice, impaired erythropoiesis and thrombopoiesis were associated with GFI1B deficiency.3 A syndrome named bleeding disorder Platelet-Type 17 (BDPLT17) is rare but already reported on the Online Mendelian Inheritance in Man OMIM database.

Method We investigated global parameters of plasmatic coagulation, elisa assays, aggregometry of Born, lumi-aggregometry, flow cytometry, thrombelastografy and immunfluorescence microscopy. Genetic diagnostic has been introduced.[1–3]

Results A 27-years old woman presented to our department with suspected von Willebrand syndrome type 2B. The patient reported pronounced tendency to hematoma after minor traumas. Severe nose bleeds occurs monthly. The young woman reported also on prolonged hypermenorrhea, which was improved by oral contraception. During the removal of her wisdom teeth and a tonsillectomy, desmopressin was given to reduce bleeding tendencies. Moreover after small injuries, for example after a small mole removal on the back, severe and prolonged bleeding occured. The ISTH (International Society on Thrombosis and Haemostasis) Bleeding Score was abnormal (14 of 56 points). Thromboctyopenia (101.000 platelets/µl), which was already known, was confirmed in our tests. In vitro bleeding time was prolonged with collagen/epinephrine and in normal range with collagen/adenosine diphosphate (ADP). No quantitative or qualitative defect of the von Willebrand protein was detected. More importantly, in the aggregometry of Born no platelet aggregation was

detected after induction with collagen, ADP and epinephrine in high and low concentration. Even arachidonic acid could not induce platelet aggregation. Aggregation after induction with TRAP-6 and ristocetin in concentration of 1.5 mg/ml was decreased. Adenosine triphosphate (ATP) release of platelets was decreased after adding ionophore in lumi-aggregometry. Giant platelets were detected by microscopy. Immature platelets were detected by elevated expression of CD34 by immunofluorescence microscopy and flow cytometry. Genetic diagnostic showed a heterozygous splice in intron 3 of the GFI1B gene.

Conclusion Inherited thrombocytopenia could cause low platelet count, platelets dysfunctions and severe bleeding in patients. Mutations in GFI1b are rare but already reported as being responsible for inherited thrombocytopenia. Immature giant platelets and platelet function disorders are the result of megakaryocytes dysfunction caused by the mutation in this transcription factor.

Conflict of Interest non

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T-24-06 Assessment of platelet abnormalities in patients with DiGeorge Syndrome by immunofluorescence microscopy on the blood smear

Authors Zaninetti C[1], Fumoso F[2, 1], Vater L[1], Freyer C[1], Hollenbach S[3], Holzhauer S[4], Meinhardt A[5], Wieland I[6], Greinacher A[1]

Institutes **1** Universitätsmedizin Greifswald, Institut für Immunologie und Transfusionsmedizin, Universitätsmedizin, Greifswald, Germany; **2** University of Pavia, Department of Internal Medicine, Pavia, Italy; **3** St. Georg Hospital, Leipzig, KfH Centres of Paediatric Nephrology, Leipzig, Germany; **4** University Medicine Berlin, Department of Pediatric Hematology and Oncology, Charité, Berlin, Germany; **5** University Hospital Giessen, Center for Pediatrics and Adolescent Medicine, Medical Center, Giessen, Germany; **6** Hannover Medical School, Department of Pediatric Hematology and Oncology, Hannover, Germany

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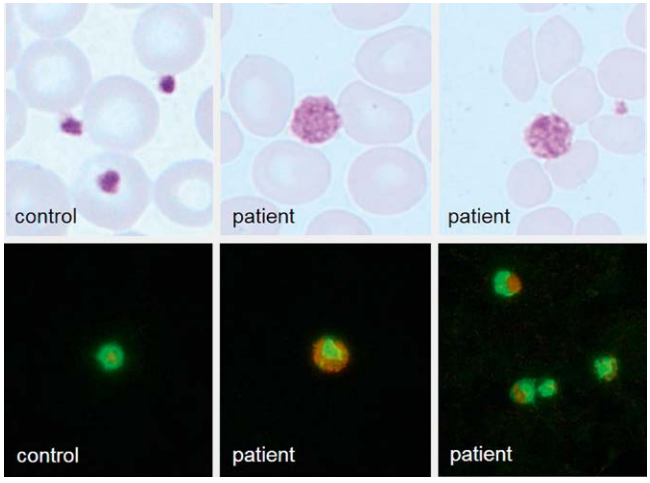
Introduction 22q11.2 deletion syndrome (22q11.2DS), also known as DiGeorge Syndrome, is caused by a chromosomal microdeletion [1]. Patients show a wide constellation of clinical features including dysmorphisms, velopharyngeal insufficiency, hypocalcemia, glands disfunction, mental retardation, and autoimmune diseases. Platelet abnormalities due to a deletion of GP1BB gene, encoding for the glycoprotein (GP) 1bβ subunit of GPIbIX, occur in the majority of patients and consist of platelet macrocytosis and mild-to-moderate thrombocytopenia. The bleeding tendency is usually mild. Although the definitive diagnosis requires genetic testing, a primary diagnostic orientation is usually performed by searching the most suggestive clinical features of the disorder in the suspected individuals.

Method We analyzed blood smears using our previously reported method for platelet phenotyping by immunofluorescence-microscopy in combination with light-microscopy [2]. In particular, 13 different primary antibodies were used to label platelet markers belonging to distinct structures (granules, cytoskeleton, surface receptors) [3].

Results The investigated subjects were 3 young adults (2 male and 1 female; mean age: 16) and a 2-year-old female child with genetically confirmed DiGeorge Syndrome. The mean platelet count was 94,000/µl (range 64-156). Three out of 4 patients had a low platelet count (50,000-100,000/µl). Two patients suffered from mild bleeding symptoms. By light microscopy, platelet macrocytosis (with mean platelet diameter measured on 200 platelets >2.6 µm) was found in 3 out of 4 subjects; typical giant platelets were seen in two of them. In one subject, only few platelets were enlarged. By immunofluorescence-microscopy, a reduced expression of GpIbX in a subpopulation accounting for 20-to-50 % of platelets was found in all patients. This reduction was independently confirmed by flow-cytometry in 3 cases. In two patients, GpIbX clustered on platelets. Clustering of GpIbIX was the prominent conspicuous change in the platelet phenotype in the subject with the lower percentage of platelets showing reduced GpIbIX expression. Overall, the GpIbIX alterations were particularly evident in large and giant platelets. Other features, which are typically associated with platelet macrocytosis (convoluted distribution of the cytoskeletal protein β1- and alpha tubulins), were observed in 3 out of 4 individuals (▶ **Fig. 1**).



▶ **Fig. 1** 22q11.2 deletion-associated platelet phenotype; Platelet macrocytosis and reduced expression of GPIbIX in two 22q11.2DS-patients with respect to control by light- and immunofluorescence microscopy.

Conclusion Immunofluorescence-microscopy on the blood smear can be used as a rapid screening tool in patients with suspected DiGeorge Syndrome. This is particularly suitable for very young children due to the small amount of required blood (<100 µL). In patients with confirmed DiGeorge Syndrome, the method can be used to assess the 22q11.2 deletion-associated platelet abnormalities.

Conflict of Interest the authors have no conflicts of interest to declare.

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T-24-07 Platelet expression of the transcription factor ETV6 points toward ETV6-related thrombocytopenia and can be detected by immunofluorescence on the blood smear

Authors Zaninetti C[1], Baschin M[2], Vater L[1], Karastaneva A[3], Holzhauer S[4], Leinøe E[5], Freyer C[1], Seidel MG[3], Greinacher A[1]

Institutes 1 Universitätsmedizin Greifswald, Institut für Immunologie und Transfusionsmedizin, Greifswald, Germany; 2 Universitätsmedizin Greifswald, Pädiatrischen Onkologie und Hämatologie der Klinik und Poliklinik für Kinder- und Jugendmedizin, Greifswald, Germany; 3 Medical University of Graz, Division of Pediatric Hematology and Oncology, Department of Pediatric and Adolescent Medicine, Graz, Austria; 4 Charité University Medicine, Department of Pediatric Hematology and Oncology, Berlin, Germany; 5 Rigshospitalet University Hospital, Dept. of Haematology and Dept. of Genomic Medicine, Copenhagen, Denmark
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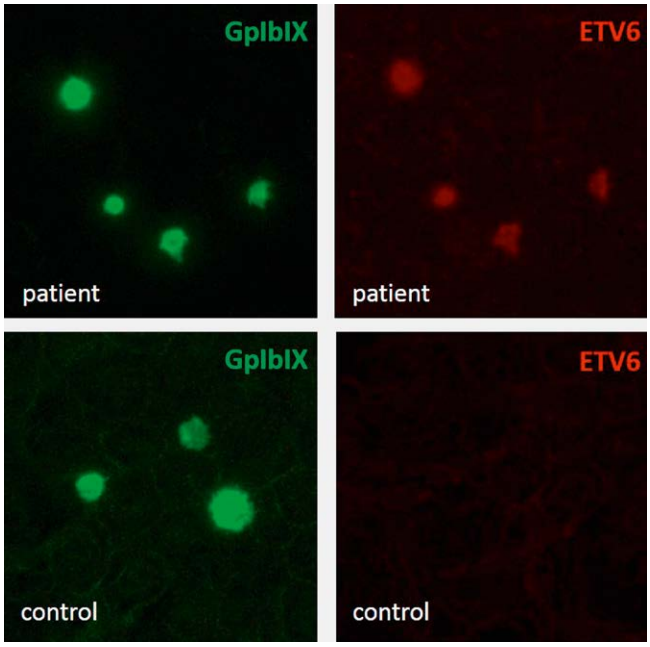
Introduction Inherited platelet disorders (IPD) present with congenital reduced or dysfunctional blood platelets and sometimes with additional, acquired manifestations affecting the morbidity of the patients more than the thrombocytopenia itself. ETV6-related thrombocytopenia (ETV6-RT), as well as IPD due to mutations in RUNX1 and ANKRD26, causes mild-to-moderate thrombocytopenia with normal platelet size and makes the patients prone to develop throughout-life hematological malignancies [1, 2]. ETV6 protein is a transcriptional suppressor, which is usually located in the nucleus of megakaryocytes but redistributes into the cytoplasm when its DNA-binding site is hit by mutations. Recognizing ETV6-RT-patients is challenging. While the diagnostic confirmation could be relevant for the interpretation of their phenotype, it could raise at a time ethical issues because of the associated risk of leukemic transformation (▶ **Fig. 1**).



▶ **Fig. 1** Platelet expression of ETV6; Platelet expression of ETV6 protein in platelets from an ETV6-RT patient with respect to a healthy control by immunofluorescence-microscopy. The platelets are counterstained for GPIbIX.

Method We analyzed blood smears by light- and immunofluorescence-microscopy upon staining with May-Grünwald-Giemsa technique and with a set of primary antibodies against 13 markers belonging to different platelet structures (granules, cytoskeleton, surface receptors) and ETV6 protein. Their expression was assessed after staining with fluorescence-labelled secondary antibodies [3, 4].

Results We investigated 11 patients belonging to 6 unrelated families with genetically confirmed ETV6-RT. In all but one patient, diverse abnormalities of dense granule markers LAMP1, LAMP2 and CD63 were reported (variable reduction of granule number; diffused distribution of the staining; granule structure presenting on the outer membrane instead inside the platelets). ETV6 protein was detectable in the cytoplasm of platelets of all patients, suggesting a pathological expression. In parallel, we assessed ETV6 expression in platelets from healthy blood donors (n = 20) and from patients affected with other forms of IPD (n = 20). We found no platelet expression of ETV6 in any of the investigated control individuals.

Conclusion We have established an immunofluorescence-based screening method for ETV6-RT on the blood smear. Typical features are: platelet expression of ETV6, normal platelet size, and evidence of dense granule abnormalities. Due to the ethical implications of this finding, i.e., the association of ETV6-RT with an increased risk for hematologic malignancies, patients have to be informed and should provide explicit consent prior to performing this investigation.

Conflict of Interest The authors have no conflicts of interests to declare

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T-24-08 A novel, homozygous mutation in GFI1B causing inherited thrombocytopenia with Glanzmann-like platelet dysfunction

Authors Zaninetti C[1], Selleng K[1], Thiele T[2], Wesche J[1], Rath M[3], Felbor U[3], Greinacher A[1]

Institutes 1 Universitätsmedizin Greifswald, Institut für Immunologie und Transfusionsmedizin, Greifswald, Germany; 2 Universitätsmedizin Rostock, Institut für Transfusionsmedizin, Rostock, Germany; 3 Universitätsmedizin Greifswald, Institut für Humangenetik, Greifswald, Germany
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Introduction Inherited platelet disorders (IPD) are rare. To date mutations in around 80 genes have been identified as causes of IPD with heterogeneous clinical manifestations [1, 2]. Recognizing IPD can be difficult but is advisable in order to provide patients with proper counseling and treatment in bleeding situations [3]. Novel mutations are frequently found and require an accurate platelet diagnostic work-up to confirm their clinical relevance and pathogenic role [4]. We report a female patient with lifelong hemorrhagic diathesis.

Method Clinical assessment, platelet phenotyping by flow-cytometry, immunofluorescence microscopy, standard aggregometry, DNA analysis as well as a platelet reactive antibody screening using MAIPA were performed.

Results The patient was a 46-year-old woman with congenital, moderate thrombocytopenia (range 55-76,000/µl) and substantial spontaneous and provoked bleeding symptoms. A diagnosis of Glanzmann Thrombasthenia (GT) had been previously made at another institution, and the patient was consequently treated on-demand with platelet concentrates. Family history strongly suggested an autosomal-dominant inherited bleeding disorder. By standard aggregometry, a GT-like platelet dysfunction (absence of platelet aggregation after stimulation with all agonists except ristocetin at high concentration) was found. However, the expression of platelet glycoprotein receptor GPIIbIIIa and GPIbIX was normal. No platelet autoantibodies potentially causing acquired GT were detected. By light-microscopy, we found partially degranulated large platelets and rarely giant platelets. A mild alpha-storage pool disorder was

detected by immunofluorescence-microscopy as well as expression of the stem cell antigen CD34 on platelet surfaces. The combination of autosomal-dominant macro-thrombocytopenia with reduced alpha granule markers and expression of CD34 strongly indicated a mutation in GFI1B gene [5]. Genetic testing confirmed a hitherto non-reported GFI1B variant, p.His181Leu, which was present in a homozygous state. Alternative splicing results in two GFI1B isoforms. The identified missense variant was predicted to affect the function of the longer isoform, which is required for megakaryopoiesis and platelet production but not of the short one which is essential for erythroid differentiation. Because of the highly consistent platelet phenotype, we interpreted this variant as pathogenic. In addition, we suggest that the particular homozygous state of the mutation can lead to the GT-like platelet dysfunction, which is unusual in patients with GFI1B-related thrombocytopenia. No mutations were present in ITGA2B and ITGB3.

Conclusion In patients presenting with platelet dysfunction immunofluorescence on the blood smear allows extended platelet phenotyping. This can guide further diagnostic steps and, together with genetic testing, result in a final diagnosis

Conflict of Interest The authors have no conflicts of interests to declare.

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T-24-09 Aggregates of non-muscle myosin IIA in the erythrocytes associate with GATA1-related thrombocytopenia

Authors Vater L[1], Zaninetti C[1], Freyer C[1], Rivera J[2], Ohlenforst S[3], Freson K[4], Greinacher A[1]

Institutes **1** Universitätsmedizin Greifswald, Institut für Immunologie und Transfusionsmedizin, Greifswald, Germany; **2** Universidad de Murcia, Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Murcia, Spain; **3** University Clinic Bonn, Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany; **4** KU Leuven, Department of Cardiovascular Sciences, Center for Molecular and Vascular Biology, Leuven, Belgium

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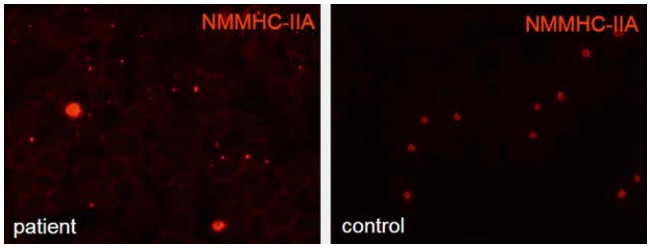
Introduction GATA1-related thrombocytopenia (GATA1-RT) is a rare X-linked inherited platelet disorder (IPD) characterized by reduced blood platelets, enlarged platelets, and dyserythropoietic features due to mutations in the GATA binding protein 1 (GATA1), which serves as transcription factor for either megakaryopoiesis and erythropoiesis [1]. We have established an immunofluorescence-based method for phenotyping platelets on the blood smear, which we have proposed as a screening tool for 10 different forms of IPD, for which typical changes of platelet structure can be detected [2, 3]. For GATA1-RT, no specific morphologic changes, which can be recognized by immunofluorescence on the blood smear, have been reported so far.

Method We stained blood smears with May-Grünwald-Giemsa method, and with a panel of 13 primary antibodies against as many markers of the main platelet structures (granules, cytoskeleton, surface receptors). The blood smears were eventually assessed with standard light- and immunofluorescence-microscopy upon staining with fluorescent-labelled secondary antibodies (▶ **Fig. 1**).



▶ **Fig. 1** Red blood cell phenotype in GATA1-RT; Aggregates of non-muscle myosin IIA (NMMHC-IIA) in the erythrocytes of a GATA1-RT patient with respect to control, by immunofluorescence-microscopy.

Results We investigated 7 genetically confirmed GATA1-RT-individuals belonging to 5 unrelated families. Subjects were 4 males and 3 females. Their mean platelet count was 80,000/µL (range 30-90,000). By light-microscopy, a heterogeneous platelet population with some large platelets in combination with red blood cell anisopoikilocytosis was observed. By immunofluorescence-microscopy, we found aggregates of non-muscle myosin II A, heavy chain (NMMHC-IIA) in the erythrocytes as the most conspicuous finding, which was present in all subjects. The platelet phenotype was also characterized by reduction of alpha granule markers in 6 patients, and of both alpha and dense granule markers in one patient. By systematic re-analysis of blood smears from a cohort of patients with 20 different forms of IPD confirmed at molecular level, we found a similar red blood cell expression of NMMHC-IIA exclusively in subjects with GFI1B-related thrombocytopenia (GFI1B-RT). This disorder also associates with dyserythropoiesis [4]. Of note, no expression of NMMHC-IIA in the erythrocytes was found in subjects affected with other IPDs including MYH9-related disorder, who present with NMMHC-IIA aggregates in the leukocytes as well-recognized pathognomonic signature. We did not observe such peculiar red blood cell phenotype also in one patient suffering from congenital erythroid aplasia type Diamond–Blackfan.

Conclusion Aggregates of NMMHC-IIA in the erythrocytes associate with GATA1-RT and, possibly, with GFI1B-RT. This finding may represent a novel marker of dyserythropoiesis, and it enables recognition of GATA1-RT by immunofluorescence microscopy on the blood smear.

Conflict of Interest The authors have no conflicts of interests to declare.

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T-24-10 In-vitro and In-silico characterization of a pathogenic variant in integrin β3 associated with hereditary macrothrombocytopenia

Authors Sereda N, Pezeshkpoor B, Loos K, Biswas A, Oldenburg J, Pavlova A
Institute University Clinic Bonn, Institute for experimental hematology and transfusion medicine, Bonn, Germany
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Introduction Congenital macrothrombocytopenia is a group of rare platelet disorders characterized by a decreased platelet count with macrothrombocytes and are associated with a varied bleeding tendency. Pathogenic variants have been reported in more than 12 genes including the genes encoding the αIIbβ3 integrin (ITGA2B and ITGB3). Pathogenic variants in these genes are usually associated with the classical Glanzmann thrombasthenia (GT) which is a rare autosomal recessive disorder characterized by the absence of platelet aggregation, normal platelet number, and volume and is usually associated with severe mucocutaneous bleeding. Very rarely, an autosomal dominant form of GT, associated with platelet dysfunction and mild macrothrombocytopenia, has been described. In the frame of this study, we aimed to evaluate the effect of such variant using in-vitro and in-silico tools.

Method Here we present a large family with congenital macrothrombocytopenia. Platelet morphology was studied on blood smears stained with May-Grünwald Giemsa. The expression of platelet membrane glycoproteins was investigated by flow cytometry. Platelet aggregation was studied with a four-channel aggregometer. Genetic results were obtained through Next Generation Sequencing with a custom Nextrera Panel. Structural and in-vitro analysis of the genetic variant were conducted. For in-vitro analysis, ITGB3 and ITGA2B genes were cloned into multiple cloning sites of pIRES vector.

Results The proband and 11 other relatives experienced lifelong mild bleeding history. The platelet count was reduced and mean platelet volume (MPV) increased. The blood smears were analyzed using May-Grünwald-staining. The analysis revealed increased platelet size under microscopy. No other visible alterations were detected. Platelet aggregation was impaired. The αIIbβ3 surface expression and activation of platelets were evaluated by CD41 and CD61 antibodies and PAC-1 binding after induction of platelets with ADP and PMA. Molecular analysis revealed a single genetic variant in Exon 14 of the ITGB3 gene (p.Leu738Arg) in a heterozygous state, which was detected among all affected members. HEK cells were transfected with the wildtype and mutated ITGB3 variant and the expression of both proteins was evaluated by western blotting and immunostaining. The structural model showed that the defective amino acid is located in membrane-proximal region of the cytoplasmic domain of β3 integrin. The change of Leucine 738 to Arginine could result in bending of the helical backbone of the β3 integrin within the membrane and result in disruption of the αIIbβ3 integrin complex.

Conclusion In conclusion, we have performed in-vitro and in-silico analysis of a pathogenic genetic variant causing macrothrombocytopenia. These findings raise interesting questions about role of integrin β3, in platelet function. Moreover, the presented case highlights the need for reconsidering the inheritance penetrance for genes associated with classical platelet disorders.

Conflict of Interest None

T-24-11 The impact of Syk-inhibition on 5B9 monoclonal HIT antibody-mediated procoagulant platelet formation

Authors Wurmbäck P[1], Zlamal J[1, 2], Bakchoul T[1, 2]
Institutes **1** Institute for Clinical and Experimental Transfusion Medicine, University Hospital of Tuebingen, Tübingen, Germany; **2** Centre for Clinical Transfusion Medicine, Tübingen, Germany
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Introduction Heparin-induced thrombocytopenia (HIT) is a prothrombotic disease caused by immunoglobulin G (IgG) antibodies (Abs) that recognize complexes of heparin and platelet (PLT) factor 4 (PF4). Formation of anti-PF4/heparin HIT IgG immune complexes can be recognized by PLT immune receptor Fc-gamma-RIIA which results in immunoreceptor tyrosine-based activation motif (ITAM) mediated activating signal downstream with a pivotal role of the enzyme spleen tyrosine kinase (Syk). While it is well recognized that HIT Abs induce PLT activation, the potential contribution of different Ab-induced PLT subpopulations to the prothrombotic condition in HIT remains elusive. 5B9 is a chimeric monoclonal HIT-antibody that resembles the pathogenesis of HIT. This study aims to characterize 5B9 HIT Ab-induced Fc-gamma-RIIA mediated PLT subpopulations and to investigate the therapeutic potential of different Syk inhibitors on 5B9 Ab-induced PLT alterations.

Method To investigate whether 5B9 HIT Ab has the potential to induce different PLT subpopulations, PLTs from healthy individuals were incubated with increasing concentrations of 5B9 in the presence of buffer, low- (0.2IU/mL) or high- (100 IU/mL) dose heparin. After incubation, PLTs were analyzed for changes in the expression level of the PLT surface activation marker P-selectin (CD62p) and phosphatidylserine (PS) externalization via double staining in flow cytometry (FC).

Results 5B9 Ab induced significant formation of CD62p and PS double positive PLTs (procoagulant PLTs) in the presence of low- (0.2 IU/mL) dose heparin while these changes were not detected under buffer conditions or in the presence of high- (100 IU/mL) dose heparin (63.01 ± 7.63 % vs. 4.66 ± 1.86 %, p value 0.0009; and 2.83 ± 0.21 %; p value 0.0011, respectively). Formation of procoagulant PLTs was 5B9 dose-dependent as higher procoagulant PLT formation was observed in the presence of increasing 5B9 concentrations (EC50:6.37 ± 1.24µg/mL). Additionally, formation of 5B9 Ab-induced procoagulant PLTs seems to be solely mediated by PLT Fc-gamma-RIIA as the inhibition of PLT protease activated receptor-1 (PAR-1) with specific PAR-1 inhibitor did not affect 5B9-induced procoagulant PLT formation. Furthermore, experiments with the Syk inhibitors Fostamatinib (R406) and Lanraplenib revealed that the formation of procoagulant PLTs seems to be dependent on Syk mediated PLT activating signaling mechanisms, as preincubation of PLTs with these Syk inhibitors resulted in significant inhibition of Ab-induced procoagulant PLT formation (IC50:14.35 ± 1.27µM and 0.22 ± 1.29 µM, respectively).

Conclusion Taken together, our findings indicate that 5B9 induces a new PLT subpopulation, namely procoagulant PLTs, via the sole engagement of PLT Fc-gamma-RIIA by anti-PF4/heparin 5B9 immune complexes. This standardized in vitro model of HIT will allow further identification of different PLT subpopulations and analysis of the potential impact of Syk inhibitors on 5B9-induced procoagulant PLT and thrombus formation.

Conflict of Interest There are no potential conflicts of interest.

T-24-12 Establishment of an in vitro assay to detect antibody-mediated procoagulant platelet formation on a single cell level in real time

Authors Böttcher L[1], Zlamal J[1, 2], Bakchoul T[1, 2]
Institutes **1** Institute for Clinical and Experimental Transfusion Medicine (IKET), University Hospital of Tübingen, Tübingen, Germany; **2** Centre for Clinical Transfusion Medicine GmbH, Tübingen, Germany
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Introduction Procoagulant platelets (PLTs), a subpopulation of PLTs that is characterized by increased externalization of phosphatidylserine (PS), are increasingly identified to promote a prothrombotic environment in different diseases. Recently we observed that procoagulant PLT formation can be induced via engagement of immune receptor Fc-gamma-RIIA by COVID-19, VITT and HIT patient immunoglobulin subclass G (IgG) antibodies (Abs). Here, Fc-gamma-RIIA engagement by patient Abs resulted in significant formation of procoagulant PLTs and loss of mitochondrial potential that was associated with high thrombin formation as well as increased thrombus formation. In the cur-

rent study, we aim to establish a PLT adhesion assay that allows investigation of PLT mitochondria during procoagulant PLT formation.

Method PLTs were spread on human serum albumin, fibrinogen or collagen precoated glass slides. Adhesion and subsequent shape change of PLTs as well as procoagulant PLT formation were investigated in real time using immune fluorescence microscopy. For the detection of PLT shape change, mitochondrial dynamics and PS externalization, PLTs were double stained with MitoTracker green, a mitochondrial dye that binds to free thiol groups of cysteine residues in the mitochondrial membrane, and Annexin-V, respectively. For the visualization of mitochondrial release from PLTs intracellular compartment, a monoclonal Ab that binds to a subunit of the translocase of the outer membrane (TOM) complex on the mitochondrial membrane, namely TOM22, was used.

Results During the observation period, a subgroup of PLTs that was spread on collagen became procoagulant as determined by an increased binding of Annexin-V on the PLT surface. Contrary, these changes were nearly absent in PLTs that adhered to fibrinogen (percentage [%] of Annexin-V positive cells: 19.80 ± 3.42 % vs. 1.92 ± 0.62 %, p value 0.0357). Interestingly, procoagulant PLT formation was associated with a significant loss of MitoTracker green signal in PLTs while it remained constant in non-procoagulant PLTs attached on both extracellular matrix coatings. Loss of MitoTracker green signal was associated with translocation of mitochondrial proteins from intracellular to extracellular, as a higher count of TOM22 Ab-positive labelled structures, most likely extracellular mitochondria were detected on collagen but not on fibrinogen coated glass slides.

Conclusion Our findings indicate, that the formation of procoagulant PLTs is associated with dramatic changes of the mitochondrial integrity in PLTs. Further attempts, that investigate the potential pathophysiological role of PLT mitochondrial release in Ab-mediated prothrombotic disorders may contribute to a further understanding of the role of PLT mitochondria in these complex diseases.

Conflict of Interest There are no potential conflicts of interest.

Late breaking abstract

Efficacy and safety of valoctocogene roxaparvovec gene transfer for severe hemophilia A: results from the GENEr8-1 three-year analysis

Authors Mahlangu J[1], von Drygalski A[2], Shapiro S[3, 4, 5], Sheng-Chieh C[6], Ozelo M[7], Kenet G[8], Peyvandi F[9, 10, 11], Madan B[14], Laffan M[12], Dunn A[13], Mason J[14, 15], Quon D[16], Leavitt A[17], Oldenburg J[18], Chambost H[19], Reding M[20], Jayaram K[21], Yu H[21], Robinson T[21], Pipe S[22]

Institutes **1** Hemophilia Comprehensive Care Center, Charlotte Maxeke Johannesburg Academic Hospital, University of the Witwatersrand and NHLS, Johannesburg, South Africa; **2** Department of Medicine, University of California San Diego, CA, USA; **3** Oxford University Hospitals National Health Service Foundation Trust, Oxford, UK; **4** Radcliffe Department of Medicine, University of Oxford, Oxford, UK; **5** Oxford National Institute for Health Research Biomedical Research Centre, Oxford, UK; **6** Division of Hematology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; **7** Hemocentro UNICAMP, Department of Internal Medicine, School of Medical Sciences, University of Campinas, Campinas, SP, Brazil; **8** The National Hemophilia Center, and Amalia Biron Research Institute of Thrombosis and Hemostasis, Sheba Medical Center, Tel Hashomer, Tel Aviv University, Tel Aviv, Israel; **9** Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milan, Italy; **10** Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy; **11** Guy's & St. Thomas' NHS Foundation Trust, London, UK; **12** Centre for Haematology, Imperial College London, London, UK; **13** Nationwide Children's Hospital and The Ohio State University College of Medicine, Columbus, OH, USA; **14** Queensland Haemophilia Centre, Cancer Care Services, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia; **15** University of Queensland, Brisbane, QLD, Australia; **16** Orthopaedic Hemophilia Treatment Center, Los Angeles, CA, USA; **17** University of California San Francisco, San Francisco, CA, USA; **18** Institute of Experimental Haematology and Transfusion Medicine and Center for Rare Diseases, University Hospital Bonn, Bonn, Germany; **19** AP-HM, Department of Pediatric Hematology Oncology, Children Hospital La Timone & Aix Marseille University, INSERM, INRA, C2VN, Marseille, France; **20** Center for Bleeding and Clotting Disorders, University of Minnesota, Minneapolis, MN, USA; **21** BioMarin Pharmaceutical Inc., Novato, CA, USA; **22** Departments of Pediatrics and Pathology, University of Michigan, Ann Arbor, MI, USA

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Introduction In August 2022, Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) received conditional marketing authorization from the European Medicines Agency for the treatment of severe hemophilia A in adult patients without a history of factor VIII inhibitors and without detectable antibodies to adeno-associated virus serotype 5 (AAV5). Stable and durable annualized bleed control superior to prior prophylaxis was previously demonstrated through 2 years post gene transfer. For the first time, we will report the latest findings from the Year 3 analysis of the ongoing GENEr8-1 study (NCT03370913).

Method GENEr8-1 is an open-label, multicenter phase 3 trial evaluating the efficacy and safety of $6x10^{13}$ vg/kg valoctocogene roxaparvovec in 134 adult men with severe hemophilia A (FVIII ≤ 1 IU/dL) without inhibitors or anti-AAV5 antibodies (ITT population). Of those, 132 were HIV negative (mITT population), and 112 enrolled from a prospective noninterventional study (rollover population), providing baseline data for annualized bleeding rate (ABR) and FVIII use. The primary efficacy endpoint was change from baseline in ABR for treated bleeds from 5 weeks post-infusion or 3 days after the end of FVIII prophylaxis, whichever was later, to the last visit by data cut-off. Change from baseline in annualized FVIII infusion rate (AFR) and FVIII activity will also be reported for week 156.

Results Updated efficacy and safety assessments from 3-year follow-up data will be shared at the GTH 2023 Congress. Presented endpoints will include FVIII activity, annualized treated bleed and FVIII utilization rates, patients who resumed FVIII prophylaxis, and adverse events.

Conclusions The 3-year data from the phase 3 GENEr8-1 study will provide the first opportunity for the hemophilia community to assess the long-term durability of valoctocogene roxaparvovec in a large cohort. These data will be of key interest to clinicians in Europe who may be considering whether to initiate gene therapy with valoctocogene roxaparvovec with their patients with severe hemophilia A.

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Disclosures

Johnny Mahlangu, MBBCh, MMed

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Late breaking abstract

Comparative transcriptome profile analysis on early T2DM-aggravated atherosclerotic mouse aortas

Authors Matulac John Mark[1, 2], Zakariah Nazatul Nurzazlin[1, 2], Kim Nari[1, 2]

Institutes **1** Inje University, NLRL for Innovative Cardiovascular Engineering, Cardiovascular and Metabolic Disease Center, Busan, Republic of Korea ; **2** Inje University, Department of Physiology, College of Medicine, Busan, Republic of Korea

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Introduction A significant challenge in understanding the atherosclerotic biomolecular alterations induced by type 2 diabetes (T2DM) is the synergistic binding of genes expressed in the early stages of diabetes. The study aims to employ a novel approach to identify pro-atherogenic differentially enriched genes and pathways under T2DM conditions by comparative analysis of transcriptome profiles.

Method The T2DM diabetic mouse model was generated by low-dose STZ induction on high-fat diet-fed male ApoE deficient mice. Diabetic and nondiabetic mice were sacrificed during plaque initiation and early progression. Atherosclerotic aortic arch regions were harvested and subjected to RNA sequencing analysis. Differentially expressed genes (DEGs) were obtained by pairwise comparison of each group relative to the control. A protein-protein interaction network was generated from each dataset, clustered into modules, and subjected to overrepresentation analysis to determine their biological roles.

Results The diabetic model exhibited hyperglycemia, dyslipidemia, and insulin resistance. The plaque area and necrotic core percentage were significantly increased in the diabetic model. More DEGs were detected in diabetes reflecting the significant contribution of diabetic factors in modifying the overall molecular landscape for plaque formation. Dataset clustering analysis showed a total of 667 DEGs are differentially enriched in diabetes and labeled as 'Enriched in DM'. The top ten enriched pathways in this dataset were related to the immune system, ECM-receptor interaction, Jak-STAT, complement cascade, NOD-like receptor signaling, leukocyte transendothelial migration, Toll-like receptor signaling, transcription from RNA polymerase II promoter, and so on. Interestingly, the enrichment of DEGs related to defense response to viruses suggests that diabetic conditions can non-canonically trigger antiviral responses even under sterile conditions which can contribute to the overall inflammatory response.

Conclusion Comparative transcriptome profile analysis of early atherosclerotic plaque from nondiabetic and diabetic mice shows that T2DM triggers increased defense response to immune signaling pathways, contributing to diabetes-aggravated atherosclerosis.

Disclosures

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