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ABSTRACT BOOK

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TABLE OF CONTENTS

Author List

Science, Fast and Furious I

SFF01-OP01

UNMASKING GALACTOSEMIA IN PATIENTS WITH UNEXPLAINED ANTITHROMBIN DEFICIENCY: A NOVEL AND REVERSIBLE CAUSE OF THROMBOSIS

M. E. De La Morena-Barrio [1,*], M. Gutierrez-Alvariño [2], M. Llamas-López [1], C. Bravo-Pérez [1], J. J. Rojo-Carrillo [1], B. de la Morena-Barrio [1], R. Cifuentes [1], J. Padilla [1], L. Entrena [3], S. Asenjo [4], A. Miñano [1], E. García [2], P. Llamas [5], I. Martínez-Martínez [1], M. L. Lozano [1], V. Jiménez-Yuste [2], J. Corral [1]

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Background: Galactosemia is a rare autosomal recessive disorder caused by mutations in *GALT*, an enzyme involved in galactose metabolism. Classic cases (<1% GALT activity) present in neonates with severe multi-organ dysfunction. Without dietary galactose restriction, patients may develop intellectual and growth delays, hepatic failure, cataracts, and other life-threatening complications. Hemostatic abnormalities—mainly bleeding attributed to liver dysfunction—are reported, although thrombotic events have also been described. The role of *GALT* in protein glycosylation (galactosemia is classified as a congenital disorder of glycosylation, CDG) may further contribute to disease pathogenesis.
Antithrombin is a key endogenous anticoagulant whose N-glycosylation is critical for its secretion and function. CDG patients often exhibit antithrombin deficiency (ATD) and increased hypoglycosylated antithrombin isoforms. Notably, up to 5% of ATD cases are caused by glycosylation defects.

Aims: To identify hidden galactosemia among patients with ATD of unknown genetic cause.

Methods: We studied 626 unrelated patients with ATD. *SERPINC1* was analyzed by sequencing and MLPA. Functional and biochemical characterization of plasma antithrombin was performed using chromogenic assays and Western blot. Glycosylation of other liver-derived proteins (e.g., α1-antitrypsin, transferrin) was evaluated by Western blot or HPLC. Whole-exome sequencing and family studies were used to analyze genes involved in N-glycosylation. Duarte 2 *GALT* variants were identified using TaqMan probes and Sanger sequencing.

Results: Among 33 patients with ATD without SERPINC1 mutations who also had proved CDG by increased hypoglycosylated forms of antithrombin, α1-antitrypsin, and transferrin, three had pathogenic GALT variants and thrombotic events.
P1, a one-year infant with severe ATD and thrombosis, showed <1% GALT activity and compound heterozygosity for GALT mutations. After dietary galactose removal, glycosylation normalized, antithrombin levels recovered, and anticoagulation therapy was discontinued with no recurrence after two years.
P2 and P3, both adults with milder ATD, carried Duarte 2 GALT variants in cis with 25% activity. Galactose removal had no impact on glycosylation or antithrombin levels in P2.
Screening of 116 healthy controls found neither glycosylation defects nor ATD among 11 Duarte 2 carriers.

Summary/Conclusion: Galactosemia should be considered in patients with thrombosis, ATD without *SERPINC1* variants, and circulating hypoglycosylated proteins. Early diagnosis enables simple dietary treatment that can restore antithrombin levels and eliminate the need for anticoagulation. However, isolated Duarte 2 galactosemia does not cause ATD or hypoglycosylation. PI24/00106;PI24/00429

SFF01-OP02

PEROXIDASIN, OBESITY AND VENOUS THROMBOEMBOLISM – RESULTS FROM THE HUNT STUDY

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Background: The risk of venous thromboembolism (VTE) increases with increasing body mass index (BMI), and approximately 25% of all VTE cases in the population can be attributed to overweight and obesity. The mechanisms by which obesity increases VTE risk are not fully understood, and there is a need to identify biomarkers that can aid risk stratification and be potential targets for intervention of obesity-related VTE. In a proteome-wide discovery study, plasma levels of peroxidasin, a heme-containing peroxidase involved in cross-linkage of collagen and facilitation of oxidative stress, were associated with increased risk of VTE. Peroxidasin is highly expressed in adipose tissue and studies have indicated a correlation between plasma peroxidasin and obesity.

Aims: To explore the association between plasma peroxidasin levels and risk of future VTE after adjustment for potential confounders, and to investigate if peroxidasin could mediate the VTE risk in overweight and obesity.

Methods: We created a case-cohort from the Trøndelag Health Study (HUNT3, n=50800), including all incident VTE cases occurring within the first 5 years of follow-up (n=294) and a randomly sampled age- and sex-weighted subcohort (n=1066). Body height and weight were objectively measured, and blood samples were collected at cohort inclusion. The participants were categorized as normal weight (BMI<25), overweight (BMI 25-30) and obese (BMI>=30). Plasma levels of peroxidasin were analyzed using the SomaScan® 7k aptamer-based platform. Correlation between BMI and peroxidasin was assessed by the Pearson coefficient. Weighted Cox-regression models were used to estimate hazard ratios (HR) with 95% confidence intervals (CI) for VTE according to quartiles of peroxidasin adjusted for age, sex, sample batch, BMI, glomerular filtration rate, C-reactive protein, history of cardiovascular disease and smoking status. Furthermore, HRs for VTE according to overweight and obesity were estimated with normal weight as reference. Regression-based causal mediation analysis (CMAverse R package) was applied to investigate whether peroxidasin mediated the association between overweight/obesity and VTE. The study was approved by the regional committee for medical research ethics and all participants provided written consent.

Results: Participants with peroxidasin levels in the highest quartile had a 2.2-fold higher risk of VTE than those in the lowest quartile after multivariable adjustment (HR 2.23, 95% CI: 1.36-3.65). There was a significant correlation between increasing BMI and peroxidasin levels (r: 0.24, p-value: 7.0×10^{-19}). Obesity was associated with 51% increased risk of VTE (HR: 1.51, 95% CI: 1.08-2.09) when compared to normal weight in a model adjusted for age and sex. After adjustment for peroxidasin, the HR was attenuated to 1.30 (95% CI: 0.93-1.81), and the mediation analysis showed that peroxidasin mediated 45% (95% CI: 24%-88%) of the VTE risk in obesity.

Summary/Conclusion: Plasma peroxidasin was associated with increased risk of future VTE and mediated 45% of the VTE risk in obesity. Our findings suggest that peroxidasin may play a role in the pathogenesis of obesity-related VTE.

SFF01-OP03

UNRAVELING C5AR1 INDUCED INTRACELLULAR SIGNALING PATHWAYS DRIVING THROMBUS FORMATION ON MICROVASCULAR ENDOTHELIAL CELLS IN ATYPICAL HEMOLYTIC UREMIC SYNDROME

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Background: Atypical hemolytic uremic syndrome (aHUS) is a complement-mediated thrombotic microangiopathy, resulting from excessive complement activation generating C5a and C5b-9. These effectors drive endothelial dysfunction and loss of thromboresistence. C5a interaction with its receptor C5aR1, along with aHUS serum exposure, induced thrombus formation on human microvascular endothelial cells (HMEC-1) via exocytosis of von Willebrand Factor (vWF) (Aiello et al, Blood Adv 2022). vWF exocytosis is typically induced by G protein–coupled receptor (GPCR) activation involving Gαs or Gαq subunits. C5aR1 is a poorly characterized GPCR, and its signaling in endothelial cells has not yet been well elucidated.

Aims: To investigate the intracellular signaling pathways activated by C5a/C5aR1 in endothelial cells and identify novel antithrombotic targets for aHUS.

Methods: vWF exocytosis was assessed by cell surface immunofluorescence on HMEC-1 stimulated with C5a, or adrenaline or thrombin (positive controls for Gαs/cAMP- and Gαq/Ca^{2+}-mediated secretagogues respectively). Stimulated cells were pre-treated or not with either an adenylyl cyclase specific inhibitor or a Ca^{2+} chelator to determine the specific Gα subunit involved in C5a/C5aR1-mediated signaling. To further characterize C5a intracellular signaling, vWF exocytosis was assessed on C5a-stimulated HMEC-1 silenced for Gαs by specific siRNA or pre-incubated with inhibitors specific for Gαs/cAMP-dependent effectors (Epac, PKA or myosin-IIa).

Thrombus formation was evaluated on ADP-activated HMEC-1 or glomerular endothelial cells (GEnC), stimulated with C5a or pre-incubated with serum from aHUS patients, and subsequently perfused with control blood under shear stress of 60 dynes/cm^2. HMEC-1 and GEnC were pre-treated or not with the same Gαs/cAMP pathway inhibitors previously used in vWF exocytosis experiments or transfected with Gαs-targeting siRNA.

N=3-5 experiments for each set.

Results: C5a increased vWF exocytosis in HMEC-1 similarly to adrenaline and thrombin ($p<0.05$ vs unstimulated cells). Inhibition of adenylyl cyclase significantly reduced C5a-induced vWF release, while calcium chelation had no effect. Comparable results were obtained in adrenaline-stimulated cells, suggesting that C5aR1 may be coupled to a Gαs subunit. Gαs silencing reduced vWF release, confirming the hypothesis that C5aR1 signals through a Gαs subunit ($p<0.05$ vs C5a-stimulated scramble cells). Inhibition of downstream cAMP effectors Epac, PKA, and myosin IIa also led to a marked reduction in C5a-induced vWF exocytosis.

C5a stimulation induced massive thrombus formation on HMEC-1 compared to unstimulated cells. This effect was completely prevented by silencing the Gαs subunit or inhibiting adenylyl cyclase, highlighting the

role of Gαs/cAMP signaling. Similarly, inhibition of Epac, PKA, and myosin IIa prevented thrombus formation, confirming the involvement of cAMP pathway.

Pre-exposure of HMEC-1 to aHUS serum also induced massive thrombus formation. Silencing Gαs or inhibiting its downstream signaling components fully prevented this effect. Similar results were obtained repeating the experiments with GEnC, representing the main target in aHUS.

Summary/Conclusion: The prothrombotic effect of C5a/C5aR1 signaling in endothelial cells relies on activation of Gαs/cAMP-dependent pathways. aHUS serum induces thrombus formation via the same mechanism. Understanding C5aR1-driven intracellular signaling may provide novel antithrombotic targets for aHUS treatment.

SFF01-OP04

OFF-TARGET EFFECTS OF EMICIZUMAB ON MONOCYTES/MACROPHAGES: PHENOTYPIC, FUNCTIONAL, AND SINGLE-CELL TRANSCRIPTOMIC DATA

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Background: Emicizumab is a humanized IgG4 monoclonal antibody used for the prophylaxis of bleeding in patients with severe hemophilia A (HA). By reducing the frequency of joint bleeds, this treatment helps to limit synovial inflammation and prevent hemophilic arthropathy. Monocyte-derived macrophages play a key role in these processes. Three subpopulations of blood monocytes are defined based on the expression of CD14 and CD16 markers: classical, intermediate, and non-classical monocytes. Macrophages also exhibit functional heterogeneity, ranging from the pro-inflammatory M1 phenotype to the reparative M2 phenotype. Since IgG4 can bind to FcγRI receptors on monocytes/macrophages, our hypothesis is that emicizumab may modulate the monocyte/macrophage phenotype through the activation of these receptors.

Aims: This study aims to assess the ability of emicizumab to bind to monocytes and to identify the activated signaling pathways as well as its effects on the phenotype of human monocytes and macrophages.

Methods: We studied a cohort of patients with severe HA. The distribution of monocyte subpopulations was analyzed by flow cytometry (FCM) and confocal microscopy in patients treated with either emicizumab or FVIII concentrates (FVIII). The phosphorylation status of Syk, a key protein in Fcγ receptor-associated signal transduction, was assessed by Western blot following short-term stimulation of monocytes with emicizumab. The phenotype of monocyte-derived macrophages (MDMs) from healthy donors, with or without emicizumab exposure, and from HA patients treated or not with emicizumab, was evaluated by quantitative RT-PCR and FCM. Finally, the impact of emicizumab on monocyte phenotype was investigated using single-cell transcriptomic analysis.

Results: In patients with severe HA, no significant differences were observed in the distribution of monocyte subpopulations (in percentages or absolute counts) based on treatment (FVIII: n=12; emicizumab: n=7). Emicizumab binds to all three circulating monocyte subpopulations (mean (standard deviation)): 71% (6) for classical monocytes, 48% (17) for intermediate, and 37% (11) for non-classical monocytes. Emicizumab induces Syk phosphorylation and stimulates the expression of both M1 macrophage markers (interleukin-1β and -6) and M2 markers (mannose receptor, CD200R) in MDMs. Single-cell transcriptomic analysis revealed that the phenotypic profile of monocytes under emicizumab was similar to that of healthy individuals. Common clusters, absent in FVIII-treated patients, as well as FVIII-specific clusters, showed differences in the expression of genes involved in cytokine production and the phagocytosis process.

Summary/Conclusion: This study highlights that emicizumab, by binding to monocytes via FcγRI receptors, induces phenotypic and functional changes in monocytes and macrophages. The results suggest that the phenotypic profile of monocytes under emicizumab treatment resembles that of healthy individuals, in contrast to monocytes from FVIII-treated patients, which display distinct characteristics. These findings

contribute to a better understanding of the immunomodulatory effects of emicizumab and its impact on the inflammatory response, which may help explain, in addition to its role in hemostasis, its benefits in preventing hemophilic arthropathy.

Science, Fast and Furious II

SFF02-OP01

HEMOSTATIC IMPACT OF ALPHA-1-ANTITRYPSIN PITTSBURGH, A PAN-INHIBITORY SERPIN

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Background: α1-antitrypsin (α1AT) is a hepatic serpin that inhibits neutrophil elastase. The P1 residue of serpins, at the reactive site, plays a crucial role in determining protease specificity. The natural variant α1AT Pittsburgh (p.Met358Arg), identified in heterozygosis in few patients with bleeding events, implies an amino acid change at the P1 residue that functionally transforms the mutant α1AT into an antithrombin.

Aims: To investigate the hemostatic impact of α1AT Pittsburgh.

Methods: The inhibitory capacity of α1AT Pittsburgh against different purified proteases of the hemostatic (FIIa, FVIIa, FXa, FXIa, FXIIa, kallikrein, and tPA) and the complement (C1S) systems were assessed by chromogenic assays, or by evaluating the formation of serpin-protease covalent complexes by Western blot. In addition, thrombin generation and fibrin degradation in the presence and absence of α1AT Pittsburgh were also studied. These analyses were conducted in plasma extracted from a heterozygous carrier of α1AT Pittsburgh, and in the supernatant from transfected HEK293T cells expressing this variant.

Results: Both plasma and recombinant forms of α1AT Pittsburgh effectively inhibited all tested proteases and formed covalent complexes, except for bovine FXa and C1S in the case of plasma α1AT Pittsburgh. However, the recombinant variant was less efficient for the inhibition of thrombin and FXIa since cleaved forms were also observed in these cases, probably by an altered N-glycan decoration in the recombinant product (vs. plasma protein) generated by the cell line resulting in a different interaction with the proteases.

The inhibitory capacity of α1AT Pittsburgh was observed in the absence of heparin. Accordingly, α1AT Pittsburgh, even at very low concentrations, caused a dose-effect reduction on thrombin generation. Analysis of the plasma derived from the patient carrying the variant revealed complete inhibition and null thrombin generation. Moreover, α1AT Pittsburgh also moderately impaired fibrinolysis.

Next, we explored the effect of heparin added in vitro. In plasma, the presence of heparin reduced the formation of complexes involving α1AT Pittsburgh, as other natural serpins -primarily antithrombin, along with C1-INH- also played a role in inhibiting the proteases. However, a similar effect was also observed in the recombinant system, suggesting that heparin, per se, is capable of impairing the inhibitory capacity of α1AT Pittsburgh.

Summary/Conclusion: Nature offers a fascinating variant, α1AT Pittsburgh, which transforms a specific molecule into a “superantithrombin”, i.e. a pan-hemostatic serpin capable of an efficient and constitutive (in the absence of heparin) inhibition of most procoagulant proteases, thus explaining the bleeding events in carriers: from FXIIa and FVIIa, at the initiation of both coagulation pathways, to the terminal effector thrombin, or to FXIa as a protease involved in the amplification loop). Furthermore, α1AT Pittsburgh also inhibits the contact and the fibrinolytic pathways. These qualities point out the potential therapeutic possibilities of α1AT Pittsburgh.

SFF02-OP02

IMMUNOTHROMBOLYTIC MONOCYTE-NEUTROPHIL AXES DOMINATE THE SINGLE-CELL LANDSCAPE OF HUMAN THROMBOSIS AND CORRELATE WITH THROMBUS RESOLUTION

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Background: Thrombotic diseases remain the major cause of death and disability worldwide, and the contribution of inflammation is increasingly recognized. Thromboinflammation has been identified as a key pathomechanism, but an unsupervised map of immune-cell states, trajectories, and intercommunication at a single-cell level has been lacking.

Aims: The aim of this project was to provide a broad, unsupervised single-cell resolved atlas of human and murine thrombosis and to subsequently provide mechanistical validation of the key identified insights.

Methods: We pioneered the use of single-cell RNA-sequencing, CITE-Seq, prime-seq as well as flow cytometry to unravel the immune-landscape of human and murine thrombosis. We subsequently used murine in vivo as well as in vitro thrombosis models to validate the key inter-cellular communication pathways identified by the previous multi-omic measures.

Results: Here, we reveal innate leukocyte substates with prominent thrombolytic properties by employing single-cell omics measures on human stroke thrombi. Using *in vivo* and *in vitro* thrombosis models, we propose a pro-resolving monocyte-neutrophil axis, combining two properties: (1) NR4A1^{hi} non-classical monocytes acquire a thrombolytic and neutrophil-chemoattractive phenotype, and (2) blood neutrophils are thereby continuously recruited to established thrombi through CXCL8-CXCR1 and CXCR2 and adopt a hypoxia-induced thrombus-resolving urokinase receptor (PLAUR)$^{+}$ phenotype.

Summary/Conclusion: This immunothrombolytic axis results in thrombus resolution. Together, with this immune landscape of thrombosis, we provide a valuable resource and introduce the concept of “immunothrombolysis” with broad mechanistic and translational implications at the crossroad of inflammation and thrombosis.

SFF02-OP03

DIRECT ORAL ANTICOAGULANT LEVELS AT TIME OF ELECTIVE SURGERY: THE DALI STUDY

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Background: Before elective surgery, direct oral anticoagulants (DOACs) are discontinued according to a standardized protocol, depending on the average DOAC half-life, kidney function and bleeding risk of surgery. However, this half-life varies between patients. This may lead to residually elevated DOAC levels and an increased bleeding risk during surgery.

Aims: To estimate the percentage of patients with preoperatively elevated DOAC levels. Secondary aims were to investigate determinants of DOAC levels, the associated surgical blood loss and the 30-days postoperative complications.

Methods: The DALI cohort included patients discontinuing DOACs (apixaban, dabigatran, rivaroxaban) for an elective procedure between 2019-2024 in two Dutch hospitals. All participants were asked for informed consent before inclusion and the study was approved by the Medical Ethical Committee of the Leiden University Medical Center. Blood was drawn just before surgery to determine DOAC levels by liquid chromatography-mass spectrometry. The primary outcome was the proportion of preoperative DOAC levels ≥30 ng/ml and its 95% confidence intervals (CI), stratified by DOAC type and bleeding risk of surgery. We identified determinants of DOAC levels through multivariable linear regression. Blood loss during the surgery and the 30-days postoperative complications - including major bleeding based on the International Society on Thrombosis and Haemostasis classification - were described, according to DOAC concentrations.

Results: The study was terminated after including 257 patients (100 apixaban, 100 rivaroxaban, 57 dabigatran) due to slow inclusion of dabigatran patients. Patients had a median age of 72 years (interquartile range [IQR] 66-78) and 173 (67%) were male. Two hundred twelve (82%) patients underwent a high bleeding risk surgery and 45 (17%) an intermediate bleeding risk surgery. Preprocedural DOAC levels were ≥30 ng/mL in 7.6% (95%CI 4.9-11.6) of patients. Dabigatran and rivaroxaban had similar proportions (3.6%, 95%CI 1.0-12.3% and 4.2, 95%CI 1.6-10.2, respectively), while higher proportions of apixaban patients had levels ≥30 ng/mL (13.1%, 95%CI 7.8-21.2). Moreover, this proportion was higher in intermediate bleeding risk surgeries (apixaban 42.9%, 95%CI 24.5-63.5, dabigatran 15.4%, 95%CI 4.3-42.2, and rivaroxaban 18.2%, 95%CI 5.1-47.7). Treatment with apixaban (9.0 ng/ml, 95%CI 4.7-13.3, compared with dabigatran and 7.0 ng/ml, 95%CI 3.2 to 10.6 compared with rivaroxaban), a lower glomerular filtration rate (0.1 ng/ml, 95%CI 0.01-0.2) and an interruption time <48h (15.9 ng/ml, 95%CI 3.6-28.3) were associated with increased preprocedural DOAC levels in the multivariable model. Median surgical blood loss was 0mL (IQR 0-100mL) which was not associated with DOAC levels. The postoperative period was complicated by an infection in 30 patients (11.6%, 95% CI 8.3 to 16.2), which all occurred in patients with DOAC levels < 30 ng/mL. An additional procedure was necessary in 21 (8.2%, 95% CI 5.4 to

12.2) patients, of whom 1 (5.3%, 95% CI 1.0 to 24.6) had DOAC level ≥ 30 ng/mL. Twelve patients, all with levels <30 ng/ml, experienced a major bleeding in the 30 days following surgery (4.7%, 95%CI 2.7-8.0).

Summary/Conclusion: The current protocol is generally effective in achieving DOAC levels <30ng/ml, although the proportion of subjects with elevated levels was high(er) for apixaban. Residually elevated DOAC levels were not associated with surgical blood loss.

SFF02-OP04

ADOPTION OF SPECIALIZED METHODS OF PLATELET ANALYSIS IN DIAGNOSTIC SCHEME OF INHERITED PLATELET DISORDERS

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Background: Inherited platelet disorders (IPD) are very rare diseases. Despite established recommended procedures, diagnostics is difficult and the exact diagnosis remains unknown in many cases. Platelet dysfunction is associated with various genetic causes and diverse clinical manifestations. Due to the clinical heterogeneity, testing of platelet structure and function should be broadened beyond standard screening assays. It is also important for correct patient selection, since the strategy for definitive diagnosis of IPDs is genetic testing, as is recommended by the international society on thrombosis and hemostasis (ISTH) guidelines.

Aims: To introduce a platelet function testing scheme that should contribute to the improved diagnosis of these conditions

Methods: In a group of 19 patients with suspected IPD, the primary screening methods (PFA, platelet count, morphology, light transmission aggregometry). VWD (von Willebrand disease) was excluded. Serotonin released from platelet dense granules after TRAP-6 activation was measured using high-performance liquid chromatography (HPLC) with fluorescence detection. Analysis of dense granules was screened by transmission electron microscopy (TEM) using the whole-mount method to distinguish defects in the secretion of dense granule content from the absence of the dense granules in platelets. Flow cytometry was used for determination of surface platelet receptors. NGS hemostasis-platelets panel with 42 platelet disease-causing genes was used. Finally, platelet RNA was isolated and transcribed cDNA was analyzed using PCR and Sanger sequencing.

Results: A causal pathogenic variant was found in 10 patients (52.6 %) in the genes *NBEAL2, GP1BB, GP1BA, HPS1, HPS4 and MYH9.* No definitive diagnosis was reached in 9 patients (47.4 %). Serotonin release assay was conducted in 18 patients, 6 of them had a pathological outcome (33.3 %). In 4 of the 6 patients with a pathological result, a pathogenic variant was subsequently confirmed (66.7 %). TEM was performed in 11 patients. In 3 cases, the result was pathological (27.3 %), in two of which a pathogenic variant was found (66.7 %). Flow cytometry was used in 12 patients. Reduced expression of at least one glycoprotein was demonstrated in 6 patients (50 %), and a pathogenic variant was found in 4 out of 6 patients with reduced expression (66.7 %).

Summary/Conclusion: Definitive diagnosis was reached in 52.6 % of suspected IPD patients. Newly introduced specialized platelet function tests have aided the diagnostic process and are suitable to be performed alongside genetic testing. These tests also contribute to a better characterization of the phenotype of the given platelet disorder in individual cases. The study confirmed that, due to heterogenous pathophysiology of IPDs, a broader range of examinations focused on secretion disorders (serotonin release assay), dense granule count (TEM), or surface glycoproteins (flow cytometry) is necessary. Therefore, expanded testing of platelet function aided the diagnostics of patients with suspected congenital thrombopathy.

Focus Symposium – Obesity and VTE

FS01-OP01

MBL levels are regulated by the ABO locus and influence ABO-related risk of venous thromboembolism - results from The HUNT Study

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Background: Non-O blood groups are well-established risk factors for venous thromboembolism (VTE) and elevated levels of von Willebrand factor and coagulation factor VIII in non-O blood groups partly explain this association. As a major proportion of the genetic risk for VTE is attributed to the *ABO* locus, it is important to reveal additional mechanisms that link the *ABO* locus with VTE risk. Mannose-binding lectin (MBL), a component of the lectin pathway of the complement system, in complex with MBL-associated serine proteases (MASPs) can activate thrombin and high MBL is associated with VTE risk. Recently, a variant genetically linked to the *O1* allele of the *ABO locus* (rs505922) was identified as a major determinant of lectin pathway activation.

Aims: To investigate whether common alleles of the *ABO* locus explained part of the plasma MBL variability, and if so, whether plasma MBL levels partly mediated the VTE risk in non-O blood groups.

Methods: The discovery study comprised a case-cohort derived from the population-based third survey of The Trøndelag Health Study (HUNT3) (n=50,800), in which plasma MBL at study inclusion was measured in 294 participants who developed VTE during 5 years of follow-up and in 1066 age- and sex-weighted subcohort participants using an aptamer-based method (SomaScan® 7K). Genotyping array data was used to distinguish between O (*O1* allele) and non-O ABO blood groups and further differentiate the five most common *ABO* haplotypes (*A1/A2/B/O1/O2*). Protein quantitative trait loci (pQTL) analysis was performed to assess genetic variants associated with MBL levels. Linear regression models were used to calculate the effect (R^2) of *ABO* haplotypes on standardized (standard deviation, SD) MBL levels in the subcohort. The mediating effect of MBL on the association between non-O ABO blood group and VTE risk was estimated using regression-based mediation analysis software (R-package CMAverse). The results from the discovery study were validated in a nested case-control study derived from the population-based Tromsø 4 study (n=27,158). The study was approved by the Regional committee for medical research ethics and informed written consent was obtained from all participants.

Results: The pQTL analysis of unrelated participants (n=1900) revealed that rs8176719, distinguishing between O and non-O ABO blood groups, was associated with MBL levels with genome-wide significance ($p=5.3\times10^{-9}$). By using rs8176719 to differentiate between non-O and O blood groups, the non-O blood group displayed 40% higher MBL levels than the O blood group. The *ABO* haplotype effects on MBL levels compared to the reference haplotype (*O1*) were largest for *A1* (0.34 SD, $p=4.910^{-10}$), and the five main *ABO* haplotypes explained approximately 4% of the variation in plasma MBL levels. The non-O blood group was associated with VTE risk (HR:1.76, 95%CI:1.37-2.36) and MBL was estimated to mediate 16.1% of the ABO-related increase in VTE risk in analyses adjusted for age, sex, C-reactive protein and renal function. These findings were replicated in the Tromsø study.

Summary/Conclusion: We found that plasma MBL is genetically regulated by the *ABO locus* and that MBL partly explained the association between ABO blood groups and VTE risk.

FS01-OP02

THE RELATIONSHIP BETWEEN (ANIMAL AND PLANT-BASED) PROTEIN INTAKE WITH THE RISK OF VENOUS THROMBOEMBOLISM; THE MAASTRICHT STUDY

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Background: Venous thromboembolism (VTE) is a major public health issue, affecting more than 10 million people globally, with higher incidence rates in Europe and United States and lower in Africa and Asia. Recent studies have shown a strong association between VTE and factors such as age, sex, cancer, type 2 diabetes, obesity and smoking. While dietary patterns and macronutrients have been recognized for their important role in cardiovascular diseases, the role of protein intake as well as the source (animal or plant-based) in relation to VTE is still poorly understood.

Aims: The current study aims to investigate the association between total protein intake and VTE risk including protein source (animal vs. plant), separately for DVT and PE.

Methods: Data from 9187 participants (aged 40-75 years, median age: 63 years) were used from the Maastricht Study, a population-based observational prospective cohort study. Associations were made using patient data from the start of the study, including all cases of VTE with at least one confirmed diagnosis during the 10 years follow up. Protein and energy intakes were assessed and estimated using a Food Frequency Questionnaire (FFQ) and the 2011 Dutch Food Composition Database. Protein intake was divided into quintiles with the lowest group (≤0,8 g/kg/d) as reference. Protein source was categorized based on dominant protein intake: animal-based (reference group) versus plant-based. Logistic regression models estimated odds ratios (ORs) and 95% confidence Intervals (CIs) for the associations between protein intake, source and risk of DVT, PE and people with both DVT and PE. Adjustments were made for age and sex (Model 2), for comorbidities (Model 3) and for lifestyle factors (Model 4).

Results: Of the 9187 participants, 118 developed DVT, 103 developed PE and 40 developed both conditions. Moderate to high protein intake (Q4:1.14-1.35g/kg/d) was associated with lower risk of DVT in unadjusted analysis (Model 1: OR: 0.425, 95%CI 0.226-0.798, p=0.008) and after adjustments for age and sex (OR: 0.488, 95% CI 0.259, 0,919, p=0.026), compared to a low protein intake (Q1;£ 0.8 g/kg/d). No significant associations were observed between protein intake and isolated PE or DVT with PE. Plant-based protein sources were significantly associated with a lower odds of DVT in comparison with animal based proteins in Model 1: OR: 0.536, 95%CI 0.305 -0.974, p=0,030; and Model 2: OR: 0.554, 95%CI; 0.315, 0.974, p=0.040), but the results attenuated after full adjustments.

Summary/Conclusion: Moderate total protein intake and plant-based protein were associated with a lower risk of DVT, compared to low protein intake. These findings support further investigation into the role of dietary protein intake and the risk of venous thromboembolism.

FS01-OP03

PLASMA BIOMARKERS OF ABDOMINAL OBESITY-RELATED VENOUS THROMBOEMBOLISM

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Background: Obesity is a well-established risk factor for venous thromboembolism (VTE), and it is estimated that 25% of the VTE incidence in the population is attributed to overweight or obesity. Waist circumference (WC), a measure of abdominal obesity, has been shown to be superior to body mass index (BMI) to predict future VTE by identifying more subjects at risk and displaying higher risk estimates for VTE. To date, no study has explored the association between the plasma proteome and WC, and to what extent such specific biomarkers could mediate WC-related VTE risk.

Aims: To identify plasma biomarkers linearly associated with WC and to determine to what extent these candidate proteins affect the VTE risk associated with abdominal obesity.

Methods: We conducted a case-cohort study derived from the third survey of the Trøndelag Health Study (HUNT3, n=50,800), including 908 objectively confirmed incident VTE events that occurred from inclusion (2006–2008) until the end of follow-up (December 31, 2019) and a randomly selected, age-weighted subcohort (n=2410). Plasma samples collected at inclusion in HUNT3 were analyzed using an aptamer-based proteomic platform (Somascan®). Protein levels were log2-transformed and standardized. WHO-criteria were used to determine abdominal obesity (WC≥102cm in men and WC≥88 cm in women) and normal weight (WC< 94 cm in men and WC<80 cm in women). Linear regression analyses for the association between WC in cm and plasma proteins level in the subcohort adjusted for age and sample batch were conducted in men and women separately. Cox regression models were applied to estimate hazard ratios (HRs) with 95% confidence intervals (CI) for VTE by WC categories in models adjusted for age and proteins related to WC with a false discovery rate (FDR) < 10%. The study was approved by the regional committee for medical research ethics and informed written consent was obtained from all participants

Results: We identified 2142 proteins in women and 1574 proteins in men, of which 1178 proteins were common for both men and women, which were correlated with WC with FDR<10%. Abdominal obesity was associated with a 1.43-fold (HR: 1.43, 95% CI: 1.13 - 1.81) and 1.68-fold (HR: 1.68, 95% CI: 1.22 - 2.3) increased VTE risk in men and women, respectively. Among the proteins positively correlated with WC in both men and women, individual adjustment for Collagen alpha-3(VI):BPTI/Kunitz inhibitor, Coagulation Factor IX, and ADAMTS-like protein 2 had the strongest attenuating impact on VTE risk. HRs for VTE by abdominal obesity adjusted for these proteins were 1.22 (95% CI: 0.94-1.57) in men and 1.34 (95% CI: 0.97-1.87) in women.

Summary/Conclusion: We identified specific proteins, such as Collagen alpha-3(VI):BPTI/Kunitz inhibitor, Coagulation Factor IX, and ADAMTS-like protein 2 involved in the pathogenesis of VTE, that might partially explain the link between abdominal obesity and increased VTE risk. Our findings might provide novel

insights into the mechanisms of obesity-related VTE and reveal potential targets for prevention of obesity-related VTE.

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FS01-OP04

BEYOND BMI: EXPLORING THE ASSOCIATION BETWEEN ALTERATIONS IN BODY FAT DISTRIBUTION AND SERUM PROTEIN LEVELS

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Background: Obesity, defined as body mass index (BMI) ≥30kg/m^2, is a major modifiable risk factor for venous thromboembolism (VTE). However, BMI does not reflect body fat distribution, and the effect of reducing body fat and specific fat tissues on the risk of VTE is unknown.

Aims: To investigate changes after 12 months in BMI, visceral fat (VAT), abdominal subcutaneous fat (aSAT), and liver fat resulting from a dietary intervention and their associations with serum levels of 159-proteins in participants of the Fasting In diabetes Treatment (FIT) trial.

Methods: 100 participants with type 2 diabetes (T2D), not using insulin, were randomized to receive either a fasting-mimicking diet program in addition to usual care, or usual care alone. At baseline and after 12 months, MRI measured VAT, aSAT, and liver fat, and serum samples were collected to quantify 159-proteins using a multiplexed targeted mass-spectrometry assay. These proteins represent various physiological pathways, including coagulation, the complement system, and lipid metabolism; some have been linked to VTE risk. Associations between 12-month protein levels and BMI or fat depots were assessed using linear regression, adjusting for baseline protein levels and baseline BMI or fat depot (model 1). Model 2 included adjusted for treatment group, and model 3 (VAT, aSAT, and liver fat only) additionally adjusted for other types of body fat to determine the dominant exposure. Results are expressed as beta coefficients per standard deviation (SD) of BMI or fat depot, with Benjamini-Hochberg adjusted p-values. Analyses were repeated stratified by sex. Ethical approval and informed consent were obtained.

Results: Among 77 patients who completed follow-up (median age 65 years [IQR 57–70]; 50.6% women), a reduction of 1 SD in BMI (4.6 kg/m²) was associated with significantly lower serum levels of 35 proteins and higher levels of one protein in model 1; in model 2, 26 proteins remained decreased and one increased. A 1 SD reduction in VAT (88.6 cm²) was associated with 35 decreased and four increased proteins; in model 3, two remained decreased and one increased. For aSAT, a 1 SD reduction (114.2 cm²) was linked to 11 decreased and one increased protein, with no significant associations in model 3. Per 1 SD reduction in liver fat (8.6%), 55 proteins decreased and three increased; results were similar in model 3 (52 decreased, two increased). Many proteins overlapped across BMI and fat depot analyses. In sex-stratified analyses, associations persisted in men but not women. In women, changes in BMI, VAT, and aSAT were not associated with protein changes, and only a few associations with liver fat were observed in model 1.

Summary/Conclusion: In patients with T2D, reductions in BMI and all fat depots were associated with lower levels of VTE-related proteins, including complement factors, coagulation factors, and alpha-2 antiplasmin, suggesting reduced thrombogenicity. For aSAT and VAT, these effects were largely explained by liver fat. These findings, predominantly observed in men, suggest that liver fat plays an important role in the causal pathway linking weight loss to reduced VTE risk, highlighting the importance of sex and fat distribution.

Focus Symposium – Vascular Remodelling

FS02-OP01

THE IMPACT OF HYPOXIC PROFILES AND INFLAMMATION ON PRIMARY MONOCYTE RESPONSES

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Background: Carotid artery stenosis is caused by a burden of atherosclerotic plaque on the vessel wall. Atheroma plaques are classified as stable or unstable (vulnerable), being vulnerable plaques more prone to rupture and causing subsequently neurological symptoms. Circulating cell-free DNA (cfDNA) is released into bloodstream as a result of cell death or active secretion. cfDNA integrity has been previously related to several pathologies, including atherosclerosis, however its relationship with plaque vulnerability has been scarcely studied.

Aims: In this study, we analyzed cfDNA fragments and cfDNA integrity as potential markers of carotid plaque vulnerability associated with ischemic neurological symptoms.

Methods: cfDNA was isolated with QIAamp® Viral RNA Mini Kit (Qiagen) from 240 µl of plasma from patients with recent symptomatic carotid stenosis (stroke, transient ischemic attack, *amaurosis fugax* < 3 weeks of evolution, N=35) and asymptomatic patients with severe carotid atherosclerosis (control group, N=66). A short (≤115 base pairs, bp) and a long (≥247 bp) DNA fragment from two repetitive genome sequences, Alu (Alu-115 bp; Alu-247 bp) and Line1 (Line1-97 bp; Line1-266 bp), as well as a gene related to atherosclerosis, HTRA1 (HTRA1-90 bp; HTRA1-250 bp), were quantified. Quantitative PCR reactions were carried out with 1 µl (Alu and Line1) and 4 µl (HTRA1) of isolated DNA, and were performed in a Lightcycler® 480 (Roche) with SYBR ® green (Roche). CfDNA integrity was calculated as the ratio between the long and short fragments of each gene. The association between each fragment studied and cfDNA integrity, and the presence of neurological symptoms was analyzed using non-parametric tests and ROC curves with GraphPad Prism 8.0.0.

Results: We observed an increase in the concentration of the following fragments in plasma of symptomatic patients: Alu-115 bp (P=0.0003), Alu-247 bp (P=0.0421), Line1-97 bp (P=0.0109), and HTRA1-90 bp (P=0.0053). Additionally, symptomatic patients showed a lower integrity of Alu (P=0.006) and Line1 (P=0.0016). The Alu-115bp fragment showed the highest discriminatory ability between groups (AUC=0.718; 95% CI (0.613-0.823); P=0.0003).

Summary/Conclusion: Our results suggest that the concentration in plasma of several cfDNA fragments, particularly Alu-115bp, and the integrity of Alu and Line1 cfDNA may serve as novel biomarkers for assessing carotid plaque vulnerability and the ischemic risk. These markers could contribute to the monitoring of patients with carotid stenosis.

FS02-OP02

THE NEEDLE IN A HAYSTACK; IDENTIFYING THORACIC AORTIC ANEURYSM (TAA) PATIENTS AT RISK OF TYPE-A AORTIC DISSECTION (TAAD)

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Background: Current guidelines for ascending thoracic aortic aneurysm (TAA) recommend elective surgical repair at a diameter of 52mm or a growth rate exceeding 3mm/year to prevent type-A aortic dissection (TAAD) *(Czerny et al., 2024)*. This approach, based on aortic diameter as a primary risk predictor, aims to reduce TAAD incidence. However, a growing body of evidence reveals that a significant number of TAAD patients present with aortic diameters below this surgical threshold *(Perez et al., 2023)*. Conversely, annual dissection rates are approximately 5% in patients with diameters above 50mm *(Solomon et al., 2024)*. Consequently, a strategy of prophylactic surgery for all patients exceeding 52mm requires open-chest intervention with cardiac arrest for a large cohort to prevent TAAD in a smaller subset (5%), while failing to address the risk in patients with smaller aneurysms *(Evangelista et al., 2016)*. This highlights the urgent need for a more targeted approach to identify individuals truly at high risk of TAAD, moving beyond diameter-centric assessment.

Aims: This study aimed to identify the molecular mechanisms driving aortic wall remodelling in patients at high risk of TAAD and to pinpoint potential druggable targets.

Methods: Aortic tissue samples from a 250+ patient biobank were histologically characterized to determine the extent and nature of aortic wall remodelling. We identified patients exhibiting hypotropic (thinning) and hypertrophic (thickening) tunica media, as well as patients with Marfan syndrome, those with TAAD, and non-dilated controls. In-depth proteomic and metabolomic (kinase activity) profiling was performed on these tissues. Patient clusters at risk of TAAD were identified using Partial Least Squares (PLS) analysis. Potential therapeutic targets identified through these analyses were subsequently validated in vitro using iPSC-derived Vascular Smooth Muscle Cells (iVSMCs).

Results: Histological evaluation revealed a heterogeneous pattern of collagen, elastin, and cellularity in the aortic tissue of TAA(D) patients. Some patients displayed active remodelling characterized by increased cell number and wall thickening (hypertrophic), while others exhibited significant degeneration and thinning (hypotropic). Proteomic analysis and subsequent PLS analysis demonstrated distinct molecular signatures associated with these remodelling patterns. Notably, hypertrophic and control tissues clustered together, whereas hypotropic and Marfan/TAAD tissues formed a separate cluster, indicating a high-risk proteomic profile for patients with hypotropic aortic walls. Further kinome profiling revealed aberrant kinase activity in pathways governing VSMC contractility and calcium handling in hypotropic aortas compared to hypertrophic aortas. NUAK1 was identified as a potential therapeutic target, with metformin suggested as a possible modulator of aortic remodelling.

Summary/Conclusion: Our findings demonstrate that distinct patterns of aortic wall remodelling are associated with different molecular profiles and potentially varying risks of TAAD. Specifically, a hypotropic remodelling pattern correlates with a high-risk proteomic signature and aberrant kinase activity in VSMC-

related pathways. The identification of NUAK1 as a potential therapeutic target, with metformin as a possible effector, offers a promising avenue for developing targeted interventions to mitigate TAAD risk in susceptible individuals, moving beyond the limitations of current diameter-based guidelines.

FS02-OP03

PI3KC2B: AN INNOVATIVE THERAPEUTIC TARGET TO PREVENT ENDOTHELIAL DYSFUNCTION

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Background: The vascular endothelium plays a central role in maintaining vascular homeostasis through its anti-aggregant, anti-coagulant, and anti-adhesive properties. Disruption of these functions leads to endothelial dysfunction, a key factor in the onset and progression of complex thrombo-inflammatory diseases such as atherosclerosis, sepsis, and ischemic stroke. The class II phosphoinositide 3-kinase beta (PI3KC2β) has emerged as a regulator of intracellular trafficking. We have recently shown that its inactivation reduces vascular permeability and inflammation in the context of ischemic stroke. This protective effect is associated with the disruption of endosomal trafficking, which promotes the recycling of VE-cadherin at the expense of its degradation, thereby reinforcing endothelial junction stability. These findings identify endothelial PI3KC2β as a promising therapeutic target for preserving vascular integrity. However, the molecular mechanisms underlying its role in endothelial cells (ECs) remain poorly understood.

Aims: This study aimed to elucidate the role of PI3KC2β in ECs within a thrombo-inflammatory context by: *(i)* characterizing its impact and mechanism of action in EC activation by pro-inflammatory cytokines; *(ii)* evaluating its effect on endothelial dysfunction *in vivo*; and *(iii)* assessing the generalizability of its protective role across various vascular beds and pathological conditions.

Methods: To assess EC activation, we used human brain microvascular ECs (hCMEC/D3) and human umbilical vein ECs (HUVECs) with PI3KC2β knockdown. Western blotting (WB) and RT-qPCR were used to analyze the activation of key signaling proteins and gene expression, respectively. The role of endosomal trafficking in cytokine-induced EC activation was investigated using pharmacological inhibitors, IF, and WB analyses. In parallel, PI3KC2β knock-in mice (catalytic inactive form) were used in two *in vivo* models of inflammation: a sterile model of endothelial dysfunction (cremaster) and a polymicrobial sepsis model (cecal slurry). Intravital microscopy and various quantitative approaches were employed to evaluate the impact of PI3KC2β on endothelial activation.

Results: PI3KC2β knockdown in ECs significantly altered signaling pathways (ERK1/2, Src, and Akt) triggered by inflammatory cytokines (TNFα, IL-6, IL-1β), while leaving responses to growth factors unaffected. These signaling changes were associated with decreased NF-κB activation and a reduction in the expression of adhesion molecules (VCAM-1, ICAM-1) and pro-inflammatory cytokines (IL-1β, IL-6). *In vivo*, using a murine model of TNFα-induced endothelial dysfunction, PI3KC2β inactivation markedly reduced neutrophil adhesion to the activated endothelium, underscoring its role in maintaining the anti-inflammatory properties of the vascular endothelium. Furthermore, PI3KC2β inactivation attenuated the progression of polymicrobial sepsis, suggesting that its protective effects extend beyond the cerebral vasculature.

Summary/Conclusion: PI3KC2β is emerging as a key regulator of endothelial inflammation through its modulation of endosomal trafficking and cytokine signaling pathways. Its inactivation appears to provide significant protection against endothelial dysfunction and systemic inflammation across several pathological

models. These findings underscore the therapeutic potential of targeting PI3KC2β to preserve vascular integrity in thrombo-inflammatory conditions such as stroke and sepsis.

FS02-OP04

FROM CLOTTING TO CALCIFICATION: GLA-DOMAINS AS GUARDIANS OF VASCULAR REMODELING

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Background: Vitamin K dependent (VKD) proteins, also known as Gla-proteins, play critical roles in coagulation, bone and matrix mineralization, and cell proliferation. These proteins undergo γ-carboxylation of specific glutamic acid residues to form Gla residues, which endow them with calcium-binding properties essential to their function. Despite differences in physiological roles, VKD proteins from both coagulation and mineralization subfamilies contribute to preventing vascular calcification, a process whose underlying mechanisms remain poorly understood. Notably, Gla residue spacing differs between subfamilies, potentially influencing calcium binding and protein folding. Crystal structures suggest cooperative calcium binding, involving multiple Gla residues per Ca^{2+} ion.

Aims: To elucidate how VKD proteins regulate vascular calcification and to define the biophysical properties of Gla residues—specifically calcium-binding affinity and pKa—in different structural contexts.

Methods: Three VKD proteins—matrix Gla protein (MGP), osteocalcin, and the Gla-domain of protein S (PSGla)—were chemically synthesized in both uncarboxylated and carboxylated forms. Their ability to inhibit in vitro calcium phosphate precipitation and calcification of human vascular smooth muscle cells (hVSMCs) cultured under calcifying conditions was assessed. Protein structures were analyzed via circular dichroism and NMR. Additionally, bulk RNA sequencing was performed on treated hVSMCs. To study individual Gla residues, model peptides with either random coil or defined secondary structure were synthesized. Residue-specific pKa values and calcium-binding affinities were determined using pH titration and Ca^{2+} titration ^{13}C NMR studies.

Results: Carboxylation influenced VKD protein secondary structure and was essential for inhibiting both in vitro calcium phosphate precipitation and hVSMC calcification. While all carboxylated proteins inhibited crystal formation (osteocalcin > PSGla > MGP), only MGP and PSGla fully prevented hVSMC calcification. RNA sequencing revealed distinct gene expression profiles in cells treated with these proteins, suggesting different modes of action. Structural studies of model peptides showed that Gla residues have two distinct pKa values affected by calcium, and that two spatially arranged Gla residues bind calcium cooperatively, significantly enhancing affinity. Calcium binding also induced increased α-helical content in peptides.

Summary/Conclusion: VKD proteins from both coagulation and mineralization pathways inhibit vascular calcification, although through distinct molecular mechanisms. Their inhibitory function is closely tied to Gla residue carboxylation, cooperative calcium binding, and associated structural changes. Understanding these differences offers new insights into how calcium-binding properties contribute to the physiological functions of VKD proteins.

Focus Symposium – Bleeding

FS03-OP01

UNBIASED ASSESSMENT OF VWD BURDEN AND HEALTHCARE CONSUMPTION USING PSEUDONYMIZED BIG DATA

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Background: The French National Health Data System (SNDS) comprises healthcare data that cover 99% of the population (over 67 million individuals) in France allowing exhaustive analysis of healthcare consumption.

Patients with von Willebrand disease (VWD) can experience bleeding symptoms with varying degrees of severity and from different bleeding sites. Some studies suggest that VWD patients experience a higher use of healthcare resources. However, there are no high-quality datasets combining complete phenotypic and genotypic VWD characterization with exhaustive and unbiased healthcare data.

Aims: Compare healthcare consumption in VWD patients and a matched control (MC) population without bleeding disorder.

Methods: The French VWD database (CRMW) was paired to the SNDS. For each VWD patient, 4 healthy matched controls (MC) were matched on sex, age at inclusion and geographical area. The incidence of hospitalisation and consultation during the follow-up period was estimated and compared between VWD cases and their MC using a generalized estimating equations (GEE) based, negative binomial model. This model takes into account for matched sets using a compound symmetry covariance structure, the follow-up period using an offset variable (after logarithmic transformation). First analysis covered the overall VWD group and further analyses were stratified on the VWD subtypes. Here we present data on the top 2 causes of inpatient/outpatient healthcare use.

Results: In the CRMW 4636 VWD patients were eligible to the study. Among them 3791 were successfully matched to the SNDS and 2791 were fully characterized. Patients without a unique SNDS number such as twins were excluded from the study. A total of 2779 VWD patients were included in the study after successful match to 4 controls each. The majority were type 2 VWD patients (N=1804, 65%), followed by type 1 (n=850, 31%) and lastly type 3 (n=98, 4%).
Less than half (1367) of VWD patients had the International Classification of Diseases (ICD) code for VWD (D68.0). This proportion was identical for all VWD types and in particular severe VWD forms such as type 3 were not better identified.

Preliminary results show a significantly higher annualized incidence rate ratio(RR) of hospitalization for gastro-intestinal (RR=3.66 [2.43 ; 5.51]) and orthopedic issues (RR=2.96 [1.75 ; 5.04]) not only in VWD type 3 but also in type 1 (RR=1.49 [1.22 ; 1.81] and RR=1.25 [0.99 ; 1.57]) and type 2 (RR=1.69 [1.51 ; 1.89] and RR=1.62 [1.43 ; 1.82]) compared to MC.

Surprisingly we found no overall increase in consults with a general practioner for VWD patients compared to MC (1.03 [0.99 ; 1.07]). Moreover, referral to psychiatrists was significantly lower when comparing overall VWD patients to MC (0.75 [0.59 ; 0.96]).

Reimbursements of outpatient care, outside of public or private hospital structures, showed no significant difference between VWD patients and their MC.

However pharmaceutical costs linked to retail pharmacies were significantly higher for VWD patients compared to their MC.

Summary/Conclusion: This is the first study on healthcare consumption matched with a nationwide VWD registry. Previous VWD healthcare studies were based on International Classification of Diseases (ICD) codes which lack precision, VWD subtyping and have reporting bias. Further analysis will evaluate healthcare consumption, bleeding phenotype according to the annualized RR of bleeding based on ICD for each VWD subtype. We will also compare impact of VWD diagnosis on these 2 outcomes.

FS03-OP02

PROTEASE NEXIN-1 (PN-1), AN ANTIPROTEASE BELONGING TO THE SERPIN FAMILY, IN HEMOPHILIC ARTHROPATHY

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Background: Prophylactic treatment of hemophilic patients with factor replacements decreases joint bleeding events, but does not prevent them entirely. Undetectable microbleeds can therefore occur in the joints making the synovial tissue and cartilage targets of proteases known to be involved in the development of hemophilic arthropathy. The balance between proteases and antiproteases plays an important role in maintaining joint homeostasis. Among these antiproteases, a serpin (serine protease inhibitor) known as protease nexin-1 (PN-1) has emerged as an important player in the regulation of proteolytic degradation at tissue level. It is produced by various cell types, notably chondrocytes and in joint synovium. Several studies carried out on similar pathologies such as osteoarthritis, rheumatoid arthritis and ex vivo models of cartilage destruction have highlighted the potential protective role of PN-1 in these pathologies.

Aims: We therefore hypothesize that PN-1 present in joint tissue may play a role in hemophilic arthropathy

Methods: To test this hypothesis, we studied the impact of PN-1 in an in vitro model of hemarthrosis, comparing the phenotype of isolated murine chondrocytes, from wild-type (WT) or PN-1-deficient (PN-1-/-) mice and exposed to blood for 48h. We also compared the joint phenotype of F8-/-/PN-1+/+ mice and double KO F8-/-/PN-1-/- mice, using an in vivo model of hemarthrosis.

Results: Our in vitro results obtained from WT chondrocyte transcripts show a loss of chondrocyte-specific markers (collagen II and aggrecan), while PN1 expression remains stable after 48 h exposure to blood. Furthermore, our results obtained in vivo with the hemarthrosis model show, at a late stage (D14 repair stage), a higher visual blood score in the F8-/-/PN+/+ mice than in the F8-/-/PN-1-/- mice, suggesting that PN-1 is involved in the tissue repair phase. However, there are no differences in knee size between the two groups.

Summary/Conclusion: Our results suggest that PN-1 expressed in joint tissue may play a protective role in hemophilic arthropathy.

FS03-OP03

SELECTIVE TSP-1/CD47 ANTAGONISM: A NOVEL ANTITHROMBOTIC STRATEGY WITH PRESERVED HAEMOSTASIS

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Background: Cardiovascular diseases remain the leading cause of death worldwide with stroke and myocardial infarctions being the most frequent. Platelets play a critical role in primary haemostasis and arterial thrombosis. Current antiplatelet agents, although effective, are limited by an elevated bleeding risk, highlighting the need for safer therapeutic strategies. Thrombospondin-1 (TSP-1), a matricellular glycoprotein released from alpha-granules during platelet activation, promotes thrombosis via CD47-dependent inhibition of the nitric oxide (NO)/cGMP/PKG signalling pathway. Selective disruption of the TSP-1/CD47 axis therefore represents a promising therapeutic opportunity. In this context, we developed TAX2, a cyclic dodecapeptide acting as an orthosteric antagonist of TSP-1/CD47 binding. Previous studies demonstrated the antithrombotic properties of TAX2 *in vitro*, inhibiting platelet aggregation and adhesion, as well as *in vivo*, reducing carotid artery thrombosis without prolonging bleeding time.

Aims: The current study aims to elucidate the mechanism of action of TAX2 in the modulation of platelet function and the prevention of thrombosis.

Methods: Human platelets from healthy donors were collected and isolated by centrifugation, then subjected to agonist-induced activation. Platelet aggregation, intracellular signalling (SDS-PAGE and western blot), production of secondary mediators (luminescent and colorimetric assays), surface activation markers (flow cytometry) were assessed under TAX2 treatment.

Results: Disruption of the TSP-1/CD47 interaction by TAX2 significantly reduced platelet aggregation in response to collagen (1-6 µg/mL) or TRAP-6 (5-30 µM) by 58.3 ± 7.0% and 36.1 ± 2.6%, respectively, compared to a control peptide. Granule secretion was only moderately affected. Interestingly, the combination of TAX2 with 10 nM of tirofiban, an antagonist of the $\alpha_{IIb}\beta_3$ integrin, achieves an inhibition comparable to that obtained with 25 or 50 nM of tirofiban alone. TAX2 also increases intracellular cGMP levels (by 2.5-fold), independently of CD36, as assessed using a CD36 blocking antibody (FA6-152). Signalling pathway analyses reveal that TAX2 markedly reduced phosphorylation of key signalling proteins upon platelet activation, including LAT (- 48.9 ± 8.7%), PI3K (- 54.9 ± 5.42%), AKT (- 67.3 ± 6.8%) and SRC (- 46.6 ± 7.4%). This led to reduced $\alpha_{IIb}\beta_3$ integrin activation and decreased fibrinogen binding to platelets. Moreover, TAX2 also diminished the platelet-dependent thrombin generation by 50%.

Summary/Conclusion: These findings support the therapeutic potential of TAX2 as an antithrombotic drug and document its mode of action. Its favourable safety profile, along with potential combination with existing antiplatelet drugs, paves the way for novel strategies in thrombosis management with reduced bleeding risk.

FS03-OP04

PHARMACOKINETICS IN THE TREATMENT OF HAEMOPHILIA – CHANGES IN PHARMACOKINETIC PARAMETERS DURING CHILDHOOD

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Background: In recent years, significant advances have been made in the application of pharmacokinetics to determine and adjust factor concentrate dosing in patients with haemophilia, thereby ensuring optimal protection against bleeding in various clinical situations. Existing evidence suggests that pharmacokinetic parameters may undergo changes in certain patient groups, particularly in children as they grow.

Aims: Our intention is to examine changes in pharmacokinetic parameters in patients with severe haemophilia A throughout childhood, and to describe these changes (trends, magnitude) at a statistically significant level.

Methods: Pharmacokinetic studies were conducted as part of standard care in a total of 38 paediatric patients with severe haemophilia A treated at University Hospital Brno and University Hospital Motol, Prague. The results were analysed using the WAPPS-Hemo pharmacokinetic model, and subsequently processed statistically. Obtained data were analysed separately for individual patients and for concentrates with both standard and extended half-lives.

Results: The results obtained show that throughout childhood, a gradual and statistically significant prolongation of the biological half-life of the administered factor VIII concentrates occurs in the observed group of patients. The average increase of half-life in our sample is by 3.9% per year ($p < 0.001$). Additionally, a prolongation of the average elimination half-life was observed in the individual drug groups. The average annual increase in half-life for EHL products was 3.5%, while for SHL products, it was slightly higher at 4.4%, Furthermore, a significant increase in the time to reach 5% factor activity was observed with age, with an average increase of 4.1% per year ($p = 0.001$).

Summary/Conclusion: We demonstrate that throughout childhood, a significant prolongation of the elimination half-life of factor VIII and other pharmacokinetic parameters occurs in paediatric patients with severe haemophilia A. The obtained data support the need for repeated pharmacokinetic assessments during childhood, which should be reflected in relevant guidelines.

Focus Symposium - Platelets

FS04-OP01

THE INFECTIOUS ENDOCARDITIS-CAUSING BACTERIUM STREPTOCOCCUS ORALIS TRIGGERS PLATELET AGGREGATION VIA THE SIALIC ACID-BINDING ADHESIN FAP1, HUMAN ANTIBODIES, AND THE PLATELET RECEPTOR GPIIB/IIIA

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Background: Infective endocarditis is a devastating infection of the heart's endocardial surface, carrying a one-year mortality rate nearing 40% and causing substantial long-term morbidity. Among the most frequent culprits are oral streptococci, particularly *Streptococcus oralis*, which can translocate into the bloodstream following dental procedures, trauma, or poor oral hygiene. Once in circulation, *S. oralis* exhibits a remarkable ability to adhere to damaged or prosthetic heart valves. This initial adhesion triggers the formation of vegetations consisting of tightly packed platelet aggregates, fibrin, and bacteria that effectively shield the pathogen from both immune clearance and antibiotic therapy. These protective niches enable persistent infection and complicate treatment, underscoring the urgent need to unravel the molecular mechanisms driving early platelet-bacterial interactions in infective endocarditis.

Aims: To dissect the molecular mechanisms underlying *S. oralis*-induced platelet aggregation.

Methods: Platelet aggregation was assessed using light transmission aggregometry in platelet rich plasma (PRP) and washed platelets from healthy donors in response to *S. oralis* strain ATCC 10557. The role of Fap1 was tested using a Fap1 mutants. Receptor blockade and reconstitution assays with pooled human IgG were used to investigate the involvement of GPIb, FcγRIIa, and plasma components.

Results: Wild-type *S. oralis* triggered platelet aggregation in an all-or-nothing manner in PRP via a GPIIb/IIIa-dependent pathway. Aggregation onset occurred after a 2.9±1.0 minute lag phase, significantly longer than the 0.1±0.09 minute lag observed with positive control TRAP-6. Despite being independent of the cyclooxygenase pathway, *S. oralis* induced dense granule secretion at levels markedly higher than those elicited by TRAP-6. The *S. oralis* adhesin Fap1 was essential for this response. Deletion of Fap1 abolished platelet aggregation, as did a single amino acid substitution (R489E) within the known sialic acid binding site, highlighting the critical role of Fap1-mediated interaction with a sialylated platelet receptor. Given that GPIbα is heavily sialylated, we demonstrated that blocking GPIbα with a site-specific antibody eliminated both platelet aggregation and dense granule secretion. Further studies confirmed that *S. oralis* directly bound glycocalicin, the extracellular domain of GPIbα, and that this binding was inhibited by the GPIbα-blocking antibody. While Fap1–GPIbα engagement was clearly required, it alone was not sufficient to trigger aggregation in washed platelets. This suggested an important role for an additional plasma-derived cofactor. Inhibitor studies in PRP implicated FcγRIIA in the aggregation response suggestive of antibody involvement. Reconstitution of washed platelets with pooled human IgG (hIgG) fully restored aggregation, identifying IgG as a necessary plasma factor. Importantly, hIgG failed to rescue aggregation in

Fap1-deficient or R489E mutant strains, demonstrating that both Fap1–GPIbα binding and IgG–FcγRIIA engagement are essential for *S. oralis*-induced platelet activation.

Summary/Conclusion: We propose that *S. oralis*-induced platelet aggregation requires sequential Fap1–GPIbα binding, IgG–FcγRIIa engagement, and GPIIb/IIIa signaling, with disruption of any step blocking aggregation. These findings reveal key mechanisms in platelet–bacteria interactions and suggest targets for early intervention in infective endocarditis.

FS04-OP02

RECOMBINANT FACTOR VIII BINDING TO PLATELETS INFLUENCES FIBRIN CLOT FORMATION AND STABILITY

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Background: Platelet activity plays a central role in clot formation, with distinct phenotypes having complementary functions. Pro-aggregatory platelets stabilize the fibrin network through adhesion, contraction and fibronectin fiber assembly, while sustained platelet activation transforms them into pro-coagulant platelets, which drives thrombin generation and fibrin polymerisation by binding factor VIII (FVIII) to their phosphatidylserine (PS)-exposing surface. Haemophilia A (HA) is a rare genetic bleeding disorder caused by a deficiency of functional FVIII. One treatment option is the prophylactic administration of recombinant FVIII (rFVIII). While a lot is known about the traditional role of FVIII in the coagulation cascade, there is a lack of further understanding FVIII's additional functions, specifically the impact of its interactions with platelets and how this might regulate specific outside-in signalling that contributes to fibrin network formation and stability.

Aims: To examine the impact of rFVIII-platelet interactions on platelet function and clot stability.

Methods: Isolated, activated platelets from healthy donors and people with severe HA were incubated with rFVIIIa. Flow cytometry was used to assess rFVIIIa-platelet interactions over time and how it impacts the phenotype shift, measured by PS exposure or Ca^{2+} levels in the presence or absence of thrombin. The role of integrins was assessed in activated platelets using the integrin αIIbβ3 inhibitor antibody 10E5. Integrin αIIbβ3 signalling was inhibited by 10E5 antibody to assess potential downstream signalling effects of rFVIIIa-platelet binding. A real-time imaging using lattice light sheet microscopy was developed to visualise the role of platelet phenotypes and their spatial positioning within a freshly forming blood clot to decipher their distinct roles in clot formation. Dynamic fibrin network formation and rFVIIIa-platelet phenotype co-localisation was assessed. 3D fibrin clots were imaged with and without inhibition of platelet contractility or platelet-mediated thrombin generation.

Results: rFVIIIa-platelet binding to platelets increased PS exposure over time in both healthy donors and HA samples. Early FVIIIa binding triggered thrombin-independent outside-in signalling cascade, upregulating intracellular Ca2+ signalling which led to an increased pro-coagulant potential (PS exposure) in both healthy and HA samples. Clusters of αIIbβ3 integrin were co-localised with rFVIIIa. Inhibition of αIIbβ3-integrin decreased the binding of rFVIIIa to the pro-aggregatory phenotype. Microstructural analysis showed enhanced fibrin density in proximity to pro-aggregatory platelets compared to the overall clot fibrin density. The addition of rFVIIIa to clots of HA platelets increased the density of fibrin co-localisation with platelets. Inhibition of the platelet phenotype shift and contractility were associated with altered fibrin networks.

Summary/Conclusion: FVIIIa-platelet interactions upregulate haemostasis by increasing the procoagulant platelet phenotype, as indicated by PS exposure, and is mediated by integrin αIIbβ3 signalling. Our preliminary data suggest that fibrin clot densification requires platelet contractility and is enhanced by rFVIIIa-platelet interaction. Deepening our understanding of the impact of rFVIIIa-platelet interactions on clotting dynamics could create opportunities for improved therapeutic strategies in HA.

FS04-OP03

HIGH FACTOR VIII CONCENTRATIONS MODULATE INTERACTIONS BETWEEN PLATELETS AND VON WILLEBRAND FACTOR IN VITRO

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Background: Platelets adhere to shear-unfolded von Willebrand factor (vWF) bound to subendothelial collagen at sites of vascular injury. This interaction occurs through vWF's A1 domain which engages platelet glycoprotein (GP) Ib-IX receptors. Coagulation factor VIII (FVIII) circulates in complex with vWF, bound to the D'D3 region located adjacent to the A1 domain. Recent studies showed that high FVIII concentrations allosterically increase vWF cleavage in the A2 domain by ADAMTS13 [1] and directly interfere with GPVI-mediated platelet activation [2].

Aims: To determine if FVIII directly or indirectly affects platelet-vWF interactions.

Methods: Platelet rich plasma (PRP) and washed platelets were isolated from healthy donor blood (RCSI_REC_1504). All assays used 0.5–50 nM B-domain deleted recombinant FVIII (simoctocog alfa) and FVIII-depleted vWF whenever applicable. Platelet agglutination with vWF (10 µg/mL) in the presence of eptifibatide (9 µM) was induced by adding ristocetin (0.5–1.5 mg/mL) and monitored using an aggregometer. Fixed platelet agglutinates were stained for GPIb and vWF, and the amount of vWF incorporated was calculated from confocal 3D image stacks using Fiji/ImageJ software. The GPIbM assay was performed using the CN-6000 analyser (Siemens/Sysmex). Platelet adhesion to vWF-coated flow channels under arterial shear (1,500 s^{-1}) was studied using whole blood. Botrocetin-facilitated platelet filopodia formation on vWF-coated glass was visualised by STimulated Emission Depletion (STED) super-resolution microscopy. vWF-collagen binding was assessed by vWF:CB3 and vWF:CB4 assays using ELISA. Statistical analysis was carried out by one-way ANOVA with Tukey correction for multiple comparisons.

Results: rFVIII dose-dependently reduced ristocetin-induced agglutination of washed platelets in solution, with effects being significant above 5.3 nM, equivalent to 10 times the physiological concentration, and reaching a 60% reduction at 53 nM. vWF incorporation into platelet agglutinates was reduced accordingly. Similar inhibitory effects were observed in PRP, whereas the GPIbM assay only showed a 25% reduction at 53 nM rFVIII. In contrast, GPIb binding and signalling in platelets interacting with surface-immobilised vWF were not significantly affected by high rFVIII concentrations, neither under arterial shear flow nor under static conditions. High rFVIII concentrations did not affect vWF-collagen III binding which involves vWF's A3 domain but significantly reduced binding of collagen IV to the vWF A1 domain in the presence of ristocetin ($p=0.0174$).

Summary/Conclusion: rFVIII did not directly block GPIb binding and signalling in assays where vWF was pre-adsorbed at a surface and thus present in an extended conformation. Interference of rFVIII with platelet GPIb-vWF interactions in solution and with selected collagen-vWF interactions supports the hypothesis that high rFVIII occupancy stabilizes a vWF conformation that helps to shield the cryptic vWF-A1 binding sites for GPIb and collagen IV. Our data support the newly postulated role that supraphysiological FVIII concentrations negatively regulate thrombus growth [1,2]. Ongoing experiments aim to clarify contributory direct effects of rFVIII on platelets and the significance of our findings for clinical situations.

FS04-OP04

CHARACTERISTICS OF PATIENTS WITH CYCLIC THROMBOCYTOPENIA: AN INTERNATIONAL REGISTRY STUDY

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Background: Cyclic thrombocytopenia (CTP) is a rare platelet disorder characterized by fluctuations in platelet counts, occurring in cycles of 3-5 weeks with values ranging from 1000 G/l. In the absence of a consensus definition that clearly differentiates CTP from immune thrombocytopenia (ITP), misdiagnosis as ITP is common. Patients respond poorly to ITP treatments including corticosteroids, splenectomy, intravenous immunoglobulin (IVIG) or thrombopoietin receptor agonists (TPO-RA). Management of CTP is not standardized and its underlying pathophysiology remains poorly understood.

Aims: To establish a comprehensive dataset of CTP patients to enhance understanding of the disease's clinical course, underlying mechanisms, and treatment responses.

Methods: A registry has been developed under the auspices of the International Society of Thrombosis and Haemostasis to enable healthcare providers to document cases of suspected or confirmed CTP (https://redcap.isth.org/surveys/?s=LJJE7T7XKLJEK3WX). The registry collects detailed clinical and laboratory data, and treatments specific to CTP. Descriptive analyses are performed on complete data sets to characterize the patient population and identify trends in disease manifestation and management.

Results: Between 2019-2025, 22 patients (age 23-68 years) were included, 72 % were female. All but 2 patients were initially misdiagnosed as having ITP with the time to correct diagnosis of CTP ranging from 3 months to 17 years. Bleeding at presentation was reported in 17 patients. In 5 patients, thrombocytopenia was detected during routine laboratory testing. 10 patients had thyroid disease, 5 patients had other autoimmune disorders (type 1 diabetes mellitus, polymyalgia rheumatica, polysynovialitis, psoriasis or antiphospholipid syndrome). At diagnosis, platelet counts ranged from 2 - 109 G/l. Renal and liver function tests, including LDH, were within normal limits. T-cell receptor beta and/or gamma gene rearrangements were detected in 11 of 17 tested patients. 20 of 22 patients initially received standard-dose oral corticosteroids. Prior to CTP diagnosis, additional treatments included IVIG (n=15), TPO-RA (n=11), rituximab (n=10), and splenectomy (n=9). Following CTP diagnosis, treatments included cyclosporine A (CSA, n=11), rituximab (n=7), danazol (n=2), and mycophenolate mofetil (MMF, n=1). A watch-and-wait strategy was adopted in 6 patients. During follow-up, 2 patients developed T-cell large granular lymphocytic leukemia, and 1 patient developed acute myeloid leukemia. 2 patients had venous thromboembolism (pulmonary embolism, cerebral vein thrombosis) during TPO-RA therapy. One patient had myocardial infarction during rituximab therapy, and one had bowel ischemia at initial CTP diagnosis. Intracranial bleeding occurred in 2 patients. Remission, defined as stable platelet counts >80 G/L, was achieved in 4 patients following treatment (CSA or rituximab) and in 4 patients spontaneously. In two patients, platelet fluctuations diminished with CSA or MMF, though remission criteria were not met.

Summary/Conclusion: CTP should be considered in patients with isolated thrombocytopenia who do not respond to standard ITP therapies. CTP may be associated with serious complications, including severe bleeding and thrombotic events, and frequently coexists with thyroid and hematologic disorders. Therapeutic responses to CSA, danazol, or MMF have been observed in individual cases, and spontaneous remissions can occur, even after prolonged disease duration

Oral Communication – Atherothrombosis

OC01-OP01

MULTIOMIC ANALYSES UNCOVER IMMUNOLOGICAL SIGNATURES IN ACUTE AND CHRONIC CORONARY SYNDROMES

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Background: Acute and chronic coronary syndromes (ACS and CCS) are leading causes of mortality. Inflammation is considered a key pathogenic driver of these diseases, but the underlying immune states and their clinical implications remain poorly understood.

Aims: Multiomic factor analysis (MOFA) allows unsupervised data exploration across multiple data types, identifying major axes of variation and associating these with underlying molecular processes. We hypothesized that applying MOFA to multiomic data obtained from blood might uncover hidden sources of variance and provide pathophysiological insights linked to clinical needs.

Methods: Here we compile a longitudinal multiomic dataset of the systemic immune landscape in both ACS and CCS (*n*=62 patients in total, *n*=15 women and *n*=47 men) and validate this in an external cohort (*n*=55 patients in total, *n*=11 women and *n*=44 men).

Results: MOFA reveals multicellular immune signatures characterized by distinct monocyte, natural killer and T cell substates and immune-communication pathways that explain a large proportion of inter-patient variance. We also identify specific factors that reflect disease state or associate with treatment outcome in ACS as measured using left ventricular ejection fraction.

Summary/Conclusion: Hence, this study provides proof-of-concept evidence for the ability of MOFA to uncover multicellular immune programs in cardiovascular disease, opening new directions for mechanistic, biomarker and therapeutic studies.

OC01-OP02

DNASE I UNVEILS THE PRESENCE AND UNLOCKS THE THROMBOLYTIC POTENTIAL OF INTRAVENOUSLYADMINISTERED, TPA IN LARGE VESSEL OCCLUSION ACUTE ISCHEMIC STROKE

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Background: DNase 1 has been shown to potentiate the thrombolytic activity of t-PA added ex vivo to ischemic stroke (IS) thrombi. However, it remains unclear whether extracellular DNA in IS thrombi is responsible for intravenous thrombolysis (IVT) failure due to large vessel occlusion (LVO).

Aims: We investigated whether biologically relevant intrathrombus concentrations of t-PA were achieved following IVT in patients with LVO-related IS, and whether extracellular DNA contributed to IVT failure.

Methods: 136 thrombi from AIS patients with LVO having received (n=69) or not (n=67) IVT prior to mechanical thrombectomy were analyzed. 83 thrombi were compared for tPA content according to IVT status, and 53 thrombi (24 non-IVT, 29 IVT) were subjected to *ex vivo* thrombolysis by addition of plasminogen, with or without DNAse 1. An additional subset of IS thrombi was used to decipher how extracellular DNA interferes with IVT.

Results: Thrombi from the IVT group contained more tPA compared to the non-IVT group ($p<0.0001$). Immunohistological analysis showed that tPA was associated with both fibrin and NETs. *Ex vivo* thrombolysis assays revealed that plasminogen alone did not induce lysis of thrombi from either group, but with DNase 1, it caused a significant decrease in thrombus weight with a stronger effect in the IVT group. A non-significant trend towards increased thrombolysis was observed in tenecteplase- vs actilyse-treated patients. Analysis of the impact of DNase 1 on thrombi showed that DNase 1 induced fibrinolysis and plasmin inhibitors release, suggesting that extracellular DNA contributes to intrathrombus retention of fibrinolysis inhibitors

Summary/Conclusion: These results indicate that intrathrombus tPA concentrations reached in failed IVT bear a therapeutic potential that is impaired by NETs, which favor intrathrombus retention of fibrinolysis inhibitors and compete with fibrin for tPA and plasminogen binding. Our results stress the interest of DNase 1 to enhance the efficacy of current IVT tPA regimens.

OC01-OP03

HIGHER CONTACT ACTIVATION IN ENDOVASCULAR TREATED STROKE PATIENTS ON PERIPROCEDURAL ANTITHROMBOTIC

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Background: Endovascular treatment (EVT) for large vessel occlusion of the anterior circulation in acute ischemic stroke patients (AIS) improves reperfusion rates. However, a considerable number of patients do not recover despite successful recanalization. It is suggested that microvascular occlusions and ischemia-reperfusion injury (IRI) are associated with these unwarranted clinical outcomes. Administration of periprocedural antithrombotics (unfractionated heparin (UFH), aspirin (ASA), or a combination (UFH+ASA) may reduce thrombotic complications including microvascular occlusions.

Aims: To assess the association between coagulation activity and clinical outcomes (modified Rankin Scale, mortality and stroke severity) in AIS patients treated with periprocedural antithrombotics during EVT.

Methods: Plasma samples of three time points (T_0 = admission; T_1 = 1 hour post-EVT; T_2 = 24 hours post-EVT) of 216 patients, from the MR CLEAN MED Trial, were included. Coagulation activity was assessed by measuring activated coagulation FXII (FXIIa), FXIa, FIXa, FXa, FVIIa and thrombin (T) in complex with antithrombin (AT), C1-esterase inhibitor (C1Inh) or alpha-1-antitrypsin (a1AT) using ELISA-methods. NETs markers (Histone-DNA, MPO, citrullinated Histone 3) were measured with ELISA. Related-Samples Friedman's two-way analysis of variance by ranks and multivariable regression was used to investigate the differences in coagulation activity and its associations with outcome measures.

Results: Patients receiving standard care (n=53) showed no changes in coagulation factor XII (FXII) activity measured by FXIIa:AT and FXIIa:C1inh over the various timepoints. Patients treated with UFH (n=60) or UFH+ASA (n=55) showed a significant increase in FXIIa:AT at T_1 ($p<.001$ and $p=0.021$, respectively), returning to baseline levels at T_2 ($p<0.001$ and $p=0.016$, respectively). Patients receiving ASA (n=48) only showed a significant peak at T_1 for FXIIa:C1Inh ($p=0.036$). Downstream coagulation of FXIIa showed a similar pattern for FXIa:C1Inh in the ASA-group, but no significant changes in the UFH-group. In the group receiving ASA+UFH, the FXIa:a1AT complexes were significantly decreased at T1, whereas they returned to baseline level at T2. Interestingly, the group receiving standard care showed no significant changes in contact activation (FXIIa or FXIa), whereas represented a significant increase in FVIIa:AT at T_1 ($p=0.045$) and a decrease at T_2 ($p=0.026$). Coagulation enzyme complexes showed low to moderate correlation with NETs markers. The baseline coagulation enzyme levels showed no associations with clinical outcomes.

Summary/Conclusion: Increased contact activation is seen in EVT-treated stroke patients receiving periprocedural antithrombotics compared to patients receiving standard of care. Despite, we found no relation with clinical outcome.

Oral Communication – Recurrent Thrombosis

OC02-OP01

THE INCIDENCE OF EARLY RECURRENT VENOUS THROMBOEMBOLISM: A SYSTEMATIC REVIEW

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Background: Patients with venous thromboembolism (VTE) usually receive anticoagulants for a minimum of 3 months. To evaluate the recurrence risk thereafter, certain strategies incorporate D-Dimer testing after stopping anticoagulation, which carries the potential of early VTE recurrence.

Aims: To evaluate the incidence of symptomatic recurrent VTE within 30 days after stopping anticoagulation.

Methods: A systematic search of EMBASE, CENTRAL, and MEDLINE was conducted to identify cohort studies and randomized controlled trials reporting on VTE recurrence. Eligible studies included adult non-cancer patients with deep vein thrombosis (DVT) of the leg and/or pulmonary embolism (PE) who had completed at least 3 months of anticoagulation. Study authors were contacted to obtain patient-level data, including number of patients, demographic characteristics (age, sex), location of index and recurrent VTE, duration of anticoagulation, and classification of VTE as provoked or unprovoked.

The endpoint was objectively verified VTE recurrence within 30 days after stopping anticoagulation (early recurrence).

Risk of bias was assessed using a modified Newcastle-Ottawa Scale.

Pooled recurrence rates were calculated using fixed- and random-effects meta-analyses. Subgroup analyses were performed to evaluate sex, age, unprovoked VTE, and location of the index event as potential risk factors for early recurrence.

Results: We identified 42 studies whose authors provided data for 24 studies (57%) comprising a total of 11.407 patients. Early VTE recurrence occurred in 115 patients (1.01%), with a pooled recurrence rate (RR) of 1.04% (95% CI: 0.8–1.4%).

Women had a lower risk than men [pooled RR 0.7 (95% CI 0.5-1.1); p=0,09], and patients with unprovoked VTE had a nearly 3-fold higher risk than those with provoked VTE [pooled RR 2.9 (95% CI 1.7-5.0); p<0.001]. The location of the index event (DVT versus PE) was not significantly associated with early recurrence risk [pooled RR 0.7 (95% CI 0.4-1.1); p=0.1]. Among 49 patients with early recurrent PE, none was fatal. The influence of advanced age as a risk factor was inconsistent across studies. Most patients had received vitamin K antagonists. Risk of bias was considered low based on the modified Newcastle-Ottawa Scale.

Summary/Conclusion: The incidence of early VTE recurrence following anticoagulation cessation is low. Strategies that temporarily interrupt anticoagulation to assess recurrence risk appear safe and may be appropriately integrated into shared decision-making regarding the optimal duration of anticoagulant therapy.

OC02-OP02

PLASMA LEVELS OF HUMAN EPIDIDYMIS PROTEIN 4 AND RISK OF RECURRENT VENOUS THROMBOEMBOLISM

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Background: Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease with severe complications, including death. Approximately 30% of VTE patients will experience a recurrent event within 10 years, which may further deteriorate VTE complications. Current risk assessment models (RAM) for VTE recurrence are inaccurate to determine the recurrence risk at an individual level. Thus, it is imperative to identify novel biomarkers for VTE recurrence for improvement of risk stratification and targeted treatment to high-risk individuals. Human epididymis protein 4 (HE4) has recently been identified as a biomarker for risk of incident VTE. No previous study has investigated whether plasma HE4 levels are associated with VTE recurrence.

Aims: To investigate whether plasma HE4 levels measured prior to the incident VTE were associated with risk of VTE recurrence.

Methods: The study population consisted of 911 patients with validated incident VTE derived from the third survey of the Trøndelag Health Study cohort (HUNT3) (n=50,800) in the period 2006-2019. Information was collected from participants at inclusion in HUNT3 through self-reported questionnaires, physical examinations and blood samples. Plasma HE4 levels were quantified using the 7k SomaScan® v4.1 aptamer-based platform. Participants were followed from the date of the index VTE until a recurrent VTE, migration, death or study end (Dec 31, 2019). Cox proportional hazards regression models were used to estimate hazard ratios (HR) with 95% confidence intervals (CI) for VTE recurrence according to quartiles (Q1-4) of plasma HE4 levels determined in a subcohort of the HUNT3 population (n=1,066). Subgroup analyses were also performed based on type of index VTE (DVT/PE, provoked/unprovoked). All participants provided written informed consent and ethical approval was received prior to initiation of the study.

Results: There were 116 validated recurrent VTE events during follow-up. High plasma HE4 levels measured prior to the index event were not associated with risk of VTE recurrence (HR 0.89, 95% CI 0.53-1.51) when compared to the lowest quartile (Q1) in the unadjusted model. Further adjustment for age and sex (HR 0.82, 95% CI 0.46-1.49), as well as body mass index (HR 0.83, 95% CI 0.45-1.53) did not reveal any significant associations. No significant associations were found between plasma HE4 levels and risk of VTE recurrence in any of the subgroups (provoked, unprovoked, DVT or PE) of the index events.

Summary/Conclusion: Plasma HE4 levels, measured prior to the incident VTE diagnosis, were not associated with VTE recurrence. Our results indicate that plasma HE4 levels measured prior to incident VTE diagnosis are not useful as a biomarker for VTE recurrence.

OC02-OP03

COFFEE CONSUMPTION IS ASSOCIATED WITH REDUCED RISK OF RECURRENT VENOUS THROMBOEMBOLISM

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Background: Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease associated with severe short- and long-term complications that can seriously impair mobility and quality of life, including VTE recurrence. VTE recurrence is known to further reinforce complications to incident VTEs. Coffee, one of the most widely consumed beverages in the world, contains constituents that downregulate plasma coagulation factor VIII/von Willebrand factor levels and inhibit platelet function. Epidemiological studies have shown that coffee consumption is associated with decreased risk of incident VTE. However, no previous study has explored whether coffee consumption influences the risk of VTE recurrence.

Aims: To investigate whether the amount of coffee consumption (cups/day) is associated with risk of VTE recurrence in a population-based cohort of incident VTEs.

Methods: The cohort consisted of 2071 patients with incident VTE recruited from four surveys of the Tromsø Study (enrolment: 1994-2008) and the third survey of the Trøndelag Health Study (enrolment: 2006-08). Patients were followed from the date of the index VTE until a recurrence, migration, death or end of study (Dec 2020). Information on coffee consumption (cups/day) and potential confounders were extracted from the last survey prior to diagnosis of incident VTE to avoid reverse causation. Cox-regression models were used to estimate hazard ratios (HR) with 95% confidence intervals for VTE recurrence across categories of coffee consumption (0, 1-2, 3-4, 5-6 and >6 cups/day) after adjustment for age, sex, body mass index and smoking.

Results: During a median follow-up of 2.8 years, 310 recurrent VTEs occurred, corresponding to an overall recurrence rate of 3.3 (95% CI: 3.0-3.7) per 100 person-years. Overall, coffee consumers had a 32% lower risk of recurrence (HR 0.68, 95% CI: 0.45-1.01) than non-consumers, but there was no linear relationship with increasing amounts of coffee intake and VTE recurrence. The recurrence risk was lowest among those consuming 1-2 cups of coffee per day (HR 0.49, 95% CI: 0.30-0.81), while the HR was 0.71 (95% CI: 0.44-1.12) for those consuming >6 cups compared with non-coffee consumers. The association between coffee consumption (no/yes) and lower recurrence risk was more pronounced in those with a provoked index VTE event (HR 0.50, 95% CI: 0.30-0.82) than those with an unprovoked index VTE (HR: 1.01, 95% CI: 0.53-1.94).

Summary/Conclusion: Coffee consumption was associated with lower risk of VTE recurrence, particularly after provoked VTE, but there was no linear relationship between increasing amount of coffee and VTE recurrence risk. Our findings suggest that coffee can be safely consumed after VTE, potentially with a beneficial effect on recurrence risk.

Oral Communication – Blood Cells

OC03-OP01

DEVELOPMENT AND IN VITRO EVALUATION OF A NOVEL UNICAR T CELL THERAPY TARGETING B AND PLASMA CELLS FOR AUTOIMMUNE DISEASE TREATMENT

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Background: Chimeric antigen receptor (CAR) T cell therapy targeting CD19 on B cells has shown promise in treating refractory autoimmune diseases (AID) such as systemic lupus erythematosus (SLE). In contrast, CAR T cell therapy directed against CD38-expressing plasma cells in AID remains unexplored. The efficacy of either approach in managing refractory immune-mediated thrombotic thrombocytopenic purpura (iTTP) is currently unknown.

Aims: In this study, we investigated the potential of the Universal (Uni)CAR T cell adaptor platform for treating refractory immune-mediated thrombotic thrombocytopenic purpura (iTTP). We assessed in vitro cytotoxicity against engineered Nalm-6 cells expressing anti-ADAMTS13 antibodies (anti-S and anti-CUB2), which endogenously express both CD19 and CD38 surface markers, representative of B and plasma cells, respectively.

Methods: The UniCAR T platform consists of either the previously described anti-CD19 target module (TM) or an anti-CD38 TM which acts as a bridge between the UniCAR T cell and the target cell (engineered Nalm-6 cells). Both TMs are composed of a single-chain variable fragment (scFv), that recognizes the target cell, coupled to a human La-derived peptide epitope (E5B9). This epitope in the TM is recognized by the anti-La5B9 scFv expressed on the UniCAR T cell. TM-mediated cross-linkage between UniCAR T cells and target cells leads to the destruction of the target cells.
TMs were produced by lentiviral transduction of 3T3 cells and purified via Ni^{2+}-NTA affinity chromatography from cell culture supernatants. Engineered Nalm-6 cells expressing anti-ADAMTS13 antibodies (anti-S and anti-CUB2) were also generated by lentiviral transduction. UniCAR T cells were derived from healthy donor T cells and transduced with UniCAR constructs. The target Nalm-6 cells were co-incubated with UniCAR T cells, with and without the respective TM, to assess the cytotoxic efficacy of this therapy. Additionally, an in-house cell-based ELISA was used to measure the binding affinity of both TMs to their respective targets.

Results: After successfully generating UniCAR T cells and both anti-CD19 and anti-CD38 TMs, the cytotoxic potential of the UniCAR T cells was evaluated. Both anti-CD19 and anti-CD38 TM bound with high affinity to the Nalm-6 target cells (Kd of 1.42 nM and 3.78 nM, respectively). Incubation of UniCAR T cells together with the Nalm-6 cells (anti-S or anti-CUB2) in a 1:1 ratio in the presence of 25 nM of either TM, significant TM-dependent cytotoxicity was observed. The anti-CD19 UniCAR T cells eliminated 78.6% of anti-S and 76.2% of anti-CUB2 Nalm-6 cells. Similarly, anti-CD38 UniCAR T cells killed 83.1% of anti-S and

82.3% of anti-CUB2 Nalm-6 cells. In contrast, in the absence of TMs, only minimal TM-independent killing occurred, 17.3% for anti-S and 20.1% for anti-CUB2 Nalm-6 cells, highlighting the specificity of the TM-mediated cytotoxic response.

Summary/Conclusion: We here showed that the UniCAR T adaptor platform is ideally suited to kill CD19 and CD38 expressing cells. We are now investigating the efficacy of the platform to selectively kill iTTP patient derived anti-ADAMTS13 antibody producing B cells in vitro.

OC03-OP02

NOVEL MOUSE MODEL FOR IMMUNE-MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Current immune-mediated thrombotic thrombocytopenic purpura (iTTP) animal models rely on injecting anti-ADAMTS13 antibodies (Abs), bypassing the immune system. There's a need for models where the immune system itself produces these inhibitory or clearing antibodies.

Aims: In this study, we aim to develop a novel iTTP mouse model in which the animal's own immune system drives ADAMTS13 deficiency.

Methods: Wild-type (WT) mice were immunized with recombinant human (rh)ADAMTS13 (n=12) or injected with saline (n=7) on days 1 and 14. On day 21, anti-rhADAMTS13 antibody and murine (m)ADAMTS13 antigen levels were assessed using ELISA, mADAMTS13 activity was measured in a FRETS-VWF71 assay and immunized and control mice were injected with rVWF (1000 U/kg) to induce iTTP. After 24 hours, blood counts and LDH levels were determined.

Results: As expected, all immunized mice (n=12) developed anti-rhADAMTS13 Abs by day 21. Interestingly, in 9 of the these mice, the anti-rhADAMTS13 Abs produced by the animal's own immune system (B cells) effectively cleared mADAMTS13, resulting in ADAMTS13 deficiency. Control mice (n=7) had no anti-rhADAMTS13 antibodies, hence normal ADAMTS13 activity and antigen levels. Importantly, after triggering with rVWF, immunized mice with ADAMTS13 deficiency (n=9) exhibited iTTP-like signs and symptoms, including severe thrombocytopenia (from $480.3*10^9$/L to $107.9*10^9$/L, $p<0.0001$) and hemolytic anemia (hematocrit: from 22.56% to 6.11%, p=0.0012; hemoglobin: from 12.19 g/dL to 9.22 g/dL, $p<0.0001$), in contrast to control mice. Confirming the presence of ADAMTS13-specific B and T cells in these mice is currently ongoing.

Summary/Conclusion: We developed the first animal model for iTTP in which ADAMTS13 deficiency is driven by the animal's own immune system. Interestingly, this unique iTTP mouse model offers a valuable platform for the development of novel immunomodulatory drugs.

OC03-OP03

THROMBOTIC THROMBOCYTOPENIC PURPURA IN QUEBEC, CANADA. A 10-YEAR FOLLOW-UP OVERVIEW (2012-2022) FROM A REFERENCE LABORATORY PERSPECTIVE

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Background: Thrombotic Thrombocytopenic Purpura (TTP) is a rare and life threatening thrombotic microangiopathy disease (TMA) caused by a severe deficiency of ADAMTS-13. Since 2013, CHU Sainte-Justine Hemostasis Laboratory (CHUSJHL) serves as a reference centre for ADAMTS-13 testing within the province of Québec (9M inhabitants).

Aims: Evaluate the epidemiological profile of TTP patients within Quebec and assess their follow-up using laboratory data.

Methods: Patients with suspected TMA whose plasma samples were sent to CHUSJHL for ADAMTS-13 testing between 2012 and 2022 were included in this study. Demographic and clinical data were obtained through questionnaires or extracted from the CHUSJ laboratory information system. TTP cases were defined as patients with ADAMTS-13 activity ≤10%, with or without detectable anti-ADAMTS-13 antibodies. Patient follow-up was assessed based on repeat ADAMTS-13 requests and compared to the 2012 British Society for Haematology guidelines for TTP management (PMID: 22624596). The study was approved by the Research Ethics Committee of CHUSJ.

Results: A total of 129 patients were identified as having TTP. Of these, 103 had anti-ADAMTS-13 antibodies, 73 were idiopathic, and 56 had an associated condition (secondary TTP). Ninety-four had at least two documented ADAMTS-13 tests during their follow-up, with a median follow-up time of 9 months (1-99). Only 19/94 had adequate ADAMTS-13 monitoring according to recommendations, with 90% of these cases diagnosed between 2020 and 2022. Seventy of ninety-four (74%) patients had no documented clinical relapses, while 24 (26%) had 1 to 5 relapses with a median time of 13 months between first episode and relapse (1-74). Twenty-two of 24 relapsing patients had no ADAMTS-13 follow-up between the initial episode and the first relapse.

Summary/Conclusion: This study provides an overview of TTP in Quebec, highlighting demographic and clinical characteristics similar to other national cohorts. Significant gaps in follow-up care were identified, emphasizing the need for better TTP management..

Oral Communication – Bloodomics

OC04-OP01

SHEDDING LIGHT ON HEPATOSTEATOSIS AND COAGULATION DEFECTS: INSIGHTS FROM SINGLE-CELL RNA SEQUENCING

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Background: The liver synthesizes most coagulation factors (CF). Chronic liver diseases (CLD) deeply affect CF synthesis and the hemostatic system but the mechanisms and impacts remain unclear. This disruption leads to imbalances in the hemostatic system, increasing the risk of both bleeding and clotting complications. While anticoagulant drugs can slow CLD progression, they also carry a heightened risk of bleeding. To improve patient care and develop targeted therapies, it's crucial to understand the molecular mechanisms driving these hemostatic abnormalities.

Aims: To decipher these mechanisms using scRNAseq in a mouse model of diet-induced metabolic dysfunction-associated steatohepatitis (MASH).

Methods: This study utilized single-cell RNA sequencing (scRNAseq) to investigate these mechanisms in a mouse model of diet-induced metabolic dysfunction-associated steatohepatitis (MASH), a common form of CLD. Mice were fed either a control diet or a MASH diet for 24 weeks, after which their liver cells were individually analyzed.

Results: We identified twenty-one cell types and focused on liver sinusoidal endothelial cells (LSECs), essential for factor VIII production. Pathway deregulation in LSECs showed “Complement and coagulation cascade” KEGG pathway upregulated (Top5, -log10[P] = 4.75) in the MD condition. Genes composing this pathway were found all related to the complex AP-1 in a Gene Regulatory Network analysis. AP-1 emerged as central in the defect observed in the mice with MASH dysregulating key transcription factors like *HSF1* and *NFκB1*, both known for their role in MASH progression and hemostasis. These results suggest MASH onset as a pro-thrombotic event in the liver, partially implicating LSECs with a central complex AP-1.

Summary/Conclusion: Mice developing liver steatosis show transcriptionally reduced and imbalanced liver hemostatic capacity. Single-cell RNAseq partially attributes this defect to LSECs with a central actor of AP-1. Deeper exploration of LSEC transcriptome and deregulated pathways will enhance understanding of the liver hemostatic impairments and may provide new diagnostic or therapeutic opportunities.

OC04-OP02

HIGH-THROUGHPUT AFFINITY PROTEOMICS REVEALS EARLY DYNAMIC CHANGES IN THE CIRCULATING PROTEOME FOLLOWING LVAD IMPLANTATION

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Background: Left ventricular assist devices (LVADs) are an established therapeutic strategy for patients with advanced heart failure, used either as a bridge to transplantation or as destination therapy. While their hemodynamic benefits are well characterized, LVAD support disrupts the hemostatic system, leading to an increased incidence of hemorrhagic complications, particularly gastrointestinal bleeding. However, the systemic molecular responses underlying these complications remain incompletely understood. High-throughput affinity proteomics platforms, such as Olink Explore HT, offer an unprecedented opportunity to simultaneously measure thousands of circulating proteins, enabling comprehensive characterization of the early biological changes following device implantation.

Aims: To characterize early proteomic changes and altered biological pathways following LVAD implantation using the Olink Explore HT platform.

Methods: Serum samples from 17 patients undergoing LVAD implantation were analyzed. Samples were collected before surgery and again seven days post-implantation. Proteomic profiling was performed using the Olink Explore HT platform, measuring over 5,000 proteins. Changes in protein levels were assessed using a linear mixed-effects model adjusting for patient variability, sex, and age. Statistical significance was determined using False Discovery Rate (FDR) correction, with FDR-adjusted $p < 0.05$ considered significant. Proteins with significant changes were further analyzed by pathway enrichment to identify biological processes modulated by LVAD implantation.

Results: Proteomic analysis identified 258 proteins with significant abundance changes seven days after LVAD implantation (FDR-adjusted $p < 0.05$), including key hemostasis-related proteins such as von Willebrand factor (VWF) and ADAMTS13. Pathway enrichment analysis revealed significant enrichment of processes related to hemostasis and platelet activation, highlighting important shifts in coagulation and hemostatic regulation following LVAD support. Additionally, pathways associated with muscle contraction, extracellular matrix remodeling, and cell adhesion were enriched, suggesting ongoing cardiac and vascular tissue remodeling.

Summary/Conclusion: Our study demonstrates that LVAD implantation induces significant early changes in circulating proteomic profiles, highlighting coordinated shifts in pathways related to myocardial and vascular structural remodeling as well as systemic hemostatic balance. This work highlights the utility of high-throughput affinity proteomics for capturing early systemic changes and identifies novel candidate biomarkers for further investigation. Further studies are warranted to validate the clinical relevance and elucidate the mechanistic implications of these findings.

OC04-OP03

GENE EXPRESSION PROFILES FROM ADIPOSE TISSUE BIOPSIES IN PERSONS UNDERGOING BARIATRIC SURGERY: EXPLORING BODY FAT DISTRIBUTION AND COAGULATION

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Background: Obesity is a major risk factor for venous thromboembolism (VTE), with a population-attributable risk of 24.6%, but the biological mechanisms remain unclear. Different adipose tissue depots may contribute in varying degrees to the risk of VTE, potentially with sex-specific differences. However, these mechanisms are not well known and may be examined through protein expression profiles.

Aims: To describe the mRNA expression of 21 coagulation-related proteins, 1) within individuals across adipose tissue depots including visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (aSAT), and the liver, and 2) between individuals across stages of liver fat accumulation, categorized as healthy, metabolic dysfunction-associated steatotic liver disease (MASLD), or steatohepatitis (MASH).

Methods: Adults aged 18–65 years undergoing primary bariatric surgery were included in the BARIA study. Participants had a BMI ≥40 kg/m² or ≥35 kg/m² with obesity-related comorbidities. Biopsies of VAT, aSAT, and the liver were collected during surgery. Liver steatosis stage was determined histologically. We assessed gene expression of 21 coagulation- and endothelium-related proteins by RNA sequencing: fibrinogen, prothrombin, tissue factor, factors V, VII, VIII, IX, X, XI, von Willebrand factor (VWF), thrombomodulin, protein C, protein S, antithrombin, TFPI, tPA, plasminogen, PAI-1, α2-antiplasmin, and E-/P-selectin. Expression was summarized using medians and interquartile ranges (IQR), stratified by sex and age (dichotomized at the median). To contextualize tissue-specific expression patterns, we compared findings to data from the Human Protein Atlas.

Results: Among 318 patients (median age 48 years [IQR 33.3–62.8]; 76.4% women), diabetes and hypertension were present in 25.8% and 37.7%, respectively; 44.7% had MASLD and 15.1% MASH. Preliminary analyses included 10 genes; data for the remaining 11 will be available by the conference. Protein C expression was liver-specific. Fibrinogen and prothrombin were mainly expressed in the liver, with low levels in VAT. Antithrombin was also liver-predominant, with low expression in VAT and aSAT. TFPI, PAI-1, and protein S showed highest expression in the liver, followed by VAT and aSAT with smaller differences. FVIII and tPA were most highly expressed in VAT, followed by aSAT and liver. VWF was primarily expressed in VAT and aSAT, with low expression in the liver. No age- or sex-related differences were observed. mRNA expression was largely similar across liver fat stages, except for PAI-1, which increased stepwise from healthy liver to MASLD and MASH.

Summary/Conclusion: Gene expression patterns in VAT, aSAT, and liver largely aligned with the Human Protein Atlas, confirming liver-specific expression of protein C, fibrinogen, prothrombin, and antithrombin. TFPI, PAI-1, protein S, F8, tPA, and VWF were prominently expressed in adipose tissue, likely reflecting expression by endothelial, adipocyte, or fibroblast cells. Expression was mostly stable across liver fat stages, except for PAI-1, which showed a stepwise increase from healthy individuals to those with MASLD and MASH, suggesting a role for PAI-1 in liver inflammation and thrombotic risk. Further analyses, including additional proteins and hormone-stratified sensitivity analyses in premenopausal women, are ongoing.

Oral Communication – Cancer & Thrombosis

OC05-OP01

WHICH FACTORS ARE ASSOCIATED BLEEDING AND THROMBOSIS DURING THE LAST PHASE OF LIFE IN CANCER PATIENTS?

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Background: Deciding whether to discontinue antithrombotic therapy in cancer patients during their last phase of life remains challenging.

Aims: This study aims to identify factors associated with arterial and venous thromboembolic events (ATE and VTE) and major bleeding (MB) in cancer patients with limited life expectancy, ultimately to support decisions on (dis)continuing antithrombotic therapy.

Methods: Data from Dutch nationwide databases, covering all registered diagnoses during hospital admissions in the Netherlands and outpatient medication prescriptions from 2013 until 2021, were analyzed, including adults with a cancer type or stage associated with a median life expectancy of three years or less. Included were cancer types primarily located in their breast, brain, female reproductive organs, liver/gall bladder, lower and upper gastrointestinal system, pancreas, retroperitoneum and urinary tract, and patients with unknown primary tumor (PTU). All patients were prevalent antithrombotic therapy users at baseline. The index date was defined as the date of cancer diagnosis. The cumulative incidences accounted for competing death risk of ATE, VTE and MB after one year were estimated, and Cox cause-specific hazard models were used to calculate hazard ratios. Hazard ratios were adjusted for sex, age group (<70; 70-85; 85+), and cancer type, with the male sex, age group below 70 and breast cancer as reference categories.

Results: 72,499 patients diagnosed with cancer between 2013 and 2022 were included. Median survival time was 160 days [IQR 45, 478]. Cumulative incidences of ATE, VTE and MB after one year are 2.3% (95% CI 2.2 – 2.4), 1.2% (95% CI 1.1 -1.3) and 2.2% (95% CI 2.1 – 2.3%), respectively. Adjusted hazard ratios (aHR) for MB are highest for urinary tract (aHR 3.1, 95% CI 2.2 – 4.4), liver/gall bladder (aHR 2.3, 95% CI 1.6 – 3.3) and upper GI cancer (aHR 1.9, 95% CI 1.4 – 2.7). Pancreatic, urinary tract and pulmonary cancer have the highest hazards of ATE (aHR 1.7 (95% CI 1.2 – 2.3), aHR 1.6 (95% CI 1.1 – 2.2) and aHR 1.5 (95% CI 1.1 – 2.0), respectively), with a protective association for cerebral cancer (aHR 0.5, 95% CI 0.3 – 0.8). aHRs for VTE are highest for brain (aHR 2.4, 95% CI 1.4 – 4.0), pulmonary (aHR 2.0, 95% CI 1.2 – 3.1) and lower GI cancer (aHR 1.6, 95% CI 1.0 – 2.7) and PTU (aHR 1.6, 95% CI 0.9 – 2.9). Age groups are most strongly associated with VTE (aHR 0.7 (95%CI 0.6 – 0.8) for 70 – 85 years, aHR 0.3 (95%CI 0.2 – 0.4) for 85+).

Summary/Conclusion: In cancer patients with limited life expectancy, cancer type was most strongly associated with MB and ATE, while age was most strongly associated with VTE. Our findings will be compared with results from three other databases, including nationwide data from Wales and Denmark, and free-text data from a Dutch primary care database. Altogether, with our results, we may identify patients at highest risk of thrombosis or bleeding and hence guide decisions regarding (dis)continuing antithrombotic therapy.

OC05-OP02

OVARIAN CANCER-RELATED BIOFLUIDS FROM PATIENTS AND CELL LINES INDUCE NEUTROPHIL REPROGRAMMING TOWARDS A PRO-TUMORAL PHENOTYPE

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Background: High-grade serous ovarian cancer (HGSOC) is the most lethal gynaecological malignancy and one of the most prothrombotic tumours. This tumour spreads following peritoneal fluid (PF) dynamics, being the most representative biofluid of the tumour microenvironment (TME). Neutrophils are actively recruited into the TME, undergoing functional reprogramming and engaging in pro-tumour activities, as the formation of neutrophil extracellular traps (NETs), which are involved in immunothrombosis, metastatic niche formation, and therapy resistance. We previously showed that HGSOC patients exhibit elevated levels of NETosis markers compared to control women, particularly within the TME. However, the potential contribution of OC cells to neutrophil pro-tumoral reprogramming remains unknown.

Aims: To evaluate whether OC cells contribute to the reprogramming of neutrophils towards a pro-tumour phenotype, promoting their migration, the expression of pro-tumour-associated genes, and the formation of NETs within the TME.

Methods: The RayBio Cytokine Array-C5 was used to assess the profile of 80 biological factors in PF samples from HGSOC patients (n=15), control women (n=9), and conditioned media (CM) from SK-OV-3 and Caov-3 cell lines. NETosis biomarkers—cell-free DNA (cfDNA), nucleosomes, citrullinated histone H3-DNA complexes (citH3), calprotectin, and myeloperoxidase—were quantified in PF samples. Neutrophils were isolated from freshly drawn blood using density-gradient centrifugation and their migration was assessed using Transwell assays upon incubation with HGSOC-PF and OC-CM. Extracellular DNA release was visualized with SYTOX Green staining. cfDNA, citH3 and calprotectin were measured in neutrophils supernatant following OC-CM stimulation. Gene expression- *ELANE, MPO, IL6, TNFA, OSM, VEGFA, MMP9, LTF, IL1B, IL8* and *MMP1*- was evaluated by RT-qPCR. Statistical analyses were performed using R (v.4.4).

Results: Advanced HGSOC patients showed deregulated levels of 20 out of the 80 analytes—18 up- and 2 down-regulated- in the PF. Several of them, highlighting IL-8 and GROα, showed a strong correlation with NETosis biomarkers levels. Notably, investigations in the OC-CM cell lines corroborated the pattern of dysregulation for 8 out of the 20 PF-altered molecules, pointing to an OC origin. Next, functional assays revealed that both biofluids (HGSOC-PF and OC-CM) induced a marked increase in neutrophil migration compared to control PF. Regarding NETs, HGSOC-PF significantly enhanced extracellular DNA release, along with increased expression of *MPO* and, notably, *ELANE*. Isolated neutrophils exposure to OC-CM triggered progressive NETosis activation, evidenced by extracellular DNA release, upregulated gene expression and secretion of soluble NETosis markers. Besides, exposure to biofluids induced neutrophil

gene expression upregulation of *IL6, TNFA, VEGFA, MMP9,* and *LTF* (both), and *IL8, IL1B,* and *MMP1* (OC-CM).

Summary/Conclusion: Our findings suggest that factors present in the TME of HGSOC, including those secreted by tumour cells, promote neutrophil reprogramming toward a pro-tumoral phenotype, promoting migration, activation and NET formation. This pro-tumoral shift may contribute to the prothrombotic and metastatic phenotype observed in HGSOC, offering new insights into the role of neutrophils in OC progression.

OC05-OP03

THE ROLE OF NEUTROPHILS IN CHEMOTHERAPY RESISTANCE AND HYPERCOAGULABILITY IN 3D CELL CULTURES OF MUSCLE-INVASIVE BLADDER CANCER

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Background: The tumor microenvironment (TME) influences cancer growth and response to chemotherapy through intricate cell interactions, a feature absent in 2D cultures. Advanced 3D models, like spheroids and organoids, better mimic *in vivo* conditions, offering platforms to study drug resistance, potentially involving neutrophil extracellular traps (NETs), according to some initial findings. However, the unexplored role of neutrophils/NETs in bladder cancer (BC)'s drug resistance within the TME presents a critical research gap for improving treatment.

Aims: To investigate how neutrophils influence BC behavior *in vitro*, including tumor proliferation and chemotherapy response, and whether it is mediated by NET formation.

Methods: 3D spheroids were generated from one muscle-invasive BC cell lines (T24, ATCC) and co-cultured with peripheral blood neutrophils isolated from healthy donors. After 24 h, cisplatin (cis) and gemcitabine (gem) chemotherapy was added to the co-culture at 3 concentrations: (1) 2 µM cis- 100 nM gem, (2) 1 µM cis-50 nM gem, and (3) 0.1 µM cis-5 nM gem, and incubated for 5 days. Conditioned media (CM) was then collected for analysis. Cell viability was assessed using 3D CellTiter-Glo Luminescent Cell Viability Assay (Promega) and the result was normalized to vehicle-treated controls (100%). NET markers (cell-free DNA, cfDNA; citrullinated histone H3, citH3; neutrophil elastase, NE) were quantified in the CM. Selected spheroids were formalin-fixed, paraffin-embedded and stained with haematoxylin and eosin. Also, the aforementioned NET markers were detected by immunofluorescence. Tissue factor (TF) levels were quantified in all CM with the Human Coagulation TF ELISA Kit (Bio-Techne R&D Systems) to evaluate the potential pro-thrombotic effects of chemotherapy response mediated by neutrophils.

Results: BC spheroids induced NETosis when co-culturing with neutrophils, as evidenced by the presence of citH3 in the CM. Although neutrophils surrounded the spheroids, these didn´t infiltrate the spheroids and no NETosis occurred within. As expected, chemotherapy reduced the viability of T24 spheroids by 24%. At the highest chemotherapy dose, neutrophils increased the viability of T24 spheroids (103.00 ± 5.04%) compared to those without neutrophils (75.98 ± 3.37%; $P<0.0001$). Similar TF levels were found in the CM independently of chemotherapy treatment or the presence of neutrophils thus suggesting that NETs did not affect BC procoagulant potential *in vitro*.

Summary/Conclusion: Neutrophils seem to mediate chemotherapy resistance in BC spheroids. BC spheroids induce NET formation, which may contribute to chemotherapy resistance *in vitro*. However, no increase in TF was observed; thus, an increase in the procoagulant activity of BC cells may be explored by other means. Our findings suggest that targeting NETs could enhance treatment efficacy and improve BC patient outcomes.

OC05-OP04

LIQUID BIOPSY FOR PEDIATRIC TUMORS: NEUTROPHIL EXTRACELLULAR TRAPS (NETS) AS NOVEL MINIMALLY INVASIVE BIOMARKERS

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Background: Neutrophil Extracellular Traps (NETs) have been implicated in cancer progression, but their diagnostic and prognostic potential in pediatric solid (PST) and hematologic tumors (PHT) remains unclear.

Aims: This study aims to evaluate NET-associated biomarkers in newly diagnosed pediatric oncology patients and to evaluate their diagnostic capacity.

Methods: We analyzed EDTA plasma samples from pediatric and adolescent patients (<18 years) newly diagnosed with acute lymphoblastic leukemia (PHT, n=18) or PST (n=115), including neuroblastoma (n=32), osteosarcoma (n=18), Ewing sarcoma (n=16), Wilms tumor (n=12), hepatoblastoma (n=10), rhabdomyosarcoma and other sarcomas (n=9), pilocytic astrocytoma (n=5), retinoblastoma (n=4), medulloblastoma (n=3), ependymoma (n=1), and other tumors (n=5). Patients were classified by risk (high-risk [HR] and low-risk [LR]). Healthy pediatric controls (C, n=10) were included. We quantified circulating free DNA (cfDNA, Life Technologies), calprotectin (Hycult Biotech), citrullinated histone H3 (H3cit)-DNA complexes (Roche and Abcam), and elastase (Abcam). Plasma DNaseI activity was measured using a single radial enzymatic diffusion (SRED) assay. The diagnostic capacity of these markers was assessed with the Area Under the ROC curve (AUC). Statistical analysis was performed using GraphPad Prism v9.

Results: Median ages were 4 years for PST (range 0-16), 3.5 years for PHT (range 0-16), 1.5 years for controls (range 0-12). Patients showed significantly elevated cfDNA levels compared to controls ($p<0.01$). PST had higher calprotectin and elastase levels than PHT and controls ($p<0.001$, $p<0.05$). PST-HR exhibited increased calprotectin versus PST-LR ($p<0.01$), PHT-HR ($p<0.01$), PHT-LR ($p<0.0001$), and controls ($p<0.01$). Elastase was higher in PST-HR and PST-LR versus PHT-HR ($p<0.01$, $p<0.05$) and PHT-LR ($p<0.0001$, $p<0.001$). DNaseI levels were significantly lower in PST-HR versus PST-LR ($p<0.05$). Furthermore, cfDNA and calprotectin showed the highest diagnostic capacity for all cancer patients (AUC=0.878, 95% Confidence Interval [0.814-0.942], $p<0.0001$; AUC=0.717, 95% Confidence Interval [0.605-0.829], $p=0.022$ respectively), but particularly for HR patients (0.890, [0.818-0.962], $p<0.0001$; 0.806, [0-706-0.903], p=0,0016 respectively).

Summary/Conclusion: Our study, the largest pediatric oncology cohort analyzing NET biomarkers, describes a dysregulation in the NETosis process and reveals cfDNA as a potential simply to measure diagnostic marker, particularly for HR patients. Elevated NETosis markers in PST-HR patients compared to PST-LR, suggest their prognostic utility that should be addressed in further studies. Decreased DNaseI in PST-HR highlights its potential as a therapeutic target, supported by treatments increasing DNaseI levels (NCT05880524, NCT05453695). These findings underscore NETosis's relevance in pediatric tumors and clinical applications.

OC05-OP05

PLASMA MIR-145 AND THE RISK OF FUTURE CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM

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Background: Cancer is associated with a transient 4- to 9-fold increased risk of venous thromboembolism (VTE) and 20-25% of all incident VTEs are cancer-related. Cancer-associated VTE (CAT) leads to increased morbidity and mortality in cancer patients. MicroRNAs (miRs) are small endogenous noncoding RNAs that downregulate gene expression and have been shown to play a critical role in several biological processes and in human diseases, including cancer and VTE. Elevated miR-145 levels have been reported to be associated with lowered risk of future incident VTE. Given that the pathophysiological mechanisms of VTE partially differ in cancer and non-cancer, and miR-145 acts as a tumor suppressor, it is important to investigate whether the association between miR-145 and VTE extends to both cancer and non-cancer groups.

Aims: To investigate the association between plasma miR-145 levels and the risk of future CAT and non-CAT in a population-based case-cohort study.

Methods: In this prospective case-cohort study derived from the third survey of the Trøndelag Health Study (HUNT3) (n=50800) in Norway, we measured plasma miR-145 levels at study inclusion in 455 participants who developed VTE during 9 years of follow-up and 1,740 age-matched subcohort participants. Plasma miR-145 levels were measured using qPCR by Qiagen Genomic Services (Hilden, Germany). VTEs were classified as CAT if they occurred within 1 year before or 2 years after a cancer diagnosis, and as non-CAT otherwise. Hazard ratios (HRs) with 95% confidence intervals (CIs) for CAT and non-CAT according to miR-145 levels were estimated using weighted Cox regression models. miR-145 was analyzed both as quartiles and as a continuous (scaled) variable, with cancer modeled as a time-dependent covariate. The study was approved by the regional committee for medical research ethics and informed written consent was obtained from all participants.

Results: Among the 455 VTE cases, 95 (21%) were classified as CAT. In a model adjusted for age, sex, BMI, and arterial cardiovascular disease, each 1 standard deviation increase in (scaled) plasma miR-145 levels was associated with a HR of 0.79 (95% CI: 0.60–1.04) for CAT and 0.81 (95% CI: 0.72–0.90) for non-CAT. Sensitivity analyses, including variations in the definition of the active cancer period, yielded consistent results.

Summary/Conclusion: Our findings suggest that the previously reported protective association between plasma miR-145 and VTE extends to both CAT and non-CAT. Combined with experimental data showing that tissue factor, factor XI, and plasminogen activator inhibitor-1 (PAI-1) are gene targets of miR-145, these results position miR-145 not only as a potential risk biomarker for CAT but also as a gateway to understand the pathogenesis of CAT and non-CAT.

OC05-OP06

BLADDER CANCER CELLS INDUCE A PROCOAGULANT STATE THROUGH TISSUE FACTOR AND NETS DRIVE THEIR MALIGNANT TRAITS

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Background: Patients with bladder cancer (BC) have an increased risk of thrombosis and also elevated levels of neutrophil extracellular traps (NETs) both systemically and within the tumor microenvironment. While NETs are suspected of contributing to thrombosis, tumor progression and metastasis, the precise mechanisms remain unclear and have yet to be definitively established in BC.

Aims: To verify if BC cells increase thrombin generation (TG), to identify whether it is mediated by NETs, to assess whether NETs influence BC hallmarks and to identify the cytokines involved. Evaluate if these effects differ when NETs originate from BC patients or healthy individuals.

Methods: Peripheral blood neutrophils were isolated from 4 BC patients and 4 healthy donors. NETs were induced using 500nM phorbol-12-myristate-13-acetate and collected for downstream assays. Two human BC cell lines—T24 (muscle-invasive, MIBC) and 5637 (non-MIBC)—cells were treated with increasing concentrations of NETs. Functional assays were performed to assess cancer hallmarks: cell proliferation (sodium phosphatase viability assay), migration (scratch wound assay), extracellular matrix invasion (transwell assay), and resistance to anoikis. Cytokine profiling of conditioned media (CM) from NET-treated T24 cells was performed using the Human Cytokine Array C5 (RayBiotech) to identify differentially secreted inflammatory mediators. The procoagulant activity of NET-treated and not treated BC cells was assessed with the TG test (Stago) without triggers, and with PRP- and MP-reagents to assess the intrinsic and extrinsic coagulation pathways, respectively, and also adding anti-Tissue Factor (TF) antibody (eBioscience). TF levels were quantified in all CM with the Human Coagulation TF ELISA Kit (Bio-Techne R&D Systems).

Results: 5637 cells secreted significantly more TF than T24 (123.4±14.08 pg/mL *vs*. 21.11±4.37 pg/mL). Accordingly, both BC cells and their CM increased TG, particularly 5637 cells, effect that was blocked with the addition of anti-TF antibody. High concentrations of NETs (500ng/mL) significantly increased proliferation and extracellular matrix invasion and reduced migration of T24 cells, more profusely with NETs derived from BC patients compared to those from healthy controls. NET treatment did not alter the behaviour of 5637 cells. After NET treatment of T24 cells, our observations revealed a cytokine profile that may explain the observed effects. Treating 5637 cells with NETs from controls slightly increased TG via the extrinsic pathway but was not mediated by a further release of TF.

Summary/Conclusion: BC cells induce a procoagulant state through the expression and release of TF. NETs modulate the behavior of high-grade BC cells by enhancing proliferation and invasion while reducing migration, potentially facilitating tumor progression and metastatic implantation. These effects are more pronounced with NETs derived from BC patients and could be attributed to the cytokine profile. While NETs did not alter the procoagulant activity of T24 cells, NETs from healthy controls modestly enhanced TG in 5637 cells via the extrinsic pathway.

Oral Communication – Vessel Wall

OC06-OP01

PLATELET INTERNALIZATION BY VASCULAR SMOOTH MUSCLE CELLS PROMOTES A PRO-COAGULANT PHENOTYPE

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Background: Vascular smooth muscle cells (VSMCs) are highly plastic cells adopting phenotypic switching in vascular diseases and accelerated aging. VSMCs migrate from the media to the intima, while platelets cross the dysfunctional endothelial barrier and can become activated and form clots due to the procoagulant conditions present in vascular disease. Previous studies have demonstrated that VSMCs, which constitutively express tissue factor (TF), support TF-induced thrombin generation (1) and can phagocytose circulating blood cells (2).

Aims: To demonstrate that platelet phagocytosis by VSMCs leads to an increased procoagulant phenotype of VSMCs, potentially contributing to the thrombotic environment and/or a fragilization of the vascular wall in vascular diseases such as instability of atherothrombosis plaques.

Methods: Human adherent VSMCs were cultured in the presence of human platelets activated with various agonists. Platelet internalization at 48 h was assessed using ELISA, cytokine arrays, and fluorescence microscopy. RT-qPCR was performed to evaluate VSMC expression of TF. Human primary VSMCs were incubated with normal plasma to assess their procoagulant status using calibrated automated thrombography (TGA). Controls included VSMCs alone and VSMCs incubated with platelets without co-culture exposition time.

Results: VSMC possess the ability to phagocytose activated platelets. This observation is supported by several experimental approaches. Firstly, immunofluorescent analysis shows the presence of specific platelet markers, such as von Willebrand factor (VWF), inside VSMCs after co-culture with activated platelets. On the other hand, a cytokine array performed after 48 h of co-culture on VSMCs proteins revealed the presence of platelet-derived cytokines like PF4, RANTES, and ENA-78. Expression of TF, the key coagulation trigger, is significantly increased in VSMCs that have internalized platelets. Thrombin generation assays showed a marked increase in thrombin production in VSMCs cultured in the presence of activated human platelets for 24, 48 or 72h, compared with VSMCs alone and VSMCs incubated with platelets without co-culture time. Lag time is shortened and thrombin peak is increased. This increase correlated directly with the number of platelets added in the co-culture, which ranged from 0.0005 to 0.05 G/L, but not with the time of exposure, underlining a quantitative impact of the phagocytosis process on the procoagulant potential of VSMCs.

Summary/Conclusion: These results reveal an active and underestimated role for human VSMCs exposed to platelets in the regulation of hemostasis, particularly in inflammatory or pathological contexts where platelet activation is frequent. Platelet internalization by VSMCs represents a novel mechanism responsible for increasing VSMC thrombin-generating capacity, with potential implications in ageing

complications, plaque instability or thrombus formation in aneurysms. The clinical interest is focused on the administration of anti-aggregant or anti-coagulant drugs in vascular pathologies.

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OC06-OP02

VKORC1-DEPENDENT ACTIVATION OF MGP DRIVES IPSC-DERIVED SMOOTH MUSCLE CELL DIFFERENTIATION

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Background: Vitamin K (VK) acts both as cofactor for the γ-carboxylation of vitamin K-dependent proteins[1] (VKDPs) and as redox-modulating molecule[2]. VKORC1, an enzyme with vitamin K oxido-reductase activity, is responsible for the reduction of VK to VKH_2, thereby enabling the activation of VKDPs. Vitamin K-dependent Matrix Gla protein (MGP) is a potent inhibitor of vascular calcification[3], and has recently been shown to regulate the differentiation of perivascular adipose-derived stem cells differentiation toward smooth muscle cells via BMP2/SMAD signaling pathway[4]. While VKORC1 is known for its role in the activation of coagulation factors in the liver[5], its role in the vasculature remains underexplored.

Aims: The aim of this work is to investigate the mechanistic roles of VKORC1 and MGP in the development and maturation of vascular smooth muscle cells (VSMC).

Methods: An induced pluripotent stem cell (iPSC)-based differentiation protocol was used to generate induced VSMC. CRISPR/Cas9 genome editing was used to knock-out VKORC1 in an iPSC control line, and to restore MGP coding sequence in a patient-derived iPSC line carrying a deletion in the MGP starting codon. Differentiation efficiency of iPSCs into iVSMCs is monitored via VSMC-marker characterization (aSMA, CNN1, pMLC and SM22a).

Results: Knock-out of VKORC1 in iPSCs resulted in a failure to differentiate into iVSMC. Similarly, the patient-derived iPSC line with a deletion in the start codon of MGP failed to undergo iVSMC differentiation. Restoration of MGP coding sequence through CRISPR/Cas9 technology was found to rescue the differentiation capability of these cells.

Summary/Conclusion: Our findings support a model in which VKORC1 promotes iVSMC differentiation, likely by ensuring sufficient levels of VKH_2 to enable MGP g-carboxylation. Carboxylated MGP may stimulates BMP2/SMAD signaling, thereby contributing to proper VSMCs differentiation. To further validate this mechanism, rescue experiments using VK and recombinant MGP supplementation in VKORC1- and MGP-deficient iPSC lines are orgoing to further validate this mechanism.

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OC06-OP03

FACTOR VIII IS A REGULATOR OF ANGIOGENESIS AND PROMOTER OF ENDOTHELIAL BARRIER STABILITY

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Background: Haemophilia A (HA) is a rare bleeding disorder caused by the absence or dysfunction of factor VIII (FVIII). The main clinical manifestation is spontaneous bleeding episodes that primarily involve hemarthroses and intracranial haemorrhages. The factors that initiate haemorrhage are not known, and the onset of haemorrhage is often a random event, occurring either spontaneously or after minimal injury. Attenuated macrovascular and microvascular endothelial function has been observed in animal models and patients with HA suggesting a dysfunction of endothelial cells (ECs) which can be rescued by recombinant FVIII (rFVIII) treatment. ECs can be isolated from peripheral blood as blood outgrowth endothelial cells (BOECs) which can be used as a model to study endothelial function. Current HA treatments include prophylaxis with rFVIII or the non-factor bispecific antibody emicizumab. However, the impact of rFVIII and emicizumab on EC function and barrier stability remains unknown.

Aims: To elucidate the impact of rFVIII modifications and the role of emicizumab on EC function.

Methods: BOECs were isolated from healthy donors and HA patients. HA BOECs was treated *in vitro* with simoctocog alfa, efmoroctocog alfa, rurioctocog alfa pegol, damoctocog alfa pegol, octocog alfa, efanesoctocog alfa or non-factor therapy, emicizumab. EC function was evaluated *in vitro* by tubulogenic, migration, permeability, proliferation and wound healing assays. The binding of rFVIII to BOEC membranes was assessed by TriCEPS technology. *In vivo*, the impact of the different treatments was evaluated in NOD/SCID γ–Null HA (NSG-HA) mice by permeability (Evans blue) and angiogenesis (Matrigel plug injection) assays.

Results: : HA BOECs showed significantly reduced migration, impaired tubulogenesis and increased permeability *in vitro* compared with healthy BOECs. HA BOEC function *in vitro* significantly improved following treatment with simoctocog alfa, which resulted in superior improvement compared to other rFVIIIs and versus emicizumab. Interestingly, *in vivo* treatment of NSG-HA mice with simoctocog alfa, unlike emicizumab, rescued EC in both permeability and angiogenesis assays. Furthermore, simoctocog alfa *in vivo* rescue was greater compared to efmoroctocog alfa or efanesoctocog alfa. These effects on ECs may be mediated by the binding and signalling of simoctocog alfa to the ECs surface.

Summary/Conclusion: A deeper understanding of the extra-coagulative role of FVIII may be crucial to understand the molecular targets associated with impaired EC functionality in HA patients. Furthermore, investigating the effects of different rFVIII products on EC function may contribute to the optimisation of therapeutic strategies, improving patient outcomes and treatment efficacy.

OC06-OP04

FACTOR VIII REGULATES EXTRACELLULAR MATRIX PROTEINS TO STIMULATE ANGIOGENESIS AND VESSEL STABILITY

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Background: Haemophilia A (HA) is an X-linked bleeding condition resulting from mutations in the coagulation factor VIII (FVIII). The main clinical manifestation is spontaneous bleeding episodes that primarily involve hemarthroses and intracranial haemorrhages. Standard replacement therapies are ineffective in preventing the bleeding episodes. It is well established that FVIII is mainly secreted by endothelial cells (ECs) but there is limited understanding of the vascular stability status of HA patients, and little is known about the relationship between FVIII and endothelial function.

Aims: To investigate the role of FVIII in maintaining EC function and homeostasis.

Methods: Blood Outgrowth ECs (BOECs) from HA patients and healthy donors were isolated. HA BOECs were transduced with a lentiviral vector (LV) carrying the B-domain deleted FVIII (LV-FVIII). The transcriptomic and proteomic profile of healthy, HA and LV-FVIII HA BOECs was assessed. Healthy ECs, HA ECs, LV-FVIII HA-ECs, were used to investigate the role of FVIII in ECs function by tubulogenesis, migration, permeability and wound healing assays both in vitro and in vivo in an immunocompromised mouse model of severe HA (NSG-HA mice).

Results: HA BOECs showed in vitro impaired tubule network formation and endothelial cell migration capability higher permeability compared to healthy BOECs. This impaired phenotype was reverted by the re-introduction of FVIII by transduction with LV-FVIII carrying the F8 transgene or by treatment with recombinant human FVIII. The endothelial function of FVIII was also confirmed in vivo in NSG-HA mice, a mouse model of HA, which showed that an altered angiogenesis and vessel permeability could be corrected by exogenous FVIII. The transcriptomic and proteomic profiles of BOECs revealed that FVIII regulates the expression of endothelial basement membrane and extracellular matrix genes and proteins. The exogenous expression of Nidogen2, identified as one of the main a FVIII regulated gene, restored the extracellular matrix integrity and EC function of HA ECs.

Summary/Conclusion: The reduced EC functionality can be explained by the downregulated genes identified in FVIII deficient HA BOECs. These findings suggest that FVIII is not solely a coagulation factor but also plays a crucial role as an endothelial cell factor which promotes vessel stability by upregulating genes involved in extracellular matrix organization. These preliminary results, provide new insights into the possible extra-coagulative role of FVIII and be crucial to understand the key molecular targets missing in HA patients at the cellular level impairing EC functionality. Such knowledge could pave the way for novel therapeutic strategies, ultimately leading to safer and more effective treatments for HA.

OC06-OP05

THE ENDOTHELIUM AS A CENTRAL REGULATOR OF HEMOSTASIS AND THROMBOSIS: AN INTEGRATIVE META-ANALYSIS OF MOLECULAR, FUNCTIONAL, AND CLINICAL EVIDENCE

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Background: The vascular endothelium plays a central role in regulating hemostasis by balancing pro- and anticoagulant factors in response to various pathophysiological stimuli. While individual studies have characterized molecular and functional mechanisms, a systematic analysis integrating these findings with robust clinical data is still lacking. This gap hinders the identification of therapeutic and prognostic targets in conditions associated with endothelial dysfunction, such as sepsis, COVID-19, and thrombotic events.

Aims: To conduct an integrative meta-analysis synthesizing molecular, functional, and clinical data on the role of the endothelium in coagulation and thrombosis, with the goal of identifying potential biomarkers and endothelium-targeted therapeutic strategies.

Methods: A systematic search was performed across PubMed, Embase, Web of Science, and the Cochrane Library up to January 2025. Included studies comprised experimental (in vitro/in vivo), clinical cohort, and randomized controlled trials correlating endothelial markers with hemostatic outcomes. Quantitative analysis was conducted using RevMan 5.4 and R (metafor package), calculating risk ratios (RR), standardized mean differences (SMD), and assessing heterogeneity using the I^2 index. The quality of evidence was assessed using the GRADE system.

Results: A total of 72 studies were included (n = 31,452 participants). Increased expression of von Willebrand factor (VWF; SMD: 1.12; 95% CI: 0.89–1.36) and P-selectin (SMD: 0.95; 95% CI: 0.62–1.28) was associated with a 2.57-fold higher risk of venous thrombosis (RR: 2.57; 95% CI: 1.98–3.19) and a 3.14-fold increased risk of disseminated intravascular coagulation (DIC; RR: 3.14; 95% CI: 2.21–4.32). Reduced levels of thrombomodulin (TM) were significantly associated with a 2.89-fold higher risk of in-hospital mortality (RR: 2.89; 95% CI: 2.10–3.45). Elevated endothelial microparticles were consistently observed in pro-thrombotic endothelial dysfunction contexts such as sepsis and severe COVID-19. Overall heterogeneity was moderate (I^2 = 48%), but subgroup analyses revealed amplified effects in COVID-19 patients, with a 3.45-fold increased risk of thrombotic events (RR: 3.45; 95% CI: 2.71–4.26).

Summary/Conclusion: This meta-analysis integrates, for the first time, molecular, functional, and clinical evidence on the role of the endothelium in hemostasis and thrombosis. The findings highlight the endothelium as a functional link between inflammation and coagulation, particularly in critical illness contexts. VWF, TM, and P-selectin emerge as potential prognostic biomarkers, while endothelial microparticles reflect relevant functional outcomes. The consolidation of this evidence enhances the understanding of pathophysiological mechanisms and supports the development of endothelium-targeted therapeutic approaches.

OC06-OP06

MODULATION OF PLATELET INTERACTION WITH COLLAGEN BY CARBAMYLATION, A NEPTM OCCURRING DURING PATHOPHYSIOLOGICAL VASCULAR AGEING

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Background: Vascular ageing is associated with extensive extracellular matrix (ECM) remodelling and leads to arterial wall stiffening and thickening. Due to their long half-life, ECM proteins including collagens are exposed to molecular ageing characterized by non-enzymatic post-translational modifications (NEPTMs) that progressively alter their structure and function. One of these NEPTMs, carbamylation, consists of binding of isocyanic acid (mainly coming from urea dissociation) to amino groups of proteins. This NEPTM is exacerbated during chronic kidney disease (CKD) due to hyperuraemia. CKD leads to cardiovascular and thromboembolic complications, and paradoxically, to an increased haemorrhagic risk. Different risk factors have been reported in the literature such as the accumulation of uraemic toxins, chronic inflammation, endothelial dysfunction, hypercoagulability or anaemia. However, whether carbamylation of collagen, the main thrombogenic ECM component of the vascular subendothelium, may impact haemostasis and thrombosis, has never been investigated so far.

Aims: This study aims to provide a mechanistic insight into the impact of collagen carbamylation on various steps of thrombus formation, including its interaction with plasma von Willebrand factor (VWF), platelet adhesion under static conditions, the signalling cascade triggered by platelet activation, and platelet aggregation.

Methods: Rat type I collagen was carbamylated with sodium cyanate and the impact of collagen carbamylation on plasma VWF binding, platelet adhesion under static and flow conditions, platelet signalling and platelet aggregation was evaluated.

Results: Carbamylated collagen reduces by two-fold its ability to bind plasma VWF compared to control collagen and leads to a drastic decrease by more than 80% of platelet interaction with collagen after 6 min perfusion (human whole blood, 1,500 s^{-1}). Washed platelet adhesion to carbamylated collagen under static condition was slightly reduced after 30 min and associated with reduced platelet activation and signalling compared to control collagen. Finally, human washed platelet aggregation was also strongly decreased by carbamylated collagen. Impact of ECM carbamylation in *in vivo* models of haemostasis and thrombosis is currently under investigation.

Summary/Conclusion: These results suggest that carbamylation of collagen may strongly affect platelet interaction with vascular subendothelium and play a critical role in haemorrhagic/thrombotic complications associated with CKD.

Oral Communication – Acquired Bleeding

OC07-OP01

INCIDENCE AND ANATOMICAL DISTRIBUTION OF RECURRENT MAJOR BLEEDING IN INCIDENT ATRIAL FIBRILLATION PATIENTS ON ORAL ANTICOAGULANTS: A POPULATION-BASED COHORT STUDY IN THE NETHERLANDS (2015-2022)

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Background: Major bleeding (MB) represents the most serious and potentially life-threatening complication among patients with atrial fibrillation (AF) receiving oral anticoagulant therapy. While the initial occurrence of MB has been widely investigated, population-based studies on recurrent major bleeding (RMB) remain scarce, particularly in direct oral anticoagulants (DOACs) users.

Aims: We aimed to estimate the incidence and characterize the anatomical sites of RMB in patients with incident AF receiving oral anticoagulation therapy.

Methods: We conducted a population-based cohort study using routinely collected health care data from Statistics Netherlands ("Centraal Bureau voor de Statistiek", CBS). As this study used de-identified data from CBS, informed consent was not required. The study cohort consisted of adult Dutch residents (aged ≥18 years) with incident atrial fibrillation who initiated oral anticoagulants and subsequently experienced at least one hospitalization for MB between 2015 and 2022. Follow-up started at the first MB admission. RMB event was defined as at least two distinct hospital admissions with MB listed as the primary diagnosis. Incidence rates of RMB were calculated per 100 person-years (PYs) with 95% confidence intervals (CIs). Analyses were further stratified by CHA_2DS_2-VA score (0-1, 2, 3, 4, ≥5) and HAS-BLED score (0-2, 3-5, ≥6), both assessed at the first MB event. The anatomical locations of (R)MB episodes were reported as frequencies and proportions.

Results: Among 468,318 Dutch residents with incident AF, 7266 adult patients developed a MB event following the initiation of oral anticoagulants. Of these patients, 1235 experienced RMB (17.0%). Among RMB patients, 40.3% were female. Prior to the first MB event, 2291 patients (31.5%) received vitamin K antagonists (VKAs) and 4975 (68.5%) DOACs. The incidence rate of RMB was 10.9 per 100 PYs (95%CI: 10.3-11.5). The risk of recurrence was highest shortly (1-3 months) after the initial MB event and declined over time. The incidence rate of RMB was similar between patients receiving DOACs (10.7, 95%CI: 10.0-11.5) compared with VKAs (11.1 per 100 PYs, 95%CI: 10.1-12.2), and the absolute difference was modest. Patients with a higher CHA_2DS_2-VA score generally saw a higher incidence rate of RMB - score 0-1: 8.3 per 100 PYs (95%CI: 6.9-10.0); score 2: 9.3 (95%CI: 8.1-10.6); score 3: 11.6 (95%CI: 10.4-12.9); score 4: 10.7 (95%CI: 9.5-12.1); score ≥5: 13.5 (95%CI: 12.0-15.2). Similarly, incidence rate of RMB increased progressively with the increase of HAS-BLED scores - score 0-2: 10.1 per 100 PYs (95%CI: 9.0-11.2); score 3-5: 11.1 (95%CI: 10.4-11.9); score ≥6: 15.7 (95%CI: 10.4-22.9). The most frequently involved anatomical site of RMB was gastrointestinal (561 MBs, 45.3%), followed by intracranial (361 MBs, 29.2%), genitourinary (162 MBs, 13.1%), and pulmonary bleeds (98 MBs, 7.9%). Among patients with recurrent events, 80.5% had bleeding at the same anatomical locations for both the first and recurrent MBs.

Summary/Conclusion: Incident atrial fibrillation patients with an initial MB had a high incidence of RMB (especially shortly after the incident event), with similar rates between DOAC and VKA users. Higher CHA_2DS_2-VA and HAS-BLED scores were associated with an increased risk of RMB. RMB often occurred at the same anatomical sites as the first MB event. These results highlight the importance of clinical monitoring following a MB episode, along with identifying and managing risk factors to mitigate the risks.

OC07-OP02

QUANTITATIVE TARGETED PROTEOMICS IDENTIFIES NOVEL BIOMARKERS FOR PREDICTION OF MAJOR BLEEDING DURING ANTICOAGULATION: A PROSPECTIVE CASE-COHORT STUDY

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Background: About 1-3% of patients on oral anticoagulants suffer a major bleeding annually. Current risk assessment models insufficiently identify high-risk patients, and addition of novel biomarkers has the potential to improve this.

Aims: We aimed to identify novel circulating biomarkers for major bleeding during anticoagulation through quantitative targeted proteomics.

Methods: The BLEEDS is a population-based cohort including 16,570 patients initiating a vitamin K antagonist (VKA) from three anticoagulation clinics in the Netherlands between 2012-2014. Inclusion followed an opt-out procedure and 99% of the elegible population was included. Patients were followed for a median follow-up of 13 months from initiation until termination of VKA, migration to an area not covered by the anticoagulation clinics, death, the occurrence of a major bleeding, or the end of the study. For efficiency reasons, we established a case-cohort study and included all 326 patients with major bleeding during follow-up and a random subcohort of 414 patients. Plasma concentration of 270 proteins was measured at baseline (i.e., before the bleeding) using quantitative targeted proteomics with internal standards. Plasma levels were compared between major bleeding patients and the subcohort with Wilcoxon rank-sum test (p-value≤0.05 adjusted for false discovery rate) and stratified by sex. Association with major bleeding was assessed through weighted Cox regression, with proteins modeled both continuously and categorical (quartiles) and further adjusted for age and sex.

Results: Plasma was available for 264 patients with major bleeding and 362 subcohort members. Patients who experienced a major bleeding were older (76 years [standard deviation 11] vs 70 years, [standard deviation 12]) and more frequently male (146, 56% vs 188, 53%) compared with the subcohort. The most frequent indication for VKA was atrial fibrillation for both the major bleeding patients and the subcohort (196, 76%, and 250, 71%, respectively). In total, 139/270 proteins passed the quantification criteria. The levels of 50 proteins were increased or decreased in the major bleeding patients compared with the subcohort. In particular, neuropilin-2, insulin-like growth factor-binding protein 2, protein AMBP, β-2-microglobulin, C-reactive protein, serum amyloid A-1 protein and leucine-rich α-2-glycoprotein were, among others, ≥15% increased in the major bleeding patients compared with the subcohort. Decreased and increased proteins were different by sex and stronger in males compared with females. Complement factor 5 levels in quartile 4 were associated with a 2.9-fold increased risk of major bleeding compared to levels in quartile 1 (95% confidence interval [CI] 1.7-5.0). Patients with elevated β-2-microglobulin had also a 2.7-fold increased risk of major bleeding (95%CI 1.5-2.7). On the contrary, patients with albumin levels in the highest quartile had a

decreased risk of major bleeding compared with patients with levels in the lowest quartile (hazard ratio 0.3, 95%CI 0.2-0.6).

Summary/Conclusion: Levels of proteins related to inflammation and vascular dysfunction were associated with major bleeding during anticoagulation and can be used to optimize its prediction. External validation of these results (in the UK biobank) is ongoing and it is expected to be ready in the fall of 2025.

OC07-OP03

DIRECT ORAL ANTICOAGULANTS IN THE POSTPARTUM PERIOD: REPORT OF REAL-LIFE EXPERIENCE IN THE HEMOTHEPP COHORT STUDY

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Background: Anticoagulants are commonly prescribed in women during postpartum, mainly to prevent venous thromboembolism (VTE), which is a potential life-threatening complication. Tolerance of low molecular weight heparin (LMWH) is variable, and compliance may not be optimal. Vitamin K antagonists (VKA) are prescribed as a relay of LMWH for VTE treatment after delivery in breastfeeding women, or in women on long-term anticoagulation, but their use requires repeated biological testing. DOA are also widely prescribed to treat VTE, except during pregnancy/postpartum, due to lack of evidence on their use in this context. In particular, there is much concern about the risk of abnormal uterine bleeding (AUB) on DOA during postpartum. However, DOA could be an alternative to LMWH and VKA during postpartum, in particular in non-breastfeeding women with side effects of LMWH, or reluctant to injections.

Aims: We report here our real-life experience of DOA prescription, tolerance and efficacy during postpartum in the women enrolled in the French “Hemorrhages and Thromboembolic Venous Disease of the Postpartum” (HEMOTHEPP) cohort study.

Methods: We compared women on oral anticoagulants during postpartum, either DOA or VKA. For this purpose, we collected clinical data on all women on DOA or VKA between delivery and 3 months postpartum.

Results: Among the 20 238 pregnant women included in HEMOTHEPP, 45 received oral anticoagulants during postpartum: 23 DOA and 23 VKA. Among the 23 women on DOA during postpartum, 15 received rivaroxaban and 8 apixaban. No woman was breastfeeding. The reason for DOA prescription was VTE treatment in 18 (78.3%) women, resumption of long-term anticoagulation in 2 women, and VTE prophylaxis in 3 women.

Among the 20 women on DOA for curative indication, 19 received a full dose, and 8/19 (42.1%) received a loading dose (6 with rivaroxaban 30 mg daily, and 2 with apixaban 10 mg daily). 2 out of 20 (10.0%) women experienced hemorrhagic events, i.e. AUB, without need for hospitalization, surgery or red blood cells transfusion: one on full-dose apixaban, and one on full-dose rivaroxaban. None of these 20 women experienced VTE recurrence during postpartum.

In 3 other cases, DOA were prescribed off-label, at half dose (rivaroxaban 10 mg/day), as a thromboprophylaxis, in women with poor cutaneous tolerance to LMWH.

The median delay between delivery and DOA start was 14 (7-41) days, with 10 (6-18) days for apixaban and 31 (10-44) days for rivaroxaban. The median duration of DOA treatment was 4 (3-7) months, with 4 (3-7) months for apixaban and 4 (2-7) months for rivaroxaban.

When compared to women on VKA during postpartum, women on DOA had a higher body mass index and were less often breastfeeding at hospital discharge. Other characteristics were similar between the 2 groups of women.

When interviewed on their experience of DOA during postpartum, several women expressed some relief to have resumed subcutaneous LMWH.

Summary/Conclusion: This is the first report in literature on a real-life cohort of 23 women treated with DOA during postpartum. DOA were prescribed mainly to treat a VTE diagnosed during pregnancy or postpartum, as a relay to LMWH. Curative treatment with DOA during postpartum was overall well tolerated and showed an efficacy similar to VKA. Non-severe AUB were reported in around 10% of women on DOA at standard dose, in line with rates observed outside postpartum. DOA appear as a potential safe alternative treatment to LMWH during postpartum in non-breastfeeding women.

OC07-OP04

MONITORING OF ANTI-XA ACTIVITY OF ENOXAPARIN IN HEMODIALYSIS PATIENTS

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Background: Anticoagulation of the extracorporeal circuit with low molecular weight heparin (LMWH) is essential for an adequate dialysis session. The concentration of LMWH during dialysis can be reflected by measuring anti-Xa activity.

Aims: Our objective was to study the kinetics of anti-Xa activity in the circuit during the session at different time points and to assess the risk of systemic passage in order to adjust the LMWH dosage for hemodialysis patients.

Methods: The study is descriptive, prospective, and single-center, involving 24 adult hemodialysis patients using enoxaparin as an anticoagulant in a single bolus dose based on weight in the circuit. Anti-Xa activity was measured before, during, and at the time of disconnection from the session. Dialysis characteristics, including the LMWH dosage, were collected and processed for biostatistical analysis.

Results: The average anti-Xa activity values at time H0, before enoxaparin injection, were 0.13 IU/ml (0.02 – 0.46), within the target range of < 0.5 IU/ml. Subsequently, the levels increased from H0 to H2 during dialysis, with 0.64 IU/ml (0.1 – 1.27) at H1 and 0.61 IU/ml (0.16 – 1.05) at H2. They then decreased until the time of disconnection, reaching 0.45 IU/ml (0.1 – 1.31). The anti-Xa activity profile was characterized by initially almost undetectable residual values, with 90 % of patients within the target range. Activity peaked during the first and second hours, with 70 % of patients reaching the desired therapeutic levels between 0.5 and 1 IU/ml, and 28 % had anti-Xa activity > 1.0 IU/ml without hemorrhagic manifestations. However, only 30 % of patients remained within the target range until the end of the session. The values then decreased by 25 % by the end of the process, with 60 % of patients within the target range of < 0.5 IU/ml.

Summary/Conclusion: Hemodialysis does not effectively eliminate enoxaparin, leading to the possibility of systemic accumulation. This highlights the need for closer monitoring of anti-Xa activity to achieve a balance between anticoagulation and the risk of bleeding.

OC07-OP05

WORSE CLINICAL OUTCOME IN YOUNG WOMEN TREATED WITH VITAMIN K ANTAGONISTS FOR MECHANICAL HEART VALVES COMPARED TO MEN: A DUTCH POPULATION-BASED COHORT STUDY

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Background: Sex differences have been established in valvular heart disease; mitral valve pathology occurs more often in women, while aortic valve pathology in men. Patients in whom the affected valve is replaced by a mechanical prosthesis require lifelong anticoagulant treatment with vitamin K antagonists (VKAs). While previous studies have reported poorer quality of VKA treatment in women, limited research has been conducted specifically in patients with mechanical heart valves (MHVs).

Aims: To examine quality of treatment and clinical outcomes, including bleeding, venous and arterial thromboembolism, separately in men and women with a MHV treated with VKAs in the Netherlands. In addition, we aim to assess differences between men and women stratified by age.

Methods: Data from 17 Dutch anticoagulation clinics were linked on an individual level to microdata from Statistics Netherlands. Patients with a MHV, identified through hospitalization data and cross-validated by anticoagulation clinic records, who received VKA treatment between 2013 and 2019 were included. The index date was defined as the first MHV hospitalization discharge and patients were followed until death, VKA discontinuation, or 31-12-2019. Quality of VKA treatment was assessed every 6 months after index by the time in, above and below therapeutic range (TTR, TAR, TBR) and INR variability (VGR). Clinical outcomes were identified by first primary or main in-hospital diagnoses and primary causes of death. Incidence rates (IR) and corresponding 95% confidence intervals (95%CI) of major/clinical relevant bleeding and arterial/venous thromboembolic events were assessed separately and expressed per 100 person-years (PYs), stratified by gender and age.

Results: In total, 3527 MHV patients (38% women, median age 67 years [IQR 58-76]) were included with a median follow-up of 3.0 years (IQR 1.3-4.9). Most patients had an international normalized ratio (INR) target range between 2.5-3.5 (50%) or 3.0-4.0 (32%). Among VKA users aged <55 years, women (N=228) demonstrated poorer quality of treatment compared to men (N=395), with lower TTR and higher TBR, TAR and VGR. The average TTR was 58.4 (95%CI 55.6-61.3) during the first six months and 64.0 (61.0-66.9) from 6-12 months after index among young women compared to 61.1 (59.1-63.1) and 67.0 (64.6-69.3)

among young men. Differences were less pronounced among patients aged ≥55 years. Women <55 years seemed to have a higher IR [95%CI] of both bleeding (2.52 [1.49, 3.98] vs 1.03 [0.51, 1.84]) and thromboembolism (2.83 [1.73, 4.38] vs 1.70 [1.01, 2.69]) compared to similarly aged men. However, IR were similar for women and men ≥55 years (bleeding 2.85 [2.27, 3.53] vs 2.81 [2.36, 3.33]; thromboembolism 3.71 [3.05, 4.48] vs 3.29 [2.80, 3.85], respectively).

Summary/Conclusion: Women with a MHV aged <55 years exhibited poorer quality of VKA treatment and higher bleeding and thromboembolic event rates than similarly aged men. These disparities may reflect differences in valve pathology and corresponding INR targets or biological factors such as menstrual cycle related fluctuations in coagulation factors, complicated anticoagulant treatment during pregnancy, and use of hormonal contraceptives. Our findings highlight the need for tailored anticoagulation strategies in younger female MHV patients.

OC07-OP06

EVALUATION OF DOAC-LEVELS IN ACUTELY ADMITTED FRAIL OLDER PATIENTS: AN EXPLORATORY STUDY (THE DOAC-FRAIL STUDY)

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Background: DOAC-use among frail older patients increases with the aging population. Extrapolating risk-benefit ratios from younger to geriatric populations may not be valid. No recommendations are available on DOACs in this population and data on DOAC levels during acute illness is lacking.**Aims:** To examine quality of treatment and clinical outcomes, including bleeding, venous and arterial thromboembolism, separately in men and women with a MHV treated with VKAs in the Netherlands. In addition, we aim to assess differences between men and women stratified by age.

Aims: This study evaluates DOAC levels in (frail) older patients acutely admitted to the hospital.

Methods: This cohort study included patients aged ≥65 years, using a DOAC and acutely admitted to the internal medicine. DOAC levels were measured as soon as possible after admission. The time interval between DOAC intake and blood sample collection was recorded.

Results: In 90.5% of the 42 patients, NVAF was the DOAC indication. Apixaban was prescribed most often(52.4%) and 95.2% had an adequate dose. 54,8% of all DOAC levels measured(54.8%) were outside the on-therapy range (40.5% above,14.3% below). Patients admitted with an infection had lower odds of a level above the on-therapy range(p 0.003). Impaired renal function<30ml/min/1.73m2 , a low BMI(<20), and rivaroxaban had higher odds of a DOAC level above the on-therapy range. Patients using a PPI had lower odds of having a DOAC level below the on-therapy range (p 0.03).

Summary/Conclusion: This is the first study that evaluates DOAC levels in frail older patients during acute hospitalization. Half of all DOAC levels were outside the on-therapy range emphasizing the importance of gaining insight into DOAC-use in the frail older population. Future research should focus on causes for deviated DOAC levels and their implications to establish guidelines for DOAC management in this population.

Oral Communication – Platelets

OC08-OP01

NATIVE ADAMTS13 N-GLYCANS INFLUENCE AUTOIMMUNE RECOGNITION IN IMMUNE-MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: ADAMTS13 is a heavily glycosylated plasma protein, carrying ten N-linked glycans with complex structures capped by terminal sialic acids. In immune-mediated thrombotic thrombocytopenic purpura (iTTP), patient autoantibodies can recognize all ADAMTS13 domains but are known to predominantly bind the glycosylated cysteine-rich and spacer (CS) domains. To prevent pathological autoantibody recognition, novel ADAMTS13 therapeutics are being designed to structurally shield the immunogenic spacer regions. Despite these advances, the precise role of native ADAMTS13 glycosylation in its autoimmune recognition remains largely unexplored.

Aims: To explore the impact of ADAMTS13's native glycosylation on its autoimmune recognition in iTTP.

Methods: Recombinant human ADAMTS13 was treated with glycosidases to study the role of glycosylation in autoantibody recognition. Neuraminidase selectively removed terminal sialic acids, while PNGaseF cleaved entire N-linked glycans. Glycan removal was confirmed by SDS-PAGE and western blot analysis. Pathological anti-ADAMTS13 IgG binding was assessed by ELISA across different ADAMTS13 domains using plasma from 20 acute iTTP patients. Subsequently, autoantibody binding against glycosidase-treated ADAMTS13 was evaluated, with all results expressed relative to binding against untreated control ADAMTS13.

Results: Enzymatic treatment of ADAMTS13 with neuraminidase and PNGaseF successfully removed terminal sialic acids and complete N-linked glycans, respectively. IgG profiling of acute iTTP patients confirmed the presence of pathological autoantibodies targeting multiple ADAMTS13 domains. Compared to untreated ADAMTS13, neuraminidase treatment minimally reduced autoantibody binding. In contrast, complete removal of N-linked glycans significantly decreased pathological IgG binding. When stratified by individual autoantibody profiles, patients with the 'anti-CS only' profile showed significant reduced binding following N-glycan removal. Similarly, patients with autoantibodies targeting multiple domains, including the CS domains, exhibited significantly lowered binding. Notably, no reduction in autoantibody binding was observed in patients lacking anti-CS antibodies, suggesting that native N-glycans may modulate epitope recognition specifically within the CS domains.

Summary/Conclusion: Our findings highlight the relevance of native glycosylation in ADAMTS13's autoimmune recognition in iTTP. Removal of N-glycans significantly decreased pathological autoantibody binding, indicating these glycans may form an integral part of the recognized epitopes. This reduction was especially pronounced in iTTP patients with autoantibodies targeting the CS domains. Notably, two of the four N-glycans on the CS domains (Asn614 and Asn667) are positioned adjacent to the immunogenic hotspots (Arg602-Ser610 and Val657-Gly666) we previously identified. Hence, these N-glycan bearing

residues may provide interesting targets for future mutagenesis when trying to evade the immune recognition in iTTP.

OC08-OP02

SPLEEN TYROSINE KINASE PHOSPHORYLATION AT Y317 NEGATIVELY REGULATES BOTH ITAM AND HEMITAM-MEDIATED SIGNALING AND FUNCTION IN PLATELETS

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Background: Spleen tyrosine kinase (Syk) is expressed in a variety of hemopoietic cells and has been known that Syk phosphorylation regulates downstream signaling events in platelets upon stimulation of glycoprotein VI (GPVI) and C-type lectin-like receptor II-type (CLEC-2) as ITAM and hemITAM receptors, respectively. This study focuses on the role of a specific phosphorylation site, Tyrosine 317, located in linker region that separates the amino-terminal, tandem pair of SH2 domains from the carboxyl-terminal catalytic domain, in the regulation of Syk function. The amino acid sequence surrounding phosphotyrosine 317 matches the consensus sequence for recognition by the phosphotyrosine-binding (PTB) domain of the protooncogene product, c-Cbl.

Aims: Characterization the role of Syk Y317, as a novel signaling molecule, provides comprehensive understanding of signaling networks in platelets and could be applicable for other cellular system which express Syk in order to pave the way for more targeted therapeutic interventions.

Methods: We generated mice expressing Syk Y317F with CRISPR-Cas9 technique then compared platelet functions including Aggregation, secretion, and integrin adhesion in WT littermates and Syk Y317F after activation with collagen-related peptide (CRP) and CLEC-2 crosslinking. On the physiological level, we determined the time to occlusion in $FeCl_3$ injurythrombosis model and tail bleeding times.

Results: Platelets from homozygous Syk Y317F mice showed enhancement of functional responses after activation with CRP and CLEC-2 crosslinking compred to WT littermates with no signifecant diffrences in PAR-4 or purinergic receptors responses. CRP- and CLEC-2 mAb- induced signaling events, including LAT and PLCɣ2, were enhanced in Syk Y317F platelets. Moreover, the time to occlusion in the $FeCl_3$ injury model was increased in Syk Y317F mice compared to WT littermates, However there was no significant difference in the tail bleeding times.

Summary/Conclusion: Syk Y317 negatively regulates GPVI and CLEC-2- mediated functional response and signaling events in platelets.

OC08-OP03

GENETIC AND CLINICAL CHARACTERIZATION OF CZECH PATIENTS WITH MYH9-RELATED DISORDER

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Background: The *MYH9* gene encodes human nonmuscle myosin heavy chain IIA, which occurs in different tissues including platelets, leukocytes, kidneys and the cochlea. Pathogenic variants in this gene cause very rare disorder, MYH9-related disorder (MYH9-RD). It is an autosomal dominant inherited disease characterized by macrothrombocytopenia and leukocyte inclusion bodies (Döhle inclusions). So far, 157 pathogenic variants of the *MYH9* gene have been found. Due to occurence in different tissues patients can manifest nonhematological symptoms like deafness, nephropathy and cataracts, which may complicate to establish an accurate diagnosis. The prevalence of the MYH9-RD is difficult to determine. Mild forms may be discovered incidentally and severe forms may be misdiagnosed as other disorders. The indicated prevalence in Italy was 4:1,000,000, the estimated prevalence was 1:25,000. Accordingly, the prevalence in the Czech Republic should be 44 or 440 patients.

Aims: Find causal variants to confirm the diagnosis of MYH9-RD and evaluate the clinical status of patients with MYH9-RD focusing on bleeding symptoms.

Methods: We have investigated 37 Czech patients and their relatives from 18 families with macrothrombocytopenia suspected of MYH9-RD. The primary screening including blood count and platelet count was performed. Further, May-Grünwald Giemsa staining of peripheral blood smears for Döhle inclusions detection was done. Light transmission aggregometry was performed in some patients. In the end, NGS coagulation-thrombotic panel and Sanger sequencing was used for *MYH9* variant detection.

Results: The pathogenic variant in *MYH9* was not found in 7 patients. These patients were therefore excluded. However, one of these patients had Döhle inclusions, another two had partly sensorineural hearing loss or easy bruising. The genetic testing confirmed the diagnosis of MYH9-RD in 30 patients from 12 families. Overall, seven different pathogenic variants in *MYH9* were detected in exon 2, 17, 27, 39. One variant found in exon 2 was novel p.Ala44fs. The phenotype within a family was usually the same. Most patients had Döhle inclusions (86%, 18 out of 21). Clinical data about sensorineural hearing loss, cataract, nephropathy was available only for 10 patients. Five patients presented sensorineural hearing loss. Only 1 patient manifested sensorineural hearing loss, cataract, nephropathy altogether. Information about bleeding manifestation was available for 23 patients with MYH9-RD; 65% patients had bleeding symptoms. Fourty five percent (9 out of 20) patients bruised easily. Most women with MYH9-RD (59%, 10 out of 17) suffered by menorrhagia. Four patients gave birth, thromboconcentrate was given 2 of them. Epistaxis had 32% patients (6 out of 19). Twelve patients underwent tooth extraction, only 4 of them suffered by an increased bleeding and had to be given antifibrinolytics or thromboconcentrate.

Summary/Conclusion: We confirmed the diagnosis of MYH9-RD at 30 out of 37 patients by the molecular genetic analysis, one pathogenic variant detected was novel. Patients with MYH9-RD were not severe bleeders. However, 65% of them manifested bleeding symptoms and 43% needed some treatment when underwent surgical procedures or tooth extraction.

OC08-OP04

EXTRACELLULAR VESICLES FROM ACTIVATED PLATELETS ARE DECORATED WITH COAGULATION-ASSOCIATED PROTEINS

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Background: Platelet activation and elevated levels of platelet-derived extracellular vesicles (PDEVs) are associated with an increased risk of deep vein thrombosis (DVT). EVs from activated platelets are enriched with both proteins and lipids and exhibit enhanced procoagulant activity. To what extent proteins displayed on the surface of PDEVs can be used as diagnostic biomarkers for DVT is unknown.

Aims: To characterize the proteome of EVs derived from unstimulated (control) and activated platelets, and to investigate whether proteins enriched on PDEVs from activated platelets can be detected on the surface of the PDEVs and be used as diagnostic biomarkers for DVT.

Methods: The proteome of EVs isolated from control or TRAP6-activated washed platelets collected from healthy volunteers was analyzed by LC-MS/MS. Immunocapture bead-based flow cytometry was applied to investigate the surface-display of selected proteins enriched on EVs derived from TRAP6-activated washed platelets. Plasma EVs were isolated from citrated plasma of healthy individuals and patients admitted to hospital (Akershus University hospital, Norway) with suspicion of DVT in which DVT was confirmed (DVT patients, n=10) or rejected (non-DVT patients, n=10). The plasma EVs were analyzed by immunocapture bead-based flow cytometry. The discriminatory ability of the proteins was evaluated by receiver operating curves. The study was approved by the Regional committee for medical research ethics and informed written consent was obtained from all participants.

Results: EVs from TRAP6-activated platelets were enriched with 129 proteins compared to EVs from unstimulated, control, platelets, many of which associated with platelet activation (CD62P), coagulation (FV, vWF) and inflammation (CCL5). Immunocapture bead-based flow cytometry analysis of the isolated EVs revealed an increased surface-display of CD41, CCL5, CD62P and CD9 on PDEVs from activated platelets. The surface-display of CD41, CD9, CD151, PF4, and CD62P, along with particle number, differentiated DVT patients from healthy controls (area under the curve (AUC) = 0.82, 95% CI: 0.63-1.00), but not from non-DVT patients (AUC = 0.55, 95% CI: 0.25-0.82).

Summary/Conclusion: EVs from activated washed platelets are enriched with proteins that play a role in inflammation and coagulation, of which some are displayed on their surface. Detection of such proteins on the EV surface may serve as a biomarker for conditions involving platelet activation.

OC08-OP05

DETECTION OF HYPERFUNCTIONING PLATELETS AND NOVEL PLATELET SUBTYPES: TOWARDS A NOVEL PRINCIPLE FOR RISK STRATIFICATION OF THROMBOSIS

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Background: Prevention of thrombosis is a critical objective in healthcare. Several risk factors for increased thrombosis risk are known, e.g. smoking, obesity and diabetes, but biomarkers for additional stratification of thrombosis risk within these patient groups are lacking. Platelets are key players in thrombus formation and represent a target for intervention to reduce thrombosis risk. Primary prevention, however, is not feasible without additional risk stratification because of bleeding risks and associated costs. But platelets are heterogeneous in size, density, and receptor content and it is not known how variations in the platelet pool affects thrombosis risk. The procoagulant subtype of platelets has been widely studied, but detailed information about their exact phenotypes is missing. In addition, while there is evidence of an "angry" platelet phenotype in diabetes, little is known about specific platelet subtypes and their detailed phenotypes. Outside of diabetes, knowledge about hyperfunctioning platelets and their exact phenotype is limited. By studying platelet function and phenotypes at high resolution, we aim to identify new biomarkers for thrombosis risk stratification.

Aims: We aim to develop a highly polychromatic panel suitable for conventional flow cytometry to detect hyperfunctioning platelets and phenotypic subtypes. Our goal is to identify clinically relevant platelet subtypes that predict thrombosis risk more accurately in high-risk individuals, to tailor anti-platelet treatment effectively.

Methods: We designed a novel multi-colour flow cytometry panel that utilises modern innovations in flow cytometers and new dyes to map distinct platelet phenotypes in hyperfunctioning platelets. This panel consists of 18 colours with antibodies against β2M, CD9, CD29, CD31, CD32, CD36, CD41, CD42a, CD42b, CD47, CD49b, CD61, CD62P, CD63, CD107a, CLEC2, GPVI and PAC-1. This panel can be adjusted to accommodate Annexin V for the detection of procoagulant platelets as well. We have measured 16 healthy individuals, of which 5 are aged above 60 years and 56% are male, with this panel and a combination of agonists at varying concentrations. All blood donors gave informed consent, and the study was approved according to the Helsinki declaration.

Results: We have designed a working panel for conventional flow cytometry that uses 18 colours for the detection of hyperfunctioning platelets. We have combined this with bioinformatic tools such as clustering and dimensionality reduction algorithms, and we have detected novel phenotypes in healthy controls. However, the clinical relevance of these is yet to be shown. In healthy controls, considerable variability was observed both in vehicle control treated platelets and agonist treated platelets, with the middle of the dose response curve varying between individuals.

Summary/Conclusion: We have developed a functioning methodology for the detection of hyperfunctioning platelets and novel phenotypic subtypes. This has the possibility to lead to better risk stratification of thrombosis risks in the future. This methodology is now ready for application to known high-risk groups such as diabetes patients.

OC08-OP06

THE CALM BEFORE THE STORM: HOME BLOOD COUNT MONITORING FOR PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: P Immune thrombocytopenia (ITP) is an immune-mediated disorder characterized by increased platelet destruction and impaired platelet production, leading to thrombocytopenia of varying severity. In some patients, the clinical course is marked by sudden, unpredictable fluctuations in platelet levels, usually manifesting as mild skin and mucosal bleeding. However, a minority of patients may develop life-threatening complications. High-risk patients (patients with unstable platelet count) must therefore be closely monitored, often requiring outpatient blood count sampling multiple times per month, which presents a significant burden for both the patient and the healthcare system. The HemoScreen device is a point-of-care hematology analyzer capable of performing a complete blood count with differential from capillary blood, making it a promising candidate for at-home monitoring of platelet levels in high-risk ITP patients.

Aims: Our goal was to assess the feasibility of blood sampling in a home setting, verify the accuracy of measurements, increase the safety of high-risk ITP patients, and reduce the frequency of outpatient visits.

Methods: Selected ITP patients with unstable platelet count were trained and, after signing informed consent, loaned the HemoScreen device for home use. Capillary blood testing was generally performed twice weekly as agreed. Results were emailed to the assigned physician, who contacted the patient remotely and adjusted treatment as needed. Upon returning the device, patients subjectively evaluated their participation using a questionnaire. Measurement precision was assessed using red blood cell parameters MCV, MCH, and MCHC that should not change significantly over time in ITP patients.

Results: A total of 10 patients with ITP were included in the project. The median age was 44 years (range 20–68), with a predominance of females (70%, 7/10). The average loan period for the device was 34 days (range 24–44), and the average number of measurements was 10 (range 8–12). All patients (100%, 10/10) successfully performed capillary blood collection and testing, with 80% (8/10) managing it without assistance. In two cases, a sudden drop in platelet count was detected remotely before bleeding symptoms developed, prompting urgent hospitalization for rescue therapy. In four patients, treatment was adjusted on an outpatient basis based on home measurements. In 50% of cases (5/10), no additional outpatient visits were needed during the study period. The measured values of MCV, MCH, and MCHC showed very low variability within the standard ranges for each parameter (mean SD for MCV: 0.79, MCH: 0.33, MCHC: 4.01).

Summary/Conclusion: Home blood count monitoring using the HemoScreen device in high-risk ITP patients is feasible, safe, and contributes to improved patient´s treatment management and reduced healthcare burden.

Oral Communication – VTE

OC09-OP01

HIGH LEVELS OF POLYUNSATURATED N-3 FATTY ACIDS IN SERUM ARE INVERSELY ASSOCIATED WITH RISK OF FUTURE INCIDENT VENOUS THROMBOEMBOLISM – THE HUNT STUDY

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Background: Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE), affects almost 10 million individuals annually world-wide. VTE is a major challenge to health care systems due to hospitalizations, comorbidities, and a high mortality rate. Marine n-3 polyunsaturated fatty acids (n-3 PUFAs), including docosahexaenoic acid (DHA), are essential fatty acids acquired by sea food consumption. n-3 PUFAs are associated with lowered inflammation, reduced thrombin generation, inhibition of platelet function as well as with less endothelial dysfunction, all of which might be implicated in VTE pathogenesis. Previous studies on the association between n-3 PUFAs and risk of VTE, mainly derived from self-reported dietary intake of n-3 PUFAs, have shown mixed results. No previous study has investigated the association between total serum n-3 PUFAs and risk of future VTE.

Aims: To investigate the association between objectively measured serum total n-3 PUFA concentrations, DHA concentrations, and risk of first-ever VTE.

Methods: The present cohort consisted of 17,087 individuals, aged 20 years or older, and without previous VTE, recruited from the third survey of the Norwegian Trøndelag Health Study (HUNT3). The participants underwent a health examination including blood sampling in 2006–2008. Serum n-3 PUFA levels and DHA levels were measured using a nuclear magnetic resonance metabolomic platform (Nightingale) in samples collected at inclusion. Participants were followed from inclusion until an objectively verified first-ever VTE event, death, migration, or December 31, 2019. All VTE events were manually validated. Cox proportional hazards regression was used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for VTE according to tertiles of n-3 PUFA and DHA levels, respectively. The study was approved by the Regional committee for medical research ethics and informed written consent was obtained from all participants.

Results: The median age at HUNT3 participation was 53 years and 46% of the study participants were men. During a median follow-up of 12 years, 340 participants experienced an incident VTE event at a median age of 71 years. PE was the most common presentation (56% of VTE events) and 65% of the events were provoked. Participants with n-3 PUFA levels in the highest tertile had 28% lower risk of VTE

(HR 0.72, 95% CI 0.54–0.96) compared with those with n-3 PUFAs in the lowest tertile in age-adjusted analysis. The inverse association was most pronounced for provoked VTE (HR 0.62, 95% CI 0.44–0.87) and for DVT (HR 0.61, 95% CI 0.40–0.93). HRs for VTE remained virtually unchanged after further adjustment for sex, body mass index, history of cardiovascular disease and cancer at baseline. The association between DHA concentrations and risk of overall VTE and VTE subgroups were similar to those observed for total n-3 PUFAs.

Summary/Conclusion: We found that higher serum concentrations of n-3 PUFAs were associated with lower risk of future VTE, and provoked VTE and DVT in particular. Our findings support the idea that a diet enriched with marine-derived constituents and supplements might protect against future VTE.

OC09-OP02

INFLAMMATION, IMMUNITY, AND ENDOTHELIAL PLASMATIC PROTEINS ASSOCIATED WITH VENOUS THROMBOSIS IN RETROVE

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Background: Venous thromboembolism (VTE) is a heterogeneous and multifactorial condition with an approximately 25% risk of recurrence within five years of the first event. There is a clinical need for high-specific plasma biomarkers to predict the risk of venous thromboembolism (VTE) and its recurrence.

Aims: To study the relationship with VTE of 88 proteins related to inflammation, immunity, and endothelium, and to build different Supervised Machine Learning (ML) algorithms to discriminate cases and controls.

Methods: RETROVE (Risk of Venous Thromboembolic Disease) is a case-control study involving 400 VTE patients and 400 controls representative of the general population, with data collected between 2012 and 2016. To date, the study included clinical variables, more than 100 plasma biomarkers, and multiple genetic markers. In addition, 88 proteins were tested in cases and controls using the OLINK platform, a proximity extension assay technology.

The bivariate analysis and supervised machine learning methods “KNN algorithm” (KNN), “Random Forest” (RF), “Supporting Vector Machines” (SVM), and “Neural Network” (NN) were implemented on the protein variable set plus age and sex using R-V 4.5.0 with the “tidymodels” library.

Results: 51/88 significantly differed between cases and controls in the bivariate analysis adjusted by age and sex (p-values<0.05).

20/51 proteins were previously described in the literature: seven previously described and related to a protector effect: ANGPT1, FGF-21, ADAMTS13, THBS2, CCL3, LEP, CD4. And 13 associated to an increased risk of VTE: IL1RA, IL6, CD40-L, TNFRSF11A, PAR-1, PDGF subunit B, IL-18, PIgR, REN, MERTK, F3/TM, ACE2, HSP27.

31/51 proteins were not previously described: ADM, AMBP, TNFRSF10B, TNFRSF10A, Gal_9, PGF, IL-4RA, CXCL1 GRO, GIF CBLIF, FGF-23, SPON2, GLO1, KIM1, XCL1, SORT1, PTX3, CCL17, MMP7, DCN, Dkk-1, PRSS8, HB-EGF, GDF-2, FABP2, THPO, CTSL1, hOSCAR, TNFRSF13B, TGM2, CA5A, HAOX1.

From the four supervised machine learning methods (KNN, RF, SVM, and NN), the best AUC of 0.803 was obtained in the test set with the SVM model, and the best accuracy of 73.3% was obtained with the RF model (Table 2).

The variables most repeated on the Variable Importance Plot (VIP) diagrams were: ADAMTS-13, NF-kappa-B essential modulator (NEMO), Chemokine-C motif-ligand (XCL1), Carbonic anhydrase 5A mitochondrial (CA5A), Receptor for Advance Glycation End Products (RAGE), Tumor necrosis factor receptor superfamily member 13B (TNFRSF13B), Tumor necrosis factor receptor superfamily member 10A (TNFRSF10A), and Kidney Injury Molecule -1 (KIM1) (Graph 1).

Only ADAMTS13 was previously described in the literature as associated with venous thrombosis from these eight proteins.

Summary/Conclusion: Thirty-one proteins related to inflammation, immunity, and endothelium, previously not described in the literature as related to VTE, may be associated with VTE and become high-specific plasma biomarkers to predict the risk of venous thromboembolism. The SVM is the model with the best AUC of 0.803. The 3 proteins with the highest relation with VTE (most repeated on the VIP plots) were ADAMTS-13, NEMO, and XCL1, although NEMO by itself did not reach statistical significance in the bivariate analysis (p-value=0.096).

OC09-OP03

ABO GENOTYPES AND RISK OF FUTURE INCIDENT VTE IN A POPULATION-BASED COHORT

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Background: The ABO blood group system is a well-established risk factor for venous thromboembolism (VTE), where individuals with non-O groups are at a 2-fold increased VTE risk, and nearly 20% of the VTE events in the population can be attributed to non-O blood groups. Most previous studies on the association between ABO genotypes and VTE risk have been conducted in case-control studies of younger populations (< 60 years). It remains unclear whether the association between ABO genotypes and VTE risk varies across age groups (< 50, 50-70, and > 70 years) and differs for deep vein thrombosis (DVT) and pulmonary embolism (PE).

Aims: To investigate the association between ABO genotypes and the risk of overall VTE, DVT and PE across age groups in a general population.

Methods: In a population-based cohort, 68,306 unique individuals from the Trøndelag Health Study (HUNT2, 1995-97 and HUNT3, 2006-08) were followed through 2019, and all first-lifetime VTEs were recorded. Genotyping was performed by Illumina HumanCore Exome array with uniform imputation for the surveys. Five polymorphisms tagging common ABO haplotypes rs2519093-T (A1), rs1053878-A (A2), rs8176743-T (B), rs8176719-delG (O1), and rs41302905-T (O2) were used to generate ABO genotypes. Hazard ratios (HRs) with 95% confidence intervals (CI) for VTE by ABO genotypes was estimated by Cox proportional hazards models with the O1O1 genotype as reference. The study was approved by the Regional committee for medical research ethics and informed written consent was obtained from all participants.

Results: In the study population, the most frequent ABO genotypes (of in total 15 genotypes) were O1O1 (39%), A1O1 (26%), A2O1 (10%), BO1 (8%), and A1A1 (5%). During a median follow-up of 22.8 years, 2,019 incident VTE events were recorded. Individuals with the A1A1 genotype had the highest HR for overall VTE (HR:1.75, 95% CI:1.46-2.12) followed by A1O1(HR:1.58, 95% CI:1.42-1.76) and A1A2 (HR:1.48, 95% CI:1.18-1.87) in analyses adjusted for age, sex and body mass index. The risk estimates by ABO genotypes were higher for DVT than for PE for all genotypes. The HRs for DVT were 2.25 (95% CI:1.75-2.89) and 1.77 (95% CI:1.51-2.07) for A1A1 and A1O1, respectively, while the corresponding HRs for PE were 1.34 (95% CI:1.01-1.78) and 1.42 (95% CI:1.22-1.66) for these genotypes. In general, the HRs of VTE by ABO genotypes declined across age groups. For the A1A1 genotype, HRs were 2.80 (95% CI:1.69-4.64), 2.41 (95% CI:1.76-3.31) and 1.32 (95% CI:1.01-1.72) for age groups <50, 50-70, and > 70 years, respectively.

Summary/Conclusion: We found that genotypes involving the A1 allele, particularly A1A1 and A1O1, were associated with increased VTE risk compared to O1O1, that these genotypes displayed higher risks for DVT than for PE, and that the relative risk declined substantially with increasing age groups. Our findings highlight the impact of A1-related genotypes on VTE risk, especially in younger individuals.

OC09-OP04

RISK FACTORS FOR RECURRENT VENOUS THROMBOEMBOLISM IN NEONATES

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Background: Recurrent venous thromboembolism (VTE) in neonates is a rare and poorly understood disease. Insight into this complication is essential to improve care.

Aims: The primary aim of this study was to determine the incidence of recurrent VTE in neonates. The secondary aim was to identify risk factors for recurrent VTE and determine the recurrent-free survival rate.

Methods: This cohort study included neonates (birth – 1 month post-term) with VTE treated at Emma Children's Hospital, Amsterdam UMC between 2000-2021. Outcomes included the incidence of recurrent VTE, recurrent-free survival and risk factors for recurrent VTE. Multivariable analysis was performed to evaluate risk factors. Confounders were identified using directed acyclic graphs (DAG).

Results: In total 165 neonates (mean age 25.6 ±24.8 days; prematurity 41.8%) were included, with a mean duration of follow up of 2.8 ± 1.6 years. Of these, four neonates (4.2%) died as a result of thrombosis, and twenty-seven (16.4%) experienced recurrent VTE. The recurrent -free survival was in 90.3%, 87.3% and 84.9% at 3 months, 1 and 2 years respectively.

Anticoagulant therapy was disrupted prior to VTE in 12/27 (44.4%) and 23/138 (16.7%) neonates with and without recurrence respectively. Risk factors associated with recurrent VTE were total parental nutrition (OR 4.3, 95% CI 1.4-12.8 , p=0.009) and disruption of anticoagulant therapy (OR 9.9, 95% CI 3.4-29.1 , p=<0.001).

Summary/Conclusion: This study demonstrated a high incidence of recurrent VTE in neonates, especially within 3 months after the initial VTE. Both total parental nutrition and disruption of anticoagulant therapy contribute to these outcomes, underscoring the importance of anticoagulant therapy, especially in the first 90 days.

Oral Communication – Cancer & Thrombosis II

OC10-OP01

CANCER MORTALITY AFTER INCIDENT VENOUS THROMBOEMBOLISM

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Background: Patients with venous thromboembolism (VTE) have a transiently elevated cancer risk. The elevated cancer risk the first year after a VTE is often attributed to the presence of unrecognized (occult) cancer at the time of VTE diagnosis. Cancers diagnosed within 1 year after VTE are typically at more advanced stages and predominantly include more biologically aggressive cancer types. Data on mortality in individuals diagnosed with cancer after VTE is scarce.

Aims: To assess the absolute and relative risks of mortality among cancer patients without VTE and with VTE ≤1 year and >1 year prior to cancer diagnosis, respectively, and to explore whether these estimates changed during two time-periods in the last 25 years.

Methods: Participants from the population-based Tromsø4-7 (1994-2016) and HUNT2-3 (1995-2008) (n=111,119) cohort surveys were followed through 2020, and all first-lifetime cancer and VTE events were recorded. Cancer patients with VTE ≤1 year and >1 years prior to cancer diagnosis were compared with cancer patients without prior VTE. We estimated cumulative survival and hazard ratios (HRs) with 95% confidence intervals (CIs) for all-cause mortality using Cox regression. Analyses were performed for the entire period and for two separate periods (1994-2007 and 2008-2020). The study was approved by the Regional committee for medical research ethics and informed written consent was obtained from all participants.

Results: Among 15,072 cancer patients, 135 and 250 experienced a VTE event ≤1 year and >1 year prior to cancer diagnosis, respectively. The 1-year overall survival after cancer diagnosis was 56.7% (47.9-64.6) for those with VTE ≤1 year, 66.6% (60.3-72.1) for those with VTE >1 year and 78.8% (78.2-79.5) for those without VTE prior to cancer diagnosis, respectively. The corresponding HRs for 1-year mortality were 2.29 (1.76-2.98) for patients with VTE ≤1year and 1.24 (0.98-1.56) for patients with VTE >1 year prior to cancer diagnosis (compared to cancer patients without VTE). HR for 1-year mortality in cancer patients with VTE ≤1year before cancer diagnosis attenuated substantially after adjustment for cancer type and stage (HR 1.19, 95% CI: 0.90-1.55). The survival of these patients improved slightly from 1994-2007 to 2008-2020 and reflected the overall trend in improved survival among cancer patients.

Summary/Conclusion: Patients with VTE ≤1 prior to cancer diagnosis had a more than 2-fold increased mortality risk, which was mainly driven by aggressive cancer types and advanced stage at cancer

diagnosis. The survival of these patients improved slightly from 1994-2007 to 2008-2020. Recent advancements in the diagnostics and treatment of cancer, along with increased awareness of occult cancer in patients with VTE, might have improved the mortality risk in patients diagnosed with cancer ≤1 after incident VTE.

OC10-OP02

INCIDENCE OF VENOUS THROMBOEMBOLISM IN PERSONS WITH CANCER – A POPULATION-BASED COHORT STUDY IN NORTHERN SWEDEN

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Background: Venous thromboembolism (VTE) is a common vascular disease. Approximately 20% of first-ever VTEs are cancer-related. Among persons with cancer, VTEs are linked to a higher mortality and VTE is the second most common cause of death. Studies of the incidence of validated VTE events in persons with cancer are needed.

Aims: Our primary aim was to calculate the overall incidence rate of objectively verified, validated first-ever VTE events in persons with cancer over different time intervals following their cancer diagnosis. We also aimed to study the association between cancer and risk of first-ever VTE.

Methods: We included a cohort of participants of the Västerbotten Intervention Programme, a health examination program aimed at middle-aged residents of Västerbotten County, northern Sweden. Health examination participants during the years 1985 to 2014 without a diagnosis of VTE or cancer before health examination were followed as a cohort until a first-ever VTE event, emigration, death or end of study on September 5, 2014. All objectively verified, validated VTE events among cohort members were registered. Data on cancer was collected from the Swedish Cancer Register. Incidence rates of first-ever VTE after a cancer diagnosis were calculated for participants with cancer with 95% confidence intervals (CIs). A Cox proportional hazards model where cancer was treated as a time-updated covariate was used to investigate the association between cancer and risk of first-ever VTE. Hazard ratios (HRs) with 95% CIs were calculated. The study was approved by the Regional Ethics Review Board, Umeå, Sweden. All participants provided informed consent.

Results: We included 105,997 participants. Among these, 9643 (9%) were diagnosed with cancer during the follow-up period. Prostate, breast, and colorectal cancer were the most common cancer sites. Among the participants with cancer, 677 participants had a first-ever VTE event after a median of 0.8 years after the cancer diagnosis (interquartile range 0.2–3.6 years). The incidence rate of first-ever VTE after a cancer diagnosis was 13.2 (95% CI 12.3–14.3) per 1000 person-years in all participants with cancer with similar incidence rates for men and women. The incidence of pulmonary embolism was 6.9 (6.2–7.7) and the incidence of lower extremity deep vein thrombosis was 4.5 (4.0–5.1) per 1000 person-years. The incidence of VTE was highest immediately after a cancer diagnosis, decreased markedly during the two years thereafter and subsequently remained relatively stable. There was an association between a cancer diagnosis and the risk of a first-ever VTE (HR 8.31 [95% CI 7.48–9.23] in a model adjusted for sex, age, body mass index, education level and smoking). A significant multiplicative interaction between sex and cancer in relation to risk of VTE was detected ($p<0.001$). When stratifying the analysis for sex, the multivariable HR was 10.48 (95% CI 9.03–12.15) in women and 6.81 (95% CI 5.87–7.90) in men.

Summary/Conclusion: The incidence of first-ever VTE in a population with cancer is high, especially shortly after the date of a cancer diagnosis. Differences between men and women in the association between cancer and risk of VTE need to be explored further.

OC10-OP03

CATHETER-ASSOCIATED THROMBOSIS: ROLE OF FACTOR XI AND IDENTIFICATION OF BIOMARKERS

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Background: The widespread use of central venous catheters is not without complications, one of the most frequent being thrombosis. Up to 66% of patients requiring central venous access develop asymptomatic thrombosis, while symptomatic thrombosis occurs in 5–41% of cancer patients. Currently, no biomarkers are available to predict this complication. Beyond interfering with chemotherapy, catheter-related thrombosis (CRT) often necessitates anticoagulant treatment, which carries a risk of bleeding. Recent phase III trials of novel anti-FXI agents have shown promising antithrombotic efficacy with minimal hemorrhagic risk.

Aims: This study aimed to investigate components of the contact pathway, FXI levels, and other hemostatic parameters to identify potential biomarkers predictive of CRT in cancer patients.

Methods: We conducted a prospective study in 15 patients with stage IV colorectal cancer receiving 5-fluorouracil (5-FU) chemotherapy and undergoing placement of a peripherally inserted central catheter (PICC). Blood samples were collected from both the catheter and contralateral arm on the day of implantation and at day 14 (D14). On D14, ultrasound imaging was performed to assess catheter-associated thrombosis. FXI, FXII, and kallikrein were evaluated by coagulometric assays and/or Western blot. Contact pathway activation was assessed via ELISA using anti-FXIIa-C1 inhibitor nanobodies. Thrombin generation was measured using low tissue factor stimulation (1 pM). Additional hemostatic and inflammatory markers were also analyzed.

Results: Seven patients (46.7%) developed thrombosis—six subclinical and one symptomatic. The PICC did not significantly activate the contact pathway, as activated forms of FXII or kallikrein were not detected, and FXIIa-C1INH complexes were only mildly elevated in four cases. A positive correlation was observed between FXIIa-C1INH complexes and FXII levels (Spearman r = 0.414, p = 0.001), but FXIIa-C1INH levels did not increase by D14 nor correlate with thrombosis. Notably, FXI and FXII levels increased by D14, with FXI levels rising more markedly in patients who developed thrombosis. D-dimer levels also increased over time. A reduction in thrombin generation was observed at D14, though it was not associated with thrombosis occurrence . No significant differences were found between measurements from the PICC and contralateral arm.

Summary/Conclusion: Our study confirms the high incidence of PICC-associated thrombosis in cancer patients. However, the device did not significantly activate the contact pathway. Thrombosis may be linked to post-insertion increases in FXI levels, which could enhance thrombin generation - as evidenced by rising D dimer - despite a decreased thrombin response to tissue factor. These findings support the therapeutic value of FXI inhibition and highlight FXI levels as potential biomarkers for catheter-related thrombosis risk.
PI24/00106

OC10-OP04

PLASMA LEVEL OF HUMAN EPIDIDYMIS PROTEIN 4 AND RISK OF NON-CANCER- AND CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM

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Background: Recently, we demonstrated that high human epididymis protein 4 (HE4) levels were associated with elevated risk of future incident venous thromboembolism (VTE) in a case-cohort with 5 years of follow-up. In addition, HE4 is known to be upregulated in various cancers and established as a diagnostic biomarker for ovarian cancer. The incidence of cancer-associated venous thromboembolism (CAT) has increased during the last decades and is associated with poor prognosis. Therefore, it is imperative to identify individuals with the highest risk of CAT to provide targeted preventive treatment. To the best of our knowledge, no study has investigated the association between plasma HE4 levels and risk of future CAT and non-CAT in a general population.

Aims: To investigate whether plasma HE4 levels were associated with risk of future CAT and non-CAT.

Methods: We created a case-cohort from the third Trøndelag Health Study (HUNT3) conducted in 2006–08, including 902 individuals with validated incident VTEs during follow-up (ending December 31, 2019) and 1,072 randomly sampled, age- and sex-matched individuals in the subcohort. Plasma HE4 levels were measured using an aptamer-based method (Somascan®) and categorized into quartiles (Q) based on cutoff values derived from the subcohort. Cancer diagnoses were obtained from the Cancer Registry of Norway. For participants diagnosed with VTE, VTEs occurring within the active cancer period (1 year before to 2 years after the cancer diagnosis) were classified as CAT while non-CAT was defined as VTEs occurring outside this period. Age-weighted Cox proportional hazards models stratified by the active cancer status were used to assess the association between HE4 quartiles and the risk of VTE. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated and adjusted for age, sex, and body mass index (BMI).

Results: Among the 902 VTE events occurring during a median follow-up of 10.8 years, 188 (21%) were classified as CAT and 714 as non-CAT. High HE4 levels (Q4) were associated with increased risk of non-CAT (HR: 2.00, 95% CI: 1.05–3.80) compared to those with low HE4 levels (Q1) in the age- and sex-adjusted analysis. Further adjustment for baseline BMI moderately attenuated the risk for non-CAT (1.71, 95% CI: 0.95–3.10). The corresponding adjusted HR for CAT was 1.40 (95% CI: 0.46-4.25) for high (Q4) versus low (Q1) HE4 levels.

Summary/Conclusion: We found that high HE4 levels were associated with a non-significant increased risk of both non-CAT and CAT in multivariable adjusted analyses. Further studies are warranted to clarify the putative role of HE4 in the pathogenesis of CAT and non-CAT.

Oral Communication – Haemophilia

OC11-OP01

IMMUNOGLOBULIN PROFILE DURING THE DEVELOPMENT AND SUCCESSFUL TREATMENT OF A LOW-TITRE INHIBITOR WITH ASSOCIATED COMPLEMENT ACTIVATION-RELATED PSEUDOALLERGY (CARPA) IN A CHILD WITH SEVERE HEMOPHILIA B.

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Background: Acute allergic reactions to Factor IX (FIX) concentrates have been extensively documented in hemophilia B patients who are positive for FIX inhibitors. However, complement activation-related pseudoallergy (CARPA) or CARPA-like reactions have predominantly been associated with hemophilia A. This study follows the immunoglobulin anti-FIX sub-profile of a 2-year-old patient diagnosed with severe hemophilia B (SHB) before and after the development of an alloantibody against both recombinant standard FIX (rFIX) and prolonged half-life FIX with a Fc fusion protein (rFC-FIX). This occurred on his seventh rFC-FIX treatment day (eleventh treatment day in total), coinciding with an acute “allergic” reaction, the development of an anti-FIX inhibitor (with a maximum titre of 1.94 BU), and the detection of peak sC5b-9 levels exceeding 2415 ng/ml. Following this, the patient was successfully treated with a desensitization and tolerization protocol using rFIX.

Aims: The primary objective of this study is to characterize the evolution of FIX concentrate-specific antibody subtypes and subclasses over time in a patient with SHB who was successfully treated for a CARPA-associated FIX low-titre inhibitor. This characterization aims to provide deeper insights into the immunological dynamics at play.

Methods: Over a span of three months, thirteen plasma samples were collected, starting from the diagnosis of SHB to the completion of inhibitor treatment. These samples were retrospectively analyzed for plasma sC5b-9 levels using ELISA (Quidel) and for the presence of IgA, IgM, IgE, IgG1, IgG2, IgG3, and IgG4 antibodies specific to two FIX concentrates using an in-house ELISA assay. This comprehensive approach allowed for a detailed examination of the patient's immunological response over time.

Results: The analysis revealed elevated IgA levels specific to rFC-FIX, which were detected beginning two weeks prior to the CARPA event. Notably, IgG2, IgG3, and IgG4 levels, but not IgG1, became noticeable before, during, and after the peak of sC5b-9 levels. Over time, IgG2, IgG3, and IgG4 levels eventually normalized, except for anti-rFC-FIX IgG4, which remained elevated (rFC-IX was not used for tolerization). IgM was detected only before the sC5b-9 peak, and no IgE antibodies were detected throughout the study period. These findings suggest a complex interplay of immunological factors that warrant further investigation.

Summary/Conclusion: This study observed a distinct progression of the immunoglobulin profile before and after the development and treatment of a CARPA-associated FIX inhibitor in a patient with SHB. These observations could significantly contribute to the future understanding of inhibitor development in SHB patients who experience acute reactions to FIX concentrates. The detailed characterization of immunoglobulin dynamics provides a foundation for further research and potential therapeutic advancements.

OC11-OP02

THE IMPACT OF PHARMACOKINETIC-GUIDED PROPHYLAXIS WITH AN EXTENDED HALF-LIFE RECOMBINANT FACTOR VIII ON CLINICAL OUTCOMES IN HEMOPHILIA A PATIENTS: A TAIWANESE MULTICENTER STUDY

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Background: Rurioctocog alfa pegol, a polyethylene glycol-modified recombinant factor VIII (FVIII) with an extended half-life (EHL), can reduce the dosing frequency for hemophilia A patients.

Aims: This study assessed the clinical benefits of PK-guided prophylaxis with EHL recombinant FVIII using two target trough levels. Personalized treatment regimens were determined via the FDA-approved myPKFiT application.

Methods: This multi-center, observational study evaluated rurioctocog alfa pegol prophylaxis in severe Hemophilia A patients aged (≥12-years) treated for ≥1 month. Patients were instructed to observe their predicted FVIII trough level from the infusion schedule and log infusions/ bleeds using the myPKFiT app throughout the study. Patients were stratified by baseline PK trough levels into trough level <3 or ≥3% groups. The primary endpoints assessed were changes in the annual bleeding rate (ABR) and annual joint bleeding rate (AJBR) during the observational period (Month 0-12) vs the 6 months prior to study enrollment. The secondary endpoints included joint health (measured by HEAD-US), physical activity levels (evaluated with the International Physical Activity Questionnaire [IPAQ]), and hemophilia-related quality of life (QoL), which were assessed at Months 0 and 12.

Results: Of the 15 subjects who completed the 12-month follow-up, were classified by myPKFiT into FVIII trough levels ≥3% (N=9; mean age 45.1) and <3% (N=6; mean age 24.2). Patients with FVIII trough levels ≥3% experienced modest reductions (0.44 ± 3.81 in ABR and 0.67 ± 3.64 in AJBR). Those with FVIII trough levels <3% experienced meaningful reductions (3.33 ± 5.65 in ABR and 3.17 ± 5.95 in AJBR). Both groups displayed increased IPAQ scores, indicating improved physical activity. QoL scores also exhibited an improving trend. The HEAD-US total score in FVIII trough levels <3% has indicated an improvement by the end of follow-up (-0.17 ± 1.33). For patients with the FVIII trough levels ≥3%, the HEAD-US total score remained stable compared with the 6 months prior to screening (mean: 24.11±7.91) and the 12-month observation period (mean: 25.44±10.74).

Summary/Conclusion: The ATTRACT-HA study is the first multi-center real-world investigation in Taiwan on PK-guided prophylaxis with rurioctocog alfa pegol. Results showed improved bleeding rates and physical activity in both patients groups achieving <3% and ≥3% trough levels, demonstrating the value of PK-guided dosing. Younger patients, with greater access to the myPKFiT tool for personalized dosing, showed more pronounced improvements.

OC11-OP03

MONITORING AND OUTCOMES OF MAJOR SURGERY IN PATIENTS WITH HEMOPHILIA A TREATED WITH EMICIZUMAB – A CASE REPORT SERIES

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Background: Hemophilia A is an inherited bleeding disorder that has traditionally been managed with FVIII replacement therapy. The bispecific antibody emicizumab offers a novel therapeutic approach, partially mimicking the effect of FVIII by bridging Factor IX and X.

Experience with major surgery in patients with HA receiving prophylactic emicizumab remains limited, with a recent review reporting 72 surgeries across 20 publications. While a majority reported using supplemental FVIII replacement therapy, few of these studies stated the targeted and achieved FVIII levels, and only one study reported the specific chromogenic assay employed. Accurate assay selection is critical because chromogenic assays using bovine-derived reagents (FVIII:CSA-B) detect only endogenous or exogenous FVIII without interference from emicizumab, whereas assays utilizing human-derived reagents (FVIII:CSA-H) also measure emicizumab activity.

Aims: Our aim was to investigate the relationship between FVIII:CSA-B and FVIII:CSA-H at higher FVIII levels, and to assess whether monitoring with FVIII:CSA-B is sufficient in a major surgery setting.

Methods: We collected clinical and laboratory data from the first major orthopedic surgeries performed at Karolinska University Hospital in patients with HA receiving prophylactic emicizumab treatment. In total, four orthopedic surgeries were performed on three patients. Supplemental treatment with efmoroktokog alfa was given in all surgeries with targeted FVIII:CSA-B levels of 80 IU/dL on the day of operation, 70 IU/dL day 2-3 and 50 IU/dL day 4-7. 5000U of dalteparin was administered daily as long as the patient had a FVIII level above 50 IU/dL. Peroral tranexamic acid, dosed after weight, was given for 7 days postoperatively. Blood samples were obtained thrice on the day of surgery (before administration of any factor concentrate, immediately after surgery, and in the evening), followed by daily sampling thereafter. Analysis with FVIII:CSA-B and FVIII:CSA-H, were performed. Additionally, isolated emicizumab activity was assessed using FVIII:CSA-H following preanalytical heat treatment, previously demonstrated to neutralize endogenous FVIII activity.

Results: One patient suffered a minor bleed in the surgery area postoperative day 5 but otherwise no adverse outcomes were reported.

Before surgery, all patients had endogenous FVIII levels of 1-3 IU/dl and estimated emicizumab concentrations 25-40 micrograms/ml. Peak levels of around 100 IU/dl were achieved with both FVIII:CSA-B and FVIII:CSA-H.

Summary/Conclusion: As monitoring with FVIII:CSA-B does not take emicizumab effect into account, there might be a theoretical risk of overcompensating the FVIII activity, however at least in vitro this does not seem to be the case as the methods converged at higher FVIII levels, in accordance with previous reports. We thus add to the literature regarding major surgery on emicizumab treated patients and further confirm that monitoring with only FVIII:CSA-B is sufficient.

OC11-OP04

INTRINSIC ACTIVATION OF THROMBIN GENERATION IS A PROMISING TOOL TO MONITOR SEVERE HEMOPHILIA A PATIENTS ON EMICIZUMAB

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Background: Severe hemophilia A (SHA) patients often receive the prophylactic non-factor treatment emicizumab, which interferes with existing coagulation assays. Conventional laboratory tests are unreliable for monitoring the emicizumab effect on coagulation in acute settings or bleeding. The present study aimed to investigate the applicability of the generally accepted extrinsic thrombin generation (TG) assay and a newly developed intrinsic activated TG assay.

Aims:

Adequately measure thrombin generation in severe hemophillia (SHA) patients

Increase the sensitivity of thrombin generation for factor VIII products or non-factor (mimetics) agents

Provide a reliable monitoring tool for coagulation in acute settings or bleeding

Methods: The TG assay was performed on institutional normal pool and congenital FVIII deficient plasma (George King) spiked with emicizumab or FVIII octocog alpha using the CAT assay and activated using extrinsic trigger reagent PPP Reagent LOW or intrinsic trigger reagent PPP Reagent INT. Both assays were also used to measure TG in patients switching from FVIII to non-FVIII replacement therapy at baseline, before switching to emicizumab (n=37) and 4 weeks (n=52), 3 months (n=42), and 1 year (n=28) after switching to emicizumab.

Results: TG in normal pool plasma triggered with PPP Reagent LOW or PPP Reagent INT (36 replicates) resulted in inter-assay and intra-assay variabilities below 10%. The maximum thrombin (peak height) in most SHA subjects was decreased compared to the normal pool. Dose-dependent correlations were found between plasma FVIII and emicizumab levels and TG measured in plasma from SHA subjects for both trigger reagents. Overall, PPP Reagent INT proved to be more sensitive to changes in the FVIII or emicizumab concentrations than PPP Reagent LOW, especially in the lower range. There was a dose-dependent correlation in the low emicizumab range (0-10 µg/ml), while a plateau was observed in the range above 10 µg/ml.

Summary/Conclusion: Compared to tissue factor, intrinsic activation enhances sensitivity for FVIII or its replacement products in plasma TG. Therefore, intrinsic activated TG is a promising tool for monitoring of SHA patients on different therapies. Further studies comparing TG parameters with clinical bleeding outcomes are needed.

Oral Communication – Thrombin Generation

OC12-OP01

MIND THE GAP: THROMBIN GENERATION DIFFERENCES ACROSS VISCOELASTIC SYSTEMS

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Background: Tissue factor (TF) is the principal activator of the coagulation system *in vivo*. In acute settings (e.g., trauma, major surgery), TF-activated viscoelastic testing (VET) offers quick, actionable data regarding a patient's coagulation status, guiding transfusion and hemostatic therapy decisions. Moreover, by focusing on the extrinsic pathway, clinicians can pinpoint deficiencies or imbalances (e.g., in factors VII, X, V, prothrombin, or fibrinogen) and evaluate how those might be affecting overall clot dynamics. However, we hypothesized that variations in TF source and concentration across various VET platforms can influence assay sensitivity and interpretation.

Aims: To compare TF reagents from multiple commercially available VET devices (QUANTRA®, ROTEM® Sigma, ROTEM® Delta, and TEG®5000) under different conditions using thrombin generation (TG) profiles.

Methods: Three plasma conditions were prepared for analysis: (1) normal plasma, (2) hemodiluted plasma (50% dilution), and (3) mild FVII-deficient plasma (11–17%). TG was initiated by adding the reagent mix from each TF-activated viscoelastic test to each plasma condition, without supplemental phospholipids. TG was then quantified using the Calibrated Automated Thrombinography (CAT) method (Stago).

Results: In normal plasma, a robust TG was observed, with peak analysis reaching up to 430 nM. Interestingly, the QUANTRA TF reagent exhibited a longer time to peak compared to TF from ROTEM Sigma and Delta. In 50%-diluted plasma, velocity of TG was reduced, indicating decreased FVII activity. Among the three conditions, FVII-deficient plasma showed the most substantial reduction in peak thrombin levels and the most pronounced prolongation of TG kinetics. Notably, QUANTRA TF appeared more sensitive in detecting dilutional effect and FVII deficiency than the ROTEM reagents, as evidenced by changes in lag time, endogenous thrombin potential (ETP), peak height, and time to peak.

Summary/Conclusion: TF source and concentration profoundly affect TG results in clinical settings. QUANTRA's greater sensitivity may offer a distinct advantage in detecting coagulation deficiencies or imbalances, thereby enhancing clinicians' ability to make informed transfusion and hemostatic therapy decisions.

OC12-OP02

INFLUENCE OF ABO BLOOD GROUP ON THROMBIN GENERATION AND SENSITIVITY TO THROMBOMODULIN

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Background: ABO blood group antigens are expressed on red blood cells, endothelial cells, epithelial cells, and platelets, and have been implicated in hemostasis regulation. Non-O blood groups are associated with a higher risk of venous thromboembolism (VTE) than group O, though the underlying mechanisms remain unclear. The thrombin generation test (TGT) provides a functional assessment of the hemostatic balance.

Aims: To evaluate the impact of ABO blood group on thrombin generation and thrombomodulin (TM) sensitivity in individuals with and without thrombotic events.

Methods: We conducted a descriptive study including 400 patients with confirmed thrombotic events and 400 independent controls from the RETROVE study. Participants were grouped into O and non-O (A1, A2, B, AB) blood groups. TGT parameters were analyzed using the ST Genesia® device (Diagnostica Stago), with inhibition by thrombomodulin (TM). Comparisons between groups were performed using Kruskal-Wallis statistics (SPSS).

Results: Of the 800 participants, 793 were evaluable: 244 in group O and 546 in non-O. Age and sex distribution were comparable between groups. Significant differences in factor VIII and von Willebrand factor levels were observed, both lower in group O ($p<0.001$).

Among the 394 cases (O: n=92; non-O: n=302), all TGT parameters differed significantly. Thrombin peak was lower in group O (77.73%) vs. non-O (89.77%) (p=0.001), as were ETP (94.76% vs. 98.61%, p=0.049) and TM-inhibited ETP (71.90% in O vs. 66.62% in non-O, p=0.018), indicating greater TM sensitivity in non-O individuals.

Among the 396 controls (O: n=152; non-O: n=244), similar differences were found. Thrombin peak was lower in group O (73.23%) than non-O (81.36%) (p=0.002), as was ETP (82.05% vs. 88.12%, p=0.007). TM-inhibited ETP was also lower in non-O controls (57.38%) compared to group O (62.50%) (p=0.019).

Summary/Conclusion: This study confirms that the ABO blood group influences thrombin generation in both thrombosis patients and healthy individuals. Group O is associated with lower thrombin generation and greater sensitivity to endogenous anticoagulant mechanisms, while non-O groups exhibit higher thrombin generation and reduced TM sensitivity, potentially reflecting a prothrombotic phenotype. These findings align with previous studies and support the utility of TGT as a functional tool to assess thrombotic risk associated with blood group.

OC12-OP03

DISRUPTION OF HEMOSTATIC PATHWAYS BY A TICK-DERIVED SERPIN

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Background: Proteolytic enzymes are key regulators of critical physiological processes such as coagulation, immunity, and inflammation. As a result, their inhibitors have attracted considerable interest for their diagnostic and therapeutic potential. Tick saliva is a rich source of such protease inhibitors, which have evolved to modulate host responses for successful blood feeding. The discovery and functional analysis of these exogenous molecules provide valuable insights into hemostatic regulation and open new avenues for developing innovative biotechnological tools to diagnose and treat coagulation disorders.

Aims: To evaluate the impact of the tick salivary serpin Iripin-3 on blood coagulation, platelet activation, and hemostatic function in a whole blood flow-based assay.

Methods: Iripin-3 was produced via recombinant expression and characterized using HPLC and mass spectrometry. Its anticoagulant properties were evaluated through calibrated automated thrombography (CAT) and enzymatic assays with purified coagulation factors. The Maastricht flow chamber system was employed to assess the effects of Iripin-3 on thrombus formation in whole blood under both coagulating and non-coagulating conditions. Platelet activation in response to various agonists was analyzed by flow cytometry.

Results: Iripin-3 inhibited thrombin generation in a dose-dependent manner via both extrinsic and intrinsic coagulation pathways. This anticoagulant activity involved the classical serpin mechanism targeting FXIIa, FXIa, and thrombin, as well as FXa exosite binding, which impaired FVa-dependent prothrombinase function. In washed platelets, Iripin-3 caused a modest but significant reduction in CRP-induced integrin αIIbβ3 activation and partially inhibited thrombin-induced activation of the same integrin. Under flow conditions in whole blood, Iripin-3 delayed thrombus formation and reduced fibrin deposition.

Summary/Conclusion: The results of this study underscore the pleiotropic activity of Iripin-3 and further support the therapeutic potential of tick salivary proteins as antithrombotic agents or modulators of thrombo-inflammatory conditions.

OC12-OP04

THE RELATIONSHIP BETWEEN CAROTID STENOSIS, PERIPHERAL ATHEROSCLEROSIS AND THROMBIN GENERATION: AN EXPLORATORY STUDY

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Background: Carotid atherosclerosis is a major cause of ischemic stroke, influenced by various risk factors including hypertension, hyperlipidemia, smoking, diabetes, and obesity, which contribute to vascular dysfunction and plaque progression. While the relation between platelets, cellular hemostasis and atherosclerosis has been intensively studied, the relationship between the extent of atherosclerosis and coagulation activation remains unclear.

Aims: This study aimed to investigate the association between the extent of carotid stenosis, peripheral atherosclerosis and hypercoagulability, as assessed by the thrombin generation assay.

Methods: In this pilot study, 30 patients from the Department of Neurology without acute thrombotic disease at inclusion were enrolled. The extent of carotid stenosis was evaluated via Doppler ultrasonography, and patients were classified into mild (1-49%) and advanced (50–100%) stenosis groups. Peripheral atherosclerosis was assessed using the ankle-brachial index (ABI). From the blood samples routine laboratory examinations were performed (ions, glucose levels, renal and liver function tests, high-sensitivity C-reactive protein (hsCRP), complete blood count, lipid profile) by standard methods. Thrombin generation was measured using platelet-free plasma. Demographic data, comorbidities and medication use were also recorded.

Results: No significant association was found between thrombin generation parameters and the severity of carotid stenosis. However, patients with advanced stenosis had significantly lower ABI values compared to those with mild stenosis (left ABI: mild vs. advanced stenosis, 1.09±0.18 vs. 0.94±0.19, p=0.039; right ABI: mild vs advanced stenosis, 1.11±0.17 vs. 0.86±0.19, p=0.001), suggesting greater systemic atherosclerosis burden. In the mild stenosis group, triglyceride levels showed a significant inverse correlation with CRP (r=-0.5581, p=0.0328). Across the entire cohort, peak thrombin and endogenous thrombin potential (ETP) positively correlated with CRP (r=0.3802, p=0.0382, and r=0.3753, p=0.0410, respectively). Additionally, time to peak positively correlated with triglyceride levels in both the advanced stenosis group (r=0.653, p=0.008) and the entire cohort (r=0.501, p=0.005).

Summary/Conclusion: These preliminary findings suggest that localized carotid atherosclerosis pathology is not directly associated with systemic thrombin generation. In contrast, ABI values, reflecting systemic atherosclerotic burden, were significantly associated with advanced carotid stenosis, underscoring its potential as a complementary diagnostic tool. The observed correlations indicate a complex interplay among lipid metabolism, inflammation and coagulation in patients with atherosclerosis.

Oral Communication – Venous Thrombosis

OC13-OP01

EVALUATION OF HEPARIN RESISTANCE IN ANTITHROMBIN DEFICIENCY: IN VITRO EVIDENCE AND IMPLICATIONS FOR TREATMENT MANAGEMENT DURING PREGNANCY

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Background: Antithrombin (AT) is a major endogenous anticoagulant that requires heparin to reach its full activity. Thus, there is controversy about the use of heparin in patients with antithrombin deficiency (ATD), especially with mutations at the heparin site (HBS). Clinical observations and robust experimental evidence accounting for the molecular defect causing ATD are needed.

Aims: To assess heparin resistance in a large and molecularly diverse cohort of ATD patients, and to correlate with clinical findings with a focus on ATD women during pregnancy, scenario where heparin virtually becomes the only therapeutic option.

Methods: We used a chromogenic anti-Xa method in plasma supplemented with escalated doses of tinzaparin (0-2.5 U/mL). The study was conducted in 92 ATD patients and 22 controls. Cases with different *SERPINC1* variants, including type I defects and all type II subtypes of ATD, were analyzed. We also studied 8 cases homozygous for II HBS variants; a hemophilic patient with AT<5% due to treatment with siRNA against AT (Fitusiran); 15 cases with *SERPINC1* variants not impairing anti-Xa activity; and 14 patients with ATD due to congenital disorders of glycosylation (CDG). We also reviewed the response to heparin in 80 pregnancies of 38 women with ATD managed in 3 tertiary centers.

Results: Heparin caused a dose-effect increase in anticoagulant activity in all cases, reaching saturation at 2.5 U/mL. However, as compared to controls, all heterozygous ATD cases, regardless of the type or the gene variant, saturated at submaximal activities (42% [37-47%] of controls P<.001). At common target concentrations of 0.3 and 1.2 U/mL, the effects of heparin in patients were 37% (33-40% P<.001) and 35% (32-39% P<.001) of controls, respectively. No variant at the HBS, nor at the reactive site with presumed heparin-sequestration effect, differed from other *SERPINC1* defects. In cases with AT<20% due to homozygous variants, or in the patient treated with Fitusiran, the effect of heparin was severely compromised even at the highest dose (11% [4-18%] of controls P<.001). Analysis of 6 cases on AT concentrates revealed complete restoration of anticoagulant potential. In carriers of *SERPINC1* variants not affecting anti-Xa activity, heparin had an efficacy equal to that of controls. However, CDG patients had a distinct response, overlapping with controls at low heparin levels (<0.2 U/mL) but diverting at higher doses (66% [51-81%] of controls P<.001). Increased high-heparin-affinity beta glycoform in CDGs could explain this behavior.

In pregnancies with ATD, heparin was used at prophylactic and treatment doses in 28% cases each, and AT concentrates in 31% cases. The rates of maternal thrombosis and obstetric complications were 9 and 26%, respectively. In treated pregnancies, anti-Xa levels were monitored in 75% cases. At starting doses, prophylactic, and treatment anti-Xa were 0.2±0.1 and 0.4±0.2 U/mL, respectively. Heparin dose was

increased in 85% cases (15-400% dose increase) with suboptimal results (0.5±0.2 U/mL) that were improved only after AT supplementation (0.7±0.2 U/mL).

Summary/Conclusion: Our study shows that heparin resistance is shared by any type of ATD. The observed ceiling effect does not recommend indefinite dose escalation, but rather the use of AT concentrates if full anticoagulation is desired. This aspect becomes particularly relevant in ATD women during pregnancy, in whom alternatives to heparin may be contraindicated and treatment failure can have serious consequences. PI24/00429

OC13-OP02

IMPLEMENTATION AND EARLY CLINICAL EVALUATION OF AN AI-ASSISTED DECISION SUPPORT SYSTEM FOR THROMBOPHILIA ASSESSMENT

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Background: Current guidelines recommend thrombophilia testing in select patient populations. Effective assessment requires interpretation of both biochemical and anamnestic data, the latter of which is often buried within unstructured electronic health records (EHRs). Manual EHR review is, however, time-consuming and error-prone. Natural language processing (NLP)-based artificial intelligence (AI) tools offer a solution by automating the extraction of data on thromboses, bleeding episodes, and use of anticoagulant or antithrombotic medication.

Aims: The objective was to assess feasibility, accuracy, and clinical utility of an NLP-based AI system for thrombophilia assessment. Specific aims included evaluating the system's ability to extract anamnestic data, its agreement with manual EHR review, its impact on clinical workflow efficiency, and its usability among clinicians.

Methods: The AI system was developed using NLP classification models trained on 299,718 Danish EHRs (2015–2022) from Odense University Hospital, comprising 1.4 billion tokens. The models were fine-tuned on clinical narratives. Pre-implementation validation was conducted on an independent dataset, achieving high sensitivity (> 99%) and specificity (> 95%). The AI system was implemented in a thrombophilia assessment workflow, where clinicians used its structured output to supplement or replace manual EHR review. The system was evaluated by comparing AI-assisted reviews with traditional manual reviews in a retrospective cohort of 50 patients undergoing thrombophilia assessment. Additionally, a usability evaluation was performed using eye-tracking technology to assess review time, accuracy, and information retrieval patterns. Clinician feedback was gathered through semi-structured interviews to explore feasibility, workflow integration, and barriers to adoption.

Results: The AI system significantly enhanced data retrieval, capturing additional relevant findings in 68% of cases compared to manual review. The AI system also improved efficiency: in complex EHRs, review time was reduced by 33%, and participants using AI-assisted review identified 20% more thrombosis related findings than those using manual review alone. Two distinct usage patterns emerged - active filtration, where users relied on AI-generated highlights, and manual scrolling. Active filtration was associated with greater time savings and improved focus on clinically relevant data. Usability evaluations showed that most clinicians found the AI system intuitive and beneficial, with minimal training required for adoption. Feasibility interviews revealed strong acceptance of AI tools, with clinicians emphasizing the system's potential to enhance accuracy and streamline assessments.

Summary/Conclusion: This study serves as a proof-of-concept for integrating NLP models into thrombophilia assessment, demonstrating high accuracy, improved efficiency, and enhanced clinical decision-making. The AI system improves risk stratification and treatment for patients by ensuring comprehensive data extraction while maintaining clinician oversight. Additionally, it enhances security and consistency by minimising human error and standardising data interpretation. Real-world implementation highlights its potential to optimise thrombophilia assessments, reduce clinician workload, and ultimately leading to better patient outcomes.

OC13-OP03

GENETIC ASSOCIATION OF THE 5P13.3 LOCUS WITH RESIDUAL VASCULAR OBSTRUCTION IN PATIENTS WITH UNPROVOKED PULMONARY EMBOLISM

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Background: Pulmonary Embolism (PE) is a frequent disease (annual incidence rate of 1 to 2/1000) and represents the third most common cause of cardiovascular death. In about 50% of cases, PE occurs in the absence of any major risk factors (termed unprovoked PE). After 3 to 6 months of anticoagulant therapy, over 30% of unprovoked-PE patients will develop chronic and irreversible residual pulmonary vascular obstruction (RPVO) due to incomplete thrombus dissolution. These patients have a higher risk of recurrent PE, chronic thromboembolic disease with or without pulmonary hypertension.

Aims: In this work, we aimed to identify molecular determinants of RPVO in unprovoked-PE patients.

Methods: Capitalizing on two French (EDITH and PADIS-PE) and one Canadian (REVERSE I) clinical cohorts comprising 586 unprovoked PE patients in whom we have RPVO measurements and genome-wide genotype data, a meta-analysis of Genome-Wide Association Studies (GWAS) on RPVO was performed. To handle the semi-continuous nature of RPVO measurement, a Compound Poisson-Gamma model was implemented in each GWAS to study the association of ~7M single nucleotide polymorphisms with RPVO, while adjusting for age, gender and key genetic principal components. Results across studies were meta-analyzed using Mantel-Haenszel methodology.

Results: A genome-wide significant locus on chromosome 5p13.3 was found as associated with RPVO, with the lead SNP rs10076945 (p=3.76x10-8) mapping to this region. The rs10076945-A allele, with frequency about 0.51, was associated with a 1.53-fold [1.32–1.78] increase in RPVO. Effect estimates were homogeneous across studies (pheterogeneity=0.11) with an increase of 1.18 [0.89-1.58], 1.60 [1.30-2.04] and 1.82 [1.23-2.69] in EDITH, PADIS-PE and REVERSE I, respectively. Fine mapping analysis of this locus identified an independent suggestive association signal (p=4.56x10-6) at rs35559997, where the T allele (frequency ~0.15) was also associated with a 1.72-fold [1.36-2.18] increase in RPVO.

Summary/Conclusion: This study presents the first GWAS exploring predictors of RPVO and identified a novel locus whose precise role in the etiology of RPVO—and potentially in PE-related complications—warrants further investigations.

OC13-OP04

PROTHROMBOTIC GENOTYPES AND THE RISK OF MORTALITY AFTER CANCER-RELATED VENOUS THROMBOEMBOLISM - THE HUNT STUDY

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Background: Cancer is a major risk factor for venous thromboembolism (VTE), and the presence of common prothrombotic genotypes is known to further increase the VTE risk in cancer patients. Cancer-related VTE is associated with a high mortality rate, and non-O blood group, which is among the prothrombotic genotypes, is reported to increase the mortality risk in specific cancer types. Still, the influence of prothrombotic genotypes on mortality risk after the diagnosis of cancer and cancer-related VTE remains to be settled.

Aims: To assess the association of five common prothrombotic genotypes, individually and as a genetic risk score, with all-cause mortality following cancer and cancer-related VTE in a population-based cohort.

Methods: Participants from the second and third surveys of the Trøndelag Health Study (HUNT2-3, 1995-2008) with genotyping data (n=66,780) were followed through 2019, and all first-lifetime cancer and VTE events were recorded. Cancer patients with a VTE occurring within one year before and two years after the cancer diagnosis were regarded as having cancer-related VTE. Five prothrombotic single nucleotide polymorphisms (SNPs) previously reported as a risk score were genotyped: rs8176719 (*ABO)*, rs6025 (*F5)*, rs1799963 (*F2)*, rs2066865 (*FGG)*, and rs2036914 (*F11*). Cox regression was used to estimate hazard ratios (HR) with 95% confidence intervals (CI) for all-cause mortality, with 0 risk allele serving as the reference category for the individual SNPs, and 0-1 risk allele as the reference category for the 5-SNP score (0-1, 2, 3 and ≥4 categories of risk alleles). Separate analyses were conducted for the cancer-only and cancer-related VTE groups. The study was approved by the Regional committee for medical research ethics and informed written consent was obtained from all participants.

Results: A total of 9,637 cancer patients were included, of whom 338 (3.5%) experienced cancer-related VTE. Among patients with cancer-related VTE, the presence of ≥1 risk allele for rs6025 in *F5* (factor V Leiden [FVL]) compared with 0 risk allele was associated with a 39% lower risk of mortality (HR 0.61, 95% CI 0.41-0.92) in age- and sex-adjusted analysis. The impact on risk estimates was minor after further adjustments for body mass index, smoking, and comorbidities (HR 0.62, 95% CI 0.41-0.92) and when adding cancer type and stage to the regression model (HR 0.67, 95% CI 0.44-1.01). There was no association of other SNPs or categories of the 5-SNP score with mortality in patients with cancer-related VTE. None of the prothrombotic genotypes, including FVL, were associated with mortality among patients with cancer only.

Summary/Conclusion: Our results suggest that FVL is associated with reduced mortality risk after cancer-related VTE. The other prothrombotic genotypes were not linked to mortality in either cancer or cancer-related VTE groups. Future studies are warranted to confirm our findings and explore the potential mechanism of decreased mortality after cancer-related VTE in FVL carriers.

Oral Communication – Structure Function

OC14-OP01

FUNCTIONAL STUDY OF SIX THBD VARIANTS IDENTIFIED IN FAMILIES WITH A HISTORY OF VENOUS THROMBOEMBOLISM

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Background: Thrombomodulin (TM) is a transmembrane endothelial glycoprotein that regulates the anticoagulant/anti-inflammatory potential of the endothelium, particularly by promoting the activation of protein C (PC) by thrombin (FIIa). Plasma levels of soluble TM (sTM) have been associated with venous thromboembolism (VTE).

Aims: The role of TM in venous thrombosis remains to be clarified. The aim of our work is to better understand the involvement of TM in venous thrombosis by reporting the results of a functional study of six TM variants identified in patients with a history of VTE.

Methods: Patients with an episode of VTE and a negative thrombophilia profile were explored with a 37-gene NGS panel. Plasma levels of sTM and thrombin generation (FIIa) were measured in patients carrying missense variants in the *THBD* gene encoding TM. These variants were introduced into the coding sequence of wild-type TM and expressed in HEK293 cells for functional studies. Expression of the mutant TMs was quantified in cell lysates and conditioned culture media by ELISA and immunoblot. The ability of TM variants to bind FIIa on the cell surface and promote PC activation on the cell surface and in vitro was evaluated.

Results: A total of 355 patients were screened by NGS. Among them, six carried variants of uncertain significance (class 3, ACMG) in *THBD*: Met1Leu (start codon), Cys265Arg, Cys310Trp, Gly313Arg, Cys329Gly, and Cys340Trp (extracellular domain). Cellular expression studies showed that the Met1Leu variant, which lacks the start codon, results in no TM expression. FIIa binding and PC activation on cells were reduced for the Cys310Trp, Cys329Gly, and Cys340Trp variants. In vitro PC activation with equivalent amounts of sTM was also reduced for these variants as well as for Gly313Arg. Functional studies of plasma TM are ongoing and will be presented at the congress. To date, family studies have been conducted for the Cys310Trp and Cys340Trp variants and demonstrated cosegregation of the variants with the thrombotic phenotype. Family investigations for the other variants are in progress.

Summary/Conclusion: Our findings support the involvement of TM in venous thrombosis. *THBD* sequencing could thus constitute a complementary investigation in patients with a history of VTE with negative BT. This study highlights the importance of functional investigations to better characterize class 3 variants. Indeed, these analyses helped uncover the likely biological mechanisms involved in thrombosis for four variants (Cys310Trp, Gly313Arg, Cys329Gly, and Cys340Trp), supporting their reclassification as likely pathogenic (class 4, ACMG).

OC14-OP02

NOVEL CRYPTIC EPITOPES UNCOVER A DISTINCT AND CONSISTENT OPEN ADAMTS13 CONFORMATION IN ITTP

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Background: Only in open ADAMTS13, an initially cryptic spacer domain epitope becomes accessible for antibody recognition. This open ADAMTS13 conformation is gaining clinical interest as a biomarker for diagnosing immune-mediated thrombotic thrombocytopenic purpura (iTTP) and monitoring remission phase patients for increased relapse risks. Yet, the exact conformation of open ADAMTS13 remains unknown and it remains unclear whether iTTP patients display one distinct or multiple different open ADAMTS13 conformations.

Aims: To identify novel cryptic epitopes in ADAMTS13 and characterize the uniformity of the open ADAMTS13 conformation in iTTP patients.

Methods: Using ELISA, over 100 monoclonal antibodies (mAbs) were screened to identify cryptic epitope-recognizing mAbs. Epitopes were considered cryptic when mAbs could not bind closed ADAMTS13, but could bind open ADAMTS13 following mAb-mediated conformational opening. ADAMTS13 antigen and conformation were evaluated for 25 healthy donors (HDs) and 25 iTTP patients. Also, the conformation of ADAMTS13 from HDs was screened upon preincubated with pathological IgGs purified from iTTP patient plasma.

Results: Four mAbs were identified to recognize novel cryptic ADAMTS13 epitopes that became accessible following mAb-mediated conformational opening. Novel cryptic epitopes were found to be distributed across ADAMTS13's D, T7, T8 and CUB1 domains, which expands our toolset to explore open ADAMTS13's conformation beyond the cryptic spacer domain epitope. In HD plasma, all novel cryptic epitopes were inaccessible indicating ADAMTS13 closure through multiple intramolecular interactions that extend beyond the well-described spacer-CUB interaction. All acute iTTP patients consistently presented one distinct conformation in which all cryptic epitopes were accessible. Furthermore, this distinct open conformation could also be induced when incubating closed plasma ADAMTS13 with anti-ADAMTS13 autoantibodies purified from acute iTTP patient plasma.

Summary/Conclusion: We demonstrated that open ADAMTS13 exposes multiple cryptic epitopes across various domains, extending beyond those defined by the spacer-CUB interaction. This highlights that closed ADAMTS13 likely adopts a folded structure stabilized by more interdomain interactions than previously recognized. Following autoantibody-mediated opening, ADAMTS13 adopts a distinct open conformation that is consistently observed in all iTTP patients.

OC14-OP03

FROM DISORDER TO FUNCTION: PROBING THE B DOMAIN OF COAGULATION PROCOFACTORS VIII AND V VIA MOLECULAR DYNAMICS

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Background: Factors (F)VIII and V are homologous coagulation procofactors that circulate in inactive states until activation to cofactors. Proteolytic activation triggers structural changes essential for cofactor function. While activation is well-studied, molecular mechanisms maintaining the inactive state and enabling activation remain unclear. In this regard, computational modeling offers atomic-level insights into these processes focusing on the functional roles of individual domains.

Aims: Augment and extend the available structural data on non-activated FVIII and FV using computational approaches emphasizing i) the dynamic interplay among structural domains, and ii) the functional role of bound ions and glycans in maintaining procofactor states and affecting folding.

Methods: Non-activated FVIII (2332 residues, R1648-cleaved heterodimer) and FV (2196 residues, monomer) models were generated using AlphaFold3, post-translational modifications, i.e. glycans, sulphotyrosines, and phosphorylation sites, were added using CHARMM-GUI, and Cu2+ and Ca2+ ions were placed at the appropriate sites. The final structures were used as starting conformations for molecular dynamics (MD) simulations. Coarse-grained (CG) models were employed to extensively explore the conformational space of both systems.

Results: The heterodimeric FVIII and monomeric FV models were predicted with high confidence scores of 0.87 (ipTM) and 0.65 (pTM), respectively. In both models, the A1-A2-A3-domains adopt the characteristic triangular arrangement. The C1- and C2-domains are positioned side by side, forming a platform for the A1-A2-A3-domains, with C1 binding to A3. The B-domains are predicted to be intrinsically disordered (pLDDT<20), which was confirmed using AIUPred and metapredict, sequence-based prediction tools for intrinsically disordered proteins. In FVIII, the B-domain is projected to extend without engaging in specific interactions. Interestingly, in FV, the relative orientation of the basic (BR) and acidic regions in the B-domain are predicted with higher confidence, with the BR interacting with the acidic region. Incorporation of post-translational modifications and ions resulted in final structures of approximately 41,000 atoms for FVIII and FV, excluding solvent and counterions. Due to the large size of the systems and the disordered nature of the B domain - which requires extended sampling to reach convergence - explicit all-atom MD simulations become computationally prohibitive, making CG simulations a computationally feasible and well-suited alternative.

Summary/Conclusion: This study provides detailed insights into the procofactor FVIII and FV structures including the disordered B-domain regions, suggesting functional roles in proteolytic cleavage and activation. These models pave the way for advanced MD studies to explore how conformational transitions and intrinsic flexibility of disordered regions facilitate activation and maintain procofactor states.

OC14-OP04

TERMINAL SIALYLATION MODULATES VON WILLEBRAND FACTOR STORAGE AND SECRETION

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Background: Von Willebrand factor (VWF), a crucial glycoprotein in hemostasis, is synthesized by endothelial cells (ECs), where it undergoes extensive multimerization and glycosylation before being stored in Weibel-Palade bodies (WPBs). Its secretion follows polarized routes, with apical release predominantly involving WPBs. Although glycan modifications have been linked to von Willebrand disease pathophysiology, the role of terminal sialylation in regulating VWF intracellular biology remains poorly defined.

Aims: In this study, we investigated the role of terminal sialylation on VWF intracellular biology.

Methods: Human umbilical vein endothelial cells (HUVECs) were treated with glycosylation inhibitors P-3Fax-Neu5Ac (STI) and swainsonine (SW). VWF glycan composition, levels, and secretion were assessed via ELISA. VWF multimerization by multimer blotting and collagen-III binding assay. WPB morphology was studied using confocal microscopy and analyzed by an automated script in ImageJ. VWF secretion was evaluated by Transwell assay and Brefeldin A treatment and VWF levels were quantified by ELISA. Stimulated secretion was evaluated by string formation under shear flow.

Results: STI and SW significantly affected the glycan composition on VWF, with STI-treated cells lacking terminal sialylation and SW-treated cells containing more high-mannoze N-glycans compared to controls. Additionally, STI and SW treatments significantly attenuated VWF release into the supernatant ($P=0.0297$ and $P=0.0006$, respectively), with simultaneously elevated intracellular levels ($P<0.0001$ and $P=0.0032$, respectively). Inhibition of sialylation led to a marked decrease in high-molecular-weight multimers (HMWM) and increased of secreted VWF dimers. STI treatment affected VWF directional secretion, with constitutive basolateral targeting being the most affected ($P=0.0391$). Sialylation inhibition also resulted in a notable increase in the total number per cell ($P=0.0105$), with a higher proportion of elongated, hemostatically active WPBs ($P=0.0319$). Despite the presence of more hemostatic-competent WPBs, stimulated VWF secretion and string formation were significantly hindered ($P=0.0318$ and $P=0.0035$, respectively).

Summary/Conclusion: In this study we provide novel evidence on the role of terminal sialylation in regulating VWF intracellular biology. Desialylation modifies VWF storage dynamics and impairs stimulated VWF hemostatic activity. These findings expand our understanding on how terminal sialylation influence VWF and contribute to VWD pathophysiology.

Oral Communication – Stroke

OC15-OP01

INVESTIGATION OF THE INTERPLAY BETWEEN HEMOSTASIS AND INFLAMMATION IN ACUTE HEMORRHAGIC STROKE PATIENTS

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Background: Non-traumatic intracerebral hemorrhage (ICH) accounts for 10-15% of all strokes and has a higher rate of mortality as compared to ischemic strokes, however the pathophysiology of ICH is still not entirely known. Pre-clinical studies have identified components of neuroinflammation that are involved in the breakdown of the extracellular matrix and the blood brain barrier, associated with the evolution of hematoma. Despite the potential link between neuroinflammation and hemostasis to the overall outcome of ICH, only few studies are available in patient cohorts.

Aims: To find out whether inflammatory cytokines measured at admission could predict outcomes in ICH patients. We also aimed to seek an association between inflammatory cytokine levels and thrombin generation in ICH patients.

Methods: In this prospective, observational study consecutive patients with ICH were included. Detailed clinical and laboratory investigations were performed from patients on admission. The evolution of the hematoma was recorded on admission, on days 14, 90 (non-contrast CT). Patients were followed, and stroke severity and long-term outcomes at day 90 were assessed by the National Institutes of Health Stroke Scale (NIHSS) and the modified Rankin Scale, respectively. Cytokine profiling was carried out from platelet-free plasma (PFP) using a bead-based multiplex fluorescent immunoassay (LEGENDPlex), allowing simultaneous quantification of 13 human inflammatory cytokines (IL1-β, IFN-α2, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33). Samples were read using flow cytometry and the data was analyzed using LEGENDplex™ Data Analysis Software. Thrombin generation assay (TGA) was performed using PFP obtained on admission.

Results: In this study, 87 patients with ICH were enrolled. The mean age was 67 years, 63% were men. Median NIHSS on admission was 13 (IQR:6-19). Mortality was 23% by day 14 after the event and 41% by day 90. Only 20% of the patients had favorable long-term outcome (mRS 0-1) by day 90. IL-6, IL-10, IL12p70, and IL-18 levels were above the reference limits in all ICH patients. The severity of stroke, as assessed by the NIHSS at admission, showed significant positive association with IL-6 levels (NIHSS 0-10 vs. >10: median:29.8; IQR:18.1-48.8 vs. 45.7; 25.6-97.1 pg/mL, p=0.0216). IL-6 and IL-10 showed significant positive correlation with admission bleeding volume (r=0.2728, p=0.0223 and r=0.2833, p=0.0175, respectively). Mortality (mRS 6) by day 90 showed significant positive association with MCP-1 (mRS 0-5 vs 6: median: 313; IQR:251-411 vs. 454; 262-526 pg/mL, p=0.0261). TGA was not significantly associated with any of the analyzed cytokine levels.

Summary/Conclusion: Elevated levels of cytokines IL-6, IL-10, IL-12p70, and IL-18 were observed across all ICH patients, which might suggest a systemic inflammatory response after the stroke. Elevated levels of IL-6 were associated with stroke severity and admission bleeding volume, while increased MCP-1 was associated with worse long-term outcomes. These findings suggest that studies on inflammatory cytokines in ICH may provide valuable information on the pathomechanism and outcomes.

OC15-OP02

ADMISSION D-DIMER LEVELS AND ARTIFICIAL-INTELLIGENCE SUPPORTED IMAGING CAN HELP PREDICT THE OUTCOME OF ACUTE ISCHEMIC STROKE THROMBOLYSIS TREATMENT

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Background: Intravenous thrombolysis with recombinant tissue plasminogen activator (rt-PA) and mechanical thrombectomy are the mainstays of acute ischemic stroke (AIS) treatment, aiming to rapidly restore cerebral perfusion and improve clinical outcomes. In AIS, early prognostic markers are crucial to guide treatment decisions, but limited options exist due to the narrow time-window of treatment.

Aims: This study aimed to assess whether quickly available test results including admission D-dimer levels and artificial intelligence (AI)-supported imaging analysis can serve as predictive tools in AIS patients treated with thrombolysis only.

Methods: We conducted a prospective observational study involving 423 consecutive AIS patients treated with intravenous recombinant tissue plasminogen activator (IV rt-PA). D-dimer levels were measured at admission, immediately after thrombolysis (n=132), and at 24 hours. Admission and 24-hour non-contrast CT scans were analyzed using AI-based software to assess the Alberta Stroke Program Early CT Scores (ASPECTS) and to quantify cerebral atrophy. The primary clinical outcomes were 90-day functional status measured by the modified Rankin Scale (mRS), mortality, and the incidence of hemorrhagic transformation (HT). Multivariable logistic regression was applied to determine independent predictors of outcome.

Results: Elevated D-dimer levels at admission (>0.5 mg/L) were associated with poor clinical outcomes and higher mortality (OR: 2.37, 95% CI: 1.19–4.74). AI-assisted imaging revealed that a 24-hour ASPECTS ≤7 strongly correlated with unfavorable outcomes, and cerebral atrophy independently predicted both poor functional outcome and death. Kaplan-Meier survival analysis showed significantly better survival rates in patients with admission D-dimer levels below threshold (<0.5 mg/L; p<0.001).

Summary/Conclusion: Admission D-dimer levels together with AI-based assessment of ASPECTS and cerebral atrophy are readily accessible tools that can contribute to early risk stratification in AIS patients undergoing thrombolysis. Their combined use may support more individualized treatment strategies and improve clinical decision-making in acute ischemic stroke care.

OC15-OP03

INCREASED THROMBIN GENERATION POTENTIAL IN PATIENTS AFTER AN EPISODE OF RETINAL VASCULAR OCCLUSION – A PRELIMINARY STUDY

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Background: Retinal vascular occlusion (RVO) encompasses a group of ocular vascular diseases characterized by a sudden interruption of retinal blood flow, which may lead to vision loss. It includes venous forms such as central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO), as well as arterial forms like central retinal artery occlusion (CRAO). Although patients are routinely screened for inherited and acquired thrombophilic factors, the underlying cause of hypercoagulability often remains unexplained.

Aims: We aimed to investigate thrombin generation potential as a global assay to identify a prothrombotic plasma alteration in these individuals.

Methods: We enrolled 27 patients diagnosed with RVO (n = 4 CRAO, n = 12 BRVO, and n = 11 CRVO) and 27 matched by age, sex, and body mass index healthy controls without a family history of thrombophilia-related conditions. In all participants, thrombin generation potential (TGP) was assessed using Calibrated Automated Thrombinography (CAT).

Results: Patients with RVO demonstrated an altered thrombin generation profile, including a 19.1% higher endogenous thrombin potential ($p = 0.004$), a 21.7% longer lag time ($p<0.001$), a 14.1% lower peak thrombin concentration ($p = 0.007$), and a 25.7% longer time to thrombin peak ($p<0.001$) compared to controls. No significant differences in TGP parameters were found between BRVO, CRVO, and CRAO subtypes. RVO patients also showed a 54.1% higher factor VIII activity ($p = 0.011$), while D-dimer and fibrinogen levels were comparable to those in the control group. A positive family history of myocardial infarction, stroke, or other thromboembolic events was reported in 59.3% of RVO patients (n = 16). These individuals exhibited a 28.3% higher peak thrombin concentration compared to RVO patients without such a family history ($p = 0.028$), while other thrombin generation parameters, as well as D-dimer, factor VIII activity, and fibrinogen levels, remained comparable between the groups. Importantly, at the time of enrollment, most patients (n = 23, 85.2%) reported impaired vision, including complete vision loss, visual field restriction, or blurred vision.

Summary/Conclusion: Our findings indicate that RVO patients are characterized by an altered thrombin generation potential with increased endogenous potential, suggesting prothrombotic plasma properties, which might partially explain the underlying hypercoagulable state in cases where conventional thrombophilia screening fails to identify a clear cause.

OC15-OP04

A POTENTIAL BIOMARKER REGARDING ISCHAEMIC STROKE

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Background: Stroke is a major cause of morbidity and mortality. About 87% of all strokes are caused by a thrombotic or embolic etiology and are referred as Ischaemic strokes(IS). Magnetic resonance imaging(MRI) is the most frequently used screening method for IS diagnosis. However, MRI is expensive and sometimes fails to identify IS early. It would be helpful to have non-invasive tests, such as biomarkers, to screen for IS. The role of b-natriuretic peptide(BNP) includes vasodilatation, reduction of cardiac preload, increase in renal blood flow and increase in natriuresis and diuresis. Many studies have shown that BNP production by cardiomyocytes is triggered by inflammation and hemodynamic stress. Thus, the studies that followed established BNP as a biomarker for heart failure. However, it is important to remember that BNP was originally discovered in the brain. Particularly, regarding IS, BNP may increase due to the activation of the hypothalamic-pituitary-adrenal axis. The utility of BNP for stroke patients triage has been reported in recent studies.

Aims: Aim of the study was to determine BNP levels in patients with IS and compare them with a control group.

Methods: 172 patients admitted to a tertiary Greek hospital were evaluated. 111 suffered an IS, 59 out of them were males, their median age was 81(21-99) years and they constituted Group A. 61 were healthy controls with a median age of 56(20-85) years (Group B). Patients with renal or heart failure or cardiovascular disease, or atrial fibrilation were excluded. BNP was determined on admission using the *UniCell DXI800* (Beckman Coulter) immunoassay analyzer with enhanced chemiluminescence(CMIA). The SPSS® v. 29 and the non-parametric Mann-Whitney U test was used to compare BNP medians between the two groups. The statistical significance level was set at $p<0.05$.

Results: Regarding Group A, median BNP on admission was 149(6-842) pg/ml. In Group B median BNP was 18(4-46) pg/ml. BNP was statistically significantly higher in Group A ($p<0.05$).

Summary/Conclusion: BNP is a natriuretic peptide produced in the heart ventricles. It is released in response to dilatation of the heart wall, increased preload, systemic ischemia and hypoxia. Therefore, BNP establishes the diagnosis and severity of heart failure. There is evidence that BNP is also released from hypothalamus and in response to cerebral ischemia. IS is a life-threatening condition that may lead to death or disability. It is associated with an acute stress reaction involving a neurohormonal and systemic inflammatory response that leads to elevated BNP levels. In this study patients with heart failure were excluded and it was shown that BNP levels were statistically significantly higher in patients with IS compared to the control group. MRI is an indispensable tool for the assessment of IS due to its high sensitivity, specificity and non-invasive nature. Nevertheless, BNP is a biomarker available in tertiary hospitals, easy to determine with CMIA on immunoassay analysers. The integration of biomarker determination may be useful to improve outcome in IS diagnosis. Therefore, BNP in combination with MRI may have a role in the management of IS. Correlation of BNP with prognosis and incorporation of this novel laboratory biomarker into ischaemic stroke management are pending.

Oral Communication – VTE-2

OC16-OP01

A MICROFLUIDIC IMMUNOASSAY TO PROBE VON WILLEBRAND FACTOR ANTIGEN LEVELS IN PLASMA USING MINIATURIZED PHOTON SENSORS

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Background: Point-of-care (PoC) or near-patient testing holds the promise to transform diagnostics. PoC devices have already been implemented to monitor chronic conditions, such as diabetes, demonstrating the role of PoC devices in advancing personalized medicine. Recently we demonstrated the use of a microfluidic setup to measure Factor VIII activity for hemophilia patients. Von Willebrand disease (VWD) is the most common inherited bleeding disorder, caused by a quantitative or qualitative deficiency of von Willebrand Factor (VWF). A reduced or complete absence of VWF is categorized as type 1 and 3, respectively, whereas type 2 is related to a qualitative defect. During this project, a novel VWF antigen assay based on chemiluminescence was implemented on a microfluidic cartridge. Each microfluidic cartridge contains 16 detection chambers where signals can be quantified by miniaturized photon sensors.

Aims: Demonstrate suitability of a quantitative microfluidic VWF test for measuring VWF antigen levels spanning the medically relevant interval.

Methods: The VWF antigen levels were measured using immunopurified polyclonal anti-VWF antibodies labelled with either one subunit of split luciferase probe. These probes compose of two subunits of the NanoLuc® Binary technology. When these anti-VWF labelled antibodies bind to the VWF protein, the subunits reassemble into a functional luciferase enzyme and produce photons in the presence of furimazine. The antibodies and substrate were printed onto a microfluidic cartridge containing 16 detection chambers aligned with 16 photon sensor arrays to quantify the luminescent signals using a handheld processor. Each cartridge can be used to measure 4 samples (4x4). Samples with different VWF levels were prepared by spiking VONVENDI®, Wilfactin®, and Haemate P® in VWF-deficient plasma. To demonstrate accuracy, the results obtained with the microfluidic setup were compared to the VWF:Ag ZYMUTEST™ from Hyphen BioMed.

Results: Photon generation measured in a volume of 0.1 µL of 15-times diluted Normal Pooled Plasma (NPP) in the microfluidic cartridge was VWF dependent, as shown by comparison with VWF deficient plasma. Samples containing different levels of VONVENDI®, Wilfactin ®, and Haemate P®, demonstrated concentration dependent VWF antigen signals in the microfluidic system (r^2 = 0.931). Moreover, samples measured with the microfluidic setup and the predicate test showed high level of agreement with a Pearson r of 0.954 and a slope of 0.75.

Summary/Conclusion: Here, we present the design and proof-of-principle of a microfluidic platform capable of quantifying VWF antigen levels in minutes amount of plasma (2 µL), aiming to advance near patient testing technologies and improve outcomes for patients with VWD.

OC16-OP02

A PROSPECTIVE STUDY OF THE ASSOCIATION BETWEEN PHOSPHATIDYLETHANOL CONCENTRATION, AND RISK OF FIRST-EVER VENOUS THROMBOEMBOLISM

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Background: Venous thromboembolism (VTE) is a vascular disease with an increasing incidence and a high rate of chronic complications and mortality. A high alcohol consumption has many negative health effects including associations with cancer, injuries and mortality. The association between alcohol consumption and risk of VTE is unclear. In previous studies of the association between alcohol consumption and risk of VTE, alcohol consumption has been measured through self-reports, usually obtained by questionnaire. Self-reports of alcohol consumption entail risks of systematic errors and underreporting. Phosphatidylethanol 16:0/18:1 (PEth) is a phospholipid formed exclusively in the presence of ethanol. PEth performs well as a biomarker for alcohol consumption as blood concentrations of PEth are correlated to an individual's alcohol intake during the last two weeks.

Aims: To study the association between alcohol consumption, measured with the biomarker phosphatidylethanol (PEth), and risk of first-ever VTE.

Methods: We conducted a population-based nested case-referent study in northern Sweden. The study is based on the Västerbotten Intervention Programme, a program in which middle-aged men and women are invited to a to a health examination including prospective blood sampling. Samples were kept frozen at -80°C until analysis. Among the health examination participants, cases of objectively verified first-ever VTE between 1985 and 2014 were registered. One referent was matched to each case. Referents were matched for age, sex, health examination date, geographical area, and number of sample freeze-thaw cycles. PEth concentrations were measured in packed erythrocytes using a liquid chromatography tandem-mass spectrometry method with a limit of quantification of 0.01 µmol/L. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated with univariable and multivariable conditional logistic regression. The study was approved by the Regional Ethics Review Board, Umeå, Sweden (approval number 06-162M §157/06). All participants provided written informed consent.

Results: The study included 1410 cases with objectively verified VTE and 1410 matched referents. At the health examination, the median age of the cases was 50.2 years, and 55.2% of cases were men. Among cases, the median time from health examination to first-ever VTE was 12.2 years. Pulmonary embolism was diagnosed in 48% of cases. We found an association between the highest PEth concentration category (>0.30 µmol/L) compared to the lowest PEth concentration category (<0.01 µmol/L) and increased risk of VTE with an OR of 1.39 (95% CI 1.05–1.85) in a univariable model and an OR of 1.44 (95% CI 1.05–1.98) in a multivariable model adjusted for education level, body mass index, smoking, hypertension, and cancer at inclusion. In separate analyses for pulmonary embolism and lower extremity deep vein thrombosis, we found an association between PEth >0.30 µmol/L and higher risk of lower extremity deep vein thrombosis with a multivariable OR of 1.91 (95% CI 1.15–3.20).

Summary/Conclusion: A high PEth concentration was independently associated with an increased risk of first-ever VTE. Furthermore, the study highlights the benefits of PEth as a biomarker to identify individuals with high alcohol intake that are at an increased risk of adverse medical outcomes.

OC16-OP03

PLASMA LEVELS OF OSTEOPONTIN ARE ASSOCIATED WITH THE RISK OF FUTURE INCIDENT VENOUS THROMBOEMBOLISM

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Background: Local hypoxia in the venous valvular pockets, promoting thromboinflammation through mechanisms that remain unclear, is suggested to be a key element in the formation of venous thrombi. Osteopontin (OPN), a protein upregulated under hypoxic conditions, has been reported to increase in the plasma of patients with acute deep vein thrombosis (DVT). However, its association with the risk of future incident venous thromboembolism (VTE) remains unknown.

Aims: To investigate the association between plasma OPN levels and the risk of future VTE.

Methods: Plasma OPN levels were measured in a case-cohort derived from the Trøndelag Health Study (HUNT3) conducted in 2006-08 in Norway. The study included 294 incident VTE cases diagnosed with VTE during 5 years of follow-up, and 1066 age- and sex-matched participants in the subcohort. An aptamer-based method (Somascan 7K) was used to determine plasma OPN levels. Weighted Cox-regression models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) in a linear model and across quintiles of plasma OPN determined in the subcohort. The study was approved by the regional committee for medical research ethics, and informed written consent was obtained from all participants.

Results: The HR for overall VTE per 1 SD increase in plasma OPN was 1.17 (95% CI: 1.04–1.31) in analysis adjusted for age, sex, and sample batch, and 1.17 (95% CI: 1.05–1.31) after additional adjustment for body mass index (BMI) and C-reactive protein (CRP) levels. Participants with plasma OPN levels in the highest quintile exhibited a 44% higher risk of VTE compared to those with the lowest OPN levels in a model adjusted for age, sex, and sample batch (HR: 1.44, 95% CI: 1.01-2.05). Notably, this association persisted after further adjustment for BMI and CRP. The VTE risk remained consistently elevated in subjects with high plasma OPN levels throughout a 5-year follow-up.

Summary/Conclusion: Our results indicate that elevated plasma OPN levels are associated with the risk of future VTE, suggesting a potential thromboinflammatory role for OPN.

OC16-OP04

EVALUATING AGE-ADJUSTED D-DIMER CUT-OFFS AND THE ROLE OF HIGHER MULTIPLIERS IN PULMONARY EMBOLISM DIAGNOSIS

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Background: Owing to the high sensitivity, the use of D-dimer is well established in PE diagnosis and is primarily used to rule out PE (3). However, other pathological or physiological factors can also elevate D-dimer levels resulting in low specificity with patients often being sent for unnecessary further tests. Ageing is one such factor; increasing age has consistently been found strongly associated with increased D-dimer levels The age-adjusted D-dimer (AADD) cut-off (age ×10 ng/mL) improves specificity in pulmonary embolism (PE) diagnosis but requires validation in heterogeneous populations.

Aims: This study evaluates AADD's performance in a diverse cohort and explores increasing the age-adjustment multiplier to enhance specificity.

Methods: A retrospective analysis included 277 patients (mean age 63.9 years; 18.1% PE prevalence) undergoing CT pulmonary angiography and D-dimer testing at Oxford University Hospitals (2018). Exclusions included trauma, perioperative states, and chronic PE. Conventional D-dimer (500 ng/mL) was compared to AADD (multipliers ×10 and ×11). Sensitivity, specificity, negative/positive predictive values (NPV/PPV), and accuracy were calculated.

Results: Both conventional D-dimer and AADD demonstrated 100% sensitivity and NPV. AADD (×10) increased specificity from 11.45% (95% CI: 7.6–16.3%) to 20.70% (15.6–26.6%), correctly reclassifying 9% of PE-negative patients. Raising the multiplier to ×11 further improved specificity to 26.43% (20.8–32.7%) without compromising sensitivity. Multipliers >12 reduced sensitivity, but ×11 maintained 100% sensitivity whilst enabling increased specificity.

Summary/Conclusion: In a heterogeneous population, AADD safely ruled out PE while enhancing specificity, reducing unnecessary imaging. Increasing the multiplier to ×11 provided additional gains in specificity (14.98% vs. conventional; 5.73% vs. ×10) without missed diagnoses. These findings support refining AADD thresholds to optimize diagnostic efficiency. While single-centre data and sample size limit generalizability, results align with prior studies, advocating for multiplier adjustments to improve clinical utility. Adoption of AADD with a ×11 multiplier could enhance cost-effectiveness and patient safety, particularly in older populations vulnerable to contrast-related complications, however further multicentre studies are warranted to validate.

Posters - Bleeding

P001

CLINICAL PRESENTATION AND LABORATORY PROFILE OF PATIENTS WITH FACTOR XI DEFICIENCY (FXID)

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Background: Factor XI Deficiency (FXID) is a rare bleeding disorder, inherited in either an autosomal recessive (1:1,000,000) or dominant (1:30,000) pattern, with a broad spectrum of clinical presentation. It is a genetically determined coagulation disorder, inherited in either an autosomal recessive (1:1,000,000) or dominant (1:30,000) pattern. The factor XI gene (F11) is located on chromosome 4, which mutations on p.Glu117X, p.Phe283Leu, and p.Pro338Leu, the most common.

Aims: To evaluate clinical presentation and laboratory characteristics of patients diagnosed with FXID, with a focus on coagulation-related laboratory findings and potential gender-based differences.

Methods: This Institutional-Review-Board-approved, retrospective, single-center, and cohort study evaluated FXID patients followed at the Coagulation Service of our institution. Data were retrospectively collected from electronic medical records (Epic Systems®, Verona, WI, USA). Variables analyzed included gender, age, age at diagnosis, factor XI (FXI) activity, platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen levels, ISTH Bleeding Assessment Tool (ISTH-BAT) scores, number of plasma protein fraction (PPF) units transfused during surgeries, number of PPF transfusion sessions, and total number of PPF units received. Statistical comparisons were made using t-tests, with a 95% confidence interval and p-values < 0.05 considered statistically significant.

Results: A total of 25 patients underwent FXID genetic testing at our institution, including 14 females and 11 males. The results were analyzed both for the entire group and separately by gender.

- Average age (years): Total – 42.2 ± 24.7; Females – 47.5 ± 23.8; Males – 35.5 ± 26.4
- Average age at diagnosis (years): Total – 18.6 ± 12.6; Females – 19.5 ± 11.4; Males – 17.7 ± 15.0
- FXI activity (%): Total – 29.2 ± 15.7; Females – 32.4 ± 14.1; Males – 25.2 ± 18.2
- Platelet count (×10⁶/μL): Total – 252.8 ± 148.7; Females – 232.0 ± 154.1; Males – 279.2 ± 144.4
- PT (seconds): Total – 12.2 ± 1.5; Females – 11.8 ± 1.3; Males – 12.7 ± 1.6
- Fibrinogen (mg/dL): Total – 320.6 ± 93.5; Females – 335.3 ± 102.7; Males – 285.6 ± 61.5
- aPTT (seconds): Total – 56.7 ± 59.1; Females – 53.8 ± 52.6; Males – 60.2 ± 68.9
- ISTH-BAT score: Total – 4.7 ± 4.2; Females – 6.1 ± 4.3; Males – 2.9 ± 3.5
- PPF units used in surgeries: Total – 1.1 ± 2.0; Females – 0.9 ± 1.2; Males – 1.4 ± 2.8
- Number of PPF transfusion sessions: Total – 1.7 ± 3.3; Females – 1.0 ± 1.4; Males – 2.6 ± 4.8

- Total number of PPF units administered: Total – 3.2 ± 6.8; Females – 1.9 ± 2.6; Males – 4.9 ± 9.1

Laboratory findings showed that the average FXI activity was below the normal range (40–70%), while the average aPTT was prolonged (normal: 25–35 seconds). All the gender analysis didn't show statistical significances between the genders and genders to the total population ($p>0.05$), exception made for ISTH-BAT score, which notably showed that females had significantly higher ISTH-BAT scores (≥6) compared to males ($p < 0.01$).

Summary/Conclusion: Patients with FXID show characteristically low FXI activity and prolonged aPTT compared to the general population. While most laboratory parameters showed no statistically significant gender differences, ISTH-BAT scores were significantly higher in females, indicating a higher bleeding burden for women. These findings emphasize the importance of early diagnosis, gender-aware clinical management, and genetic counseling for patients and their first-degree family members.

P002

OUTCOME OF EMICIZUMAB IN MANAGEMENT OF EGYPTIAN HEMOPHILIA A PATIENTS WITH AND WITHOUT INHIBITORS: A SINGLE CENTER PROSPECTIVE STUDY

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Background: The safety and efficacy of Emicizumab have been demonstrated in people with congenital hemophilia A from infancy to adulthood, with and without inhibitors, in the HAVEN clinical trial program and many real-world studies. In addition to the bleeding rate it is important to assess other outcome measures as the role of Emicizumab on resolution of target joints and its impact on chronic pain and quality of life

Aims: So our study evaluates these outcome measures in 41 Egyptian severe HA patients (PwHAs) 14 with high titre inhibitors(Group 1) and 27 inhibitor negative(Group 2) with a mean age of 9 and 12 years respectively. Enrolled patients had 3 to 4 target joints. They received subcutaneous Emicizumab 3 mg/kg/2 weeks for 4 yrs and 1 yr respectively following a 4-week induction

Methods: Outcome measures of Emicizumab in PwHAs included bleeding rate, target joint resolution, joint physical examination (WFH musculoskeletal score) and imaging (HEAD- US and MRI Denver score of target joints) , chronic pain resolution and quality of life assessment by Haem-QO

Results: On baseline bleeding rate ranged from 6-8 events/ year as patients were on secondary prophylaxis and dropped to zero bleeds after receiving Emicizumab for 6 months . Few patients experienced a breakthrough bleed afterwards but none into target joints and none required hospitalization .The mean musculoskeletal score was 17 on initial assessment and dropped to 4 with resolution of target joints and relief of chronic pain on treatment with Emicizumab . Haem-QOL and radiological changes on HEAD US showed significant improvement (p= <0.001

Summary/Conclusion: Long term outcome of Emicizumab in our described cohort of PwHAs with and without inhibitors showed significant improvements in bleeding rate , resolution of target joints and chronic pain with subsequent significant impact on HRQOL.

P004

APPLICATION OF ROTATIONAL THROMBOELASTOMETRY IN THE MANAGEMENT OF INTRAOPERATIVE AND POSTOPERATIVE BLEEDING IN CARDIAC SURGERY PATIENTS

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Background: Hemostasis is essential for maintaining homeostasis by preserving blood in a fluid state and preventing excessive bleeding after vascular injury. Standard coagulation tests are limited by their delayed results (up to 90 minutes) and inability to reflect real-time coagulation status. As such, Point-of-Care (POC) methods like rotational thromboelastometry (ROTEM) are increasingly recommended. ROTEM provides a real-time, graphical assessment of coagulation from initiation to fibrinolysis, enabling rapid diagnosis and targeted therapy.

Aims: To analyze the use and results of ROTEM testing (specifically EXTEM and FIBTEM), and to review the preparation and issuance of plasma components for cardiac surgery patients at the Clinical Center of Montenegro (KCCG), based on data from the Blood Transfusion Institute of Montenegro

Methods: A retrospective analysis was conducted from October 2022 to May 2024, involving ROTEM tests and transfusion records. Data were collected from the ROTEM test protocol and transfusion issuance logs. EXTEM and FIBTEM were mandatory; additional tests were performed based on clinical indication. Data were analyzed using standard statistical methods.

Results: A total of 1,581 patients underwent ROTEM testing, with 558 (35.3%) being cardiac surgery patients. Testing was intraoperative in 414 (74.2%), postoperative in 131 (23.4%), and preoperative in 13 cases (2.3%). Among intraoperative cases, 205 (49.5%) received no plasma therapy. Others received: Cryo 64 (15.4%), SPC 59 (14.2%), Cryo+SPC 109 (26.3%), DDAVP 54 (13%), FFP 24 (5.7%). Postoperatively: Cryo 19 (14.5%), PCs 6 (4.5%), Cryo+PCs 14 (10%), DDAVP 10 (7.6%), FFP 4 (3%). In total, 2,085 Cryo, 1,482 PCs, and 92 FFP units were issued.

Summary/Conclusion: ROTEM has significantly improved the quality of care in cardiac surgery by enabling individualized, precise transfusion strategies through multidisciplinary collaboration. Its rapid and dynamic insights into coagulation facilitate efficient resource use and patient-specific therapy.

P005

PRENATAL DIAGNOSIS FOR CONGENITAL BLEEDING DISORDERS IN IRAN

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Background: Congenital bleeding disorders present significant clinical, psychological, and socioeconomic burdens. Access to genetic counseling, carrier detection, and prenatal diagnosis (PND) is essential for informed reproductive decision-making.

Aims: This study reports the implementation and outcomes of an eight-year genetic counseling and PND program for families in Iran affected by inherited bleeding disorders. Following informed consent, fetal DNA was extracted from chorionic villus samples (CVS).

Methods: Fetal sex determination for X-linked disorders was performed using SRY and amelogenin PCR. To exclude maternal DNA contamination, QF-PCR was applied. Intron 1 and intron 22 inversions in severe hemophilia A (HA) were detected via inverse PCR, while other pathogenic variants were identified by Sanger sequencing. Gene deletions were assessed using quantitative real-time PCR for gene dosage analysis.

Results: A total of 60 first-trimester PNDs were performed: 37 for severe HA, 12 for severe hemophilia B (HB), 3 for type 3 von Willebrand disease (VWD), 5 for severe factor VII deficiency, and one case each of factor XIII deficiency, Glanzmann's thrombasthenia, and prothrombin (factor II) deficiency.

Among 30 male fetuses tested for hemophilia, 15 were affected—two with intron 22 inversion type I, one with a deletion, and 12 with other pathogenic variants in F8 or F9. No familial mutations were found in the remaining 15 male fetuses (including five without intron 22 inversion type I). Additionally, 19 female fetuses from hemophilia families were identified.

In type 3 VWD, one affected fetus and two heterozygotes were detected. In factor VII deficiency, results included one heterozygote, two unaffected, and two affected fetuses. In the Glanzmann case, involving a known exon-level deletion, no fetal mutation was detected via gene dosage analysis. No familial mutations were identified in the cases of factor XIII or factor II deficiency. Three additional PNDs are planned for families with factor X, factor XI, and Bernard–Soulier syndrome.

Summary/Conclusion: This study demonstrates that genetic counseling and PND for inherited bleeding disorders are both feasible and effective. Early molecular diagnosis supports timely, informed reproductive choices and may reduce the incidence of severe bleeding disorders in future generations. The findings highlight the urgent need to incorporate genetic services into national healthcare systems, particularly in regions with high rates of consanguineous marriage and limited treatment options

P008

HAEMOSTATIC PROFILE IN ELDERLY PATIENTS UNDERGOING CARDIOPULMONARY BYPASS DOES NOT DIFFER FROM YOUNGER POPULATION.

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Background: There are some data that haemostatic profile of elderly patients is different than normal younger population, especially the trend to hypercoagulation in perioperative period in elderly patients exists. Thromboelastography (TEG) is reliable and useful method to describe global haemostatic profile in cardiac surgical patients.

Aims: To describe differences in hemostatic profile between elderly and younger patients scheduled for surgery in cardioulmonary bypass.

Methods: In prospective study two groups of elective adult cardiac surgical patients undergoing cardiopulmonary bypass (CPB) were studied: Group A – young patients (age < 70), group B- elderly patients (age ≥ 70). Standard CPB with mild hypothermia, capillary oxygenator and heparine 3mg/kg was used. Chronic anticoagulation medication was withdrawn according to common guidelines. No prophylactic antifibrinolytics were used. Patients were monitored with standard haemocoagulation testst (Quick, aPTT, thrombin time, fibrinogen, platelets) and thromboelastography (TEG® Haemoscope 5000, kaolin activated), Four blood samples for TEG were tested: 1st after induction (native), 2nd after aortic X-clamp release (heparinase) 3rd and 4th 10min after protamine administration (nativ and heparinase). Blood los volume and number of transfusions were measured too. Statistical analysis was performed by SPSS v.15, using non-parametric Mann.Whitney and Shapiro-Wilk, p= 0,05.

Results: Mean age of Group A 61,1±6,6, group B 75,6±3,4 years, there was more men (79,5% vs 59,1%) in younger population. The other demographics and distribution of procedures type were comparable. There was no difference in CPB duration (74,8 vs 80,9 min). No difference in laboratory tests before (Quick 85,7 vs 83,5, aPTT 26,85 vs 28,89, TT 13,43 vs 13,46, FBG 3,48 vs 4,40, plt 229 vs 212) and after (Quick 58,4 vs 56,5, aPTT 30 vs 31, TT 18,85 vs 18,31, FBG 2,46 vs 2, 61, plt 145 vs 135) as well as TEG parameters before (R 5,06 vs 5,37, MA 68 vs 69, CI 2,29 vs 2,15) and after (R 4,83 vs 5,13, MA 62 vs 63, CI 1,59 vs 1,45) were found. Perioperative blood loss volume (356 vs 360 ml) was not different as well as postoperative blood loss volume (802 vs 906 ml). Number of RBC (0,55 vs 1,14 p=0,011) but not FFP (0,23 vs 0,41 p=0,175) transfusion were higher in the group B.

Summary/Conclusion: No difference in haemostatic profile measured with standard laboratory tests and TEG were found in immediate perioperative period.

P009

LONG TERM FOLLOW UP OF CEREBRAL BLEEDING IN EGYPTIAN HAEMOPHILIA A AND B CHILDREN AND ADOLESCENTS WITH AND WITHOUT INHIBITORS AND THE ROLE OF PROPHYLAXIS

M. Abdelwahab

Background: Intracranial haemorrhage (ICH) is one of the potential life threatening complications in person with haemophilia (PWH) .There are very few reports of long term follow up of children and adolescents in Africa and Egypt especially in PWH with inhibitors in general nonetheless those on prophylaxis whether with FVIII concentrate or Emisuzimab

Aims: Our study will assess the incidence, risk factors , management , and outcome of ICH in PWH with and without inhibitors over the past 18 years and the role of prophylaxis

Methods: A CRF was used to collect data on all children who had presented with ICH to Cairo University Paediatric Hospital . ICH was confirmed by clinical and computed tomographic imaging data. Clinical data were extracted at baseline and during the **f**ollow-up.

Results: 19/ 250 (7.6 %) Egyptian PWH (16 HA, 13 severe, 1 moderate, 2 mild; 3HB, 1 mild, 2 severe with age ranging from 10 to 20years who developed cerebral bleeds at a mean age of 3 years were enrolled. ICH was the initial presenting symptom in 33.3% .6 children had inhibitors (3 high titre) that 5 developed after the onset of the cerebral bleed. Our studied cohort developed 26 episodes of cerebral breeds mainly post-traumatic (70%), including 18 subdural, 7 intracerebral/intraventricular, and 1 brainstem. Mortality and morbidity are usually associated with late presentation to hospital .82 % were managed conservatively with replacement therapy for 1 to 3 weeks. 22.2% died of severe brain insult, 15.4% developed a cognitive impairment with hydrocephalic changes and visual impairment in one, 1 squint, and one developed concomitant thrombosis. Nine children started biweekly secondary prophylaxis with FVIII concentrates where 7 converted to Emisuzimab (5 with inhibitors and 2 without). None had recurrent cerebral bleeds while on prophylaxis .

Summary/Conclusion: ICH is not a rare complication in the paediatric Egyptian cohort with an unfavourable outcome. Young age, lack of primary prophylaxes and trauma are the risk factors. Secondary prophylaxis whether with FVIII concentrate or Emisuzimab prevents recurrence

P011

GENETIC ASSESSMENT IN VON WILLEBRAND DISEASE SUBTYPING: INSIGHTS FROM TWO IRANIAN CASE STUDIES

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Background: Von Willebrand Disease (VWD) is the most common inherited bleeding disorder and a significant underlying cause of heavy menstrual bleeding (HMB) in women. Genetic analysis of the VWF gene is complicated by the presence of a highly homologous non-coding pseudogene on chromosome 22, which shares approximately 97% sequence identity with the VWF gene from exons 23 to 34. Gene conversion events between the VWF gene and its pseudogene can alter VWF function and complicate molecular diagnosis. Accurate subtyping of VWD is essential for guiding appropriate treatment, minimizing blood loss, preventing anemia, and avoiding potentially life-threatening complications.

Aims: To demonstrate the utility of molecular genetic testing in accurately subtyping VWD in women with unexplained HMB being considered for hysterectomy.

Methods: Two unrelated female patients with chronic menorrhagia unresponsive to hormonal therapy were selected for genetic evaluation. One patient had a clinical diagnosis of type 2A VWD, while the other presented with inconclusive results from standard VWF laboratory panels. After obtaining informed consent, genomic DNA was extracted, and targeted Sanger sequencing of exon 28 of the VWF gene was performed to detect pathogenic variants.

Results: Pathogenic VWF gene variants were identified in both patients, including silent, missense, and nonsense mutations in the heterozygous state. The findings were consistent with gene conversion events between the gene and its pseudogene, particularly highlighting the **Q1311X** mutation. Missense mutations **included**, **Pro1266Gln**, **Val1279Ile**, **Ile1343Val**, **Val1360Ala**, **Phe1369Ile** and **Ser1378Phe**. Additionally, Case 1 exhibited a **Ser1506Leu** mutation, consistent with type 2A VWD. These results enabled definitive molecular subtyping of VWD in both cases and accordingly improved patients personalized treatment. Both patients showed significant clinical improvement and case 1 is now planning for pregnancy.

Summary/Conclusion: Molecular diagnosis provided a basis for individualized, non-surgical treatment strategies, resulting in significant clinical improvement and the avoidance of hysterectomy. These cases underscore the critical role of integrating genetic analysis into the diagnostic workflow for VWD, especially when standard laboratory findings are ambiguous. Genetic subtyping not only enhances diagnostic precision but also facilitates personalized therapy and improved patient outcomes.

P012

CHALLENGING DIAGNOSIS AND MANAGEMENT IN ACQUIRED VON WILLEBRAND SYNDROME: A CASE SERIES

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Background: Von Wilebrand disease (VWD) is a bleeding disorder caused by abnormalities in the Von Wilebrand Factor (VWF). There is a form known as Acquired Von Willebrand syndrome (AVWS) where there is a structural or functional alteration of the VWF that is not congenital. This acquired condition leads to bleeding symptoms, typically as a result of an underlying disorder, such as autoimmune diseases, certain types of cancer, or cardiovascular diseases.

The prevalence of AVWS is estimated to be between 0.04% and 0.10%. However, it is believed to be significantly underdiagnosed. This condition often presents in individuals older than 60 years of age, typically without a personal or family history of bleeding disorders. The bleeding episodes in acquired von Willebrand syndrome are commonly associated with the presence of a secondary underlying disease, which may contribute to the abnormal behavior of the von Willebrand factor.

Aims:

- Evaluate the clinical characteristics of the patients.
- Establish recommendations to improve diagnosis

Methods: Review of patients diagnosed with acquired von Willebrand disease in a Spanish province over the last 15 years.

Results: We present 8 cases of patients diagnosed with acquired von Willebrand syndrome in a Spanish region over the last 15 years. In our study, we identified 8 cases in a population, about 1.54 cases per 100000 inhabitants. The average age of the diagnosed patients was 64.5 years. The most prevalent cause was identified as hematological, accounting for 62.5% of cases, followed by autoimmune causes 25%, and the remaining 12.5% due to other neoplasms. Most patients presented with mild hemorrhagic symptoms and a prolonged aPTT in laboratory tests. The acquired von Willebrand syndrome was corrected in all patients except one, who passed away due to gastrointestinal bleeding.

	Symptoms	Fvw:CoR/FvW:Ag	FVIII	Resolution after treatment of underlying disease
Waldenström's macroglobulinemia (WM) (2)	Spontaneous epistaxis (2)	-26%/23.1% -35%/43%	-43% -30%	Yes (2)
Other neoplasms (1)	No suggestive clinic	-11%/30%	46.3%	Yes
SMPc (2)	Hematomas (2)	-31.5%/ 101.7% -26%/70%	-69% -89%	Yes (2)
LLC (1)	Severe anemia due to gastrointestinal bleeding	9%/19%	24%	No
Autoinmune deseases (2)	-Spontaneous hematomas -Epistaxis	-30%/11% -<10%/20%	-12% -40%	Yes (2)

Summary/Conclusion: The findings indicate a substantial correlation between EVWA and elderly patients afflicted with multiple comorbidities, encompassing hematologic, autoimmune, neoplastic diseases. The clinical observations demonstrate that the treatment of the underlying disease leads to the resolution of EVWA in the majority of patients, thereby suggesting a potential reversibility of this pathological condition. EVWA is an underdiagnosed entity that can manifest in various clinical contexts, wich underlines the importance of increasing its visibility to include it in the differential diagnosis of patients with unexplained bleeding in the context of other diseases, thus allowing an appropiate diagnosis and treatment.

P013

HETEROGENEITY OF FXI FORMS: STRUCTURAL AND FUNCTIONAL INSIGHTS FROM THE NOVEL P.PHE295CYS VARIANT

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Background: Factor XI (FXI) is a coagulation protein that has garnered increasing attention due to its role in linking coagulation with the contact pathway and its involvement in sustaining thrombin generation. FXI circulates in plasma as a 160 kDa disulfide-linked homodimer. It contains 36 cysteine (Cys) residues: 34 formed 17 intrachain disulfide bonds, Cys339 forms the interchain disulfide bond, and Cys29 seems to remain free.

Aims: To investigate the structural, functional, and clinical implications of a novel FXI variant that introduces an additional Cys residue

Methods: We studied a family with FXI deficiency. Plasma FXI activity was measured using standard coagulometric assays (FXI:C). Western blot analysis of plasma FXI, FXII, FIX and Kallikrein were performed under reducing and non-reducing conditions, both at baseline and after activation of the contact pathway using silica or ellagic acid. Genetic analyses included next-generation sequencing (NGS), MLPA, long-read nanopore sequencing, and short tandem repeat (STR) analysis. In addition, wild-type (WT) and mutant FXI were expressed in HEK293T cells.

Results: The proband, evaluated preoperatively, exhibited prolonged APTT and markedly reduced FXI activity (FXI:C 9%). He received prophylactic treatment with FXI concentrate (Hemoleven 20IU/kg) and experienced no bleeding. NGS revealed a novel variant, c.884T>G (p.Phe295Cys), in apparent homozygous state. The mother carried the variant in heterozygous state and presented mild FXI deficiency (65%) with no bleeding history, while the father and sister with normal FXI levels (100-120%) had no pathogenic variants. STR analysis confirmed paternity. MLPA and nanopore sequencing identified a *de novo* 7Mb deletion on the paternal allele, encompassing 30 genes including *F11* and *KLKB1*, establishing the proband as hemizygous for p.Phe295Cys. Remarkably, all p.Phe295Cys carriers showed two distinct FXI dimers, one resembling the WT and another of apparent higher molecular weight. In the proband, contact pathway activation selectively triggered the activation of the larger dimer, which retained the ability to activate FIX. Recombinant expression of the p.Phe295Cys variant secreted 2 distinct dimers and revealed 2 different monomeric forms under non-reducing conditions. All forms collapsed into a single FXI band under reducing conditions, ruling out other covalent post-translational modification than a different disulfide bond rearrangement. To investigate whether the introduced Cys295 formed an interchain disulfide bond or a novel intrachain bond with the native Cys29, we generated double mutants (p.Phe295Cys/Cys339Ala and p.Phe295Cys/Cys29Ala). These experiments excluded both mechanisms as the cause of the observed dimer heterogeneity. Additionally, the proband also presented kallikrein deficiency (50%) due to the large *de novo* deletion.

Summary/Conclusion: We identified a novel *F11* variant, p.Phe295Cys, associated with FXI deficiency in both heterozygous and hemizygous states—the latter due to a *de novo* deletion also causing kallikrein deficiency without apparent clinical impact. This variant promotes the formation of structurally and

functionally distinct FXI dimers, highlighting that disulfide bond rearrangements can occur without impairing secretion but with significant functional consequences. These findings underscore the need to explore disulfide heterogeneity in other genetic or environmental contexts, given its potential pathophysiological relevance.

P014

UNVEILING THE CLINICAL SPECTRUM AND BURDEN OF RARE BLEEDING DISORDERS: A DECADE OF DATA FROM A SOUTH ASIAN LOWER MIDDLE-INCOME COUNTRY (LMIC)

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Background: Rare bleeding disorders (RBDs) are heterogeneous with increased prevalence in consanguineous populations. In South Asia, diagnostic limitations and inadequate haemostatic infrastructure delay recognition and management, with scarce regional data despite a likely higher burden.

Aims: To define the prevalence, spectrum, severity and clinical profile of RBDs in a South Asian cohort and evaluate treatment strategies, addressing a critical data gap in LMICs to guide diagnostic prioritization, improve clinical awareness and inform policy for bleeding disorder management in resource-limited settings.

Methods: A cross-sectional study (Jan 2014 - Dec 2023) at Armed Forces Institute of Pathology Pakistan included patients with suspected inherited coagulation disorders, excluding acquired and platelet function defects. After informed consent, clinical and demographic data were recorded. Coagulation screening (PT, aPTT, TT) was done on Sysmex CA-1500, followed by mixing studies, Clauss fibrinogen assay and factor XIII urea solubility test. Factor assays used CS-5100 with Siemens factor-deficient plasma. Patients were followed for bleeding episodes and management. The study was conducted in accordance with the Declaration of Helsinki. No funding received for this study.

Results: Among 6190 patients assessed for suspected coagulation disorders, 1848 (29.9%) had inherited bleeding disorders: Haemophilia A (721; 39.0%), Haemophilia B (205; 11.1%), von Willebrand disease (536; 29.0%), and RBDs (372; 20.1%). Of RBD patients, 205 (55.1%) were males (M:F = 1.2:1). Median age at first bleeding was 1.5 years (IQR: 0.25–6.0) and at diagnosis 9.25 years (IQR: 2.0–17.0), indicating a mean diagnostic delay of 7.75 years. Parental consanguinity was observed in 266 (71.5%) and 41 (11.0%) had a positive family history. The most prevalent RBD was factor VII deficiency (96; 25.8%), followed by fibrinogen (86; 23.1%), factor V (61; 16.4%), factor XIII (46; 12.4%), factor X (42; 11.3%), factor XI (26; 7.0%), factor II (4; 1.1%), and factor XII (3; 0.8%) deficiencies; 8 patients (2.2%) had combined factor V and VIII deficiency. Female predominance was noted in fibrinogen and factor XIII deficiencies. Bruising (61.9%) and gum bleeding (55.1%) were most common, followed by epistaxis (45.9%), trauma/surgical bleeding (45.7%), and menorrhagia (22.3%)—notably in females with fibrinogen, factor VII, and X deficiencies. Umbilical bleeding (5.6%) was linked to fibrinogen and XIII deficiencies; intracranial haemorrhage (1.3%) occurred in factor X (n=3), VII (n=1), and XIII (n=1) deficiencies. Severe phenotypes were associated with X, VII, and XIII deficiencies. Recurrent bleeding occurred in 64.8%. During follow-up, local measures sufficed in 12.1%, while antifibrinolytics were primary treatment in 29.6%. Transfusions were required in 53.2%; FFPs were administered once in 24.7%, and intermittently in 21.8%. Cryoprecipitate was used in 6.5%. Recombinant FVIIa was utilized in 3% for delivery, postpartum haemorrhage, trauma and laparotomy. No treatment was required in 1.8% of cases.

Summary/Conclusion: This study from Pakistan shows RBDs comprise 20.1% of inherited bleeding disorders, underscoring high consanguinity, diagnostic delays, and urgent need for equitable diagnostics, counselling, and treatment access.

P015

ACQUIRED COAGULOPATHY AS AN INITIAL PRESENTATION OF RARE DISEASE : A CASE STUDY IN AN EGYPTIAN TERTIARY HOSPITAL

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Background: Hypoprothrombinemia can be congenital or acquired as the result of another disease process as liver disease in severe inborn metabolic disorder, anticoagulant drugs, SLE or vitamin K deficiency or an adverse effect of drugs. Clinical presentation is by easy bruising, postoperative bleeding, epistaxis, menorrhagia, miscarriage, postpartum hemorrhage, hemarthroses and intracranial bleeding usually in severe deficiency.

Aims: Highlight that cerebral bleeding due to a rare coagulopathy can be secondary and the initial presenting symptom of rare metabolic disorder

Methods: A 1-year-old boy of consanguineous marriage presented at the age of 4 months by fits mismanaged as hypocalcemia then started developing bleeding per rectum and passed into coma. CT scan brain was done showing intracranial hemorrhage for which he received replacement therapy in the form of fresh frozen plasma and blood transfusion twice. Patient was investigated for an underlying cause of his bleeding tendency Patient then developed another episode of intracranial hemorrhage, continued replacement therapy and was discharged with no neurological sequelae. Two months later, patient developed red urine with very mild jaundice accidentally noted but with normal stools. Patients had hepatomegaly. Liver function tests and coagulation profile were done.

Results: Patient had hyperbilirubinemia, increased alkaline phosphatase, prolonged PT and PTT and low prothrombin less than 10%. There was high alphafetoprotein . CTscan brain showed subarachnoid hemorrhage and left frontal and temporal cortical cerebral hematoma with adjacent subdural hemorrhage and pnemocepahly.. Patient then developed another left cerebral cortical and subcortical frontotemporoparieto-occipital hemorrhagic infarct. Sonarshowed mild hepatomegaly and prominent renal pelvicalceal system. Succinly acetone, tyrosine and methionine was done and patient was diagnosed as tyrosinemia type 1

Summary/Conclusion: Spontaneous intracranial hemorrhage in infancy CAN be the initial presenting symptom of a bleeding tendency whether primary or secondary .Well-established liver failure with coagulopathy is one of the common presentations of tyrosinemia but a severe coagulopathy in the absence of significant signs of liver disease has very rarely been described as a presenting feature of this condition.

P016

ACQUIRED HEMOPHILIA BY THE PRESENCE OF ANTIFACTOR VII AUTOANTIBODIES ASSOCIATED WITH AN ANTIPHOSPHOLIPID SYNDROME

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Background: Acquired hemophilia is a rare disease characterized by the presence of neutralizing autoantibodies against hemostasis factors, most often factor VII. However, a few cases of anti-factor VII (FVII) autoantibodies have been reported in the literature.

Aims: We report a case in this regard.

Methods: A 28-year-old woman with no family or personal history presented with a severe hemorrhagic syndrome made of very abundant metrorrhagia with multiple compressive cervical hematomas, associated with pulmonary embolism and thrombosis of the right external iliac vein. On the biological assessment, the patient presented an undetectable PT with a factor VII titer less than 6%. The hemorrhagic syndrome was aggravated by infusions of activated factor VII (FVIIa) with the appearance of hemoptysis and hematomas in all four limbs.

Results: Etiological assessment concluded the presence of anti-factor VII autoantibodies as well as circulating lupus anticoagulant. Other antiphospholipid syndrome (APS) antibodies as well as antibodies against other hemostasis factors were absent. A treatment combining corticosteroid therapy (1mg per kg per day of prednisone) and Mycophenolate mofetil (MMF) (3g) was initiated. Faced with the persistence of the hemorrhagic syndrome, rituximab was administered according to the rheumatological protocol (1g on D1 and 1g on D15). Metrorrhagia improved initially but recurred with the resumption of menstruation with a drop in hemoglobin from 9 to 7 g/dl. Plasmapheresis sessions were attempted but thromboses on the catheters occurred each time. Immunoglobulin (IVIg) courses were administered. No anticoagulant was administered. TP increased to 23%. Levels of neutralizing antibodies fell from 512 to 2 IBU at the last follow-up at five months from the bleeding event. The circulating lupus anticoagulant was present on the follow-up test after 12 weeks, thus confirming the diagnosis of APS.

Summary/Conclusion: Our patient presents an extremely rare case of acquired haemophilia. The combination of corticosteroid therapy, conventional and biological immunosuppressive, IVIG and plasmapheresis saved the patient. In the absence of consensus on the treatment, it remains adapted according to the severity of the hemorrhagic syndrome and the associated comorbidities.

P017

PERIOPERATIVE MONITORING OF THE BASIC PARAMETERS OF HEMOSTASIS AND BLEEDING IN PATIENTS WITH MAJOR ORTHOPEDIC OPERATIONS

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Background: Major orthopedic operations are classified as surgical inteventions with a moderate hemorrhagic perioperative risk. In order to reduce bleeding complications, special care is needed in the perioperative monitoring of these patients.

Aims: The aim of our research is to determine is there a correlation of the basic parameters of hemostasis and perioperative bleeding intensity in patients with major orthopedic operations.

Methods: In our instutution, 90 patients with planned total hip arthroplasty (THA) or total knee arthroplasty (TKA) and total hip arthroplasty after femoral neck fracture were monitored. We followed an average concentrations of hemoglobin, hematocrit, fibrinogen and platelet count before and after surgery. It was investigated is there a correlation of these parameters with perioperative bleeding intensity.

Results: The mean preoperative concentration of fibrinogen was statistically significantly higher in the HFS patients group compered to THA and TKA patients (4,29 g/L vs. 3,49 g/L for THA and 3,77 g/Lfor TKA). Mean preoperative hemoglobin concentrations (118,92 g/L for HFS vs. 137,54 g/L for THA and 130,31 g/L for TKA, *P* 0,001) and mean hematocrit value (0,35 L/L for HFS vs. 0,41 L/L for THA and 0,39 L/L for TKA, *P* 0,001) were statistically significantly lower, while no statistically significant differences were recorded in postoperative follow-up. The mean postoperative platelet count was statistically significantly higher in the HFS group. The mean platelet count in HFS patients group was $242,33x10^9$ on the first postoperative day; $181,64x10^9$ fot THA and $204,43x10^9$ for TKA, *P* 0,002, and $243,75x10^9$ on the second day vs. $180,38x10^9$ for THA and $187,56x10^9$ for TKA, *P* 0,008. It was obtained a statistically significant negative correlation of fibrinogen concentration and a drop in hemoglobin concentrations (Spearman's correlation coefficient -0,217) and hematocrit value (Spearman's correlation coefficient -0,249) in all patients. Also, it was found a statistically significant negative correlation of a mean platelet count postoperatively with a drop in hemoglobin and hematocrit concentrations.

Summary/Conclusion: It is possible that homeostatic mechanisms lead to a change in the hemostatic coagulation system, due to wich patients bleed less than expected. However, in order to make more precise conclusions it is necessary to conduct a study on a larger group of patients.

P018

ELEVATED BLOOD ALCOHOL CONCENTRATION AT STROKE ONSET IS AN INDEPENDENT PREDICTOR OF POOR CLINICAL OUTCOMES AND MORTALITY IN PATIENTS WITH INTRACEREBRAL HEMORRHAGE

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Background: Spontaneous intracerebral hemorrhage (ICH) is associated with high mortality and poor functional outcomes. While chronic alcohol use is a recognized risk factor for ICH, the impact of acute alcohol intake at the time of ICH onset remains unclear.

Aims: This study aimed to evaluate the association between elevated blood alcohol concentration (BAC) at hospital admission and clinical outcomes in patients with ICH.

Methods: We conducted a retrospective cohort study of 1,081 adult patients admitted with spontaneous ICH between 2000 and 2023 at a tertiary stroke center in Hungary. Patients with traumatic, vascular, or malignant causes of ICH were excluded. BAC was measured at admission when alcohol use was suspected. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS) and the Glasgow Coma Scale (GCS). Assessment of short-term outcomes included 7-day neurological deterioration (ND) and early mortality, long-term outcomes included 90-day mortality and modified Rankin Scale (mRS) scores. Logistic regression models were used to assess the independent effect of acute alcohol intake on clinical outcomes.

Results: Among the 1,081 patients, 31 (2.9%) had elevated BAC. These patients were significantly younger, more likely to be male, and exhibited larger hematoma volumes and more frequent midline shifts (≥5mm). They had significantly worse clinical presentations (higher NIHSS, lower GCS) and less frequent use of chronic medications. The alcohol group showed a markedly higher rate of ND (58.1% vs. 27.6% p< 0.001) and mortality at 7 days (51.6% vs. 23.7%), p< 0.001) and 30 days (71% vs. 37.6%, p<0.001) compared to controls. Elevated BAC was an independent predictor of ND (OR: 3.6, 95%CI: 1.02-12.73, p = 0.047) and 30-day mortality (OR: 3.9, 95%CI: 1.01-21.66, p = 0.049). Kaplan–Meier analysis showed significantly worst 90-day survival in patients with elevated BAC as compared to those without alcohol consumption at the time of event (log-rank p< 0.001).

Summary/Conclusion: Acute alcohol intake at the time of ICH onset is associated with worse functional outcomes, including neurological deterioration and mortality. BAC measurement may support early risk stratification in ICH patients.

P019

ROLE OF THROMBIN GENERATION ASSAYS IN RECLASSIFYING MODERATE HEMOPHILIA A

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Background: Hemophilia A is classically categorized by residual factor VIII activity (FVIII:C) into severe (<1%), moderate (1–5%), and mild (5–40%) disease. However, clinical heterogeneity within the moderate hemophilia group is increasingly recognized, with some patients experiencing early-onset joint bleeding and hemophilic arthropathy despite similar FVIII:C levels. Recent international studies, including the MoHem and Nordic cohorts, have highlighted that moderate hemophilia A patients—particularly those with FVIII:C ≤3 IU/dL—often present with joint damage and reduced quality of life comparable to severe cases. Thrombin generation potential, an emerging global assay of hemostatic function, may provide deeper insight into this phenotypic variability, yet its clinical relevance in moderate hemophilia remains underexplored.

Aims: This study aims to evaluate the frequency of impaired thrombin generation among patients with moderate hemophilia A (FVIII:C 1–5%) and to investigate its association with hemophilic arthropathy. By identifying subgroups at higher risk of joint deterioration, we seek to inform more individualized and proactive therapeutic strategies for this under-characterized population.

Methods: Following Institutional Review Board (IRB) approval, whole blood samples will be collected from patients with moderate hemophilia A (FVIII:C 1–5%) confirmed via electronic medical records. Participants will undergo a wash-out period appropriate to their FVIII product half-life (≥36 hours for standard and ≥45 hours for extended half-life products) prior to sampling. Plasma will be prepared for coagulation assays including FVIII:C, aPTT, and clot waveform analysis (CWA), with thrombin generation assay (TGA) performed by a central laboratory. Hemophilic joint health will be assessed using the Hemophilia Joint Health Score (HJHS) and plain radiographs of target joints (elbow, knee, ankle). Data will be analyzed to determine correlations between thrombin generation capacity and joint disease severity.

Results: Study results are yet to be delivered at this point, as table listing figures are expected by the end of September 2025 and the final report is to be completed by the end of 2025. Expected hypothesis is that patients with lower thrombin generation, particularly those with reduced ETP and peak thrombin values, will exhibit a higher frequency of joint bleeding and worse HJHS scores. In contrast, patients with relatively preserved thrombin generation are expected to be demonstrate lower bleeding rates, even within the same FVIII:C range. Lag time may demonstrate weaker correlation, but ETP may emerge as a key indicator, inversely associated with clinical bleeding episodes.

Summary/Conclusion: If the above expected results hold true, thrombin generation assay parameters, especially ETP and peak thrombin, will correlate with bleeding tendency and joint health status in patients with moderate hemophilia A. These findings will support the utility of TGA as a complementary tool to FVIII:C levels for individual bleeding risk assessment. Incorporating TGA into clinical practice may enable more personalized treatment strategies, potentially improving outcomes for this heterogeneous patient population.

P020

ANALYSIS OF COMORBIDITIES IN PATIENTS WITH HEMOPHILIA: BEYOND JOINT DISORDERS TO COMPREHENSIVE COMPLICATION MANAGEMENT

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Background: Hemophilia is caused by deficiency or dysfunction of clotting factors. While the hallmark clinical feature is recurrent bleeding into joints and muscles leading to chronic joint damage and bone disease, patients with hemophilia A (PwHA) often experience a range of additional comorbidities that extend beyond the musculoskeletal system. These include cardiovascular disease, chronic kidney disease, liver disease often related to viral infections or treatment complications, and neurocognitive or mental health disorders. The chronic inflammation and repeated bleeding episodes inherent in hemophilia, along with treatment-related factors, contribute to these systemic complications. Despite advances in prophylactic treatment, the overall burden of these comorbidities remains poorly characterized in PwHA. Real-world epidemiological data from National Health Insurance Service database and Korea Hemophilia Foundation Annual Report can provide valuable insights into the prevalence and impact of these associated conditions..

Aims: This study aims to evaluate the broad spectrum of comorbidities in Korean patients with hemophilia A—beyond the well-known musculoskeletal complications—using national claims data.

Methods: We conducted a retrospective analysis of data from two different sources: (a) Korea National Health Insurance Service (NHIS) Sample Cohort dated from January 1, 2023 to December 2023, which comprises claims data from one million anonymized individuals, representing about 2% of the Korean population; and (b) Korea Hemophilia Foundation Annual Report 2024, which comprises data from diagnosed and registered patients with any bleeding disorders including PwHA. PwHA from the Sample Cohort was identified using the Korea Standard Classification of Diseases (KCD) Code D66 (coagulation FVIII deficiency) documented as primary or secondary disease. Of these predefined PwHA, comorbid conditions were extracted and categorized to assess the prevalence of common comorbidities in this population. The data were further weighted and extrapolated to represent the total Korean population of approximately 50 million.

Results: 1,550 PwHA were included and analyzed in the NHIS sample cohort, whereas 1,835 registered PwHA were included in the KHF Annual Report. Essential (primary) hypertension (I10) were reported in 16% (250/1,550) of PwHA, indicating a significant prevalence of cardiovascular risks. Liver disease (K76) was reported in 3% (50/1,550), indicating complications of HCV. HIV was reported in less than 1% (3/1,835) according to the KHF Annual Report, showing no change since first reported in 1991.

Summary/Conclusion: Although joint disorders are the most prevalent comorbidities in PwHA, cardiovascular diseases, liver diseases, and HIV also contribute notably to the disease burden. This multifaceted complication profile emphasizes the multifactorial nature of hemophilia complications and the importance of comprehensive, multidisciplinary care approaches addressing not only bleeding control and joint health but also cardiovascular, liver, and infectious diseases to improve the overall patient outcome.

P021

THROMBIN GENERATION ASSAY IN PHARMACOKINETIC MONITORING OF PATIENTS WITH HEMOPHILIA A ON DIFFERENT FVIII CONCENTRATES AND PATIENTS ON EMICIZUMAB PROPHYLAXIS

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Background: Monitoring FVIII activity is often insufficient to describe the full hemostatic process in patients with hemophilia A on replacement therapy. Thrombin generation assay (TGA) is a promising method, as it is a global test monitoring the entire coagulation process and providing reflection of the hemostatic capacity of the patient. Since in most published studies TGA was measured by the Calibrated automated thrombogram (CAT) system, additional data for the Ceveron S100 system would be helpful.

Aims: First, to measure TGA on FVIII samples from pharmacokinetic measurements from a selection of different patients on different FVIII concentrates. Next, to evaluate the correlation of TGA results with FVIII activity values. Lastly, to verify TGA measurements in patients on emicizumab prophylaxis with the Ceveron S100 system.

Methods: We tested 18 patients and their samples from the pharmacokinetics determination of 11 different FVIII concentrates (full-length FVIII, B-domain deleted, extended half-life – EHL) and also 21 samples of patients on emicizumab prophylaxis without factor therapy. FVIII activity was measured on these samples by the reagents recommended by the product manufacturers. TGA measurement was performed on the Ceveron S100 system (Technoclone), which is a fully automatic coagulometer with fluorimetric detection. The reagent selected was the TGA RB kit and the parameter used was endogenous thrombin potential (ETP, area under curve – AUC).

Results: In samples of 6 patients on full-length FVIII concentrates, thrombin generation followed the FVIII pharmacokinetic curve. In patients on FVIII concentrates with deleted B-domain (n = 3) and in EHL concentrates (n = 9), ETP reached higher values, and persisted for a longer time than FVIII activity. The peak of ETP in TGA did not correspond to the peak of FVIII activity after administration, thrombin generation peaked at a later time than FVIII activity. In the samples of patients on emicizumab prophylaxis, ETP did not correlate with emicizumab levels, which is consistent with previous data from CAT analyses.

Summary/Conclusion: Pharmacokinetics of EHL FVIII concentrates showed increased and prolonged ETP compared to FVIII activity in our patients. The reason for this is unclear, however it may be due to the testing duration of TGA compared to the end-point measurement of FVIII. Further study on a larger number of patients is needed to evaluate if there is a relationship between TGA results and the efficacy of FVIII therapy in these patients. We also confirmed that TGA does not correlate with emicizumab levels, but it can be useful in determining the hemostatic state in patients on emicizumab in combination with other factor or non-factor therapies.

P023

RECURRENT VENOUS THROMBOSIS IN A PATIENT WITH SEVERE FV DEFICIENCY

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Background: Congenital FV deficiency is a rare, autosomal recessive bleeding disorder (incidence 1:10^6). Symtomatic patients show very low or no measurable plasma FV levels and are usually homozygous or compound heterozygous for FV gene mutations. The available therapy is fresh frozen plasma (FFP) with published experience using recombinant activated FVII. Prevalence of venous thromboembolism (DVT) is 0,3% (Agrawai et al, MP_19_WFH2024). There are no published guidelines to treat VT in this setting

Aims: Report our experience about treatment of DVT in FV deficiency

Methods: Report an unusual case of severe FV deficiency with 2nd episode of DVT after 1st episode of jugular vein thrombosis after insertion of central venous catheter (CVC) 5 years ago (presented in EAHAD, 2025 Milano) and the management performed.

Results: Woman, 83-year-old, severe FV deficiency (FV:C <1 %) and bleeding phenotype. Multiple admissions for bleeds of varying severity. Personal history includes TRALI (transfusion-induced lung injury) in 2003 and 2016. As cardiovascular risk factors, hypertension on treatment, dyslipidaemia and overweight. Jugular vein thrombosis in 2020, after insertion of central venous catheter resolved after 10 days of treatment with FFP and LMWH (Low molecular weigth heparin). She was admitted for gastrointestinal haemorraghe and completed 10 days with therapy with FFP to maintain trough FV level around 15-20%. 72 hours after discharge, she went to hospital emergency department with swelling in her neck and right arm, Doppler Ultrasound (US) showed thrombosis of right internal and external jugular veins, right cephalic and subclavian veins without catheter involvement In that moment, we decided repeat the same treatment done in 2020: infusion of FFP (to mantain through FV level around 15-20 IU/dl) and parenteral anticoagulation with Enoxaparin sodium, first dose at 1 mg/kg followed by 0.75 mg/kg each 12 h, maintaining AntiXa level close to 0.5 IU/ml (mean level 0.61 IU AntiXa). We treated 14 days, with good tolerance, no adverse events (especially bleedings) and good healing. Before discharge, we repeated the Doppler ultrasound and showed complete resolution of thrombus less in the external jugular that would be partially recanalized.

Summary/Conclusion: DVT is unusual in severe FV deficiency with bleeding phenotype and more unusual is that the same patient has 2 episodes. We didn´t find recommendations to treat this problem, so it is interesting to report it since it's difficult to generate scientific evidence to establish management recommendations. We decided to repeat the same treatment that on the first occasion: therapy with FFP, in addition to Low Molecular Weight Heparin near therapeutic doses,higher than that used in 2020 (0.5 mg/kg/12 h), due to good previous experience and adjusting for a borderline' AntiXa. Finally we wait for the patient's natural fibrinolytic system to complete resolution of thrombus.

P024

NORMALIZATION OF HEMOSTASIS AND CARDIOVASCULAR RISK IN HEMOPHILIA: A CASE REPORT

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Background: The evolution of treatments in haemophilia makes it possible to cover, at different stages, the different needs of our patients to the point that, at the present time, the life expectancy of a haemophiliac patient is similar to that of any person of the same age and general condition."Normalization of hemostasis" refers to restoring the body's ability to clot blood properly, similar to that of a person without this disorder. The question is whether this normalisation will not lead to an increase in thrombotic risk, due to the loss of protection derived from its basal hypocoagulability

Aims: To report a clinical case of a patient with moderate hemophilia B and high cardiovascular risk who suffers a lacunar stroke in the postoperative period of TAVI (Transcatheter Aortic Valve Implantation)

Methods: Electronic medical record data collection

Results: Male, 73 years old, moderate HB (baseline FIX 3.2%), on prophylaxis with Nonacog beta Pegol 40 IU/Kg every 10 days. CVRF presents: hypertension to treatment, overweight, dyslipidemia to treatment. Liver disease secondary to HCV. Severe aortic stenosis. Antigregated with Adiro 100. Hemophilic arthropathy of the knee I (need for a cane). Proposed for TAVI that is performed with prior administration of Nonacog Beta Pegol (80 IU/Kg) according to the technical data sheet. Procedure performed with use of a 5000 IU unfractionated heparin bolus, reversed at the end of the procedure. Antiplatelet therapy was not discontinued at any time, combined with non-pharmacological antithrombotic prophylaxis. 24 hours after the procedure, with chromogenic F9 levels of 87%, the patient presented dysarthria and choreoic movements. Evaluated by neurology, it concludes that it is an NHSS (National Institutes of Health Stroke Scale) 9 lacunar stroke. In this moment we decided associate low molecular weight heparin (Enoxaparin sodium at 40 mg/day (sc)), in addition to other symptomatic measures. Discharge to day +14 receiving a total of 9000 IU of Nonacog Beta Pegol and resuming his usual prophylaxis to day +14., maintaining the previous antiplatelet therapy. The patient started rehabilitation physiotherapy with little improvement.

Summary/Conclusion: Currently, with the treatments available, the goal of spontaneous bleeding 0 for our patients is very close. The normalization of hemostasis is a key objective in order to achieve equity for our patients, but, at the same time, normalizing hemostasis can facilitate the clinical expression of cardiovascular comorbidities, whether in the form of stroke, ischemic cardiac attacks or deep venous thrombosis. With the increase in the life expectancy of people with hemophilia, even in the most severe forms, we must be extremely vigilant in our patients, maximizing their cardiovascular health and probably opt for prophylaxis strategies that avoid factor peaks in patients with high cardiovascular risk, such as anti-TFPI rebalancing therapies or others. However, in this case, it was inevitable to avoid the peak level given that it was a surgery with a high bleeding risk.

P025

S EVALUATING THE IMPACT OF PROPOFOL-INDUCED LIPEMIA AND HIL ON ROUTINE COAGULATION TESTS: A COMPARATIVE STUDY OF OPTICAL AND MECHANICAL METHODS

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Background: Propofol is a rapidly-acting intravenous anesthetic that is increasingly used in continuous infusion for mechanically ventilated patients in intensive care units (ICUs). Additionally, surgical patients may sometimes be exposed to propofol infusions over several hours during complex surgeries. In both types of situations, urgent coagulation profile testing may be required. Due to its high lipophilicity and formulation in a 10% oil-in-water lipid emulsion, however, prolonged propofol infusion is a primary cause of high lipemic index in clinical laboratory specimens. This lipemia can significantly interfere with the accuracy of routine coagulation tests, posing a challenge for timely and accurate clinical decision-making.

Aims: The primary objective of this study is to compare the effects of propofol-induced lipemia and hemolysis, interference with lipids, and interference with hemolysis (HIL) on routine coagulation tests using two distinct methodologies: mechanical and optical. This comparison aims to determine the reliability and accuracy of each method under conditions of high lipemia and hemolysis.

Methods: Prothrombin time (PT) activated partial thromboplastin time (aPTT), and fibrinogen (Clauss method) were measured in parallel on an optical analyzer (CS-5100, Sysmex America, Inc.) and a mechanical analyzer (Compact, Diagnostica Stago, Inc.). Pooled normal plasma or plasma from healthy donors was spiked with propofol to reach lipemia levels expected after 4-hour and 8-hour infusions. Comparative assays were also performed on samples from ICU and emergency patients selected for high HIL index (n=51). This comprehensive approach allowed for a detailed examination of the impact of lipemia and hemolysis on coagulation test results.

Results: The optical method failed to measure PT at lipemia levels expected after a 4-hour propofol infusion. At lipemia levels approximated after an 8-hour infusion, the optical method could not produce results for PT, aPTT, and fibrinogen. Additionally, it failed to measure PT in 40% of patient samples (90% with lipemia), aPTT in 29% (93% lipemia), and fibrinogen in 29% (73% lipemia, 60% hemolysis). In contrast, the mechanical analyzer produced results for all samples under all HIL conditions, demonstrating its robustness and reliability in the presence of high lipemia and hemolysis.

Summary/Conclusion: These findings highlight the significant limitations of the optical method in the presence of high propofol concentrations and underscore the reliability of the mechanical method for accurate routine coagulation testing in critical clinical scenarios. This study is particularly relevant for surgeries requiring prolonged propofol infusion, where timely and accurate coagulation assessment is crucial for optimal patient management and outcomes. The results of this study provide valuable insights for clinical laboratories and healthcare providers, emphasizing the importance of selecting appropriate methodologies for coagulation testing in patients receiving propofol infusions.

P026

FACTOR VIII ACTIVITY MEASUREMENT OF RECOMBINANT EHL FVIII PRODUCTS WITH ONE–STAGE CLOTTING AND CHROMOGENIC SUBSTRATE ASSAY: A SINGLE–CENTRE STUDY

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Background: Routine post–administration monitoring of haemophilia replacement therapy relies on the one-stage clotting assay (OSA) and chromogenic substrate assay (CSA). Discrepancies in FVIII activity (FVIII:C) measurements have been observed when assessing extended half-life FVIII products, due to structural and chemical modifications.

Aims: Evaluation of FVIII:C measurement using different assays in spiked and post–infusion samples containing rurioctocog alfa pegol, turoctocog alfa pegol, and efmoroctocog alfa.

Methods: Commercial FVIII-deficient plasma was spiked with defined levels of rurioctocog alfa pegol, turoctocog alfa pegol, or efmoroctocog alfa. Spiked and post-infusion samples were tested using CSA (Biophen™ FVIII:C and Siemens FVIII Chromogenic) and OSA with Pathromtin® SL, Actin® FS, Actin® FSL, CK Prest®, on CN 3000 or STA R MAX analysers. Target levels were based on labelled potencies, and acceptable recovery was defined as 100 ± 25%.

Results: Rurioctocog alfa pegol

All OSA and CSA methods showed acceptable recovery, with the closest match to target values using CSA Biophen™ FVIII:C (92–99%). No significant differences were observed among the OSA methods, indicating consistent results. The OSA recovery for sample with 104 IU/dL ranged from 84–93%, while CSA was 96%. For samples with 52 IU/dL and 25 IU/dL, OSA recovery ranged from 89–97% and 86–95%, respectively, while CSA ranged from 90–99%, and 91–99%. The OSA recovery for the 6.58 IU/dL sample was 79–105%, compared to CSA 89–92%. Overall OSA methods showed comparable performance to the CSA methods across all concentrations.

Turoctocog alfa pegol

OSA Pathromtin® SL showed the most accurate recovery (92–109%). OSA Actin® FS, CSA Biophen™ FVIII:C and CSA Siemens demonstrated acceptable recovery (87–95%, 109–119% and 107–116%, respectively). Actin® FSL had lower but still sufficient recovery (76–88%). CK Prest® achieved acceptable recovery in samples with 53, 27 and 6.6 IU/dL, but not in 106 IU/dL (70%). Differences among OSA methods were observed at 106 IU/dL ($p = 0.0039$). The OSA results in samples with 53 and 27 IU/dL were on average 30% and 26% lower than those with CSA.

Efmoroctocog alfa

Both CSA methods showed higher recovery (133–141%). OSA Actin® FS had the closest recovery to the target (122–124%) across all concentrations. Acceptable recovery was observed for OSA Pathromtin® SL (112–123%) in samples with 101, 50 and 25 IU/dL, though the 6.3 IU/dL sample showed recovery 141%. Acceptable recovery was also observed for OSA Actin® FSL a CK Prest® in samples with 101 and 50 IU/dL (117–118% and 109–125%, respectively), but not at 25 and 6.3 IU/dL (126–136% and 135–153%, respectively).

Summary/Conclusion: Assay performance varied across extended half-life FVIII products, with CSA generally providing higher recovery. Certain OSA reagents–particularly Actin® FS and Pathromtin® SL–showed acceptable and consistent results depending on the product and concentration. No significant differences were observed between spiked and post–infusion samples, indicating comparable behaviour.

P027

AN AUDIT ON ANDEXANET ALFA FOR REVERSAL OF BLEEDING WITH DIRECT FACTOR XA INHIBITORS

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Background: Direct oral anticoagulants (DOACs) are the preferred anticoagulants used in the prevention and treatment of venous thromboembolism and stroke prevention in non-valvular atrial fibrillation due to its favourable efficacy and safety profiles, compared to vitamin K antagonists. DOAC-related bleeding remains a major concern, which may cause adverse outcomes.

Andexanet alfa is a recombinant, inactive form of factor Xa acting as a competitive binder of DOACs. Andexanet alfa is a specific reversal agent for apixaban and rivaroxaban in adults with life-threatening or uncontrolled gastrointestinal bleeding[1]. An audit was performed to assess local use, efficacy and safety of andexanet alfa.

Aims:

- To review use of andexanet alfa for reversing anticoagulation from apixaban or rivaroxaban in adults with life-threatening or uncontrolled gastrointestinal bleeding, and assess compliance to national and local guidance.

- To determine the efficacy of andexanet alfa by measuring clinical outcomes.

Methods: Retrospective data collection (inclusion and exclusion criteria applied) was performed from January 2023 to December 2024 via electronic patient records and pharmacy dispensing records for the issue of andexanet alfa across two hospital sites in an acute London hospital.

Patient demographics, medical documentation, pathology/radiology results, and electronic medication chart were reviewed to assess performance against audit standards, agreed by the multidisciplinary team with local targets set, to evaluate the use of andexanet alfa.

Patients followed up for 90 days to identify any thrombotic (venous and/or arterial) event(s) post discharge.

Results: 15 adult hospital inpatients (12 patients on apixaban and 13 patients on rivaroxaban) included in data analysis.

Performance against audit standards on use of andexanet alfa for reversing anticoagulation with apixaban or rivaroxaban in adults with life-threatening or uncontrolled gastrointestinal bleeding during hospital admission:

- 93% of hospital adult inpatients were prescribed andexanet alfa according to the NICE approved indication for reversing anticoagulation from apixaban or rivaroxaban with life-threatening or uncontrolled bleeding in the gastrointestinal tract

100% of hospital adult inpatients prescribed andexanet alfa had approval by Haematology on its use for the NICE approved indication

93% of hospital adult inpatients were prescribed the correct dose of andexanet alfa according to when the last DOAC dose was taken or if unknown last DOAC administration

2 thrombotic events (2 ischaemic strokes) occurred during hospital admission

No thrombotic events occurred within 90 days of recent hospital admission

Summary/Conclusion: Andexanet alfa is an effective, specific reversal agent for apixaban and rivaroxaban for life-threatening or uncontrolled gastrointestinal bleeding.

Andexanet alfa was used appropriately according to NICE approved indication[1] and local guideline with Haematology approval, correct prescribing and administration.

Ongoing education and awareness on DOAC-reversal is important for timely treatment and safe monitoring to ensure patient safety.

Clinicians have pivotal roles in:

- Ensuring appropriate guidance is followed with Haematology approval

- Immediate access and supply (including out-of-hours)

- Appropriate andexanet alfa dose prescribed according to DOAC agent and last dose taken

- Clinical advice/support on dosing, reconstitution, administration and monitoring

P028

ACQUIRED VON WILLEBRAND SYNDROME IN MONOCLONAL GAMMOPATHY – A SINGLE CENTRE EXPERIENCE

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Background: Lymphoproliferative disease is diagnosed in 30-48% of patients with acquired von Willebrand syndrome (AVWS), and in more than ⅓ of these patients, monoclonal paraprotein is found. AWWS is diagnosed in 7% of all lymphoproliferative diseases.

Aims: Describe the diagnosis and treatment of patients with AVWS and monoclonal gammopathy at our institution over the past 17 years.

Methods: Patients were examined for bleeding after invasive procedures and/or prolonged aPTT, which was prolonged in all patients. The activity of von Willebrand factor (VWF) was determined as ristocetin cofactor (VWF:RCo) and later as activity (VWF:Ac) using the Innovance set, Siemens®. Antigen (VWF:Ag), collagen binding (VWF:CB), VWF propeptide (VWFpp) and factor VIII activity (FVIII:C) were determined.

Results: AVWS was diagnosed in six patients with monoclonal gammopathy of undetermined significance (MGUS) with serum IgG kappa paraprotein 2.7–11.1 g/l and in one patient with Waldenström macroglobulinemia (WM) with paraprotein IgM in serum 49 g/l. VWF:RCo/VWF:Ac was < 15% (4-15%) in all patients, FVIII:C level was 5-46%. We did not detect any VWF:RCo/VWF:Ac or FVIII:C inhibitor in vitro in any patient. After application of VWF/FVIII concentrate, VWF:RCo/VWF:Ac recovery was < 50% with return to basal level within four hours, a similar finding was found for FVIII:C except for three patients where FVIII recovery was normal with a later decline to basal level. VWFpp/VWF:Ag ratio was in a wide range of 2-47 (median 12).

AVWS required treatment only for invasive procedures. In two patients, a higher dose of VWF/FVIII concentrate in combination with tranexamic acid was used for dental extractions.

In four patients with IgG paraprotein, high-dose immunoglobulins 1 g/kg two consecutive days were used 12x before minor and major surgery. In three patients, VWF:RCo/VWF:Ac and FVIII levels normalized 7x for a period of 2-4 weeks.

In one of these patients (VWF:Ac 10%, FVIIIC 20%) after the administration of immunoglobulins in a standard dose 2x1 g/kg the level of VWF:Ac and FVIII:C increased above 50% for 4 weeks (3x for surgeries), and therefore we then administered only one dose of immunoglobulin 1g/kg before two minor surgeries and the level of VWF:Ac and FVIII:C increased above 50% for 4 weeks too.

In the fourth patient with a high VWFpp/VWF:Ag ratio = 47, VWF:RCo/VWF:Ac normalized 5x only for 4-6 days and FVIII for 6-8 days. In two procedures, treatment for VWF decrease was supplemented with Haemate P® administration.

The patient with WM was prepared for trephine biopsy by applying VWF/FVIII concentrate followed by plasmapheresis, without further decline in level of VWF:RCo and FVIII:C below 50% during chemotherapy and WM remission. After several years, WM relapsed and VWF:Ac levels dropped to 38% and FVIII:C to

42%, and before further plasmapheresis, a single administration of Haemate P® was sufficient, and after further chemotherapy, remission was achieved again, and normalization of VWF and FVIII is ongoing.

Summary/Conclusion: Application of immunoglobulins in IgG gammopathies ensured haemostatic levels of VWF:Ac and FVIII:C for at least 2 weeks, but in a patient with a very high VWFpp/VWF:Ag ratio only for 4-8 days.

Posters - Clotting

P030

FITTING OF AN AMPUTATION OF THE COVID POSTARTERITIC LIMB, CASE REPORT.

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Background: Introduction: limb amputation is the removal of a limb or limb segment, leaving a stump in place. Post-COVID-19 arteriopathy is a rare cause of this amputation.

Aims: Presentation of a rare clinical case

Methods: We report the case of a 41-year-old patient with no previous history of covid 19 pneumonia who presented with total occlusion of the entire arterial axis of the right lower limb on angioscanner. Three successive unsuccessful attempts to unblock the occlusion necessitated a salvage amputation of the right thigh.

Our examination of this patient's thigh amputation stump did not reveal any particularities, and he was fitted with a femoral prosthesis, followed by physiotherapy sessions to help him learn to walk.

Results: The patient was placed in a standing position with an aesthetic gait.
Although post-covid 19 limb amputation is rare, preventive antithromboembolic treatment is necessary to avoid complications. This patient lost his thigh when it could have been preserved by instituting preventive treatment, and the management of this limb with a prosthesis is no different from that for other aetiologies.

Summary/Conclusion: In the case of a post-covid 19 limb amputation, all that is required to achieve a satisfactory result is a good quality stump in good general condition.

P031

PLATELET GLYCOPROTEIN VI GENETIC POLYMORPHISM IN NEONATAL SEPSIS ASSOCIATED COAGULOPATHY

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Background: Neonatal sepsis is a global burden, being a leading cause of neonatal morbidity and mortality worldwide. Platelet glycoprotein VI (GPVI) affects sepsis at multiple stages of the inflammatory response. The expression of the GPVI receptor is genetically determined, thus influencing the coagulation processes

Aims: The aim of the study is to investigate the role of platelet GPVI genetic polymorphism *T13254C* (rs1613662) in neonatal sepsis in relation to other risk factors, laboratory tests, sepsis progression, and outcome.

Methods: Detection of platelet GPVI *T13254C* polymorphism using the TaqMan allelic discrimination method by the real-time polymerase chain reaction technique in 50 neonates with early onset sepsis.

Results: The results showed that GPVI mutant polymorphic group was associated with higher D-dimer levels (*P*=0.032). Moreover, septic neonates with mutant homozygous type showed poor survival (*P*=0.047). However, GPVI mutant polymorphic types were not significantly related to other demographic, laboratory data, and different scoring systems, such as sepsis-induced coagulopathy score, International Society on Thrombosis and Hemostasis score, and Score for Neonatal Acute Physiology.

Summary/Conclusion: Septic neonates with mutant homozygous type showed poor survival, which indicates further studies with larger number of neonates. Moreover, another studies in adults to investigate the effect of this polymorphism in sepsis.

P032

SIGNIFICANT ASSOCIATION HYPERFIBROGENEMIA AND SEVERITY OF AXIAL SPONDYLARTHRITIS.

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Background: It has been reported, that the activity of some inflammatory diseases can induce an activation of the coagulation system, leading to hypercoagulable states and a potential thrombotic risk.

Aims: we aimed to evaluate the correlation between the shortness of aPTT, the elevation of Fibrinogen and the severity of axial spondyloarthritis (axSpA).

Methods: Fifty-one patients with axSpA were enrolled in this study. Clinical data were recorded at the day of admission. Coagulation tests, including PT, aPTT, Fibrinogen, were performed on an automated coagulation analyzer. Patients were classified according to ASDAS score (Ankylosing Spondylitis Disease Activity Score), into two groups (Group1 with ASDAS < 2.1; Group 2 with ASDAS ≥ 2.1).

Results: Fibrinogen levels were significantly elevated in patients of group 1 compared to group 2: 2.65 gr/L [2.15-3.16] Vs 4.00 gr/L [3,60-4,40] respectively, p = 0.001. whereas for the aPTT, no statistically significant difference was found between these two groups 30.42" [26.40"-34.43"] Vs 29.15" [27.86"-30.43"], p = 0.412. Patients with a hyperfibrogenemia, were at a higher risk of having a severe form of axSPA with an OR of 1.952 [1.448 – 2.632], p = 0.002. On the other hand, no association was observed with the shortening of aPTT.

Summary/Conclusion: This study highlights the potential of Fibriongen, used by clinicians upon admission as an accessible and affordable marker to determine the clinical course and better management of patients with axSpA. Further investigations are needed to confirm these findings and deepen our understanding of the underlying mechanisms involved.

P033

HYPOXIA-INDUCED SICKLING OF SICKLE CELL TRAIT (SCT) RED BLOOD CELLS CONTRIBUTES TO THE ENHANCED EXPERIMENTAL VENOUS THROMBOSIS IN SCT MICE

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Background: Individuals with sickle cell trait (SCT) are heterozygous carriers of the sickle beta-globin gene. Analyses of large cohort studies showed that SCT is a risk factor for venous thrombosis (VT). We previously reported that enhanced experimental VT observed in humanized SCT mice was attenuated by the treatment with Gardos channel inhibitor, Senicapoc (SEN).

Aims: Determine if sickling of SCT red blood cells (RBCs), triggered by hypoxia and/or dehydration, contributes to VT and if SEN reverse these changes.

Methods: Ex vivo clot contraction assay was performed on whole blood (WB) from control mice expressing two copies of normal human hemoglobin beta globin (AA) or heterozygous for mutated sickle hemoglobin beta globin (AS) under hypoxia (pO2 = 20 mmHg) or after exposure to 7.5 μM ionophore (ION) in the presence or absence of SEN (200 ng/mL). RBC deformability, as a surrogate of sickling, was measured by the Laser Optical Rotational Red Cell Analyzer (LORRCA).

Results: During clot contraction, excess RBCs are extruded from the thrombus. To evaluate whether SEN attenuates enhanced VT in AS mice via reducing retention of sickled RBC in the clots, we measured the number of RBCs extruded from the WB clots. To mimic venous oxygen concentration, WB from AA (n=11) and AS (n=12) mice was preincubated in hypoxia for 4 hours, clotting was then initiated, and clot weight and serum RBC content were assessed 1 hour later. Under hypoxia conditions, the number of AS RBC ($1.0\pm0.4x10^6$/μL) extruded from clot was reduced compared with the number of AA RBC ($1.6\pm0.35x10^6$/μL; $p < 0.001$). Consequently, the weight of AS clots (100±9.3 mg) was significantly increased compared to AA clots (85±8.2 mg; $p < 0.001$). Importantly, compared to vehicle treated WB, SEN pretreatment during hypoxia significantly increased the extrusion of AS RBC during clot contraction (0.92±0.29 vs $0.68\pm0.23x10^6$/μL; $p < 0.001$; n=15).

Stimulation with ION also attenuated RBC extrusion (1.0 ± 0.1 vs $2.3\pm0.5x10^6$/μL) and increased weight (107±7 vs 78±9 mg) of AS clots compared to AA controls (n=3; $p<0.05$ for both parameters). Next, AS WB was incubated with vehicle (n=8) or SEN (n=8) prior to incubation with ION. Compared to vehicle treated blood, SEN promoted the extrusion of AS RBC from these clots (1.1 ± 0.4 vs $1.7\pm0.5x10^6$/μL; $p < 0.001$).

Lastly, we used LORCA to evaluate whether the effect of ION on RBC extrusion was mediated by changes in RBC deformability (sickling). Blood from AS mice (n=5) was incubated with ION in the presence or absence of SEN. Compared to unstimulated blood, ION led to dehydration (shown by reduction of maximum elongation index Elmax; 0.495±0.01 vs 0.212±0.05; $p<0.05$) and sickling of AS RBCs, identified by a clear point of sickling (PoS; 76±4.8 mmHg). Pre-treatment with SEN decreased the PoS (26±5.4 mmHg; $p < 0.01$) and increased the Elmax (0.445±0.025; $p < 0.0001$) demonstrating that SEN partially prevented ionophore-induced AS RBC dehydration and rendered them less prone to sickling.

Summary/Conclusion: These findings demonstrate that Gardos channel inhibition partially reverses the hypoxia- and dehydration-induced retention of RBCs within AS clots.

P035

CHOLESTEROL EMBOLISM DISEASE FOLLOWING VALVE REPLACEMENT UNDER VITAMIN K ANTAGONIST THERAPY

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Background: Cholesterol embolus disease is an etiology of acute vascular nephropathies, it often occurs in polyvascular males following a triggering event.

Aims: The aim of this work is to highlight a still poorly understood and frequently underdiagnosed condition, characterized by a wide range of clinical manifestations, by illustrating its evolutionary course.

Methods: We report the case of this acute vascular nephropathy following an aortic valve replacement with a St. Jude N1 prosthesis under anticoagulant therapy.

Results: Mr. CY, a 76-year-old smoker with hypertension and polyatheromatosis, presented with severe aortic stenosis for which he underwent mechanical valve replacement and was placed on vitamin K antagonists. This was complicated by an ischemic stroke associated with rapidly progressive renal failure that was not investigated and progressed to the need for extracorporeal renal replacement therapy after three months. The patient was referred to our facility for the management of critical ischemia of both lower limbs in the context of general health deterioration, severe resistant hypertension, anuria, distal necrotic skin lesions on the toes, hypereosinophilia, and a biological inflammatory syndrome. Cholesterol embolism disease was confirmed by a skin biopsy revealing the presence of cholesterol crystals. The patient was treated with statins and corticosteroid therapy for 21 days, along with hypertension control. The progression was marked by persistent skin lesions and dialysis-dependent renal failure.

Summary/Conclusion: The delayed etiological diagnosis of acute vascular nephropathy compromised both the renal functional prognosis and the vital prognosis, underscoring the importance of thorough investigation of any postoperative acute kidney injury in the context of polyatheromatosis.

P037

ASSESSMENT OF THROMBOTIC RISK AND ASSOCIATED FACTORS IN PATIENTS WITH SEVERE KNEE OR HIP OSTEOARTHRITIS IN THE PREOPERATIVE PERIOD.

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Background: Osteoarthritis is the most prevalent pathology in rheumatology. It is one of the leading causes of chronic pain and disability in the elderly. Among osteoarthritis, lower limb osteoarthritis is the most disabling, often requiring knee and hip prosthetic surgery. However, this surgery is not exempt from complications, particularly the risk of deep vein thrombosis (DVT) or pulmonary embolism (PE).

Aims: To assess the prevalence of thrombotic risk in patients with severe knee or hip osteoarthritis who are candidates for prosthetic surgery, and to determine the associated factors.

Methods: This was a descriptive, cross-sectional study with prospective recruitment including patients with severe primary knee or hip osteoarthritis scheduled for joint replacement surgery. All patients underwent a thorough history and clinical examination. Thrombotic risk was assessed using the modified Padua score. Associations with a score ≥4 were studied using logistic regression.

Results: Over a 2-year period, 300 patients were recruited (mean age: 67.97 ± 6.78 years; 69% women); 163 (54.3%, 95% CI [48.6–59.8]) were at high thrombotic risk. Identified factors were obesity (49.3%), age ≥70 years (42.3%), history of DVT (15.7%), and chronic venous insufficiency (21.1%). Multivariate analysis revealed a significant association between high risk and age ≥70 years (OR = 2.18; 95% CI [1.32–3.58], p = 0.002) and obesity (OR = 1.91; 95% CI [1.19–3.05], p = 0.007).

Summary/Conclusion: Through this study, we demonstrated that patients with severe knee or hip osteoarthritis have a high thrombotic risk. Age ≥ 70 years and obesity are the main associated factors. These results encourage systematic assessment of this risk in these patients with reduced mobility before surgery and consideration of prevention to optimize thromboembolic events in the preoperative period.

P040

PROPHYLACTIC VS INTERMEDIATE TINZAPARIN DOSAGE FOR THE THROMBOPROPHYLAXIS OF ACUTELY ILL MEDICAL PATIENTS AT HIGH RISK OF VENOUS THROMBOEMBOLISM

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Background: Venous thromboembolism (VTE) is the third most common cardiovascular condition, with high rates among hospitalized patients. The limited efficacy of universal prophylaxis strategies have led to individual VTE risk assessments approaches, including the Padua Prediction Score (PPS).

Aims: We aimed to assess outcomes in high risk patients for VTE, who receive prophylactic vs intermediate, weight-adjusted doses of tinzaparin for thromboprophylaxis.

Methods: This was a retrospective study, assessing adult patients hospitalized with acute medical disease, in a tertiary university hospital from January 2022-2024. Patients were included if found at high risk for VTE (PPS≥4) – and received prophylactic versus intermediate dosage of tinzaparin. Data were collected from patients' files and analyzed using appropriate statistical methods.

Results: In total, 286 patients were included, of whom 160 received prophylactic and 126 intermediate tinzaparin dosage. Patients receiving prophylactic doses exhibited significantly higher mortality rates (20.62 vs 7.14, p=0.002), increased length of stay (LOS) (6 vs 4, p<0.001), and prolonged treatment durations (5 vs 3, p=0.003), compared to patients receiving intermediate dosages. Univariate analysis revealed significant associations between mortality and tinzaparin dose (OR = 3.38, p = 0.002), age (OR = 1.03, p = 0.017), LOS (OR = 1.07, p = 0.001), PPS (OR = 1.62, p < 0.001), CCI (OR = 1.27, p < 0.001), and prior thrombotic events (OR = 2.27, p = 0.028). In multivariate analysis, tinzaparin dose (OR = 2.58, p = 0.035), age (OR = 1.04, p = 0.033), LOS (OR = 1.10, p < 0.001), and PPS (OR = 1.33, p = 0.038) remained independent predictors of mortality.

Summary/Conclusion: Intermediate tinzaparin dosing improves safety and effectiveness in high-risk hospitalized patients, supporting personalized VTE management approaches.

P041

SPECTRUM OF INHERITED THROMBOPHILIA: CLINICAL AND MOLECULAR INSIGHTS IN A PAKISTANI COHORT

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Background: Inherited thrombophilia comprises genetic predispositions to thromboembolic events, including Factor V Leiden (FVL), Prothrombin G20210A mutations, and deficiencies of natural anticoagulants (antithrombin, protein C, and protein S). These abnormalities are particularly relevant in younger individuals and those with recurrent or familial thrombosis. Data from low middle income countries (LMICs) remain scarce.

Aims: To determine prevalence and spectrum of inherited thrombophilia in patients referred for thrombophilia screening, evaluate clinical presentations, correlate findings with family history, and assess treatment duration, recurrence and morbidity in a large tertiary care cohort from Pakistan.

Methods: This observational study was conducted at Department of Haematology, Armed Forces Institute of Pathology (AFIP), Pakistan. Patients with confirmed thrombotic events referred for thrombophilia screening between January 2017 and December 2024 were included. Clinical data, family history and treatment records were reviewed. Laboratory investigations included chromogenic assays for antithrombin and protein C, clot-based assay for protein S, and PCR-based detection of FVL and Prothrombin G20210A mutations. Clinical outcomes including recurrence, complications and anticoagulation duration were documented.

Results: Over an eight-year period, 7,576 patients were screened for thrombophilia, with 537 (7.1%) diagnosed with inherited thrombophilia. Deep vein thrombosis (DVT) was the predominant presentation (317; 58.9%), followed by pulmonary embolism (91; 17%), pregnancy loss (38; 7.1%), superficial venous thrombosis (32; 6%), arterial thrombosis (21; 3.9%), portal vein thrombosis (16; 3%), and mesenteric and dural sinus thromboses (11 each; 2%). FVL was the most frequent defect (313; 58.3%), with 252 heterozygous (80.6%) and 61 homozygous (19.4%) cases. Antithrombin deficiency was identified in 104 (19.3%), strongly linked to recurrence. Protein C and Protein S deficiencies were found in 74 (13.7%) and 32 (5.9%) patients, respectively, while the prothrombin G20210A mutation was seen in 14 (2.7%), including two homozygotes. A family history of thrombosis was present in 19.4% of FVL and 24.6% of antithrombin-deficient individuals. Anticoagulation was administered for <6 months in 50.3%, 6–12 months in 26.5%, >12 months in 14.6%, and lifelong in 8.6%. Thrombotic recurrence occurred in 5.4%, predominantly among antithrombin and prothrombin mutation carriers (mean interval 12.6 ± 6.9 months). Thrombosis-related morbidity was documented in seven patients.

Summary/Conclusion: Factor V Leiden predominated among inherited thrombophilias, followed by antithrombin, protein C, and protein S deficiencies. Findings emphasize importance of thrombophilia screening in selected high-risk individuals, particularly in LMICs with limited diagnostic infrastructure. Incorporating structured diagnostic algorithms and expanding access to molecular testing may enable risk-adapted anticoagulation strategies, thereby reducing recurrence and improving clinical outcomes.

P045

DISTINCT THROMBIN GENERATION PROFILES IN PEDIATRIC AND ADULT INFLAMMATORY BOWEL DISEASE PATIENTS

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Background: Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory conditions associated with an increased risk of thrombosis. The thrombin generation (TG) assay provides a sensitive, global assessment of hemostasis and is useful for identifying hypercoagulable states in IBD. However, little is known about differences between adult and pediatric IBD patients in terms of hypercoagulation and the extent of TG.

Aims: To compare TG parameters in pediatric and adult IBD patients versus age-matched healthy controls, and to evaluate associations with clinical disease activity and inflammatory markers.

Methods: Plasma samples from 194 adults (CD: $n = 43$; UC: $n = 27$; age-matched controls: $n = 127$) and 124 children (CD: $n = 27$; UC: $n = 29$; age-matched controls: $n = 68$) were analyzed. TG was assessed in platelet-poor plasma using calibrated automated thrombography. Key TG parameters - lag time, peak thrombin, endogenous thrombin potential (ETP), and time to peak - were assessed alongside standard hematologic and inflammatory markers (e.g. hsCRP, complete blood count, PT, APTT, fibrinogen, ferritin). Disease activity was evaluated using PCDAI, PUCAI, CDAI and pMayo scores.

Results: Adult IBD patients exhibited significantly elevated ETP and peak thrombin compared to controls, indicating a prothrombotic tendency (ETP in adult groups: CD: 1945 [1649-2223] vs. UC: 2044 [1718-2279] vs. *controls:* 1700[1427-2015] *nM**min, p=0.0004). In contrast, no significant differences were observed in pediatric patients, ETP and peak thrombin did not differ significantly across groups (ETP in children: CD: 1570 [1419-2145] vs. UC: 1747 [1369-2091] *vs. controls:* 1581 [1459-2008] *nM**min, p=0.9880). In both age groups, ETP correlated positively with inflammatory markers and disease activity.

Summary/Conclusion: ETP is associated with disease activity and systemic inflammation in both adults and in children with IBD.However, the absence of significantly elevated ETP in pediatric patients as compared to healthy controls suggests a lower disease-associated thrombotic risk. Thrombin generation testing may be a valuable adjunct for assessing thrombotic risk and monitoring disease activity, particularly in adult IBD patients.

P046

TEN-DAY LOW MOLECULAR WEIGHT HEPARIN THROMBOPROPHYLAXIS : A SAFE STRATEGY FOLLOWING ANTERIOR CRUCIATE LIGAMENT, RECONSTRUCTION IN YOUNG PATIENTS WITHOUT THROMBOTIC RISK FACTORS

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Background: Surgical reconstruction of the anterior cruciate ligament (SRACL) of the knee is the most commonly performed ligament surgery in Algeria. Thromboembolic complications are rare after SRACL. In the literature, SRACL is considered an orthopedic surgery with moderate thromboembolic risk, and continued prophylaxis after the tenth postoperative day is not considered necessary. Thromboprophylaxis after SRACL in Algeria is not codified; it is used systematically and for a variable duration

Aims: Reduce the duration of thromboprophylaxis after SRACL especially in young subjects without risk factors.

Methods: Our study involves a series of 178 patients who underwent SRACL. The study population was divided into two groups: 104 patients received postoperative treatment with low molecular weight heparin (LMWH) for 21 days and 74 patients received treatment with LMWH for 10 days. Informed consent was obtained from all patients. Regular clinical follow-up was carried out in consultation and clinical screening for symptomatic thromboembolic events was performed in all patients. Statistical analysis was performed using the Statistical Package for Social Sciences version 20 software.

Results: At the beginning of the data analysis, the homogeneity of the two groups of patients subjected to the two protocols was tested. No significant difference was noted regarding the variables studied: age (P=0.227), smoking status (P=0.650), BMI (P=0.284), laterality (P=0.260), operated knee (P=0.290), educational level (P=0.660), professional status (P=0.460), sporting level (P=0.110), socioeconomic level (P=0.760), seniority of the operating surgeon (P=0.210), procedures associated with RCLCA (P=0.190), duration of the surgical procedure (P=0.590). This homogeneity found in our study allowed us to compare the results obtained in the two protocols while avoiding measurement bias. No cases of symptomatic clinical thromboembolic incidents were found in any of the patients in either group.

Summary/Conclusion: In the literature, the incidence of symptomatic clinical thromboembolic events after SRACL varies greatly between studies. In our study, no thromboembolic events were reported in either group despite the reduction of the duration of LMWH treatment to 10 days in the second group. These results require us to review our practices regarding thromboprophylaxis and its duration after SRACL, especially in young subjects without risk factors. These results raise questions about the duration of preventive thromboprophylaxis of thromboembolic accidents already used in orthopedic surgery departments after SRACL and to propose a reduction of the duration to less than 10 days, especially in young subjects without risk factors.

P047

INCREASED THROMBIN GENERATION IN KIDNEY TRANSPLANT RECIPIENTS WITH DONOR-SPECIFIC ANTIBODIES DIRECTED AGAINST HUMAN LEUKOCYTE ANTIGENS

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Background: The development of de novo anti-HLA donor specific antibodies (DSAs) may lead to antibody-mediated rejection (ABMR), a common cause of graft loss with unknown underlying pathomechanism in kidney transplant recipients.

Aims: We hypothesized that in kidney transplant patients the presence of DSAs induce endothelial damage and hemostasis alterations, including hypercoagulability, as assessed by the thrombin generation assay (TGA), that may contribute to ABMR.

Methods: In this observational study, 27 kidney transplant recipients with DSAs (DSA+ group) and 16 without DSAs (DSA– group) were enrolled. Venous blood samples were obtained, and besides TGA, von Willebrand factor antigen (VWF), FVIII activity, soluble E selectin (sEsel), soluble P selectin (sPsel), and inflammatory cytokine profiling were carried out. Cytokine profiling was performed using a bead-based multiplex immunoassay allowing the simultaneous quantification of 13 human inflammatory cytokines (IL1-β, IFN-α2, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33). To correlate results with potential changes in DSA status over time, patients were followed and reassessed 6±1.5 months later.

Results: Endogenous thrombin potential (ETP) was significantly higher in DSA+ vs. DSA– patients (median: 1666; IQR: 1438-2012 vs. 1230; IQR: 1097-1659 nM*min, p=0.0019). TNF-α and IL-6 were significantly increased in the DSA+ vs. DSA– group patients (median TNF-α: 32.7; IQR: 17.4-82.3 vs. 8.5; IQR: 4.8-28.3 pg/mL, p=0.042 and median IL-6: 34.1; IQR: 22.9-58.4 vs. 21.5; IQR: 19.5-33.0 pg/mL, p=0.005, respectively). The anti-inflammatory IL-10 levels demonstrated a significant negative correlation with TGA parameters. VWF and sPsel were increased in the majority of patients. Follow-up measurements indicated that the observed alterations were not transient. The extent of anti-HLA II DSA positivity correlated positively with ETP, while the extent of immunosupression, as observed by tacrolimus levels, negatively correlated with ETP, VWF and FVIII levels.

Summary/Conclusion: Our findings provide new insights into the pathomechanism of antibody-mediated kidney graft rejection, demonstrating that the presence of DSAs are associated with an inflammatory and hypercoagulable state. TGA may serve as a valuable tool for monitoring patients, guiding the timing of graft biopsies, and assessing antibody-mediated graft injury. These findings raise the possibility that targeted anticoagulant therapy in selected patients may help mitigate graft injury and reduce the risk of rejection, although this hypothesis warrants further confirmation in future studies.

P048

INVESTIGATION OF THE EFFECT OF ETHANOL ON CLOT LYSIS IN ACUTE ISCHEMIC STROKE PATIENTS AND HEALTHY VOLUNTEERS

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Background: Intravenous thrombolysis with recombinant tissue plasminogen activator (t-PA) is the standard pharmacological treatment for acute ischemic stroke (AIS). However, the therapy is effective in only approximately 30–40% of cases. Retrospective data from our research group suggest that acute alcohol intake may improve thrombolysis outcomes dose-dependently in AIS patients.

Aims: To explore the effect of ethanol on global fibrinolysis assays, including the *in vitro* clot formation and lysis assay (CLA), using plasma samples from AIS patients (with and without alcohol intake) and healthy volunteers.

Methods: CLA assay was performed using admission plasma samples from 16 AIS patients with detectable blood alcohol concentration (BAC), and 26 age, sex, and stroke severity-matched AIS patients without alcohol consumption. Thrombolysis outcome was assessed based on the change in the National Institutes of Health Stroke Scale (NIHSS) score on day 7 and the modified Rankin Scale (mRS) at day 90. To further examine the effects of ethanol, citrated whole blood and plasma samples of healthy volunteers were spiked with increasing doses of ethanol (1-2-4‰) and CLA, ROTEM and Clot-Pro measurements were performed.

Results: Significantly shorter 50% clot lysis time (50 % CLT) was observed in AIS patients with detectable BAC compared to those with no previous alcohol intake (median: 33 min, IQR: 23–48 vs. 47 min, IQR: 28–67; p=0.045). Admission BAC of AIS patients showed a significant negative correlation with 50 % CLT (r = -0.605, 95% CI: -0.851 to -0.1409, p=0.015). In healthy plasmas with 50 %CLT >30 min, *in vitro* added ethanol dose-dependently reduced 50 % CLT, while in those with 50 % CLT<30 min, added ethanol had no significant effect.

Summary/Conclusion: Ethanol significantly and dose-dependently shortened t-PA-induced clot lysis both *in vivo* and *in vitro*. These findings suggest the potential application of ethanol as an adjuvant to thrombolytic therapy and warrant further clinical and mechanistic studies to evaluate safety and therapeutic feasibility.

P049

CCL5 AND ADAMTS-13 ARE PREDICTORS OF OUTCOME AFTER THROMBOLYSIS IN ACUTE ISCHEMIC STROKE

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Background: Tissue plasminogen activator (t-PA) is a well-established and proven treatment for acute ischemic stroke (AIS), however, some patients still have poor clinical outcome after the treatment, possibly due to factors that are not yet fully explained. Inflammation plays a key role in stroke pathophysiology, contributing to both early tissue damage and later repair mechanisms.

Aims: Our aim was to assess peripheral inflammatory cytokine/chemokine levels, their association with acute phase hemostasis parameters in AIS patients before thrombolysis and their predictive value for functional outcomes.

Methods: In this prospective observational study, 132 AIS patients receiving t-PA therapy were enrolled in a single stroke center. For each patient, demographic and clinical characteristics were registered on admission. Routine chemistry tests, CRP, blood count, hemostasis screening tests, fibrinogen, D-dimer, von Willebrand factor (VWF) antigen, FVIII and ADAMTS13 activity were determined from blood samples taken prior to thrombolysis. Inflammatory cytokine/chemokine levels (CD54, IL-6, MCP-1, CD62P, CCL5, IL1R1, IL1R2, angiogenin) were measured using cytometric bead array from citrated plasma. Stroke severity was determined by the National Institutes of Health Stroke Scale (NIHSS) on admission. Long-term clinical outcome was defined 3 months post-event according to the modified Rankin Scale (mRS).

Results: Stroke severity (admission NIHSS was associated with baseline D-Dimer (median: 0.68, IQR: 0.35-1.45 vs median: 1.31, IQR: 0.91-3.36 mg/L, respectively, p= 0.0280), IL-6 (median: 5.3, IQR: 3.63-9.14 vs median: 7.31, IQR: 5.73-13.29 pg/ml, respectively, p= 0.0162), CCL5 (mean ± SD: 1863 ± 416.6 vs 2124 ± 414 pg/ml, respectively, p=0.0180) and IL1RI levels (mean ± SD: 460.9 ± 167.7 vs 602.1 ± 291.5 pg/ml, respectively, p=0.0049). A positive correlation was identified between CCL5 and FVIII levels (Pearson r=0.3582, 95%CI:0.1397 to 0.5435, p=0.0019) while IL-6 concentrations correlated positively with FVIII (Spearman r=0.3093, 95%CI:0.0850 to 0.5038, p=0.0062), VWF levels (Spearman r=0.3052, 95%CI:0.0789 to 0.5016, p= 0.0073) and negatively with ADAMTS13 activity (Spearman r= -0.3700, 95%CI: -0.5566 to -0.1479, p= 0.0012). In a multiple logistic regression analysis, increased CCL5 levels and decreased ADAMTS13 activity emerged as independent predictors of poor (mRS 3-6) outcome, alongside increased NIHSS and age.

Summary/Conclusion: In AIS patients, more severe strokes are associated with enhanced inflammatory response, potentially contributing to poor clinical outcomes. The observed correlations between inflammatory markers and hemostatic parameters suggest that these pathways are closely interconnected in AIS. CCL5 and ADAMTS13 were identified as independent predictors of poor long-term functional

outcome after intravenous thrombolysis, supporting their role as potential biomarkers to guide treatment decisions in acute ischemic stroke care.

P050

HORMONE INDUCED THROMBOSIS: A GLOBAL PROJECT

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Background: Hormone-induced thrombosis is a significant concern in women's health, particularly during pregnancy but also in association with the use of combined oral contraceptives (COCs) or other oestrogen-based hormonal therapies. Currently, no effective tool exists to reliably estimate individual thrombotic risk before and during these hormonal fluctuations. In 2019, the normalized Activated Protein C sensitivity ratio (nAPCsr) was standardized following ICH guidelines and emerges as a promising biomarker assessing VTE risk associated with COCs.

Aims: The HIT24 project aims to evaluate the relevance of the nAPCsr in assessing hormone-induced thrombotic risk, and its utility as decision-support tool for prescriptions across various hormonal conditions throughout a women's life.

Methods: The HIT24 project, supported by the Walloon Region, includes several clinical studies focusing on women in various hormonal contexts. Among other, women treated for endometriosis, women using hormonal replacement therapy for menopausal symptoms, undergoing fertility treatment, will be studied. Blood samples will be collected at intervals defined by clinical practice and in correlation with the standard of care protocol of participating hospitals (e.g., before treatment initiation, and at 4, 12, 24, … weeks post-treatment). Thrombin generation assay will be performed and nAPCsr will be evaluated, alongside conventional assays. Comparative and longitudinal analyses, in addition to correlation with clinical parameters (e.g. VTE events occurrence), will focus on the ability and the reliability of the nAPCsr to estimate the individual thrombotic risk across these different hormonal contexts. The project has started in September 2024, with first results expected in September 2025.

Results: NA

Summary/Conclusion: The HIT24 project explores the potential of nAPCsr in measuring hormone-induced thrombotic risk in diverse hormonal states. Preliminary results are expected to provide insights into its clinical relevance, potentially leading to further validation studies and integration into routine care for personalized risk management in women's health.

P051

DEFICIENCY OF THROMBOPHILIA MARKERS (ANTITHROMBIN III, PROTEIN C AND PROTEIN S) AS POTENTIAL RISK FOR PREGNANCY LOSS IN THE FIRST TRIMESTER

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Background: Antithrombin III (ATIII), Protein C (PC) and Protein S (PS) are naturally occurring anticoagulant proteins. ATIII inhibits the serine proteases (fIIa, IXa, Xa, XIa and XIIa) and activated PC and PS inhibite fVa and f VIIIa. Thrombophilia is characterized by a clinical tendency to thrombosis due to ATIII, PC or PS deficiency. The pregnancy is a hypercoagulable state and if it is affected by thrombophilia, the hypercoagulable state becomes worse and may impair blood flow through the maternal veins, leading to DVT and fetal growth restriction.

Aims: To measure the concentration of ATIII, PC and PS in pregnant women in the first trimester and determine the correlation of deficiency of ATIII, PC, PS and pregnancy loss.

Methods: *175 pregnant women in the first trimestar were screened from March 2023 to May 2023 at the Institute for Transfusion Medicine of RNM. Women were screened for AT III (Berichrom Antithrombin III Chromogenic Assay) and PC, PS (Berichrom Protein C Assay and Coagulometric Protein S Ac) on BCS-XP coagulometar. The tests were performed on citrated plasma and the results obtained are the biological activity of ATTIII, PC and PS, shown as %. Normal values are 50-150 %.*

Results: Deficiency of PC was detected in 11 (6.28%) patients (43.36 % + 6.17 v.s. 106 %+22.4). PS deficiency was detected in 7 (4%) patients (37.14% + 9.08 v.s. 108.49+16.51) and for ATIII there was no detected deficiency (93.82 % +11.9).

Summary/Conclusion: Even though the pregnancy is in a hypercoagulable state and the activity of the ATIII, PC and PS is low during the pregnancy, there is a correlation between deficiency of PC, PS and regnancy loss in women with anamnesis of miscarriage. For better newborn outcomes, women should be screened for genetic mutations.

P054

INTRA-INDIVIDUAL VARIABILITY OF DIRECT ORAL ANTICOAGULANT LEVELS IN FRAIL OLDER PATIENTS UPON, DURING AND AFTER ACUTE HOSPITALISATION; THE DOAC-FRAIL STUDY

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Background: Utilization of Direct Oral Anticoagulants(DOACs) among frail older patients has increased. Recent studies raise concerns, particularly in relation to bleeding risk potentially caused by increased DOAC levels. Data on intra-individual variability of DOAC levels remains scarce and addressing this is essential for optimizing DOAC management.

Aims: We aimed to explore the intra-individual variability of DOAC levels in frail older patients during the peri-hospitalisation period.

Methods: This prospective exploratory cohort study included patients aged 65 years or older, using a DOAC and acutely admitted to the department of internal medicine between February 2022 and December 2022. DOAC levels were measured upon, during and after hospitalisation.

Results: In 22 patients 63 DOAC levels were measured: 22(34.9%) were above and 4(6.4%) were below the on-therapy range. Seven patients(31.8%) had levels within the on-therapy range during all time points. Ten patients(45.5%) had an SD of levels of >100ng/ml and in 11 patients(50%) the coefficient of variation(CV) was >35%. Rivaroxaban was associated with an average of 97.28 units higher SD ($p<0.001$). A reduced dose was associated with an average of 49.53 points lower SD ($p=0.09$).

Summary/Conclusion: Intra-individual variability of DOAC levels was observed in this frail older population. Rivaroxaban was associated with higher intra-individual variability, while an reduced DOAC dose showed lower intra-individual variability. Given the risk of thromboembolic and bleeding events with deviant DOAC levels, high intra-individual variability may have clinical consequences. Future research should focus on this topic as it is essential for guiding DOAC management in the frail older population.

P055

PERCEPTIONS AND CONSIDERATIONS OF HEALTHCARE PROFESSIONALS IN PRESCRIBING DOACS TO FRAIL OLDER PATIENTS; INSIGHTS FORM THE DOAC-FRAIL QUESTIONNAIRE

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Background: DOACs are increasingly prescribed for atrial fibrillation and venous thromboembolism due to their ease of use and lack of monitoring requirements. Prescribing DOACs to frail older patients remains challenging due to their higher bleeding and thrombo-embolic risks, comorbidities, polypharmacy, and underrepresentation in clinical trials.

Aims: This study explored prescribing behavior of healthcare professionals (HCPs) in this context.

Methods: An online questionnaire was distributed to HCPs involved in the care of frail older patients. The survey consisted of 25 items divided into four sections: demographics, factors influencing anticoagulant choice, DOAC management and case-based scenarios. We used descriptive statistics to describe outcomes of the survey.

Results: 355 HCPs the questionnaire. HCPs considered frailty (56.9%), fall risk (47.1%) and cognitive impairment (40.0%) when choosing an anticoagulant. Apixaban was the preferred DOAC (56.6%). 74.9% of HCPs did not measure DOAC levels in clinically stable patients, whereas 62.8% measured DOAC levels during acute hospitalizations. A large group of HCPs (46.4%) actively switched from VKA to DOAC, but expressed reservations due to fear for an increased risk. Almost half (48.1%) of HCPs indicated the need for clearer, tailored guidelines for DOAC use in frail older patients.

Summary/Conclusion: This study highlights the complexity of prescribing DOACs to frail older patients. Indeed, HCPs consider various factors when prescribing DOACs to this population, but lack a consistent approach. In addition, almost half of HCPs expressed a need for guidelines regarding DOAC use in frail older patients. Hence, more research is needed to fill this knowledge gap and guide HCPs in clinical practice.

P056

BETA-ANTITHROMBIN LEVELS IN PATIENTS WITH VENOUS THROMBOEMBOLISM

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Background: Antithrombin (AT) is a critical inhibitor of the coagulation cascade and exists in two isoforms: alpha-antithrombin (α-AT) and beta-antithrombin (β-AT). These isoforms differ in their glycosylation pattern. β-AT comprises 5-10% of total AT in the normal plasma. β-AT has a higher affinity for heparin than α-AT, making it a more effective inhibitor. There is no data about how β-AT activity levels change in thrombotic disorders.

Aims: This study aimed to determine total and β-AT activity levels in patients with venous thromboembolism (VTE) compared to age-matched healthy controls.

Methods: We analysed citrated plasma samples from controls (n=208) and patients with VTE (n=208) collected at least 3 months after the acute event. Total antithrombin heparin cofactor activity was measured using a chromogenic anti-factor Xa assay in the presence of heparin. To measure β-AT, we modified this test by increasing the NaCl concentration of the reagent to 1,1 M to inhibit the heparin binding of α-AT.

Results: The total AT activity (%) level (median (IQR)) did not show a significant difference between the control and VTE groups (100.0 (92.0-109.0) % and 99.0 (92.0-108.75) %, respectively; p=0.541). The β-AT activity levels (median (IQR) %) showed a significant elevation in the VTE group compared to controls (93.3 (89.3-97.3) % vs. 89.2 (83.5-95.0) %; p<0.001). The ratio of β-AT within total AT (%) (median (IQR)) was also significantly elevated in the VTE group compared to controls (9,3 (8,8-9,8) % vs. 8,9 (8,3-9,4) %; p<0,001).

Summary/Conclusion: In our study, plasma levels of β-AT and its ratio within total AT were elevated in VTE patients. Since total AT levels did not differ from those of the controls, a decrease in the α-AT form can be presumed. Further studies are needed to clarify the pathophysiological significance of these changes.

P057

EFFECT OF ALPHA2-PLASMIN INHIBITOR C-TERMINAL HETEROGENEITY ON CLOT LYSIS AND CLOT STRUCTURE

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Background: Alpha2-plasmin inhibitor (α2PI) forms an irreversible complex with circulating plasmin and is cross-linked to the fibrin network by activated factor XIII. This interaction plays a crucial role in regulating fibrinolysis. The N-terminal of the molecule is involved in cross-linking, while the C-terminus contains the plasminogen binding site. α2PI is cleaved in plasma at both ends of the molecule. The C-terminally intact form can bind to plasmin and plasminogen (PB-α2PI, plasminogen binding form). The truncated form loses the plasminogen binding site (NPB-α2PI, non-plasminogen binding site), leading to a significantly slower plasmin inhibition. Proteolytic cleavage has functional implications, but the impact of heterogeneity on fibrinolysis is less understood. In our previous work, we found that plasma α2PI activity and total α2PI antigen levels in venous thromboembolism (VTE) patients were significantly higher than in the control group. While the PB-α2PI level did not differ between the two groups, NPB-α2PI levels were elevated and associated with an 8.4-fold increased risk of thrombosis.

Aims: This study aimed to investigate the incorporation of PB- and/or NPB-α2PI forms into the fibrin clot and their association with clot lysis and clot structure.

Methods: α2PI-deficient plasma was supplemented with varying amounts of recombinant PB- (rPB-α2PI) and/or NPB-α2PI (rNPB-α2PI). Clot formation was induced by adding thrombin and $CaCl_2$. The formation and dissolution of the clot were detected by turbidimetric measurement, and the clot structure was investigated using confocal laser scanning microscopy.

Results: Lysis time was increased by 80% in the presence of 100% rPB-α2PI, and by 30% in the presence of 100% rNPB-α2PI, compared to the α2PI-deficient plasma. Increasing the amount of rNPB-α2PI added to a constant amount of rPB-α2PI led to a further elevation of the lysis time in a concentration-dependent manner. The highest amount of NPB-α2PI (with PB-α2PI: NPB-α2PI ratio of 46:54%) caused a 21% increase in CLT50 compared to a PB-α2PI: NPB-α2PI ratio of 73:27%. Substituting with rPB- and rNPB-α2PI significantly affected the clot structure, resulting in a denser fibrin network, increased area coverage, decreased pore size, and thicker fibrin fibers compared to the α2PI-deficient clot.

Summary/Conclusion: Although to a lesser extent than the PB-α2PI form, NPB-α2PI also contributes to the modification of fibrinolysis. Further studies are needed to explore the extent and effects of C-terminal truncation in various pathological conditions.

P058

PLASMA KALLIKREIN AND FXII IN COVID-19, A PROSPECTIVE COHORT STUDY

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Background: Pro-coagulant disorders, such as thrombosis and hyperinflammation, are consequences of severe COVID-19 and are associated with increased mortality and morbidity. Several clinical laboratory tests – including complete blood count, CRP, and D-dimer – are indicators of disease severity and coagulation dysfunction in COVID-19. The kallikrein-kinin system, involving proteins such as plasma kallikrein and FXII, plays a role in inflammation and coagulation, and has also been suggested to contribute to COVID-19 disease severity.

Aims: The aim was to compare plasma kallikrein and FXII in hospitalized and non-hospitalized patients with COVID-19 from disease onset up to six months after infection.

Methods: This study includes 220 COVID-19 patients from the prospective CoVUm study cohort. Informed consent was obtained from patients when enrolled in the study. Patients were sampled in the acute phase (25 days within disease onset) and at three- and six-months follow-up. Enrollment information includes basic patient characteristics such as age, sex, and comorbidities. Data on routine tests such as D-dimer, CRP, leukocytes, neutrophils, monocytes, and thrombocytes are available for the majority of patients. The total protein concentration of plasma kallikrein and FXII was quantified using ELISA. A total of 628 plasma samples were analyzed. Differences in protein levels between 78 hospitalized and 142 non-hospitalized patients were evaluated using the Mann-Whitney U test.

Results: Hospitalized patients were older compared to non-hospitalized patients (mean age 56.6 vs. 47 years), included more males (64.1 vs. 42.9%), had a higher BMI (mean 30.35 vs. 24.9 kg/m^2), and had more comorbidities such as hypertension (35.9 vs. 14.8%) and cardiovascular disease (14.1 vs. 4.2%).

At the acute phase, laboratory tests showed higher CRP, leukocytes, neutrophils, D-dimer (all $p < 0.001$), and thrombocytes ($p = 0.005$) in hospitalized patients compared to non-hospitalized patients. In contrast, hospitalized patients had lower lymphocytes ($p < 0.001$). Hospitalized patients also had lower levels of kallikrein (median 146 vs. 196 mg/L, $p < 0.001$) and FXII (median 35 vs. 43 mg/L, $p = 0.04$) in plasma.

At three months, hospitalized patients had higher levels of kallikrein (median 219 vs. 200 mg/L, $p = 0.011$) and FXII (median 43 vs. 36 mg/L, $p = 0.04$). At six months, kallikrein (median 206 vs. 203 mg/L) and FXII (median 39 vs. 40 mg/L) did not differ between groups.

Similar patterns were observed for males regarding kallikrein, but not FXII, which differed only during the acute phase between hospitalized and non-hospitalized. For females, including one non-binary individual, kallikrein differed only during the acute phase, and no differences were observed for FXII.

Summary/Conclusion: Lower plasma protein concentrations of kallikrein and FXII in hospitalized patients during the acute phase suggest increased activation and possible consumption in the kallikrein-kinin system. At the non-acute three-month follow-up, hospitalized patients had higher kallikrein and FXII, compared to non-hospitalized patients, which could be an inflammatory response. At six months, levels had decreased in the hospitalized group, and no difference was seen between the two groups.

P059

IMPACT OF HEMOSTASIS POLYMORPHISMS ON THE OUTCOME OF ACUTE ISCHEMIC STROKE THROMBOLYSIS TREATMENT

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Background: Thrombolysis therapy with the administration of intravenous recombinant tissue plasminogen activator (IV rt-PA) is the standard treatment for acute ischemic stroke (AIS), aiming to rapidly restore blood flow to brain regions deprived of circulation. However, predicting therapeutic outcomes remains challenging, as approximately 6–8% of patients experience hemorrhagic transformation (HT), a potentially fatal complication as side-effect. Genetic factors may modulate clot structure and thrombus susceptibility to lysis, influencing treatment response.

Aims: To evaluate the association between selected hemostasis-related single nucleotide polymorphisms (SNPs) and thrombolysis outcomes in AIS.

Methods: In this single-center, prospective, observational cohort study, DNA samples of AIS patients treated with thrombolysis were analyzed. The FII G20210A and FV Leiden mutations were detected using the LAMP Human FII&FVL Duplex kit. Additionally, 12 SNPs were examined in the genes of protein C (PROC), endothelial protein C receptor (PROCR), protein S (PROS1), and antithrombin (SERPINC1) using the SNaPshot Multiplex method (PROC: IVS1-1641 G>A, IVS-1654 C>T, IVS1-1476 A>T, IVS7+111 G>T, 3'UTR C>T; PROCR: p.Ser219Gly, 5'UTR C>T, 5'UTR G>A; PROS1: IVS11+54 T>C, p.Ser501Ala Heerlen; SERPINC1: IVS+141 G>A, p.Ala384Pro). Thrombin generation assay was performed using admission platelet-poor plasma samples. HT was confirmed via CT imaging 24 hours post-thrombolysis. A favourable outcome was defined as an improvement of ≥4-point in the National Institutes of Health Stroke Scale (NIHSS) by day 7.

Results: A total of 118 AIS patients treated with rt-PA were divided into three groups based on outcomes (28 with HT, 40 with favourable outcomes, and 50 with unfavourable outcomes), matched in terms of age, sex, and baseline NIHSS. The FV Leiden mutation, PROS1 IVS11+54 T>C and PROC IVS1-1476 A>T polymorphisms were more prevalent in the study cohort compared to the reference European population. The PROC IVS1-1641 G>A variant was significantly more common in HT patients, increasing the risk 3.96-fold for heterozygous (95% CI: 1.19–13.19) and 5.55-fold for homozygous carriers (95% CI: 1.59–19.31). This variant was also linked to decreased endogenous thrombin potential in carriers compared to wild types (1629 ± 429 vs. 1926 ± 489 nM*min, respectively, p=0.0148).

Summary/Conclusion: The higher frequency of FV Leiden mutation, PROS1 IVS11+54 T>C and PROC IVS1-1476 A>T SNPs in our cohort suggests that these variants potentially play a role in AIS susceptibility. The PROC IVS1-164 G>A variant was associated with hypocoagulability and increased the risk of HT following thrombolysis therapy.

P060

CLONAL HEMATOPOIESIS OF INDETERMINATE POTENTIAL, ESPECIALLY THOSE ASSOCIATED WITH DNMT3A MUTATIONS, PROMOTE THE FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS

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Background: Clonal hematopoiesis of indeterminate potential (CHIP) are the consequence of the acquisition by hematopoietic stem cells (HSC) of mutations in leukemia-associated genes. They have been associated with an increased risk of cardiovascular events and increased mortality. Most studies on CHIP have focused on the pro-inflammatory properties of monocytes/macrophages. Although neutrophils could also contribute to atherothrombotic events, notably via thrombo-inflammatory processes, their role in CHIP-associated cardiovascular events is unknown.

Aims: This study aimed to investigate whether neutrophils in the context of CHIP were more prone to generate neutrophil extracellular traps (NET) and contribute to CHIP complications.

Methods: The CHAth study (NCT04581057) enrolled patients who suffered from a first myocardial infraction after the age of 75 years. In these patients, CHIP-associated mutations were screened by high throughput sequencing. Inflammatory markers (hsCRP, interleukines 1ß and 6), atherosclerotic burden (carotid stenosis >50%, multivessel disease, total plaque volume via 3D ultrasound), and plasmatic NET markers (MPO-DNA complexes and citrullinated histone H3 [H3Cit], measured by ELISA) were assessed.

To study the causal role of CHIP in promoting NETosis, 2 mouse models allowing specific and inducible invalidation of *DNMT3A* or *TET2* in HSC were used: MAP17Cre;DNMT3Afl/fl and MAP17Cre;TET2fl/fl. Plasmatic level of H3Cit-DNA complexes (H3Cit-DNA) was assessed by ELISA to study basal NETosis. Neutrophils isolated from these mouse models were treated by ionomycin to explore stimulated NETosis. NET formation was then visualized and quantified by immunofluorescence.

Results: We studied plasmatic NET biomarkers in 81 patients. A CHIP was detected in 54% of them, mainly associated with *DNMT3A* (50%) and *TET2* (39%) mutations. CHIP carriers had significantly higher levels of MPO-DNA (3.1% VS 2.5%, p = 0.004) and H3Cit (0.19 VS 0.15, p=0.038). This effect was not linked to a particular mutated gene nor it was associated with the clone size (estimated by the VAF) or the number of mutations detected. No association was observed between CHIP and/or elevated NET markers and inflammation, atherosclerotic burden or post-infarction complications.

In mouse models, H3Cit-DNA levels did not differ between mice with and without CHIP, in either MAP17Cre;DNMT3Afl/fl or MAP17Cre;TET2fl/fl. However, neutrophils from MAP17Cre;DNMT3Afl/fl mice showed significantly increased NETosis upon stimulation compared to control mice (25,78% VS 47,10%, p = 0.0467). Such an effect was not observed in MAP17Cre;TET2fl/fl animals.

Summary/Conclusion: These findings demonstrate for the first time an association between CHIP and increased NET markers. Furthermore, our results indicate that CHIP have a causal role in enhancing NETosis when a stimulus is present, especially with *DNMT3A* mutations. In patients, such a stimulation could be due to inflammation or cardiovascular risk factors. This work suggests that neutrophils could

represent a new pathophysiological factor in CHIP-associated cardiovascular events. Larger cohorts are needed to confirm those findings. Transcriptomic analyses of neutrophils are currently in progress to further elucidate the causal mechanisms linking CHIP to NETosis.

P061

PROGNOSTIC VALUE OF VON WILLEBRAND FACTOR ANTIGEN FOR MORTALITY PREDICTION IN ISCHEMIC STROKE

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Background: Ischemic stroke, a leading cause of morbidity and mortality worldwide, is often accompanied by a prothrombotic state characterized by hypercoagulability in the acute phase. Endothelial dysfunction, a critical component of stroke pathophysiology, contributes significantly to this hypercoagulable milieu, leading to the increased secretion of von Willebrand factor antigen (VWF-Ag) from endothelial cells. Elevated VWF-Ag levels have been implicated in the severity of various cardiovascular events. Consequently, we hypothesized that increased VWF-Ag levels in the acute phase of ischemic stroke may serve as a valuable prognostic marker, particularly for predicting mortality in these vulnerable patients.

Aims: This study aimed to investigate the prognostic value of VWF antigen levels, measured in the acute phase of ischemic stroke, for predicting all-cause mortality in these patients.

Methods: This single-center prospective observational study included 34 patients admitted to the neurology department of the Regional Military University Hospital of Oran with a confirmed diagnosis of ischemic stroke based on CT-scan findings. VWF-Ag levels were measured in the acute phase (at admission) using an immuno-turbidimetric assay. Patients were subsequently followed up, with the primary endpoint being all-cause mortality.

Results: Of the 34 patients included in the study, 3 (8.8%) died during the follow-up period. Mean (VWF-Ag) levels were significantly higher in dead (491% [95% CI: 287-694%]) compared to survivors (215% [95% CI: 167-262%]; $p < 0.0001$). ROC curve analysis demonstrated a significant AUC of 0.935, indicating excellent discriminatory power for mortality prediction. A VWF-Ag threshold value of 311% was identified. Kaplan-Meier survival analysis revealed a significantly lower survival rate in patients with VWF-Ag levels ≥ 311% compared to those with levels < 311% ($p = 0.003$, Log-rank $\chi^2 = 15.35$, Hazard Ratio [HR] = 2.0 [95% CI: 0.9-4.45]).

Summary/Conclusion: Our study demonstrates that elevated levels of VWF-Ag, are significantly associated with increased mortality. A VWF-Ag threshold of 311% exhibits excellent discriminatory power for predicting death in these patients. These findings suggest that VWF-Ag may serve as a valuable early prognostic biomarker for risk stratification and potentially guide clinical decision-making in the management of ischemic stroke.

P062

TUBERCULOSIS AND THROMBOSIS : A COMPLICATED AND HETEREGENOUS ASSOCIATION

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Background: Thromboembolic complications associated with infection by Mycobacterium tuberculosis have been reported in the literature, that occurred in 1.5–3.4% of tuberculosis infection .

We report a 22-year-old man who presented with deep venous thrombosis , pulmonary arterial embolism and an attributable to pulmonary tuberculosis.

Aims:

To investigate the underlying pathophysiological mechanisms linking tuberculosis to thrombotic events.

To assess the prevalence and incidence of thrombosis in patients diagnosed with active.

To review current diagnostic and management strategies for thrombosis in the context of tuberculosis.

To raise awareness among healthcare providers about the thrombotic risks associated with tuberculosis.

Methods: A 22 -year-old man, student, presented with 07 days' history of with a painful swelling of the right leg, venous ultrasound showed a deep vein thrombosis extending from the gastronemius and proximal posterior tibial veins to the distal third of the superficial femoral vein and he was started on anticoagulants, with low molecular weight heparin, complicated after 5 day by an acute pulmonary embolism, when he presented a chest pain or haemoptysis , The CT pulmonary and abdominal angiography showed a bilateral segmental and sub-segmental pulmonary embolism accompanied by bilateral peripheral basal pulmonary infarct and moderate stenosis of the proximal portion of the celiac trunk, left adrenal infarction with calcification

The patient presented a general signs : loss of appetite, weight loss, fever, night sweats, weakness, coughing for longer than 3 week, At the physical examination his pulse rate was 112/minute

The auxiliary examination showed : hypereosinophilia, increased ESR , and high levels of CRP , A positive skin test result for tuberculosis infection, Acid-fast bacilli staining test for sputum sample was positive.

a complete thrombophilia, antiphospholipide profile, AAN and ANCA (Anti Neutrophil Cytoplasmic Antigen), All were within normal limits .

morning serumcortisol level was normal .

Results: The diagnosis of pulmonary tuberculosis was confirmed, and an antitubercular therapy was initiated with: isoniazid, ethambutol, rifampicin, and pyrazinamide. The patient's symptoms started improving.

Summary/Conclusion: Patients with tuberculosis are at risk of developing thromboembolic complications, as the disease is increasingly associated with systemic inflammatory responses and a hypercoagulable state. Early detection and treatment are essential to prevent complications and improve patient outcomes.

P063

LOCALIZED RETINAL ISCHEMIA REVEALING AN ANTIPHOSPHOLIPIDS SYNDROME

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Background: Antiphospholipids syndrome is a rare autoimmune disorder whose clinical manifestations are very wide and different. Vaso-occlusive manifestations are the most frequent ones. Vessels of all organs can be involved. Ocular involvement is among the rarest.

Aims: We report a case of retinal arterial thrombosis in the context of APS.

Methods: A 46-year-old patient with no previous medical history was admitted to the ophthalmology department for visual field amputation of the right eye without other associated signs. On the fundus, the left side was without abnormality and the right side was the site of a mixed arterial and superior temporal venous occlusion. Retinal angiography revealed a retinal focus in the superior temporal region with mixed occlusion of arterial and venous branches with ischemia of the superior temporal quadrant on the right and a retinal focus in the superficial inferior temporal region without occlusion on the left. Optical coherence tomography revealed ischemia of the superficial layers with alteration of the macular microvascularization in the right superior temporal quadrant.

Results: Immunological work-up showed antinuclear antibodies to be positive at 160 and anti-bgp1 positive with an IgG level of 118, IgM higher than 118, and IgA at 101. The patient was put on effective anticoagulation with good clinical evolution.

Summary/Conclusion: Ocular involvement is rare in APS and associated with poor prognosis, it seems to be difficult to diagnose but also to manage. Larger studies are required to establish guidelines on how and when to screen asymptomatic APS patients for ocular damage, but also on how to prevent and treat it.

P064

THE IMPACT OF PLASMA EXTRACELLULAR VESICLES ON COAGULATION FACTOR VIII-DEPENDENT THROMBIN GENERATION

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Background: High plasma levels of coagulation factor VIII (FVIII) are associated with an increased risk of venous thromboembolism (VTE). This risk is further elevated when high FVIII levels are combined with large platelets (e.g., high mean platelet volume, MPV) and elevated plasma levels of extracellular vesicles (EVs), known to express negatively charged phospholipids and facilitating coagulation activation. Parameters of the thrombin generation assay (e.g., lag-time and peak thrombin concentration) are associated with VTE risk.

Aims: To investigate the impact of varying levels of endogenous FVIII and EVs, along with exogenous supplementation of purified FVIII and EVs, on parameters of thrombin generation in plasma obtained from haemophilia A (HA) patients depleted of EVs.

Methods: Platelet-free plasma (PFP) was prepared using two consecutive centrifugations at 2,500 x g for 15 min and EV-depleted plasma (EVDP) was obtained by ultracentrifugation of PFP at 100,000 x g for 1 hour. EVDP was prepared and pooled from healthy volunteers and from patients with severe HA (<1% FVIII). Normal and HA plasmas were mixed to obtain final FVIII levels of 0%, 25%, 50%, 75% and 100% of normal levels with either normal or depleted EV levels. EVs were purified using size-exclusion chromatography, and purified FVIII was purchased from Haemochrome Diagnostica, Germany (ref. 5154007. The Calibrated Automated Thrombogram (CAT) assay, a global method to monitor the physiological dynamics of initiation (lag-time) and amplification (peak and endogenous thrombin potential (ETP)) of coagulation activation was used and intrinsic coagulation was triggered by adding 5 µL APTT reagent (SynthASil) obtained from HemosIL Inc (Werfen, UK) to the total (120 µL) thrombin generation mixture.

Results: Silica failed to induce thrombin generation in plasmas from patients with severe HA (< 1% FVIII). In EVDP, increasing FVIII levels (25% to 100%) induced a concentration-dependent linear decrease in lag-time from 16.8±1.9 min to 9.4±0.4 min. In the presence of normal EV levels, the corresponding lag-times decreased from 13.5±1.1 min to 7.7±0.6 min. Peak thrombin and ETP were not affected by increasing FVIII levels (25%-100%), neither in the absence nor in the presence of EVs. Exogenous supplementation of purified FVIII to EVDP from HA patients shortened lag-time in a dose-dependent manner; however no additional shortening was observed when supraphysiological FVIII levels were supplemented in the presence of EVs (i.e., HA plasma without EV depletion).

Summary/Conclusion: We found a FVIII-level-dependent shortening of lag-time when intrinsic coagulation was triggered by silica, an effect that was reinforced by the presence of EVs. However, at supraphysiological FVIII levels (achieved via exogenous supplementation), the impact of EVs was limited.

P065

THROMBOTIC STORM MANIFESTED BY MASSIVE ARTERIAL AND VENOUS THROMBOSIS AFTER DIAGNOSED THREATENING ABORTION

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Background: Thrombotic storm is a rare, acute hypercoagulable condition with rapid progressive formation of multiple arterial and/or venous thrombi, often localized in unusual positions, which occurs primarily in young women, often associated with inflammation, multiple and severe trauma, other causes of tissue necrosis, infection, surgery or pregnancy. In addition to known causes such as catastrophic antiphospholipid syndrome, certain myeloproliferative diseases, special thrombophilic conditions, malignancies, paroxysmal nocturnal hemoglobinuria, autoimmune diseases, abuse of cocaine and some other psychotropic substances, a significant part of patients with thrombotic storm does not have a clearly defined prothrombotic factor and trigger for the occurrence of multiple thrombosis.

Aims: to show rapid diagnosis and therapy in a patient with massive and multiple arterial and venous thrombosis

Methods: routine clinical, radiographic, echosonographic, biochemical, immunological and genetic methods

Results: We present a 36-year-old patient in the 21st week of pregnancy (with anamnestic data on 2 spontaneous abortions in the 7th and 12th weeks of gestation, 3rd pregnancy at term - stillbirth with fetal hydrocephalus)) who after being diagnosed with an imminent abortion, felt fainting and pain in both lower extremities immediately after getting up from the gynecological table. At the time of presentation, the patient was tachycardic and had unmeasurable arterial tension. The left arm was swollen and cold, without palpable pulses, as well as was the left lower extremity, and soon the right lower extremity became cold, edematous, and livid, without palpable pulsations in typical places.

Pulmonary thromboembolism was detected by MSCT pulmonary angiography, massive central thrombosis of the abdominal aorta infrarenal with propagation in both iliac arteries, both femoralis communis and femorales superficialis arteries, subocclusive thrombosis of the superior mesenteric artery, ischemic changes on the kidneys, and occlusive thrombosis of the left brachial artery were registered by MDCT.

Echocardiographic examination indicated an enlarged right ventricle with hypokinesia of the free wall. NT proBNP (695 pg/mL), troponin T (64 ng/L), and D-dimer (20 mg/L) values were elevated.

CDS ultrasound examination of the venous system revealed acute occlusive thrombosis of the femoral veins and veins of the lower leg on the left.

After thrombolytic therapy with tissue plasminogen activator and heparin anticoagulant therapy, the patient was better. On the first hospital day, profuse vaginal bleeding occurs, the patient complains of contractions, and soon a spontaneous abortion of the stillborn fetus and placenta occurs; an instrumental revision is performed in the operating room. In the further course, with the continuation of anticoagulant parenteral therapy combined with antiplatelet therapy, and later with vitamin K antagonist therapy, the condition further improves.

Thrombophilia analyzes registered only the heterozygous MTHFR C667 gene. Tumor markers antiphospholipid antibodies and other thrombophilia tests were normal.

Summary/Conclusion: Timely thrombolytic therapy, the application of antiplatelet and anticoagulant therapy can lead to the complete recovery of a patient with a thrombotic storm, the simultaneous occurrence of arterial and venous massive thrombosis.

P066

OVERUSE OF GENETIC THROMBOPHILIA TESTING? INSIGHTS FROM FV LEIDEN AND FII G20210A SCREENING IN REFERRED PATIENTS

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Background: Thrombophilia represents an increased predisposition to thrombosis, which may result from both inherited and acquired disorders of hemostasis. Among inherited causes, mutations in the Factor V Leiden and prothrombin (FII G20210A) genes are considered the most clinically significant. Although international guidelines define clear criteria for thrombophilia testing, these are frequently not adhered to in daily clinical practice.

Aims: To determine the prevalence of two genetic variants FV Leiden and FII G20210A associated with thrombophilia in a cohort of patients with clinically suspected hypercoagulable states, but without confirmed thrombotic events. This allows estimation of mutation prevalence in a population with suspected, rather than confirmed, thrombophilic conditions.

Methods: This retrospective study included 725 patients (aged 18–73 years; female and male) who underwent genetic testing for FV and FII mutations between 2017 and 2024. Indications for testing were based on clinical suspicion of thrombophilia. Genetic analysis was performed using the Genexpert® Xpert FII & FV assay (Cepheid) on the GeneXpert Dx System. Patient data were retrieved from electronic records.

Results: Among 725 patients, 646 (89.10%) were female and 79 (10.90%) male. Female patients were significantly younger than males (mean age 36.9 ± 8.5 vs. 47.5 ± 10.3 years, $p < 0.001$). The overall mean age was 38.1 ± 9.2 years.
For the FII G20210A variant, genotype distribution was: GG – 93.24%, GA – 6.62%, and AA – 0.14%. The mutation prevalence was 6.76% (n = 49), with a mutant allele frequency of 0.03. The cohort was in Hardy-Weinberg equilibrium ($p = 0.88$). In females and males, mutant allele frequencies were 0.03 and 0.04, respectively.

For FV Leiden (G1691A), genotypes were: GG – 89.52%, GA – 9.79%, AA – 0.69%. The mutation prevalence was 10.48% (n = 76), with a mutant allele frequency of 0.056. The cohort showed significant deviation from Hardy-Weinberg equilibrium ($p = 0.046$), although subgroup analyses for females and males separately showed equilibrium.

Summary/Conclusion: This study confirmed the presence of FV Leiden and FII G20210A variants in a clinically referred population, with a higher prevalence of FV mutations and deviation from Hardy-Weinberg equilibrium, suggesting referral bias. The predominance of younger female patients is likely due to frequent testing in pregnancy-related hypercoagulable states. Notably, a substantial number of patients underwent testing without meeting established clinical criteria, underscoring the need for stricter adherence to diagnostic guidelines and a more evidence-based approach to genetic thrombophilia screening.

P067

CEREBRAL VENOUS SINUS VEIN THROMBOSIS AFTER TRAUMATIC BRAIN INJURY: ANTICOAGULATION MANAGEMENT OF A PARTICULAR CLINICAL CASE

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Background: Acute cerebral venous sinus vein thrombosis (CVST) management after traumatic brain injury (TBI) is complex (up to 7% of all skull fractures). Despite the current European guidelines recommendation of CVST anticoagulation (Ferro JM et al., European Stroke Organization guideline for the diagnosis and treatment of cerebral venous thrombosis, 2017), this subset of patients may present concomitant large cerebral contusions and intraparenchymal haemorrhage.

Aims: Our aim was to report the management of an 8-year-old female child who suffered TBI complicated by extra-parenchymatous haemorrhage, epicranial hematoma and CVST.

Methods: We reviewed the clinical records of an 8-year-old female with CVST and TBI.

Results: Eight-year-old female with no significant past medical history admitted to a tertiary hospital due to TBI following accidental fall from approximately 1.5 meters height (day 0/D0). Child complained of headache and had vomited once. She denied loss of consciousness or amnesia. Upon admission at emergency room, Glasgow Coma Scale (GCS) was 15. She presented bilaterally symmetric reactive pupils, no identified motor or sensory deficits.

Head computerised tomography scan (CT) showed homolateral temporal and mastoid scale fractures, hemotympanum, thin extraparenchymal and gaseous hematic content in the left temporal region, small fractures in the left mastoid, extensive parietal and left temporo-occipital epicranial hematoma. She was admitted to the Paediatric Intensive Care Unit.

At 24 hours reassessment patient maintained severe frontal headache (no concomitant symptoms). She kept the same neurological status. Venous head CT (veno-CT) exhibited stable intracranial hematoma (residual) but demonstrated thrombosis of the left transverse and sigmoid sinuses.

Thus, following multidisciplinary team discussion (Neurology, Otorhinolaryngology, Pediatrics, Immunohemotherapy), considering veno-CT findings, it was decided that day (D1) to start anticoagulation with unfractioned heparin (UFH) 18 IU/kg/h, due to rapid reversal if needed.

At D3 UFH was switched to therapeutic low molecular weight heparin (LMWH).

On D4 revaluation head CT showed no evidence of acute ischemic or haemorrhagic lesions at the intraparenchymal level. On D9 post-TBI she was switched to warfarin. The team decided to maintain anticoagulation for 3 months.

Patients were discharged at D16 (asymptomatic).

Summary/Conclusion: Currently, there are no established guidelines regarding the initiation of anticoagulation in patients with traumatic CVST. Hence, the decision to start anticoagulation should be personalised, considering intracranial injuries.

Despite presenting both thrombotic and haemorrhagic risk factors, this child was successfully managed in a multidisciplinary team. The decision to start anticoagulants proved to be beneficial (no further thrombosis progression).

To date, optimal duration of anticoagulation stays uncertain. We chose to maintain it for 3 months.

P070

MANAGEMENT OF HEREDITARY ANTITHROMBIN DEFICIENCY IN PREGNANCY - SINGLE CENTER EXPIRIENCE

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Background: Antithrombin deficiency, although the rarest thrombophilia, carries the highest risk of thromboembolism. Physiological hypercoagulable state in pregnancy combined with antithrombin deficiency significantly increases the thrombogenic risk by 5 - 10 times. Thrombogenic tendency is associated with placental dysfunction, causing placental thrombosis. This leads to major gestational complications such as miscarriage, intrauterine growth restriction or fetal death, placental abruption, preeclampsia, low platelets (HELLP) syndrome.

Aims: The aim of this work is to show the relationship between the applied low molecular weight heparin (LMWH) thromboprophylaxis in pregnancy, depending on the type of antithrombin deficiency and the thrombotic history, on the outcome of pregnancies.

Methods: Data were collected through a retrospective analysis of the medical database of the Clinical Institute for Transfusion Medicine, University Hospital Centre Osijek, in the period 2013 - 2024. In that data base 12 women were identified with diagnosed AT deficiency. Four patients were monitored for up to total of 6 pregnancies.

Results: Four patients diagnosed with hereditary AT deficiency were monitored in our institution during six pregnancies. The median age of the patient at time of diagnosis was 32 years. None of the patients had thromboembolic events or any other form of thrombophilia. Hemostasis testing in one patient was performed due to family history, the patient's sister has proven AT deficiency. In two patients, hemostasis testing was performed after repeated early miscarriage (more than two). In the fourth patient, antithrombin deficiency was diagnosed as part of a bleeding tendency treatment. The range of measured antithrombin activity was 51 – 70 % before and during pregnancy. Three patients had antigen determinations, which were normal. Molecular testing confirmed the heterozygote Padua I and Budapest III mutations in two patients, while molecular testing was not performed in the remaining patients. All four patients received LMWH thromboprophylaxis in all 6 pregnancies. Earliest LMWH therapy started around 13th week of gestation and continued for 4 - 6 weeks postpartum. The patients received prophylactic doses of LMWH, enoxaparin 40 – 60 mg or dalteparin 5000 - 7500 IU with monitor anti-Xa activity at least once a month. The anti-Xa value was in the prophylactic range of 0.1 - 0.2 IU/ml. Five of the six pregnancies had good outcome, the patients were delivered at term with a normal birth weight of the newborn. One patient had a spontaneous miscarriage at 15th week of gestation, the use of LMWH had just been started previously and the course of the pregnancy was complicated by COVID-19 infection. None of the patients had any bleeding complications.

Summary/Conclusion: Our study group had a milder type of antithrombin deficiency (two patients had proven heterozygous type IIb mutation) who had no thrombotic events during their lives. During pregnancies, they had a good outcome with the use of prophylactic doses of LMWH. None of the patients received AT concentrate substitution. Individual assessment is crucial when deciding on the form of the treatment. The type of deficiency, personal and family history should be taken into account, together with additional risk factors like obesity, comorbidities, etc.

P071

THE DOAC-FRAIL STUDY: PREVALENCE OF DEVIANT DOAC LEVELS IN FRAIL NURSING HOME RESIDENTS

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Background: Direct Oral Anticoagulants (DOACs) are increasingly preferred for managing atrial fibrillation and venous thromboembolism (VTE) due to their superior safety and efficacy compared to Vitamin K Antagonists (VKAs). However, their use in frail nursing home residents poses challenges due to multimorbidity, polypharmacy, and altered pharmacodynamics, potentially leading to suboptimal DOAC levels.

Aims: This study aims to assess the prevalence of deviant DOAC levels in nursing home residents.

Methods: This prospective cohort study evaluates DOAC levels in nursing home residents receiving DOAC therapy. An additional blood sample was collected each time a routine blood test was ordered by the attending physician at the nursing home, which began on July 1, 2024 and is ongoing. Deviant DOAC levels were defined as a DOAC level outside the expected ranges as described by the Dutch antithrombotic guideline (and in line with ranges proposed by Douxfil et al).

Results: Up until March 2025, a total of 88 nursing home residents were approached, with 73 providing informed consent. Blood samples were collected from 41 participants (mean age 83.8 ± 7.65 years, 41.5% male, mean BMI 23.5 ± 4.89 kg/m²). Of the participants, 82.9% were on DOAC therapy for atrial fibrillation, 7.3% for deep vein thrombosis, and 9.8% for pulmonary embolism. Among participants with known kidney function (85.4%), 48.57% had impaired renal function (eGFR <60ml/min/1,73m²). Apixaban was used by 25 participants, edoxaban by 4 and rivaroxaban by 12 participants. Overall, 41.5% of participants had DOAC levels outside the expected range; with 7.3% below the lower limit and 34.1% above the upper limit. Among apixaban users 44% had levels above the range. For rivaroxaban 25% had levels below the expected range and 25% had levels above. All edoxaban users had levels within the expected range.

Summary/Conclusion: The current study indicates that a significant proportion (41.5%) of nursing home residents exhibit DOAC levels outside the expected range. Deviations in DOAC levels were observed both above and below the expected ranges, potentially increasing the risks of bleeding and thrombosis. Larger, prospective studies are essential to investigate whether deviant DOAC levels are truly associated with an increased risk of thrombotic or bleeding complications.

P072

COMPARISON OF PT-DERIVED FIBRINOGEN AND THE CLAUSS-BASED FIBRINOGEN ASSAYS IN CRITICALLY ILL PEDIATRIC PATIENTS

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Background: Based on the International Council for Standardization in Hematology, the Clauss-based fibrinogen activity assay is the initial step in diagnosing fibrinogen disorders in routine hemostasis laboratories. If fibrinogen activity is low, a fibrinogen antigen assay should be performed to exclude dysfibrinogenemia. ELISA is the gold standard method for testing fibrinogen antigen; nevertheless, it is not frequently used in clinical practice and is expensive and time-consuming. A recent study showed a high correlation between PT-derived fibrinogen and ELISA-based fibrinogen antigen levels, indicating that PT-derived fibrinogen testing can detect dysfibrinogenemia patients. The fibrinogen PT-derived/Clauss-based ratio is significantly greater than 1.43 in individuals with congenital dysfibrinogenemia. However, the PT-derived fibrinogen result may be overestimated due to oral anticoagulant medication, high fibrinogen, and elevated INR values, and shouldn't be used routinely. Furthermore, the importance of the PT-derived fibrinogen assay in the case of acquired dysfibrinogenemia, hyperfibrinogenemia, or hypofibrinogenemia in critically ill patients is poorly understood.

Aims: This study was the first to examine the role of PT-derived fibrinogen assay in different pediatric age groups in critically ill patients.

Methods: All patients were recruited using the ICD code from the hospital database. Patients with oral anticoagulant medication, high fibrinogen levels, and elevated INR values were excluded. The study population was divided into four groups depending on admission unit: emergency services (n=28), neonatal intensive care units (n=32), pediatric intensive care units (n=110), and healthy controls (n=60). In those groups, the Clauss methods were used to measure clot-based fibrinogen activity using Dade® Trombin Reagent (Dade; Siemens Healthier, Marburg, Germany). The Thromborel® S reagent was used to assess PT-derived fibrinogen. Both methods were analyzed using the Sysmex® CS-2500 automated coagulation analyzer.

Results: All 230 patients were under 18 years old. There were 123 (54%) males and 107 (46%) females. In comparison to Group 4 (250mg/dL and 343 mg/dL), Group 1 (260mg/dL and 397 mg/dL) had significantly higher mean values of Clauss-based fibrinogen and PT-derived fibrinogen, while Group 2 (186mg/dL and 288 mg/dL) and Group 3 (222mg/dL and 335 mg/dL) had lower mean values (p<0.05). The mean PT-derived fibrinogen/Clauss-based fibrinogen ratio was 1.54 in Group 1, 1.75 in Group 2, 1.58 in Group 3, and 1.33 in Group 4. PT-derived fibrinogen had a strong correlation with Clauss-based fibrinogen in Group-1 (r=0.89), Group-2 (r=0.96), Group-3 (r=0.86), and Group-4 (r=0.75) (p<0.05).

Summary/Conclusion: Our findings suggest that elevated levels of PT-derived fibrinogen/Clauss-based fibrinogen ratio may be linked to abnormalities in fibrin(ogen) and fibrinolysis in critically ill neonates and children in intensive care and emergency departments.

P073

INFLUENCE OF VARIANTS OF THE C-TERMINAL DOMAIN OF THE BB CHAIN OF FIBRINOGEN ON ITS STRUCTURE AND FUNCTION IN CZECH PATIENTS

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Background: The function of the C-terminal fibrinogen-related domain of the Bβ chain of fibrinogen (BβFReD) is not yet fully understood. It is involved in lateral aggregation of fibrin protofibrils, although this interaction is not essential for fibrin polymerization. Other functions are not reported. The importance of certain amino acid for protein stability and function can be studied by characterization of its variants.

Aims: We have identified and characterized five new variants in BβFReD. The aim of our work was to investigate the influence of these variants, together with information about other variants recorded in BβFReD in Czech patients, on the structure and function of this domain.

Methods: Genetic analyses were performed to identify the variant and proteomic analysis was used to detect the presence of variant molecules in plasma. Fibrin clot was studied using scanning electron microscopy and turbidimetry. Methods of bioinformatics and *in silico* structural biology were used to predict an impact of the variant on protein structure and function.

Results: In Czech patients, 11 unique variants were identified in BβFReD, five of which have not yet been published. The newly found variants were p.Asp350His, p.Leu416Ter, p.Arg436Lys, p.Tyr446Ter and p.Tyr447Cys, all heterozygous. All variants were associated with quantitative fibrinogen disorders, except for the dysfibrinogenemic p.Arg267Ser. Only two of these variants are symptomatic. The p.Arg267Ser carrier had both thrombotic and bleeding problems. A p.Trp433Ter carrier who suffered from antiphospholipid syndrome with similar clinical manifestations to fibrinogen disorders had thrombotic complications and recurrent miscarriages.

The new variants p.Asp350His and p.Arg436Lys were not detected in carrier's plasma and the optical density of fibrin clot formed from plasma of these variant carriers was lower than for the same clot formed from plasma of healthy donor. While the thickness of the fibrin fibers of the fibrin clot produced from the p.Asp350His carrier plasma resembled that of a healthy donor, the fibrin clot produced from the p.Arg436Lys carrier plasma had thinner fibers.

Summary/Conclusion: BβFReD variants in Czech patients are usually associated with hypofibrinogenemia and are asymptomatic. This indicates the importance of BβFReD for fibrinogen folding, assembly, and secretion, rather than for its function.

P074

LEPTIN IS A MAJOR MEDIATOR OF OBESITY-RELATED VENOUS THROMBOEMBOLISM

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Background: Leptin, predominantly produced by adipocytes, is elevated in obesity and exerts regulatory effects on both metabolism and immune function. Emerging evidence suggests leptin might contribute to the prothrombotic state associated with obesity by promoting platelet aggregation and upregulating inflammatory and hemostatic factors. While obesity is a recognized risk factor for venous thromboembolism (VTE), it is unclear to what extent leptin mediates this risk.

Aims: To investigate the association between plasma leptin levels and the risk of incident VTE, and to explore the potential role of leptin as a mediator of the increased VTE risk observed in obesity.

Methods: In this case-cohort derived from the third Trøndelag Health Study (HUNT3, conducted in 2006-08) in Norway, we measured plasma leptin from blood samples collected at study inclusion in 294 participants diagnosed with VTE during the first 5 years of follow-up and 1066 randomly sampled age- and sex-matched subcohort participants using an aptamer-based method (Somascan 7K). We applied correlation analysis (Pearson) and age- and sex-weighted Cox regression models to estimate hazard ratios (HRs) with 95% confidence intervals (CIs). Mediation analysis (CMAverse R package) was conducted to determine whether leptin contributed to the elevated VTE risk observed in individuals with obesity. The study was approved by the regional committee for medical research ethics, and informed written consent was obtained from all participants.

Results: Plasma leptin levels were higher in women than in men and were strongly correlated with body mass index (BMI) ($r=0.55$, $p<0.001$). In the age-adjusted model, individuals with plasma leptin levels within the highest quartile had 84% higher risk (HR: 1.84; 95% CI: 1.27-2.65) of overall VTE compared to those in the lowest quartile. Obesity (BMI ≥ 30kg/m2) was associated with a 53% higher risk of VTE (HR 1.53, 95% CI: 1.05-2.23) compared to lean individuals (BMI < 25kg/m2). This association disappeared after adjustment for leptin levels (HR: 1.01, 95% CI 0.62–1.66). In the mediation analysis estimating the impact of leptin on the association between obesity and VTE risk, the HR for the indirect effect of leptin was 1.41 (95% CI: 1.12-1.84), and leptin was estimated to mediate 87% (95% CI: 0.17-0.97) of the total effect.

Summary/Conclusion: Our findings confirm that plasma leptin is strongly correlated with BMI and show that plasma leptin levels were associated with VTE risk. The mediation analysis suggested that leptin is a major mediator of the VTE risk in obesity and might be a target for the prevention of obesity-related VTE.

P075

FUNCTIONAL AND MOLECULAR CHARACTERISTICS OF GENETIC VARIANTS IN INHERITED FIBRINOGEN DISORDERS

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Background: The severity of clinical manifestation of inherited fibrinogen disorders (IFDs) is highly heterogeneous. Such heterogeneity causes difficulty in the determination of clinical progression in patients with IFDs. It has become clear that genomic research alone does not fully explain the variability of clinical manifestation. Therefore, to better understand the role of individual residues and their overall impact on the fibrinogen molecule.

Aims: The aim of this study was molecular and functional characteristics of fibrinogen variants in *FGG* gene to better understand their behavior within the coagulation.

Methods: To better understand the role of genetic variants and their overall impact on the fibrinogen molecule, the patients with IFDs were investigated with specialized biochemical methods.

Results: We have described 11 mutations in the FGG gene in 20 patients from Czechia and Slovakia, of which 6 of them were newly discovered—γp.W3*, γp.T60A, γp.G191R, γp.E275*, γp.G310E, and γp.H333Y. Functional assessment revealed no differences in mutations that were followed by lower levels of both, functional and total fibrinogen—γp.W3*, γp.T60A, γp.E275*, and γp.R401W combined with γp.G191R. Polymerization of patients fibrinogen with mutations—γp.A108G, γp.R301H, γp.Y306C, and γp.G310E—showed abnormal development of fibrin. Furthermore, all patients had significantly different fibrin fiber diameters and clots composed of more fibers than those of the controls. Clinically relevant were 40 % of mutations, of which 30 % were associated with thrombosis. Our observations of lower levels of fibrinogen, abnormal morphology of the clots and prolonged fibrinolysis indicate thrombotic phenotype of these mutations in our patients.

Summary/Conclusion: Study shows that a combination of methods is needed to acquire a complex and complete picture of what is happening in a patient with IFDs. Our approach has enabled us to identify and characterize several new fibrinogen variants. Properties and behavior of all described fibrinogen variants were analyzed and discussed, thereby deepening insight into IFDs.

P076

VENOUS THROMBOEMBOLISM IN AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION - A SINGLE CENTER EXPERIENCE

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Background: Autologous hemopoietic stem cell transplantation (autoHSCT) is associated with serious vascular complications, including venous thromboembolism (VTE). Its estimated incidence is up to 15%. VTE events are frequently atypically localized or associated with central venous devices. The placement of the devices is mandatory for the peripheral blood stem cell (PBSC) collection and

Aims: The evaluation of VTE events and their characteristics in adult patients undergoing PBSC collection and autoHSCT.

Methods: A retrospective single-center analysis of the course of autologous PBSC collection (from the start of the mobilizing regimen up to day 30 post-collection) and subsequent autoHSCT (from the initiation of the conditioning regimen up to day 100 post-HSCT) was conducted, focusing on antithrombotic treatment and thromboembolic complications. All consecutive adult (> 18 years) patients undergoing stem cell collection and autoHSCT at the center during 12 years (2013-2024) were included. Underlying disease, transplant, clinical, and laboratory characteristics were analyzed.

Results: 111 patients (44 female, 67 male; mean age 52.5 (19-70) years at the time of HSCT) with 112 PBSC collections (2 patients with repeated procedures; 1 collected at a different center) and 112 transplantations (1 patient with repeated HSCT) were analyzed. 109 (98.2%) suffered from hematologic malignancy (myeloma 67, other non-Hodgkin lymphoma 26, Hodgkin lymphoma 12, acute leukemia 4), and 2 (1.8%) from multiple sclerosis. The conditioning regimen varied according to the underlying disease (mel200, BEAM, BendaEAM, BuMel, Cy-ATG). Thromboprophylaxis with LMWH during the hospital stay, modified due to procedural thrombocytopenia, was given in 75 (67.5%) cases of PBSC collection and 75 (66.9%) cases of HSCT. Eight patients (7.3%) suffered from VTE during the collection period (3 males and 5 females; 4 with myeloma, 2 with Hodgkin and 2 with non-Hodgkin lymphoma; 28-55 years; 1 pulmonary embolism, 7 CAT); 4 (3.6%) during HSCT period (3 males and 1 female; 2 with myeloma, 1 with Hodgkin and 1 with non-Hodgkin lymphoma; 23-67 years; all CAT). All events in HSCT occurred within 25 days after the procedure in patients with no previous VTE. Three received LMWH prophylaxis; however, LMWH was reduced or stopped at the onset of thrombosis due to severe thrombocytopenia. No event resulted in death.

Summary/Conclusion: CAT is a relatively frequent complication in both PBSC collection and early HSCT. Antithrombotic prophylaxis with LMWH seems to be an effective preventive measure in most cases. Its optimal administration and duration remain challenging.

P078

UNVEILING NETOSIS IN ENDOMETRIAL CANCER: A STUDY OF CIRCULATING BIOMARKERS

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Background: Endometrial cancer (EC) is the most prevalent gynaecological malignancy in developed countries. About 10–25% of EC patients experience venous thromboembolic events. NETosis has recently been described as a novel neutrophil immune response mechanism, through which neutrophils release extracellular traps (NETs) composed of DNA, histones, and proteins. So far, NETs have been implicated in immunothrombosis, tumour progression, metastasis, and immune evasion. However, their specific role in EC remains poorly understood.

Aims: To characterize the profile of NETosis biomarkers in plasma from patients with EC and to explore their potential association with thrombosis-related parameters.

Methods: Citrated plasma samples from EC patients (n=65) and healthy women (n=65) were analyzed. Levels of three NETosis biomarkers were quantified: cell-free DNA (cfDNA), citrullinated histone H3-DNA complexes (citH3), and calprotectin. Coagulation and thrombosis-related parameters were retrieved from clinical records. Statistical analyses were performed using R software (v.4.4).

Results: Compared to healthy controls, EC patients exhibited significantly elevated levels of cfDNA [(median 1954.21 ng/mL; Q1-Q3, 1841.68–2170.42) *vs.* (1534.33; 1425.71–1725.21), p<0.001], citH3 [(0.64 AU; 0.41–0.83) *vs.* (0.45; 0.35–0.64), p=0.016], and calprotectin [(1532.19 ng/mL; 1040.70–1974.78) *vs.* (1042.00; 748.82–1534.25), p=0.002]. All three biomarkers demonstrated discriminatory power between the groups: cfDNA (AUC = 0.90; p<0.001), citH3 (AUC = 0.63; p = 0.011), and calprotectin (AUC = 0.64; p = 0.008). Besides, significant correlations were observed among the NETosis biomarkers (Spearman's r ≥ 0.221; p ≤ 0.014). Regarding thrombosis-related markers, EC patients showed increased levels of derived fibrinogen (491.5 mg/dL; 435.5–570.0) compared to controls (425.0; 385.0–510.0) (p = 0.021). Notably, calprotectin levels significantly correlated with derived fibrinogen (Spearman's r = 0.326; p = 0.001).

Summary/Conclusion: Plasma levels of NETosis biomarkers (cfDNA, citH3, and calprotectin) are significantly elevated in EC patients compared to healthy controls. All three markers demonstrated diagnostic potential, especially cfDNA, which is the simplest to measure. The observed elevation of NET markers, alongside increased derived fibrinogen levels, suggests the activation of the immunothrombotic pathway in the tumour context. Moreover, the correlation between calprotectin and derived fibrinogen supports a possible interaction between NETosis and the prothrombotic state in EC. These findings underscore the clinical relevance of NETs as potential biomarkers and contributors to thrombotic pathophysiology in EC.

P079

EXPLORING THE ROLE OF NEUTROPHIL ACTIVATION AND NETOSIS IN VENOUS THROMBOEMBOLISM RECURRENCE FOLLOWING LOW MOLECULAR WEIGHT HEPARIN DISCONTINUATION IN ONCOLOGIC PATIENTS

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Background: Neutrophil activation through NETosis plays a key role in cancer progression, thrombosis, and cancer-associated thrombosis. While its involvement in these conditions is well established, its specific role in venous thromboembolism (VTE) recurrence after anticoagulation discontinuation in cancer patients (CAT) remains unclear and poorly described.

Aims: To ascertain whether plasma of CAT patients has an increased ability to activate neutrophils and/or NETosis, which may mediate the risk of VTE recurrence after low molecular weight heparin treatment discontinuation.

Methods: Plasma samples were collected at the time of anticoagulation discontinuation, as well as at 21 and 90 days thereafter, from CAT patients with (N=21) and without (N=39, patient controls) VTE recurrence. Patients were matched by age, sex, and tumor type. Additionally, we analyzed plasma samples from 22 healthy controls matched by age and sex with CAT patients. We isolated neutrophils from a healthy donor and assessed the ability of plasma from patients, patient controls and healthy controls to induce neutrophil activation after 4h incubation with Sytox (Life Technologies). Furthermore, in each sample we assessed different NETosis biomarkers: calprotectin (Hycult Biotech), cfDNA (Life Technologies) and DNA-H3Cit complexes (Roche & Abcam). Next, we analyzed the plasma DNaseI activity with the single radial enzyme-diffusion (SRED) assay.

Results: Interestingly, plasma from CAT patients with VTE recurrence was able to induce a higher activation of neutrophils compared to that of the healthy controls ($P<0.05$). Furthermore, comparing patients and patients controls with healthy controls we found an increase in plasma calprotectin ($P=0.0084$ and $P=0.023$, respectively) and cfDNA ($P=0.018$ in both comparisons). Interestingly, we found a negative correlation between plasma cfDNA and plasma DNaseI activity in the analyzed samples ($P=0.005$).

Summary/Conclusion: We have evidenced that plasma of CAT patients that will suffer a VTE recurrence is able to induce a greater NETosis and has a higher level of NETosis markers. It is important to emphasize that various tumor types were investigated, which may contribute to the heterogeneity of the results; thus, further studies focusing on those tumor types that induce a higher degree of NETosis should be conducted. The identification of the molecules and/or biological mechanisms prompting a higher degree of NETosis and specific predictive biomarkers may lead to the discovery of new therapeutic interventions to reduce the risk of VTE recurrence in CAT patients and predictive biomarkers.

P080

ANTI-COMPLEMENT FACTOR H ANTIBODY SUBTYPE PROFILING IN SUSPECTED AUTOIMMUNE ATYPICAL HEMOLYTIC UREMIC SYNDROME

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Background: Atypical hemolytic uremic syndrome (aHUS), also known as complement-mediated thrombotic microangiopathy (CM-TMA), is a rare and serious disease caused by a deregulation of the alternative complement pathway (ACP). Clinically, it manifests as microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. Unlike Shiga toxin-associated HUS, aHUS is primarily related to genetic mutations or acquired immunological abnormalities affecting complement regulation. Mutations in proteins such as CFH, CFI, CFB, or C3 are identified in 50 to 60% of cases; in the remaining cases, acquired forms are found, particularly with anti-factor H autoantibodies (AFHA), which are found in 11% of European patients to 50% of Indian patients.

Early diagnosis is essential because treatment varies depending on the etiology: genetic forms benefit from complement inhibitors (e.g., eculizumab), while acquired forms require immunosuppression. However, commercial ELISA tests exclusively detect immunoglobulin G isotypes, missing potential IgA or IgM antibodies, which could contribute to case misclassification. Broader detection of all three isotypes (IgG, IgA, IgM) could improve diagnosis and treatment.

Aims: This study aimed to retrospectively evaluate AFHA isotypes (IgG, IgA, IgM) in suspected cases of acquired aHUS to determine whether IgG assays alone miss a subset of patients and to explore the immunological heterogeneity of AFHA.

Methods: At CHU Sainte-Justine, 559 plasma samples from patients suspected of autoimmune aHUS (2018-2023) were tested using an in-house ELISA targeting total immunoglobulins (IgG, IgA, IgM) against human factor H. Control wells coated only with buffer were used to exclude nonspecific binding. Samples with an optical density 3 standard deviations (OD) higher than that of the controls were considered positive and retested using an isotype-specific ELISA.

Fifty-seven false positives were excluded due to the absence of a differential signal with and without factor H coating.

Results: Of the 559 samples, 73 (13%) were confirmed positive. Among these, immunoglobulin profiles varied:

IgG alone: 54 (74%)

IgG + IgM: 9 (12%)

IgG + IgA: 3 (4%)

IgG + IgM + IgA: 1 (1%)

IgM alone: 4 (5%)

IgM + IgA: 2 (3%)

IgA alone: 0

In total, 8% of cases involved IgM and/or IgA without IgG, which would not have been detected by standard IgG-targeted ELISA tests.

Summary/Conclusion: This study reveals significant heterogeneity in AFHA immunoglobulin profiles in patients with suspected autoimmune aHUS. IgG was predominant, but 8% of IgM and/or IgA cases would have been missed by commercial IgG tests alone. These findings have important implications:

Improved diagnosis: Including IgA and IgM in standard protocols could reduce missed diagnoses and enable earlier and more personalized treatment.

Therapeutic considerations: Non-IgG profiles could reflect different immune responses, requiring tailored immunosuppressive strategies.

Pathophysiological information: Isotype diversity (e.g., IgM related to acute immunity, IgA to mucosal immunity) could reflect distinct disease triggers.

Future Research: Studies are needed to correlate isotype profiles with clinical features, genetics (e.g., CFHR1-3-4 deletions), disease severity, treatment response, and IgG subclasses (e.g., IgG3).

This work supports the expansion of AFHA testing beyond IgG to improve diagnosis, reduce delays, and optimize care for patients with aHUS.

P081

THROMBIN GENERATION ASSAY IN PATIENTS WITH SELECTED THROMBOPHILIC STATES

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Background: Thrombin is a key enzyme that plays an important role in the hemocoagulation process. Most routine coagulation tests specifically focus on either the procoagulant or anticoagulant component of hemocoagulation and therefore do not provide a comprehensive picture of these two opposing physiological systems. In contrast, the thrombin generation test (TGT) is a “global” test that provides a picture of the balance of hemostasis and thrombin inhibition through continuous and simultaneous measurement over time. The test was developed to mimic physiological coagulation mechanisms and allow the measurement of the entire kinetics of thrombin generation not only during the initiation phase but also during the down-regulation phase of thrombin generation and inactivation of the generated thrombin. Thrombin generation is typically triggered by the addition of tissue factor and phospholipids, and TGT results depend on the composition of these reagents. The use of appropriate reagents with different amounts of tissue factor and phospholipids depends on the clinical indication.

Aims: The aim of this work was to highlight the importance of determining the thrombin generation test in patients with selected thrombophilic conditions using the new Ceveron S100 coagulation analyzer.

Methods: First experiences with TGT examination on the Ceveron S100 device.

Results: The heterogeneous group of patients with various thrombophilic states (platelet hyperaggregability, FV Leiden, etc.) was investigated. In a selected group of patients, we monitored the effect of increased tissue factor concentration on thrombin generation. In patients with platelet hyperaggregability, we additionally monitored the effect of platelets themselves on the overall course of thrombin generation.

Summary/Conclusion: The Ceveron S100 is a fully automated analyzer that can analyze the thrombin generation profile in patients with thrombophilic or bleeding conditions or patients on anticoagulant therapy. This is a time-consuming but useful examination when an increased risk of bleeding or thrombosis is suspected.

P082

MONOCLONAL IGG POTENTIATES THE INHIBITORY EFFECT OF DABIGATRAN ON THROMBIN GENERATION

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Background: In monoclonal gammopathy of undetermined significance (MGUS) pathological plasma cells are producing an excess of monoclonal immunoglobulins and this state can progress to multiple myeloma (MM). The paraproteins may participate in hemostatic processes. We have recently demonstrated that monoclonal IgG interacts with thrombin and exerts an inhibitory effect on both fibrin and thrombin formation.

Aims: The effect of the direct thrombin inhibitor drug, dabigatran was investigated on thrombin formation in plasma of patients diagnosed with MGUS and MM. We hypothesized that monoclonal IgG can potentiate the inhibitory effect of dabigatran.

Methods: Untreated patients who had IgG-type paraproteins and were diagnosed with either MM (n=7), or MGUS (n=8) were investigated and their results were compared to those of healthy controls (n=9). From all participants citrated peripheral blood samples were drawn and the obtained platelet poor plasmas (PPP) were treated with 200 µg/L final concentration of dabigatran that corresponds to the peak concentration in patients taking the regular dabigatran dose. Subsequently a fluorimetric thrombin generation assay was carried out and the time and quantity parameters of the thrombogram were compared to untreated PPP in both patients and controls.

Results: We confirmed that untreated MM patients display significantly shortened time parameters (lagtime: p=0.016; time to peak: p=0.003) compared to controls. Upon dabigatran exposure, in case of MM patients we have observed a significant attenuation in thrombin formation compared to controls, by endogenous thrombin potential and peak thrombin parameters (p=0.038 and p=0.028 respectively). In case of the MGUS group no difference was observed in quantitative thrombin parameters. The elevated monoclonal IgG content of the plasmas showed a significant negative correlation with the peak thrombin parameter (r = -0.824, p=0.012).

Summary/Conclusion: In these studies we could verify that in patients with elevated monoclonal IgG, the inhibitory effect of dabigatran is significantly augmented.

P083

THE NAPCSR, THE BIOMARKER FOR CHC-ASSOCIATED THROMBOTIC RISK EVALUATION

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Background: Thrombotic events related to combined hormonal contraceptives (CHC) use remain a critical concern. The “nAPCsr” shows promising results to become the biomarker for CHC-associated thrombotic risk evaluation.

Aims: To determine nAPCsr thresholds above which the thrombotic risk should be taken into consideration when assessing the suitability of a woman’s contraception.

Methods: nAPCsr was assessed on plasma samples from women using either no contraception (n=220), progestin-only pill (POP) (n=14), natural estrogen (estradiol or estetrol)-based CHC (n=43), ethinylestradiol (EE) 20 or 30µg with levonorgestrel (n=70), EE 20µg with desogestrel, gestodene or drospirenone (n=105) and EE ≥30µg with desogestrel, dienogest or cyproterone acetate (n=32). Thresholds were determined as the 95th percentile of each of the above populations.

Results: nAPCsr thresholds were 3.0 for non-users and users of POP, 4.2 for natural estrogen-based CHC users, 5.0 for EE/levonorgestrel users, 6.5 for EE20/desogestrel, gestodene or drospirenone users and, finally, 7.0 for EE30/desogestrel, dienogest or cyproterone acetate users.

For non-users or women using POP, nAPCsr >3.0 indicate a procoagulable state, which could be attributable to thrombophilia (e.g. FV Leiden), meaning the pill should be contraindicated. For women already on CHCs, nAPCsr above respective thresholds indicate that the benefit-risk balance of the contraceptive formulation used is questionable. Either the user has an undetected inherited mutation, or an over-response to the estrogen component. In both cases, this suggests switching towards a safer contraception.

Summary/Conclusion: The nAPCsr biomarker could be used by gynecologists and general practitioners to identify at-risk patients to opt for the safest contraceptive therapy.

P084

COST-EFFECTIVE SCREENING STRATEGY TO PREVENT VENOUS THROMBOEMBOLISM IN COMBINED ORAL CONTRACEPTIVE USERS

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Background: Venous thromboembolism (VTE) is a leading cause of global morbidity and mortality, with significant societal and economic burden. Combined oral contraceptives (COCs) increase VTE risk by 2-to 6-fold, resulting in approximately 22,925 cases annually in Europe.

Although the associated societal financial burden reaches 2.58 billion EUR, current international guidelines discourage routine thrombophilia screening prior to COC prescription; citing low cost-effectiveness, low prevalence of thrombophilia, and potential unintended consequences (e.g. reduced contraceptive use).

The normalized Activated Protein C sensitivity ratio (nAPCsr) assay, a recent low-cost tool capable of detecting inherited thrombophilia and acquired COC-induced activated protein C (APC) resistance, offers a promising strategy for targeted screening

Aims: To examine how a new screening functional coagulation strategy can minimize the burden of COC-associated VTE from medical and economic perspectives.

Methods: We first extracted from the literature the direct and indirect costs of a VTE event in Europe.

Using the estimated number of COC users, we calculated the total burden of COC-associated VTE events at the European level.

We then assessed the potential annual savings from VTE cases avoided by identifying thrombophilia prior to COC prescription through the implementation of the nAPCsr algorithm.

In parallel, we estimated the cost of applying the nAPCsr screening strategy to new COC users across Europe.

Based on these figures, we determined the net economic benefit of the nAPCsr algorithm in optimizing COC prescription and reducing the societal burden of VTE.

Results: Economic models estimate that implementing nAPCsr-based screening could prevent up to 13,500 VTE cases annually, leading to 1.5 billion EUR in annual healthcare savings. The cost of nAPCsr algorithm for screening new COC users has been estimated between 300 and 500 million EUR, still leading to a reduction of the societal cost burden between 1.02 and 1.17 billion EUR.

By addressing both direct healthcare costs and indirect costs related to productivity loss and long-term complications, such strategy could improve patient safety, reduce the financial burden, and promote equitable access to safer contraception. Furthermore, targeted screening could alleviate the underrepresentation of high-risk women in current cost estimates and significantly mitigate the societal impact of COC-associated VTE.

Summary/Conclusion: Reconsidering current policy recommendations to integrate innovative screening tools like the nAPCsr assay appears essential to facilitate evidence-based, cost-effective prevention of COC-related thrombotic events, ultimately enhancing public health outcomes.

P085

VALIDATION OF THE SIEMENS INNOVANCE® DIRECT THROMBIN INHIBITION ASSAY TO MONITOR ARGATROBAN

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Background: Argatroban is direct thrombin inhibitor used as an anticoagulant in critically ill patients with heparin-induced thrombocytopenia (HIT). Due to its short half-life and hepatic metabolism, precise monitoring of Argatroban levels is essential to ensure therapeutic efficacy and minimize bleeding risk. The activated partial thromboplastin time (aPTT) has historically been recommended to monitor dosing of Argatroban, but increasing evidence is supporting monitoring with specific assays of direct thrombin inhibition (DTI). DTI assays are based on the diluted thrombin time, Ecarin clotting time or chromogenic (anti-Factor IIa) assays. DTI assays are increasingly available in clinical labs, for analysis of dabigatran.

Aims: To verify whether the automated Innovance® DTI assay can be used to monitor Argatroban using the Hemoclot® Argatroban Calibrator.

Methods: Normal pooled plasma samples spiked with Argatroban were analyzed with Hyphens Hemoclot® thrombin inhibitor assay (HTI) and Siemens Innovance® DTI on Sysmex CS5100. Fifteen patient samples from HIT-patients treated with Argatroban were subsequently compared between HTI and Innovance® DTI by regression analysis and Bland-Altman plot.

Results: Both assays showed good correlation to the spiked Argatroban concentration, with coefficient of determination (R^2) 0.990 for HTI and 0.994 for Innovance DTI. In patient samples, the correlation between the two assays had an R^2 of 0,878 with slightly lower results in the Innovance assay (absolute bias -0,05 and relative bias -7,4%).

Summary/Conclusion: We verified that the automated Innovance® DTI assay that is primarily used for measurement of Dabigatran can also be used to monitor Argatroban using the Hemoclot® Argatroban Calibrator. This could enable more clinical labs to monitor Argatroban by a specific DTI assay instead of the aPTT, which has been shown to be unsuitable in critically ill patients.

P086

THE EFFECT OF DIFFERENT TYPES OF HEPARIN ON THROMBIN GENERATION IN A PREGNANT WOMAN WITH HOMOZYGOUS TYPE II ANTITHROMBIN DEFICIENCY

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Background: Thrombophilic conditions may be accompanied by increased thrombin generation (TG), especially during pregnancy when the increase in thrombin generation results from a series of changes in the haemostatic system. During administration of anticoagulant treatment, it is desirable to reduce the increase in thrombin generation.

Aims: The aim was to assess thrombin generation in pregnant women and their response to LMWH administration during severe thrombophilic conditions.

Methods: Sixty women with a prophylactic dose of LMWH were included in the group of pregnant women. In this study, we used chromogenic method for determination of factor Xa (FXa) inhibition for enoxaparine, nadroparine, dalteparine, fondaparinux and unfractionated heparin (UFH). Kit Technothrombin® TGA RC Low and RC High and analyser Ceveron® Alpha were used to monitor the thrombin generation (TGA).

Results: TG was significantly increased in pregnant women at thrombophilic risk and these women responded to prophylactic doses of LMWH with lesser reduction in thrombin generation than predicted. We found that the degree of inhibition is very different, when comparing the effect of the mentioned heparins. Nadroparin reduces TG the most, in comparison with low molecular weight heparins (LMWH).

Summary/Conclusion: Thrombophilic conditions associated with the risk of VTE should be accompanied with the monitoring of thrombin generation, especially in cases where the ineffectiveness of anticoagulant treatment is suspected. Consistent monitoring of TG in pregnant women at thrombophilic risk can improve the general condition of pregnant women and the quality of their care.

P088

CAPLACIZUMAB TREATMENT IN PATIENTS WITH NEWLY DIAGNOSED ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA: SINGLE CENTRE EXPERIENCE

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Background: Acquired thrombotic thrombocytopenic purpura (aTTP) is rare life-threatening disorder, which manifests as severe thrombocytopenia, microangiopathic hemolytic anemia, renal failure, neurological impairment, and other ischemic organ dysfunction. The cause is acquired severe ADAMTS13 deficiency. The current standard of the treatment for aTTP is a triple combination consisting of therapeutic plasma exchange (TPE), immunosuppressive therapy (e.g. corticosteroids or rituximab) and caplacizumab – an anti-vWF nanobody which disrupts the interactions between vWF multimers and platelets.

Aims: To inform about the efficacy and safety of caplacizumab in the treatment for newly diagnosed aTTP.

Methods: We have analyzed medical records and management of four patients with newly diagnosed acute form of aTTP who were hospitalized at our institution between December 2022 and April 2025.

Results: Four patients (3 female and 1 male, median age at onset 52 years) with newly diagnosed severe thrombocytopenia with bleeding manifestations and severe anemia, one with acute renal insufficiency and two with neurological symptoms were admitted to our department for suspected TTP. All patients had ADAMTS13 activity less than 2%, laboratory signs of hemolysis and positive ADAMTS13 antibodies, which confirmed the diagnosis of aTTP. We examined the presence of ultra large high molecular weight vWF multimers in our laboratory and we promptly initiated the TPE treatment in combination with high-dose corticosteroids. Shortly after, caplacizumab was added to the treatment, always administered after the TPE. Next days the platelet count was stabilized and ADAMTS13 activity increased. Due to the results, TPE was terminated and corticosteroids were slowly reduced, but the administration of caplacizumab in the standard dosage continued. Two patients experienced exacerbations and one patient had relapse with mild thrombocytopenia and reduced ADAMTS13 activity. Two of them were successfully treated with rituximab and one with cyclophosphamide. ADAMTS13 antibodies were later negative and ADAMTS13 activity progressively improved. All our patients achieved clinical remission.

Summary/Conclusion: Data of our single centre experience showed that the addition of caplacizumab to the standard treatment of aTTP helps to accelerate the time for platelet count normalization, reduce the number of TPE sessions, reduce the possibility of thromboembolic complications, length of hospital stay, and overall mortality rate. However, we need to develop larger multicenter studies to get adequate conclusions.

P089

CARDIOVASCULAR RISK ASSESSMENT AND INMUNE THROMBOCYTOPENIA (ITP) WITH CHATGPT4O; EXPERIENCE OF A CENTER

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Background: Rodoghiero noted prothrombotic nature of ITP [Am J Hematol (2016) https://doi.org/10.1002/ajh.24234]. There is increased risk of thrombosis in ITP compared to general population, but little is known about risk factors (RFs) for thrombosis in these patients, except in splenectomies. Lambert [Eur J Haematol. 2023, DOI 10.1111/ejh.14154] report RFs common to those of general population and highlight the importance of personalized approach, with adequate control of comorbidities in these patients.

Aims: To evaluate cardiovascular RFs (CVRFs) in a cohort of adults with ITP.

Methods: Retrospective collection of data from electronic medical records. Data analysis with ChatGPT40, which proposes statistical analysis.

Results: 55 patients: 30 males, mean age 61.74 years (± 22.5) and 25 females, mean age 69.8 years (± 17.87) (similar mean age, *p* 0.161, t-Student), representative of sex distribution in mixed ITP cohorts. Only significant difference between sexes: mean age (*p* 0.043, Fischer-t). For other items, no statistically significant differences. By age group, significant differences in arterial hypertension (HTN) (*p* 0.006), PLD and DM (*p* 0.0064 and 0.0035, respectively) in favour of > 65 years, similar to general population (Fischer-t). No differences in use of different TPO agonists by age (*p* 0.342) or sex (*p* 0.334). Greater use of Romiplostim® especially in patients with arterial hypertension (60% vs 45%, *p* 0.032) without scientific reason that justifies this. 23.6% patients were on antiplatelet therapy. When analyzing use of antiplatelet agents, the only variables with statistically significant difference (Fischer) were sex (*p* 0.021) and age (*p* 0.044). There are statistically significant differences between women and men and between >65 years and ≤65 years. 21.8% receive oral anticoagulation, preferably for AF (16.36%), frequency higher than that reported in spanish general population (in > 65 years, prevalence of AF between 4-9%). Greater proportion of anticoagulants among >65 years, with more use of antiplatelet agents among ≤65 years. For age, statistically significant differences between anticoagulants and antiplatelet agents (≤65 years: *p* 0.0432 and >65: *p* 0.0237, Fischer-t). For hypertension and PLD, there are highly significant differences between groups, reflecting different risk profiles (HTN: $p = 4.004 \times 10^{-15}$ and PLD: $p = 1.517 \times 10^{-10}$). No statistically significant differences for diabetes (*p* 0.4985), obesity and smoking (*p* = 0.2462 and 1, respectively). 3 patients suffered DVT, only 2 under TPO treatment (platelet count around 100000/ml). The 3rd patient was receiving dexamethasone (platelet count 8000/ml). One patient presented DVVRT weak positive, hypertension, smoking and overweight.

Summary/Conclusion: Our cohort has a moderate to high cardiovascular risk, although the analysis is limited by sample size and lack of longitudinal data. In our series, the high prevalence of arterial hypertension is highlighted, probably due to the age of most patients. Anticoagulated patients are more numerous if they are ≥ 65 years of age compared to antiplatelet patients. There was no increase in incidence of thrombotic events among TPO receptor agonist users. A comprehensive approach is necessary for management of CVRF in patients with ITP, prioritizing the control of comorbidities. Larger, more fine-tuned studies are needed to validate these findings and strengthen their clinical applicability. More studies are required to validate the use of AI for these purposes

P090

HOMOCYSTEINE AS A MODIFIABLE BIOMARKER FOR ISCHAEMIC STROKE. A PRELIMINARY REPORT

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Background: Stroke is the second cause of morbidity and mortality worldwide. Strokes caused by thrombotic or embolic etiology are referred as Ischaemic Strokes(IS). IS etiology is multifactorial. Risk factors involved in IS pathophysiology are smoking, obesity, diabetes mellitus, dyslipidemia, alcohol abuse and can be modified for the patient's benefit. It is important to identify more risk factors that can be modified and contribute to IS prevention and management. Hyperhomocysteinemia(HH) may be one such factor, modified by vitamin B12(B12) and/or Folic Acid(FA) administration. Homocysteine(HCY) is an intermediary sulphur amino acid. HCY's unique source is methionine, one of the essential aminoacids. Methionine's conversion to cysteine produces HCY. HH has been associated with cardiovascular and thromboembolic diseases, including IS. Nevertheless, conflicting results have been reported.**Aims:** Aim of the study was to determine HCY in patients with IS, including Transient IS (TIS), compare with a control group and correlate with B12 and FA.

Methods: 95 patients admitted to a tertiary Greek hospital were evaluated. 50(52.63%) suffered IS or TIS, median age 61.6(33-78)years and they constituted Group A. 45(47.37%) were healthy controls with median age 47(19-75)years (Group B). Patients with renal or heart failure, or cardiovascular disease, including atrial fibrillation were excluded.

HCY was determined by competitive chemiluminescence immunoassay(CLIA) on IMMULITE® 2000XPi immunoassay system (Siemens-Healthineers) routinely used at the hospital's core laboratory. Blood samples were collected on admission, after at least 12 hours overnight fasting. Samples were transferred to the laboratory on ice within one hour after venipuncture because HCY is still produced by red blood cells after venipuncture. Serum samples not immediately analysed were stored at -80°C. Lipemic or hemolyzed blood samples were rejected. B12 and FA were determined on UniCell DXI800 (Beckmann-Coulter) with enhanced chemiluminescence(CMIA).

The SPSS® v. 29 and the non-parametric Mann-Whitney U test compared HCY medians between the two groups. Regarding Group A, the Spearman's nonlinear correlation coefficient ρ was calculated between HCY and age, B12 and FA. The statistical significance level was set at $p<0.05$.

Results: Regarding Group A, HCY median was 12.35(5.1-42.2)μmol/L. In Group B, HCY median was 6.8(3.3-10)μmol/L. HCY of Group A was significantly higher($p<0.001$) and correlated negatively with B12($\rho=-0.528$, $p<0.001$) and FA($\rho=-0.570$, $p<0.001$).

Summary/Conclusion: IS is a major cause of death worldwide and its consequences have a significant economic burden. Preventive and therapeutic strategies suggest lifestyle, dietary modification and drug administration. Several studies suggest that HCY is a causative factor in atherogenesis and thrombus formation by causing endothelial dysfunction, vascular smooth muscle proliferation and coagulopathy and increases IS-risk two to three times. HCY was statistically significant higher in IS patients compared to the

control group on admission in the context of the present study. Some patients had low B12 levels. Findings of this study are considered extremely important because HCY levels can be modified. HCY levels decrease when administrating appropriate treatment. Simultaneous determination of B12 and FA and subsequent supplementation depending on the findings is recommended.

P092

INFLAMMATION AND THROMBOSIS: EVALUATION OF AN INFLAMMATION BIOMARKER IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background: Acute coronary syndrome (ACS) can be divided into subgroups ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI) and unstable angina(UA). ACS has significant morbidity and mortality. Prompt and accurate diagnosis and appropriate treatment are essential to reduce mortality. Biomarkers such as Troponin-I (hs-cTnI) are available for diagnosis. It is also recommended not to rely on only one biomarker, but on several biomarkers for more reliable diagnosis and management. hsCRP is an acute phase protein and a biomarker for the early inflammatory response. Several studies established hsCRP as a simple marker of the inflammatory response due to myocardial ischemia. Procalcitonin (PCT) is a biomarker of sepsis. PCT may also reflect local inflammatory process in the coronary artery. Novel studies indicated PCT elevation as a biomarker for clinical risk stratification in patients with ACS or myocardial infarction survivors. Nevertheless, the results remain controversial.

Aims: Aim of the study was to investigate if there is an association between serum PCT levels and ACS. PCT levels were determined in patients with STEMI, NSTEMI and UA on admission and were compared with a control group.

Methods: 284 patients admitted to a tertiary hospital with ACS were evaluated. 123 patients were diagnosed with STEMI, 101 were male, median age 65(43-91) years and constituted Group A. 48 with NSTEMI, 37 males, median age 69(42-92) years (Group B). 45 with UA, 34 males, median age 66(40-83) years (Group C). 68 were healthy controls, 41 males, median age 56(20-85) years (Group D). PCT was determined using the *UniCell DXI800* (Beckman Coulter) immunoassay analyzer with enhanced chemiluminescence (CMIA). Patients with infection were excluded. The SPSS v. 29 and the non-parametric Kruskal-Wallis test were used to compare PCT medians between Groups: A-D, B-D, C-D, A-B, A-C. The level of statistical significance was set at $p<0.05$.

Results: In Group A, median PCT was 0.07(0.01-2.04)ng/ml. Regarding Group B, PCT median was 0.05(0.01-1.13)ng/ml. In Group C 0.03(0.01-0.28)ng/ml and in Group D 0.03(0.01-0.13)ng/ml. PCT median of Group A was significantly higher than those of Groups C and D($p<0.05$ and $p<0.001$, respectively).

Summary/Conclusion: Myocardial tissue damage after ACS triggers a systemic inflammatory response, necessary for efficient repair of the damaged myocardium. As part of this inflammatory response, a variety of cells are activated and molecules are released. Hence, the common inflammation biomarkers may be of clinical significance in aseptic inflammation of ACS. They are potentially useful as biomarkers for ACS risk stratification. In critically ill patients, PCT is a biomarker of sepsis and a valuable tool for antibiotic stewardship. Nevertheless, limited and controversial previous data cannot clarify the clinical significance of PCT in ACS. This study demonstrated that PCT of patients diagnosed with STEMI was significantly higher than PCT of patients with UN and healthy individuals. Findings of the study suggest the potential of PCT to identify severe myocardial injury following STEMI. However, a larger number of patients is required to draw safe conclusions and randomized trials are needed to evaluate the clinical benefit of a procalcitonin-guided strategy.

P093

A REVIEW OF ANTICOAGULATION MANAGEMENT AND OUTCOMES IN BURNS PATIENTS

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Background: Burns patients have unique pathophysiology and at increased venous thromboembolism (VTE) risk exhibiting all factors of Virchow's triad.
Burns patients have altered coagulopathy, vascular integrity compromised, damage from multiple operative procedures, intravenous lines and other VTE risk factors. Limited information is known on effective anticoagulation in burns patients.

Aims: To:
Assess anticoagulant regimens for severe burns hospital inpatients
Determine VTE incidence among burns inpatients
Evaluate anti-factor Xa monitoring (sample testing, results and dose adjustments)

Methods: Retrospective electronic data collection for burns inpatients admitted to hospital from 31 October 2019 to 31 October 2024.
Patient demographics, total burns surface area (TBSA), burn lesions characteristics, anticoagulant medication, transfusions, presence of indwelling central venous catheters, hospital duration, complications, and mortality reviewed.
Data collection tool developed to facilitate data collection to assess compliance to local management.

Results: 114/116 (98%) burns patients prescribed anticoagulation.
2/116 patients had bleeding, thus not offered anticoagulation.
Routine anti-Xa monitoring was not performed in all patients on thromboprophylaxis.
55% (n=39) patients with measured anti-Xa had sub-prophylactic levels (below 0.2units/mL), with anticoagulant dose adjustment.
19% (22/116) patients developed VTE, and received anti-Xa monitoring which resulted in 15 patients with sub-therapeutic levels (below 0.5units/mL).
Enoxaparin dose increases did not cause major haemorrhage. No thrombocytopenia, or heparin sensitivity experienced.

Summary/Conclusion: This study expands understanding of thromboprophylaxis and importance of anti-Xa monitoring in burns patients to enhance their care and outcomes. Appropriate thromboprophylaxis is pivotal to reduce VTE and its complications. Low anti-Xa levels observed suggests inadequate routine enoxaparin prophylaxis dosing.
Burns management requires haematology and pharmacy input for appropriate thromboprophylaxis management, review and interpretation of anti-Xa results with appropriate dosing advice and monitoring.

This study supports routine anti-Xa monitoring with appropriate anticoagulant dose adjustments in burns patients, with multidisciplinary input into optimal VTE prevention.

P094

QUARTERLY AUDIT TO ASSESS VENOUS THROMBOEMBOLISM PREVENTION MEASURES FOR HOSPITAL INPATIENTS

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Background: Venous thromboembolism (VTE) remains a major leading cause of preventable death in hospital. It is important to ensure appropriate and accurate VTE risk assessment completion for adult hospital inpatients and offer appropriate thromboprophylaxis to inpatients at increased VTE risk for VTE prevention.

VTE stewardship support VTE risk assessment processes, ensure appropriate and timely prescribing of thromboprophylaxis, provide clinical advice, with an aim to enhance patient safety, quality of care and prevent adverse outcomes.

Aims: To evaluate whether adult hospital inpatients have appropriate VTE prevention measures, and assess compliance to national requirements.

Objectives: To establish if:

≥95% VTE risk assessment completion on admission within 14 hours and 24 hours of admission

≥90% appropriate pharmacological thromboprophylaxis prescribing during admission

≥90% appropriate mechanical thromboprophylaxis prescribing during admission

Methods: Audit standards agreed by multidisciplinary team, with local targets set, and in accordance with national/local guidance.

Quarterly audits occurred during 2024-25, of which quarter 4 audit involved 328 adult inpatients across two hospital sites (inclusion and exclusion criteria applied).

Retrospective data collection on ten random inpatients on each adult ward to assess VTE risk assessment completion, prescribed pharmacological and mechanical thromboprophylaxis (unless contraindicated) from admission, and any omitted doses.

Electronic records reviewed for patient demographics, clerking/admission documentation, VTE risk assessment forms, pathology results including renal function assessment, medication chart to assess performance against agreed standards to evaluate VTE prevention management.

Results: VTE risk assessment completion within 14 hours and 24 hours of admission was 81% and 96%, respectively

99% inpatients were prescribed appropriate pharmacological thromboprophylaxis during admission, unless contraindicated

77% inpatients were prescribed appropriate mechanical thromboprophylaxis during admission, unless contraindicated

Summary/Conclusion: Good compliance for prescribed pharmacological thromboprophylaxis despite lack of risk assessment. Further awareness on mechanical thromboprophylaxis is required, with prescribing careset.

Quarterly VTE dashboard was developed to summarise performance, highlight key messages for shared learning, with dissemination at hospital groups to improve patient safety.

Shared learning included:

Accurate/timely VTE risk assessment on admission to identify thrombosis and bleeding risk factors to support decision-making on thromboprophylaxis

Re-assessment and after-birth weight for maternity inpatients after delivery for appropriate postpartum management

Thromboprophylaxis dosing according to body weight and renal function

Prescribe mechanical thromboprophylaxis to surgical, maternity and critical care inpatients, unless contraindicated

Document reason when suspending thromboprophylaxis for identification and appropriate follow-up

Review patients who refuse anticoagulant for appropriate management, with escalation and documentation

Awareness on hospital-associated VTE event(s) and contributory factors, with action to prevent recurrence

VTE prevention measures can help reduce mortality, morbidity and costs of acute/long-term VTE effects to healthcare systems.

Audit highlighted importance of embedding VTE prevention measures in clinical practice, ongoing education and increased VTE stewardship.

P095

THERAPEUTIC UNFRACTIONATED HEPARIN MANAGEMENT IN HOSPITAL ADULT INPATIENTS

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Background: Venous thromboembolism (VTE) can be a life-threatening medical condition. Despite advances in the use of direct oral anticoagulants, VTE management with unfractionated heparin (UFH) has benefits e.g. quick onset of action, easily reversible due to short half-life, use in renal impairment patients, but also has practical challenges due to its narrow therapeutic window and high-risk profile.

UFH requires monitoring with appropriate dose adjustments for safe and effective anticoagulation treatment for patient safety.

Aims: To evaluate therapeutic UFH management (intravenous [IV] infusion or subcutaneous [SC] injection) for hospital adult inpatients and assess compliance to local guidance.

Objectives: To establish if:

≥95% VTE risk assessment completion on hospital admission within 14 hours and 24 hours of admission

≥95% of inpatients have an appropriate clinical indication for UFH treatment

≥90% of inpatients initiated on therapeutic UFH IV/SC have appropriate baseline blood tests, prescribed appropriate UFH loading and maintenance dosing and dose adjustments, with appropriate APTTR monitoring (for IV infusion), unless contraindicated

Methods: Audit standards agreed by the multidisciplinary team with local targets to assess compliance with local guidance.

Retrospective data collection (inclusion and exclusion criteria applied) using electronic patient records on adult inpatients on medical, surgical and critical care wards during 1st June 2024 to 30th November 2024 across two hospital sites.

Patient demographics, medical documentation, VTE risk assessment forms, pathology/radiology results including review of renal function status and APTTR test results, and medication chart were reviewed to assess performance against audit standards to evaluate therapeutic UFH IV and SC management.

Results: 63 adult inpatients were included in audit.

VTE risk assessment completion within 14 hours and 24 hours of admission was 87% and 90%, respectively

97% of inpatients had an appropriate clinical indication for UFH treatment

98% of inpatients initiated on UFH treatment had appropriate baseline blood tests

88% of inpatients initiated on UFH IV or SC treatment had appropriate loading dose, if clinically indicated, unless contraindicated

91% of inpatients on UFH IV or SC treatment were prescribed appropriate maintenance dose, unless contraindicated

68% of inpatients initiated on UFH IV infusion had appropriate APTTR monitoring at 4-6 hours after starting UFH IV infusion, and 60% of inpatients had APTTR monitoring at 4-6 hours after UFH IV dose adjustment

Summary/Conclusion: UFH, a high-risk medication, requires closing monitoring, regular dose adjustment, and clear management plans. Anticoagulation management may require specialist input.

Audit highlighted further education and awareness is required particularly on APTTR monitoring at appropriate timing before dose adjustments, and clear handover for follow-up, particularly amongst multidisciplinary staff e.g. medical staff for appropriate prescribing, nursing staff for correct infusion set up and administration, pharmacy staff to support dosing and clinical advice.

Clinicians have pivotal roles in:

Ensuring timely and appropriate VTE risk assessment completion

Supporting clinicians with appropriate prescribing of therapeutic UFH

Clinical advice/support on interpretation of APTTR results and appropriate dose adjustment

Ensure therapeutic drug monitoring electronic reminder is prescribed for UFH monitoring

P096

VENOUS THROMBOEMBOLISM PREVENTION MANAGEMENT FOLLOWING TOTAL KNEE AND HIP REPLACEMENT SURGERY

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Background: Total hip (THR) or knee (TKR) replacement surgery may be associated with serious post-operative complications such as venous thromboembolism (VTE), which can be fatal. VTE is an important cause of long-term morbidity and represents a preventable cause of mortality.

Pharmacological thromboprophylaxis helps reduce risk of VTE following this surgery type. Thromboprophylaxis options include low molecular weight heparin (LMWH) or rivaroxaban, an oral anticoagulant offering convenience, effective prophylaxis, similar bleeding profile to LMWH and cost-effective. Thromboprophylaxis should be offered for 2 weeks for TKR and 5 weeks for THR surgery. Effective thromboprophylaxis is required to prevent VTE.

Aims: To assess pharmacological thromboprophylaxis management during admission and on discharge following total hip and knee replacement surgery, and assess compliance to guidance.

Objectives: To establish if:

adult surgical inpatients have a completed VTE risk assessment on hospital admission

baseline full blood count tests on admission

adult surgical inpatients are prescribed appropriate pharmacological thromboprophylaxis on discharge

adult surgical inpatients receive verbal and/or written counselling information on anticoagulant agent

any patients developed a thrombotic event within 90 days of recent hospital admission

Methods: Retrospective data collection (inclusion/exclusion criteria applied) performed from January 2024 to March 2024 via electronic patient records across two hospital sites at acute London hospital.

Information team identified inpatients who had a total hip or knee replacement surgery via procedure coding data.

Patient demographics, medical documentation, pathology results, and electronic medication chart reviewed to assess performance against audit standards, agreed by the multidisciplinary team with local targets set, to evaluate pharmacological thromboprophylaxis during admission/on discharge for patients with total hip or knee replacement surgery.

Patients followed up for 90 days to identify if any thrombotic event(s) occurred post hospital discharge.

Results: 142 adult hospital inpatients included in data analysis.

- 97% and 100% of hospitalised adult surgical inpatients undergoing THR/TKR surgery had a completed VTE risk assessment within 14 hours and 24 hours of admission, respectively

51% of adult surgical inpatients undergoing THR/TKR surgery had baseline full blood count tests requested within 24 hours of admission

98% of adult surgical inpatients who have had a THR/TKR surgery were prescribed appropriate pharmacological thromboprophylaxis, unless contraindicated

94% of adult surgical inpatients who have had a THR/TKR surgery were prescribed correct duration of pharmacological thromboprophylaxis on discharge, unless contraindicated

1/142 patient developed a VTE event within 90days of recent admission

Summary/Conclusion: Patients received pharmacological thromboprophylaxis complying with guidance to help prevent VTE following major orthopaedic surgery. Surgery is a VTE risk factor in addition to expected immobility and other patient-related risk factors for thrombosis

It is important to ensure appropriate prescribing of pharmacological thromboprophylaxis during admission, correct duration on discharge to prevent hospital associated VTE events, and receive verbal and written counselling information on anticoagulant agent

A multidisciplinary action plan is in progress to implement recommendations from audit findings within 6 months, with planned feedback.

P097

PRELIMINARY REPORT: NEUROHORMONAL ACTIVATION AND A CARDIAC-DERIVED HORMONE IN THROMBOSIS

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Background: Acute coronary syndrome(ACS) is a leading cause of death worldwide and includes ST-segment elevation myocardial infarction(STEMI), non-ST-segment elevation myocardial infarction(NSTEMI) and unstable angina(UA). The underlying pathophysiology includes atherosclerotic plaque rupture and thrombus formation. ACS has been associated with complex neurohormonal activation. Using neurohormonal biomarkers for diagnosis, risk stratification and therapy gains ground in clinical practice. Although novel biomarkers have been identified and evaluated, only a few have been found to provide clinically useful information. b-natriuretic peptide (BNP) is the most promising biomarker for routine use as a supplement to Troponin-I(hs-cTnI). Nevertheless, the evidence to support the relationship between BNP and ACS is limited.

Aims: Aim of the study was to determine and evaluate BNP levels in patients diagnosed with ACS (STEMI, NSTEMI and UA) on admission. Also, comparison of BNP levels between the subgroups and a control group with regard to investigate if there is an association between BNP and ACS.

Methods: 291 patients admitted to a tertiary hospital with ACS were evaluated. 130(44.2%) patients were diagnosed with STEMI, 107 male, median age 65(43-91)years (Group A). 48(16.32%) were diagnosed with NSTEMI, 37 males, median age 69(42-92)years (Group B). 45(15.3%) with UA, 34 males, median age 66(40-83) (Group C). 68(23.12%) were healthy controls, 41 males, median age 56(20-85)years (Group D). BNP was determined using the *UniCell DXI800* (Beckman Coulter) immunoassay analyzer with enhanced chemiluminescence(CMIA). The SPSS v. 29 and the non-parametric Kruskal-Wallis test compared BNP medians between Groups: A-D, B-D, C-D, A-B, A-C, B-C. Level of statistical significance was set at $p<0.05$.

Results: In Group A, median BNP was 226.5(9-4886)pg/ml. Regarding Group B, BNP median was 125(9-2756)pg/ml. In Group C, 76(12-712)pg/ml and in Group D, 18(4-46)pg/ml. BNP median of Group A was significantly higher than those of Groups C and D ($p<0.001$). BNP median of Group B was significantly higher than those of Groups C and D ($p<0.05$ and $p<0.001$, respectively). BNP median of Group C was also significantly higher than Group D BNP median($p<0.001$).

Summary/Conclusion: Myocardial tissue damage after ACS triggers a systemic inflammatory response, necessary for efficient repair of the damaged myocardium. As part of this inflammatory response, a variety of cells are activated and molecules are released. Hence, the common inflammation biomarkers may be of clinical significance in aseptic inflammation of ACS. They are potentially useful as biomarkers for ACS risk stratification. In critically ill patients, PCT is a biomarker of sepsis and a valuable tool for antibiotic stewardship. Nevertheless, limited and controversial previous data cannot clarify the clinical significance of PCT in ACS. This study demonstrated that PCT of patients diagnosed with STEMI was significantly higher than PCT of patients with UN and healthy individuals. Findings of the study suggest the potential of PCT to identify severe myocardial injury following STEMI. However, a larger number of patients is required to draw safe conclusions and randomized trials are needed to evaluate the clinical benefit of a procalcitonin-guided strategy.

P098

GUIDELINES FOR THROMBOPHILIA TESTING FROM VARIOUS SOCIETIES – SIMILARITIES AND DIFFERENCES

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Background: Thrombophilia testing is a controversial issue. There is not a single correct approach to it and no uniform guidelines.

Aims: The aim was to evaluate the common features and the differences among the most cited guidelines for thrombophilia testing.

Methods: The three most cited guidelines were evaluated – American Society of Hematology (ASH) 2023 Guidelines; The British Society for Haematology (BSH) 2022 Guidelines; European Venous Forum (EVF) 2024 Guidelines. A comparative analysis was focused on recommendations for particular patients‘ groups.

Results: All the guidelines agree on basic principles, i.e. the appropriate selection of patients for thrombophilia testing and ordering the tests only if it could impact the patient´s management, or, eventually if it could have a benefit for thromboprophylaxis in the 1st degree relatives. There is an agreement among the guidelines concerning the first provoked venous thromboembolism – VTE (not to test) or concerning cancer patients (to test ambulatory cancer patients receiving systemic therapy, with a family history of VTE). Both BSH and EVF Guidelines recommend testing for antiphospholipid syndrome in the case of an arterial thrombosis in the absence of other vascular risk factors or after an unprovoked VTE or in the case of multiple thrombotic events. However, there is some disagreement concerning testing the patients with the first unprovoked thrombosis; asymptomatic individuals with a family history of VTE; women with a family history of VTE and/or family thrombophilia; thromboses in unusual sites.

Summary/Conclusion: Among the three most cited guidelines for thrombophilia testing, there is an agreement concerning basic principles of selecting patients for thrombophilia testing. However, there are also some differences in the recommendations for respective patients‘ groups. It might be confusing for the clinicians. Thrombophilia tests should be ordered selectively and with an individualized approach.

P100

ETP-BASED APC RESISTANCE ASSAY TO IDENTIFY LUPUS ANTICOAGULANT PHENOTYPE IN ANTIPHOSPHOLIPID-POSITIVE PATIENTS

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Background: Antiphospholipid syndrome (APS) is an autoimmune disorder defined by thrombotic and/or obstetrical complications in the presence of persistent antiphospholipid antibodies (aPL). Among them, lupus anticoagulant (LA) is the most thrombogenic and is typically detected through phospholipid-dependent clotting tests. However, LA is not a single type of antibody but a heterogeneous group of aPL that interfere with coagulation through various mechanisms, including acquired resistance to activated protein C (APC). This acquired APC resistance may result from two mechanisms: first, altered competition for anionic phospholipid surfaces by aPL–protein complexes (e.g., anti-β2GPI, anti-prothrombin, anti-protein S), second, direct interference with APC-mediated inactivation of FVa and FVIIIa. The normalized APC sensitivity ratio (nAPCsr), based on thrombin generation assay, may offer a novel and physiologically relevant functional readout of LA activity.

Aims: This study aims to assess whether the nAPCsr can help overcome the limitations of conventional LA testing, particularly false positives due to analytical interferences, and thereby support a more accurate diagnosis of both APS and LA.

Methods: Seventy-five plasma samples from patients with persistent aPL positivity (isolated APS or APS with systemic lupus erythematosus), stored in the CHU Clermont-Ferrand biobank, have been included. All were characterized for clinical presentation (thrombosis, obstetrical morbidity, and/or autoimmune manifestations) and standard aPL antibodies (aCL, anti-β2GPI, LA). The nAPCsr will be performed according to the validated ETP-based APC resistance methodology from QUALIblood s.a. Factor levels and routine LA testing will also be compared. Ethical approval was obtained and informed consent for biobanking and research use was secured.

Results: Testing is scheduled to start in June 2025. We hypothesize that LA-positive samples will show significantly elevated nAPCsr, consistent with acquired APC resistance. We will also carefully assess potential analytical artefacts to ensure the reliability of the assay. Additionnaly, we expect nAPCsr levels to correlate with clinical thrombotic or obstetric events and/or with LA positivity as determined by classical clotting assays.

Summary/Conclusion: This study will be the first to investigate nAPCsr as a supplementary functional marker, to support and enhance current diagnostic methods for LA detection. If successful, the ETP-based APC resistance assay may offer a robust, functionally integrated, and less interference-prone complementary tool to traditional clotting tests for the diagnosis of APS, particularly LA.

P101

GLOBAL HEMOSTATIC TESTS IN EVALUATION OF COAGULOPATHY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Specific types of acute myeloid leukemias, especially the acute promyelocytic leukemias (APL) are associated with the increased risk of severe coagulopathy. Predominantly at the time of diagnosis, the patients are threatened by developing ofdisseminated intravascular coagulation with different ways of manifestation. Bleeding symptoms occur more frequently, but thrombotic complications are not also rare. Routine coagulation tests usually do not reflect the real haemostatic potential in the body, they do not correlate with clinical outcomes. Typically, the therapy of coagulopathy is aimed tonormalisation of markers of routine coagulation by massive substitution of missing factors. This approach dramatically increases the therapy costs despite the fact that the global hemostatic potential is maintained. The role of global tests of haemostasis in the treatment strategy is still a matter of discussions. Among the most commonly used global tests are thrombin generation assay (TGA) and rotational thromboelastometry (ROTEM). Results of TGA unlike the ROTEM are not affected by thrombocytopenia, on the other hand, small changes in platelet count in vivo significantly affect the outcomes. ROTEM is performed individually in each patient in fresh whole blood and the results are available immediately. They reflect the changes in intrinsic and extrinsic pathway and in contribution of fibrinogen to clot formation.

Aims: The aim was to find the right global haemostatic test to evaluate the type and severity of coagulopathy, which would reflect the conditions of patients. To compare the results and advantages and disadvantages of two global test based on different principles, monitor the laboratory changes in parameters during the course of the disease and its treatment. To propose safe and effective methods of correction of developed coagulopathies.

Methods: Two test were used to assess the haemostatic conditions in patient – thrombin generation assay perfomed in platelet poor plasma on Ceveron s100 and rotational thromboelastometry performed in whole blood using ROTEM delta. Citrated blood was examined 1st, 7th and 21st day after starting treatment of acute leukemia. Basic coagulation parameters including antithrombin and fibrinogen were monitored concurrently.

Results: The authors present two case reports and haemostatic changes in patients admitted to hospital with bleeding symptoms associated with suspected APL. The diagnosis of APL was confirmed by molecular analysis only in one case, in second was negative for APL. Both of them underwent the chemotherapy induction according the type of acute leukemia. TGA and ROTEM showed significant changes in the monitored parameters. Trombin generation was evaluated mainly using two parameters - area under the curve and peak of thrombin generation, results were given as a ratio to pool plasma. In ROTEM was observed stronge effect of hypofibrinogenemia and thrombocytopenia. Changes were most prominent at the time of diagnosis, treatment of the underlying disease led to their gradual improvement.

Summary/Conclusion: Both tests are able to detect the impairment of global haemostatic potential. Sometimes they are more sensitive in comparison to routine tests. The main disadvantage is the problem of standardization and interlaboratory comparison. The next step should be the enlargement of the group of patients with this specific diagnosis to assess its influence to coagulation system.

P102

NEUTROPHIL TO LYMPHOCYTE RATIO IN ISCHAEMIC STROKE. A PRELIMINARY REPORT

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Background: Among different stroke subtypes, Ischaemic Stroke(IS) is the most common, accounting for about 80%. IS is mainly caused by atherosclerosis. Accumulating studies demonstrated that atherosclerosis is an inflammatory process mediated by activation of inflammatory markers and infiltration of various inflammatory cells, especially activated macrophages and T cells, leading to atherosclerotic plaque instability or rupture, ischaemia and infarction. A novel haematological parameter of systemic inflammation and stress is called Neutrophil to Lymphocyte Ratio(NLR). NLR is easily calculated from Complete Blood Count(CBC). The NLR reflects the dynamic relationship between innate cellular immune response that neutrophils induce and adaptive cellular immune response that lymphocytes induce during various pathological disorders. A normal range of NLR is between 1-2. NLR higher than 3.0 and below 0.7 in adults is pathological. NLR in a grey zone between 2.0-3.0 may serve as early warning of pathological conditions such as cancer, atherosclerosis, infection and inflammation. hsCRP is a common inflammatory marker with clinical significance in vascular atherosclerosis. Nevertheless, data regarding NLR and IS are limited.

Aims: Aim of the study was to determine CBC and hsCRP, calculate and evaluate NLR in patients diagnosed with IS, including Transient IS(TIS) on admission, compare with a control group and correlate hsCRP with NLR.

Methods: 95 patients admitted to a tertiary Greek hospital were evaluated. 50(52.63%) suffered IS or TIS, median age 61.6(33-78)years and they constituted Group A. 45(47.37%) were healthy controls with median age 47(19-75)years (Group B). Patients with cardiovascular disease, including atrial fibrillation were excluded. Patients with hematological diseases, inflammatory or infectious diseases were also excluded. CBC was determined using *UniCell DXH800* (Beckmann-Coulter) which is routinely used in the hospital's core laboratory. The SPSS v. 29 and the non-parametric Mann-Whitney U test was used to compare the NLR medians between the two groups. The Spearman's nonlinear correlation coefficient ρ was calculated between NLR and hsCRP. The statistical significance level was set at $p<0.05$.

Results: Regarding Group A, median NLR was 2.7(1.4-4.8) and median hsCRP 5.2(1.3-25.1)mg/L. In Group B median NLR was 1.8(1-4) and hsCRP 3.8(1.9-14.0)mg/L. The NLR and hsCRP medians of Group A were significantly higher than those of Group B($p<0.001$, $p<0.05$, respectively). NLR correlated positively with hsCRP($\rho=0.411$, $p<0.05$).

Summary/Conclusion: Neutrophils represent the first type of cells that infiltrate Central Nervous System, crossing the blood–brain barrier within a few hours after IS. The role of lymphocytes has also been studied in IS. Lymphocytes produce various cellular mediators that modulate the inflammatory response. NLR is determined by the division between the absolute number of neutrophils and lymphocytes in peripheral blood, and can be determined by a CBC. Novel studies demonstrated that NLR may be a prognostic marker for several diseases with inflammatory pathophysiology. Recent studies indicated that high NLR was correlated with IS severity, poor functional outcome, and recurrent ischaemic events. This study demonstrated that patients with IS have higher NLR compared to healthy individuals and that NLR is

positively correlated to hsCRP. NLR is a nonexpensive, fast and easily accessible marker for IS on admission. NLR may serve as a prognostic marker and potentially assist in therapeutic decisions.

P103

THE PROGNOSTIC POTENTIAL OF THE VWF/ADAMTS13 RATIO IN COVID-19, ANTIPHOSPHOLIPID SYNDROM, AND PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Patients with COVID-19, antiphospholipid syndrome (APS), and Ph-negative myeloproliferative neoplasms (MPN) share a significantly high risk of thrombosis, contributing to increased morbidity and mortality. The pathophysiology of thrombosis is similar among these disorders and includes endothelial dysfunction, platelet activation, complement dysregulation, and neutrophil extracellular trap formation. In COVID-19, an elevated antigen of von Willebrand factor (VWF)-to-ADAMTS13 ratio has emerged as a prognostic marker of disease severity and adverse outcomes, including thrombotic complications. Given the similar mechanisms that may contribute to thrombosis in APS and MPN, the VWF/ADAMTS13 ratio may represent a potential biomarker of thrombosis in these patients.

Aims: This study investigates the VWF/ADAMTS13 ratio in patients with COVID-19, APS, and MPN in comparison to a healthy population, in order to explore its potential role in characterizing thrombotic risk.

Methods: Between 2020 and 2024, a total of 145 patients were enrolled in this study, including 42 patients hospitalized with severe COVID-19, 55 with newly diagnosed APS, and 48 with newly diagnosed MPN prior to cytoreductive therapy. A control group of 30 blood donors was also included. Informed consent was obtained from all participants. Laboratory measurements included VWF activity (VWF:Ac), VWF antigen (VWF:Ag), and ADAMTS13 activity. Ratios of VWF:Ac/ADAMTS13 and VWF:Ag/ADAMTS13 were calculated and compared across groups.

Results: COVID-19 patients had significantly higher VWF:Ac and VWF:Ag levels and lower ADAMTS13 activity (all with$P<0.001$), resulting in markedly increased VWF/ADAMTS13 ratios compared to healthy controls ($P<0.001$). APS patients showed a borderline increase in VWF:Ac ($P=0.051$) and a significant increase in VWF:Ag ($P=0.01$), with no difference in ADAMTS13 activity; however, the VWF:Ag/ADAMTS13 ratio was significantly higher than in controls ($P=0.039$). In contrast, MPN patients had significantly lower VWF:Ac ($P=0.014$) and no significant differences in VWF:Ag or ADAMTS13 activity. After adjusting for age and sex, ADAMTS13 activity was significantly higher in MPN patients compared to controls ($P=0.038$). Consequently, the VWF:Ac/ADAMTS13 ratio was borderline lower in MPN patients compared to healthy controls ($P=0.058$), but significantly lower compared to COVID-19 and APS patients (both with $P<0.001$). In multivariate analyses, after adjustment for age and sex, and compared to the control group, patients with MPN showed a significantly lower VWF:Ac/ADAMTS13 ratio ($P=0.025$), while patients with APS retained a significantly higher VWF:Ag/ADAMTS13 ratio ($P=0.049$). Similarly, COVID-19 patients continued to exhibit significantly elevated VWF:Ac/ADAMTS13 and VWF:Ag/ADAMTS13 ratios (both with $P<0.001$).

Summary/Conclusion: The VWF/ADAMTS13 ratio differs significantly among patients with COVID-19, APS, and MPN compared to healthy individuals. Higher ratios in COVID-19 and APS may reflect endothelial dysfunction and thus an increased thrombotic risk. The thrombotic mechanism in MPN is complex and involves additional contributing factors. These findings highlight the potential of the VWF/ADAMTS13 ratio as a biomarker for thrombotic risk. Further studies are needed to validate its clinical application in monitoring disease course.

Posters - Platelets

P104

ISCHEMIC RENAL FAILURE SECONDARY TO ESSENTIAL THROMBOCYTHEMIA: A CASE REPORT

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Background: Essential thrombocythemia is a myeloproliferative syndrome characterized by an excessive proliferation of megakaryocytes, leading to an elevated number of circulating platelets. This condition is associated with an increased risk of thrombotic events.

Aims: The aim of our work is to demonstrate that essential thrombocythemia, although primarily a hematological disorder, can lead to severe renal involvement through thromboembolic or vascular mechanism.

Methods: We present here a case of acute kidney injury occurring in the context of a renal infarction, discovered at the time of the initial diagnosis of Essential thrombocythemia.

Results: This is the case of a 74-year-old female patient with a history of hypertension treated with an angiotensin II receptor blocker and a thiazide diuretic, who was admitted for nausea, vomiting, diarrhea, and persistent bilateral abdominal pain in the flank regions. On clinical examination, the patient presented with low-grade fever and uncontrolled hypertension measured at 160/95 mmHg. Laboratory tests revealed leukocytosis at 20 000 cells/mm³, anemia at 8 g/dl, thrombocytosis at 812 100 cells/mm³, and acute kidney injury with a serum creatinine level of 33 mg/l. LDH levels were moderately elevated at 318 IU/l, while the coagulation profile showed no abnormalities. Urinalysis showed positive hematuria and proteinuria (24-hour proteinuria at 200 mg/day). Immunological tests (antinuclear antibodies, anti-DNA, ANCA, anticardiolipin antibodies) and viral serologies were negative. Bone marrow biopsy revealed megakaryocytic hyperplasia suggestive of essential thrombocythemia. Abdominal CT scan revealed an occlusion of the left renal artery with near-complete infarction of the left kidney, infarction sequelae in the right kidney and spleen, and a few necrotic latero-caval adenopathies. Renal MR angiography confirmed the occlusion by showing an absence of enhancement in the left renal artery, located 20 mm from its origin. Treatment with aspirin and hydroxyurea was initiated. After one month of follow-up, there was a partial improvement in renal function (creatinine at 17 mg/l) and a reduction in thrombocytosis (250 000 cells/mm³). However, the patient retains renal sequelae, with chronic kidney disease classified as stage IIIB, secondary to ischemic-type vascular nephropathy. Renal scintigraphy revealed hypoperfusion of the left kidney, with residual function estimated at 10%, while the right kidney maintained satisfactory function.

Summary/Conclusion: Ischemic renal failure secondary to essential thrombocythemia is a rare but severe complication. Prompt management aimed at controlling thrombocytosis through cytoreductive therapy is essential to prevent the development of irreversible renal damage.

P105

DEFINING MAXIMAL AGGREGATION RESPONSES IN PATIENTS WITH LOW PLATELET COUNTS: A STUDY ON LIGHT TRANSMISSION AGGREGOMETRY

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Background: Pakistan has a high burden of inherited Platelet Function Disorders (PFDs) due to consanguinity. Light transmission aggregometry (LTA), the diagnostic gold standard, requires a platelet-rich plasma (PRP) count of 200–300×10⁹/L, limiting its use in thrombocytopenia. However, many patients suspected of having PFDs often present with thrombocytopenia (<150×10⁹/L) which may underestimate platelet function. It is essential to study platelet function at low counts to distinguish true platelet dysfunction from count-related effects.
Hayward and colleagues established reference intervals (RIs) for LTA in thrombocytopenia, but their RIs can't be directly applied to our setting because of differences in agonists, equipment and population. While advanced tools like flow cytometry & whole blood aggregometry have significant diagnostic implications in thrombocytopenia, they are unfortunately unavailable in low-resource settings. It is imperative to study platelet aggregometry in patients with thrombocytopenia in local settings because of high prevalence of PFDs and LTA being the only available diagnostic modality.

Aims: To determine Maximal Aggregation Responses (%MAR) using LTA in samples with low platelet counts. To explore the correlation between clinical history, findings of LTA, and platelet size.

Methods: This prospective, descriptive study is conducted at Aga Khan University, Pakistan in 2024-2025, after getting ethical approval. We took 120 healthy controls with normal platelet count and 126 patients with platelet count of <150×10⁹/L. Platelet function is analyzed by LTA using 4 agonists i.e., ADP (10uM), collagen (2 ug/mL), epinephrine (10uM), and ristocetin (1.25 mg/mL) and compared with healthy controls using Chi-square for parametric and Mann-Whitney for non-parametric results.
Using non-parametric method, 90% Confidence Intervals are computed from 120 healthy controls for each agonist at normal platelet count.
To establish RIs for thrombocytopenic samples, the data of healthy controls is extrapolated below 150 to predict MAR using regression analysis. Additionally, samples from 20 healthy controls are manually diluted with buffer to achieve platelet count from 150 to 25 x10^9/L and platelet aggregation is assessed at each dilution thus getting real-time data from controls.
Data analysis is conducted via SPSS version 22 with p-value of <0.05 considered significant.

Results: The mean age (mean±SD) of patients is 19.7±14.7 years with M:F ratio of 0.85:1. The platelet count [median(IQR)] of patients for whole blood and PRP are 72.5×10⁹/L(41-113) and 69×10⁹/L(38-110) respectively.
Bleeding time exceeded 7 minutes in 47% of patients. LTA results were normal in 40%, while 20% and 8% were suggestive of Bernard-Soulier Syndrome and Glanzmann Thrombasthenia, respectively.
Platelet aggregation (%MAR) [median(IQR)] in patients were reduced & showed wider ranges than healthy controls for all agonists. (p<0.001)
62.5 (36-75) vs 80 (70-88) for ADP, 52 (23-70) vs 75 (69.5-82) for Collagen, 59 (24-75) vs 81 (74.5-91) for Epinephrine, and 57 (11-71) vs 75 (67-84) for Ristocetin.
Platelet aggregation had positive correlation while Bleeding time and BAT (Bleeding Assessment Tool) score were inversely correlated with platelet count. (p<0.001)

Summary/Conclusion: Patients with thrombocytopenia & suspected PFDs should undergo platelet function testing regardless of the degree of thrombocytopenia.
This study addresses a key diagnostic gap by establishing much-needed reference intervals for LTA in thrombocytopenia.

P107

ROLE OF TYROSINE 130 IN SPLEEN TYROSINE KINASE SIGNALING IN PLATELETS

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Background: Syk is a non-receptor type protein-tyrosine kinase (PTK), which is associated with platelets surface receptors, glycoprotein VI (GPVI) and C-type lectin-like receptor II-type (CLEC-2). Beside platelets, Syk is expressed in most haemopoietic lineage cells and other tissues as fibroblasts, hepatocytes, and neuronal cells. Syk has two tandem SH2 motifs and a C-terminal kinase domain which are interrupted by interdomains A and B that have various tyrosines perform distinct functions upon phosphorylation. In B cells upon Antigen crosslinking, the activated Syk is dissociated from B cell Antigen receptor after phosphorylation of Y130 which triggers Syk release through long distance conformational uncoupling from the Antigen receptor.

Aims: This study aims to evaluate the role of Y130 in Syk signaling in murine platelets.

Methods: We generated Syk Y130F with CRISPR-Cas9 technique, then compared the platelet functions, aggregation, secretion, and integrin activation in wild type littermates and Syk Y130 mice. At the physiological level, we determined the time to occlusion of the carotid artery after injury in thrombosis model and bleeding time in wild type littermates and Syk Y130 mice.

Results: Platelets from homozygous Syk Y130F mice showed inhibition of functional responses after activation with CRP and CLEC-2 crosslinking compared to WT littermates with no significant differences in PAR-4 or purinergic receptors responses. CRP- and CLEC-2 mAb- downregulating signaling events, including LAT and PLCγ were diminished in Syk Y130F platelets. Moreover, the time to occlusion in the FeCl3 injury model was delayed in Syk Y130F mice compared to WT littermates which associated with prolonged bleeding time.

Summary/Conclusion: Syk Y130 regulates GPVI and CLEC-2- mediated functional response and signaling events in platelets affecting thrombosis and hemostasis.

P109

EFFECTS OF COVALENT BRUTON TYROSINE KINASE INHIBITORS (IBRUTINIB, AKALABRUTINIB AND ZANUBRUTINIB) ON PLATELET AGGREGATION

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Background: Platelet aggregation is an essential step by which platelets adhere to each other when exposed to agonists, such as collagen, adenosine diphosphate (ADP) or epinephrin. Dysregulation of this mechanisam due to antiplatelet drugs can result in bleeding. Bleeding is a frequently reported adverse event after the treatment with irreversible Bruton tyrosine kinase inhibitors (BTKi) in B cell malignancies, reported in up to 55% patients taking ibrutinib. Newer generation of covalent BTKi, acalabrutinib and zanubrutinib, were designed with a greater selectivity for BTK with the premise of decreasing associated toxicities and similar overall bleeding risk (40% vs. 45%). BTKi have been observed to inhibit downstream signaling of platelet collagen receptor glycoprotein (GP)VI which is responsible for platelet activation and aggregation in response to collagen and collagen-related peptides. Comparative studies have shown that acalabrutinib and zanubrutinib are less potent inhibitors of GPVI-mediated platelet aggregation than ibrutinib.

Aims: To determine whether there is a difference in platelet aggregability with collagen, ADP and epinephrine agonists in patients treated with different covalent BTKi.

Methods: Platelet light transmission aggregometry on Sysmex CS2500 analyzer (Siemens Healthineers, Germany) was determined using agonists collagen, ADP and epinephrin in twenty seven patients diagnosed with chronic lymphatic leukaemia. The informed consent was obtained before collecting blood samples. The patients were divided in three groups regarding the type of BTKi used for treatment (ibrutinib n=10, akalabrutinib n=10 and zanubrutinib n=7). The standard dose of BTKi were as follows ibrutinib 420 mg, akalabrutinib 200 mg and zanubrutinib 360 mg daily. The patients who used concomitant antithrombotic agents and with platelet number below 100x10*9/L were excluded.

Results: Median of platelet aggregation values with collagen, ADP and epinephrine were within the reference interval for ibrutinib and acalabrutinib (>60%), while median aggregation values with zanubrutinib were below the lower limit of the reference interval for all three agonists. In patients treated with zanubrutinib, there was a tendency to the lower values of aggregation with collagen (16%) compared to ADP (51%) and epinephrine (53%), but this difference was not statistically significant (P>0.05), possible due to small number of patients. Median levels of platelets were not significantly different between different BTKi (ibrutinib 167x10*9/L, akalabrutinib 186x10*9/L and zanubrutinib 181x10*9/L). Median ages were 70, 71 and 74 years (P>0.05), respectively. Statistical analysis was done in programme MedCalc v14.8.1.

Summary/Conclusion: Platelet aggregability with the agonists ADP, epinephrine and collagen did not show statistically significant differences between evaluated BTKi. Given that in patients on zanubrutinib, platelet aggregability with the agonist collagen showed a tendency to lower values compared to the other two BTKi (ibrutinib and acalabrutinib) as well as the other two aggregation agonists (ADP, epinephrine), it is necessary to conduct further investigation of the impact of these drugs (especially zanubrutinib) on platelet aggregability in a larger number of patients.

P110

A NOVEL B1-TUBULIN MUTATION ASSOCIATED WITH A SECRETION-DEPENDENT PLATELET HYPERACTIVITY AND PROTHROMBOTIC RISK

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Background: Microtubules (MTs) play a crucial role in platelet biogenesis and morphology. While mutations in β1-tubulin (TUBB1) have previously been linked to macrothrombocytopenia, their effect on platelet function remains poorly understood. Our research group has identified a novel homozygous TUBB1 mutation, p.P160L, associated with thyroid dysgenesis, platelet hyperactivation, and the presence of macroplatelets.[1]

Aims: To investigate the impact of the *TUBB1* p.P160L mutation on platelet morphology and function.

Methods: A knock-in mouse model harboring the *Tubb1* p.P160L mutation was used to evaluate the hemostatic phenotype *in vivo* and *in vitro*. Wild-type (WT), heterozygous (HET), and homozygous (KI) mice were compared.

Results: **Morphological abnormalities and MT disorganization:** Despite normal platelet counts in both HET and KI mice, platelet size was significantly increased in HET and KI (WT: 5.05 fL; HET: 5.22 fL; KI: 5.37 fL; $p<0.001$). Electron microscopy revealed abnormal platelet morphology, with a more rounded shape and disorganized MT structure. Western blot analysis showed a significant reduction in TUBB1 expression by 23 ± 4% in HET and 43 ± 5% in KI mice compared to WT ($p<0.01$). MT sedimentation by ultracentrifugation demonstrated a decrease in TUBB1 incorporation into MTs by 34% in HET and 58% in KI mice relative to WT ($p<0.05$), indicating a detrimental effect of the *Tubb1* p.P160L mutation on MT organization and platelet structure.

Enhanced platelet activation: Flow cytometry analysis revealed significantly increased activation of integrin $\alpha_{IIb}\beta_3$ in response to convulxin, an agonist of the collagen receptor GPVI (mean fluorescence intensity increased by 39 ± 3% in HET and 123 ± 4% in KI compared to WT; $p<0.001$), while responses to ADP and thrombin were unchanged. Inhibition of ADP and thromboxane A_2 amplification pathways normalized integrin activation. P-selectin exposure, a marker of α-granule secretion, was also significantly elevated in response to convulxin (mean fluorescence intensity increased by 20 ± 3% in HET and 53 ± 3% in KI compared to WT; $p<0.001$), suggesting enhanced GPVI-dependent activation and secretion in p.P160L platelets.

Prothrombotic phenotype: Collagen-induced platelet aggregation was markedly increased in HET and KI mice compared to WT (WT: 22%; HET: 49%; KI: 69%). Under flow conditions in a collagen-coated perfusion chamber at arteriolar shear rates, thrombi formed by HET and KI mice were significantly larger than those from WT mice (mean fluorescence intensity: WT: 233 ± 19 AU; HET: 460 ± 76 AU; KI: 482 ± 66 AU; $p<0.05$). This phenotype was reversed by inhibition of ADP and thromboxane A_2 pathways. Additionally, in an *in vivo* thrombosis model, arteriolar occlusion time was significantly shortened in HET and KI mice (WT: 38 ± 4 min; HET: 25 ± 2 min; KI: 22 ± 2 min; $p<0.01$), indicating a prothrombotic tendency associated with the mutation.

Summary/Conclusion: This study demonstrates for the first time that a mutation in TUBB1 can lead to a secretion-dependent increase in prothrombotic risk, likely due to microtubule disorganization and subsequent platelet hyperactivation.

[1] Stoupa A *et al. TUBB1 mutations cause thyroid dysgenesis associated with abnormal platelet physiology.* EMBO Mol Med. 2018;10:e9569

P111

GLANZMANN THROMBASTHENIA - A NOT SO RARE PLATELET FUNCTION DISORDER IN PAKISTAN

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Background: Glanzmann Thrombasthenia (GT) is a rare inherited autosomal recessive platelet function disorder characterized by qualitative and/or quantitative abnormalities in the GPIIb/IIIa integrin complex, resulting in defective platelet aggregation. It manifests primarily with mucocutaneous bleeding and is more prevalent in populations with high consanguinity. In resource-limited settings like Pakistan, diagnosis is often delayed or missed due to limited awareness, lack of diagnostic facilities and variable clinical presentation.

Aims: To determine the frequency of Glanzmann Thrombasthenia among patients with suspected platelet function disorders in Pakistan, describe the clinico-haematological features, and assess current diagnostic and treatment practices to guide evidence-based management.

Methods: This descriptive cross-sectional study included 2542 patients referred to the Haematology Department of a national tertiary referral institute between March 2014 and February 2024 for suspected platelet function disorders. Patients with coagulation factor deficiencies, von Willebrand disease, acquired bleeding causes, or recent transfusions were excluded. After informed consent, bleeding history, family history, and consanguinity were recorded. Complete blood counts, coagulation profiles, bleeding time (Ivy's method), and light transmission aggregometry (LTA) were performed. GT was diagnosed based on absent response to ADP, collagen, epinephrine, and arachidonic acid with preserved response to ristocetin. Data were analyzed using SPSS v24. The study was conducted in accordance with the Declaration of Helsinki. No funding was received for this study.

Results: Out of 2542 patients, 408 (16.0%) were diagnosed with platelet function disorders; Glanzmann Thrombasthenia (GT) comprised 327 (80.1%) cases, representing 13.3% of the total cohort. GT showed female predominance (65.8%) with median age at diagnosis of 9 years 2 months (range: 8 months–26 years) and median age of first bleeding at 4 years 3 months. Most patients were from Punjab (53%) and Khyber Pakhtunkhwa (39.4%). Consanguinity was noted in 85.3%, with positive family history in 28.1%. Common symptoms included bruising (85%), epistaxis (78.6%), and gum bleeding (56.3%), while haemarthrosis was rare. Anaemia, predominantly iron deficiency, was seen in 70.6%. All patients had normal platelet counts and morphology with prolonged bleeding time. Light transmission aggregometry confirmed classical GT patterns. Most received antifibrinolytics (88.9%); however, 16.8% received inappropriate FFP/cryoprecipitate, and only 0.6% received rFVIIa.

Summary/Conclusion: This study, the largest from Pakistan, highlights a significant burden of Glanzmann Thrombasthenia in a population with high consanguinity. Delayed diagnosis, reliance on inappropriate therapies, and limited availability of definitive diagnostics contribute to suboptimal care. Increased clinician awareness, use of targeted diagnostics such as LTA and flow cytometry, and avoidance of unnecessary transfusions are critical. Our findings emphasize the need for national guidelines, patient education, and strategic planning to improve early diagnosis and rational treatment, particularly in resource-constrained settings.

P113

T-MEM - COMPLEX IMUNOMODULATORY TREATMENT FOR IMMUNE THROMBOCYTOPENIA

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Background: The therapy of immune thrombocytopenic usually consists of a sequence of monotherapies as they were gradually put into practice. A combined regimen can reach a larger number of treatment responses and drug-free remissions.

Aims: These are the results of the pilot project of combined T-MEM therapy for corticoresistent patients. Therapy consists of a combination of TPO mimetics, mycophenolate mofetil (1000mg), cyclosporin (200mg) and low -dose rituximab (200mg/m2). In the case of remission, TPO therapy is terminated after 3 months and the remaining immunosuppression within 6 months. Number of CR,R or SD are noted in predefined time-points (M1,3,6,12,24). The main objective is to get the majority of patients in the sustained remission off-treatment (SROT) by month 6 or 12 after initiating the treatment.

Methods: Between 2022-2025, T-MEM was given 30 patients with follow-up-up 6-24 months. Of the 30 patients, 14 (46%) ITP patients had diagnosis for more than 1 year.

Results: In the remission of the disease it is 96%in the 1st month,90% in the 3rd month ,90% in the 6th month and 80% in the 12th month.Of these, (month 12) 73% is in CR, 23% in R and 4% are refractory. In 53% of patients, therapy can be completely terminated within the first year.

The administration was usually complicated only by mild side effects not requiring hospitalization. In the event of adverse reactions, the intensity of therapy was reduced.

Summary/Conclusion: Comprehensive immunomodulatory therapy may be the basis of ITP therapy with promising results.

The administration of such a complex cycle is still only experimental therapy, which still needs to be verified in a prospective study.

P115

INVESTIGATING A CRITICAL ROLE FOR PAR1 ON MEGAKARYOCYTE-LIKE CELLS IN THE EARLY MOUSE EMBRYO

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Background: Activation of human platelets by thrombin occurs through protease-activated receptor 1 (PAR1). However, early studies demonstrated that adult murine platelets do not respond to PAR1-specific agonist peptide, and platelets from PAR1-deficient mice respond normally to thrombin. Genetic knockout of *F2r* (encoding PAR1) in mice leads to partial embryonic lethality around E9.5, regardless of genetic background. Phenotypic *F2r -/-* embryos frequently exhibit bleeding into the pericardial space. Lethality can be rescued by overexpressing PAR1 under the control of the *Tek* promoter/enhancer. This result has been interpreted as indicative of a midgestation requirement for PAR1 in the endothelium, despite nearly uniform activity of similar constructs in primitive blood lineages.

Aims: The aim of this study is to understand the precise cellular mechanism for embryonic lethality in PAR1-deficient mice.

Methods: *Cdh5-T2A-Cre* (*Cdh5 Cre*) knock-in mice were generated in our lab. Mice developed by our lab harboring a Tango-based GPCR activation reporter knocked into the *F2r* locus (*F2r Tango*), as well as an Ai139-derived *tetO-GFP* allele were used to identify cells with active PAR1 signaling by immunostaining. The thrombin-cleavage deficient R41Q mutation was made via CRISPR/Cas9 microinjection in zygotes. *F2r fl/fl* mice were provided by Stephen Traynelis. *F2r -/-*, *Sox2-Cre*, and *F3 fl/fl* mice have been described previously.

Results: To determine if mice require PAR1 in endothelial cells, we used a knock-in *Cdh5-Cre* driver. In crosses of *Cdh5 Cre/Cre; F2r +/-* males to *F2r fl/fl* dams, we found *Cdh5 Cre/+; F2r fl/-* pups born at mendelian ratios. This result suggests that endothelial PAR1 activity is not necessary for embryonic development. Mining of published single-cell RNA-sequencing databases revealed very high levels of *F2r* transcript in megakaryocyte-like cells in midgestation mouse embryos. We found high and nearly uniform GFP expression in CD41+ and PF4+ blood cells in E9.5 *F2r Tango/+; tetO-GFP* +/0 embryos, indicative of PAR1 protein expression in primitive thrombopoietic cells. In control embryos at E9.5, PF4+ cells can be found closely opposed to the endocardium. However, *F2r R41Q/-* embryos exhibit hemopericardium with concentration of PF4+ cells in the pericardial space, phenocopying *F2r -/-* embryos. Similarly, epiblast tissue factor-deficient (*Sox2-Cre; F3 fl/-*) embryos exhibited collections of PF4+ cells in the pericardial space at E10.5 (lethal timepoint). Apart from the pericardial sac, no other potential spaces appear to fill with PF4+ cells, indicating that the developing heart is uniquely susceptible to hemorrhage in coagulation cascade mutants.

Summary/Conclusion: The present study suggests that endothelial PAR1 is unlikely to be required for embryo survival, and has uncovered expression of PAR1 in thrombopoietic cells in the mouse embryo. PF4+ cells are frequently found apposed to endocardium at E9.5, but extravasate into the pericardial space in *F2r* and *F3* mutants. While existing data suggests that platelets are dispensable for development, our results indicate that a requirement for PAR1 in a developmental megakaryocyte-like lineage requires further investigation.

P118

CHARACTERIZATION OF PLATELET FUNCTIONS IN A PATIENT WITH ARHGEF1 DEFICIENCY

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Background: ARHGEF1 (Rho Guanine Nucleotide Exchange Factor 1), primarily expressed in hematopoietic cells, is a specific activator of RhoA, a GTPase involved in cytoskeletal remodeling. Its role in human platelet function remains unclear. In an ARHGEF1 knockout (ARHGEF1-/-) mouse model, prolonged bleeding time and severe platelet activation defect have been observed.

Aims: The aim of this study is to assess the role of ARHGEF1 in platelet function in a 36-year-old patient carrying two compound heterozygous variants in the ARHGEF1 gene (R300X and Glu557Lysfs34Ter), which are associated with a primary immunodeficiency.

Methods: *Platelet count and functions (aggregation using light transmission aggregation test, glycoprotein exploration by flow cytometry (FC), granular study (mepacrine test, expression of CD63 and P-selectin after activation with TRAP 50µM and ADP 10µM in FC, serotonin and PAI-1 intraplatelet assays, nucleotide quantification), spreading on a fibrinogen matrix after activation with ADP 10µM, clot retraction), and platelet content of ARHGEF1 by Western blot were evaluated. Thrombin generation in the presence of 1 pM tissue factor was measured on platelet-poor and platelet-rich plasma. Platelet, erythrocyte, granulocyte, and monocyte coagulating microvesicles were quantified by Annexin-V labeling by FC. The experiments were conducted in parallel on platelets from two healthy controls.*

Results: The patient did not exhibit thrombocytopenia (platelet count at 279 x10^9/L, with no macroplatelets). Western blot showed no ARHGEF1 expression in the patient's platelets, unlike in control platelets. Platelet tests, including response to different agonists, glycoprotein analysis, granule studies, spreading, and clot retraction, were normal. Thrombin generation was also normal. The analysis of circulating microvesicles (Annexin V+) showed no differences in number or origin, and platelet microvesicle production after activation with thrombin and collagen was normal. Importantly, the patient did not experience any spontaneous or provoked bleeding.

Summary/Conclusion: Platelet functions are not affected by the absence of ARGHGEF1. Additional investigations are underway to understand the regulatory/compensatory pathways involved in platelet RhoA activation in the absence of ARGHGEF1.

P119

THE INFLUENCE OF DRUGS WITH ACETYL-SALICYLIC ACID ON PLATELET AGGREGATION TESTED WITH ADENOSINE DIPHOSPHATE AS AN AGONIST

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Background: Light transmission aggregometry (LTA) is the gold standard in testing the function of platelet aggregation. Arachidonic acid (AA) as an agonist is recommended for monitoring the effect of antiplatelet therapy with acetyl-salicylic acid (ASA) drugs , while the agonist adenosine diphosphate (ADP) is used to monitor the effect of thienopyridine therapy.

Aims: The aim of the study was to evaluate the effect of drugs with ASA on platelet aggregation with ADP as an agonist, with PAP 8E aggregometer and reagents from BIO/DATA CORPORATION .

Methods: The study included 78 patients of both sexes (23 male and 55 female) who were divided into two groups.The first group (G1) consisted of 38 patients who were on antiplatelet therapy with ASA drugs. The effectiveness of the therapy was confirmed by testing platelet aggregation with arachidonic acid as the agonist. In the same group of patients, platelet aggregability was also tested using ADP as an agonist. The second group (G2) was the control group. G2 consisted of patients who were not on antiplatelet therapy. Blood samples were collected into tubes with citrate as an anticoagulant in the laboratory of the University Clinical Center of Vojvodina (UKCV). From citrated blood, platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared for testing.

All tests were performed using the PAP 8E aggregometer, which operates based on the principle of LTA. The agonist concentrations used followed the manufacturer's recommendations (BIO/DATA CORPORATION). The concentration applied were: ADP 20 µM and AA 500 µg/ml.

Results: The one-way analysis of variance (ANOVA) was used to determine the difference between groups in variables.The statistical analysis was performed using the SPSS 20 (SPSS Inc., Chicago, IL, US)

The following results were obtained on the basis of the tests performed.

Average values of Group 1 (G1): ADP: 66.08± 15.10%; AA: 5.87± 12.56%.

Average values of Group 2 (G2): ADP: 74.76± 18.97%; AA 71.56± 8.05%.

The results indicated a statistically significant difference($p<0.05$) in platelet aggregation with ADP between the two groups. As expected, a statistically highly significant difference($p<0.001$) was observed in platelet aggregability with AA between the groups.

Summary/Conclusion: Based on the results of this study, it can be concluded that ASA therapy affects platelet aggregation when ADP is used as the agonist.

P120

STICKY PLATELET SYNDROME AND PREGNANCY COMPLICATIONS - ONE CENTER RESULTS

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Background: Pregnancy complications (such as recurrent miscarriage, preeclampsia, intrauterine growth restriction, placental abruption, etc.) are a very frustrating and complex area of reproductive medicine. The occurrence of pregnancy complications is reported to be 10-12% or even 15-25% among pregnant women. Sticky platelet syndrome (SPS) is an inherited thrombophilia characterized by platelet hyperaggregation following stimulation with precisely defined subthreshold (subphysiological) concentrations of adenosine diphosphate (ADP) and/or adrenaline (epinephrine).

Aims: 1. Identify a group of women with complications in pregnancy and possible causal connections with diagnosed SPS. 2. Try to optimize the management of care and treatment procedures during pregnancy for these women. 3. Evaluate the ratio of effectiveness and safety of the chosen procedure.

Methods: A 10-year retrospective analysis of a cohort of 418 women who were examined at the hematology outpatient clinic of the University Hospital Martin (UHM) for pregnancy complications. Within this study, SPS was diagnosed in 142 women, of whom 84 women with SPS were assessed for thromboprophylaxis during subsequent pregnancies. Pregnancy complications in this cohort included repeated miscarriages in 81 women, stillbirth in 4 women, infertility (failed IVF) in 10 women, preeclampsia in 5 women, and VTE (venous thromboembolism) in 4 women.

The exclusion criterion in the study was a different procoagulant disorder than SPS, as well as other possible causes of pregnancy complications. Out of 84 women with SPS, only 61 women came to the clinic/pregnant, in which 72 pregnancies were retrospectively assessed.

Results: Out of 72 pregnancies, 60 (83%) pregnancies ended at term (after 38 weeks of pregnancy). Preterm birth (32nd-37th week of pregnancy) was recorded in 9 women (13%). Only in 3 (4%) pregnancies did a stillbirth occur. Mild bleeding and intrauterine hematoma occurred in 5 women (7%). The highest number of births at term (37; 51%) occurred in the group of women who were on thromboprophylaxis with ASA (acetylsalicylic acid) from the beginning of pregnancy (up to the 36th week) and on low molecular weight heparin (LMWH) s.c. (miniheparinization) from the 36th week of pregnancy. Fewer births on time (18; 25%) were in the group of women who were on a combination of thromboprophylaxis with ASA (up to the 32nd-36th week) and on LMWH s.c. (miniheparinization/adjusted dose) from the beginning of pregnancy to delivery. The least number of births on time (5; 8%) was in the group of women who were on a combination of thromboprophylaxis with ASA (up to 32nd-36th week) and on LMWH s.c. (miniheparinization/adjusted dose) in the 10th-25th week of pregnancy.

Summary/Conclusion: A retrospective 10-year analysis of Clinic of Hematology and Transfusiology of JFM CU and UHM confirmed a frequent causal relationship between hyperaggregability of platelets and gestational complications. Out of 418 women with gestational complications 34% were diagnozed as SPS. The results of the analysis demonstrated a clear benefit of ASA - 83% pregnancy rate at the due date. No episode of severe bleeding was recorded.

P121

EVALUATION OF PRETEST CLINICAL SCORE (4TS) FOR DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA - A RETROSPECTIVE STUDY OF A TERTIARY HOSPITAL

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Background: Heparin induced thrombocytopenia (HIT) is a pro-thrombotic and potencially life-threatening complication of heparin therapy. HIT is characterized by a drop in platelet count, usually beginning 5-14 days after heparin exposure. HIT diagnosis relies on clinical suspicion determined by 4Ts score (degree of thrombocytopenia; timing of platelet decline after heparin administration; presence of thrombosis or other HIT sequelae; and the probability of other causes of thrombocytopenia) and immunoassays through testing for anti-PF4/heparin antibodies (HITAb). Current guidelines recommend use of the 4Ts score before ordering laboratory tests as a measure of pretest probability.

Aims: The aim of our study was to evaluate the utilization of the 4Ts score before ordering HITAb testing for HIT diagnosis at São João University Hospital.

Methods: A retrospective observational study was performed on patients admitted to our hospital between May 1, 2024 and April 30, 2025 that had HITAb test ordered. Laboratory tests included commercial lateral flow or chemiluminescence immunoassay kits. Given the retrospective nature of our study, confirmatory testing was not performed on all patients, and therefore all patients with a positive HITAb test were assumed to be truly positive.

After calculating the 4Ts score retrospectively, we calculated the proportion of HITAb tests which were not indicated due to a low risk 4Ts score. Scores of 0-3, 4-5 and 6-8 were classified as low, intermediate and high risk, respectively.

Results: A total of 90 patients that underwent HITAb testing were analysed (52 male and 38 female). Mean age was 63,8 ± 19,4 years. 50% of the patients were at the intensive care unit (ICU), 26,7% and 23,3% were at medical and surgical departments respectively.

Pre-test probability using the 4T score revealed 39 patients (43,3%) in the low risk category, 36 (40,0%) in the intermediate risk category and 15 (16,7%) in the high risk category. Analysis of testing trends showed that only 2 of the 39 patients (14,3%) with low 4Ts score tested positive for HITAb, while 11 of the 15 patients (78,6%) with high risk score tested positive for HITAb. 64,3% of the patients who had a positive HITAb test were receiving treatment with unfractionated heparin (UFH).

Summary/Conclusion: HIT is one of the most common immune-mediated adverse drug reactions. However, several clinical scenarios may cause thrombocytopenia in a patient receiving heparin, namely sepsis or liver disease, commonly seen in critical patients at ICU. Our study showed that in our hospital, the 4Ts score is not always accounted for when managing a patient with suspected HIT. This seems to result in unnecessary laboratory testing causing great financial burden. The use of 4Ts score for assessing the pretest probability of HIT has the potential to simplify and improve the process of identifying patients at different risk of HIT. In particular, a low risk 4Ts score is suitable for ruling out HIT in most clinical settings. We hope to improve practice trends in our institution through a multidisciplinary approach.

Posters – Vessel Wall

P124

PARTICULARITIES OF ARTERIAL LIMB AMPUTEES; A CASE REPORT OF 200 PATIENTS

B. Abdellatif*

Background: Amputation in arterial disease: is considered to be a life-saving operation, which has a functional purpose, RIPPSTEIN says that 'it is reconstructive surgery creating a new functional organ'. Obliterative arteriopathy of the lower limbs is a widespread condition. In Algeria, the prevalence of stage 2 intermittent claudication is 1 to 2% of the general population.

Aims: describe the particularities of physical medicine and rehabilitation care for limb amputees of vascular origin, as well as their orthopaedic devices

Methods: We report on a series of 200 patients with lower limb amputations, fitted in the fitting department of Staoueli Hospital - Algiers. We collected clinical particularities (scarring, associated lesions, vascular status of the limb contralateral to the amputation, functional capacities, hazards to standing and walking, as well as the type of orthopaedic device, etc.).

Results: The average age of our patients is 62 years, the level of amputation (leg 70%, and thigh 30%), the average length of hospitalisation during surgery is 03 weeks, and in 90% of cases the operation is performed cold, followed by re-education to combat post-operative oedema, vicious attitudes, the main objective is progressive verticalization which must be achieved within a few days. The average duration of rehabilitation is 14 MONTHS (3 months - 2 years). We fitted these patients with appliances, whatever their general capabilities. 20% of patients did not progress beyond the temporary prosthesis stage, due to their general condition and the progress of their pathology,
Leg amputees were fitted with contact-type prostheses, and thigh amputees were fitted with contact-type prostheses with a suspension belt to make walking safer.

Summary/Conclusion: The average length of time for treatment and fitting is longer than for patients amputated for other reasons (trauma, tumour....), and the progressive nature of vascular pathologies often leads to repeat amputation operations, each time moving up a level (30% of the patients in our series were returned for surgery). The association of arteriopathy of the limbs with coronary pathologies delays and limits the functional possibilities of amputee patients.
Prevention through the application of healthy lifestyle rules remains essential, as does smoking cessation, regular physical activity and screening by vascular Doppler of any intermittent claudication in order to avoid dreadful complications such as limb amputation.

P125

KANK3 IS A SHEAR STRESS REGULATED ENDOTHELIAL PROTEIN WITH A ROLE IN CELL MIGRATION AND TISSUE FACTOR REGULATION

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Background: The endothelium is the innermost layer of all blood vessels. Endothelial cells (EC) play a central role in the regulation of vascular processes, such as coagulation, inflammation, and angiogenesis. Proteins with endothelial-specific expression often play pivotal roles in specific vascular functions. In our previously published bioinformatic-based analysis of bulk RNAseq, we predicted that *KANK3,* which encodes an uncharacterised protein, had body wide enriched expression highly specific to human EC, a unique feature among the KANK protein family. The KANK family consist of four members with strong conservation across the evolutionary tree. Indeed, they share a unique structure, consisting of a variable number of coiled-coil motifs in the central regions, five ankyrin repeats in the C-terminal region and a talin-binding KN-motif domain at the N-terminus. KANK1 and KANK2 are widely expressed and well characterized, particularly for their roles in cytoskeletal interactions and focal adhesion dynamics. In contrast, KANK3 and KANK4 remain less thoroughly investigated. KANK3 has no reported function in a vascular system in vertebrates.

Aims: To investigate the role of highly specific KANK3 protein in EC function.

Methods: Human umbilical vein EC were transfected with siRNAs targeting KANK3 or a scrambled control, and then cultivated under static or shear stress conditions. Cellular localization of KANK3 was evaluated by immunohistochemistry imaging and KANK3 expression by qPCR and Western blot. Cell proliferation and migration was measured in an *in vitro* scratch assay. Tissue factor expression level and activity were investigated by qPCR, FACS and thrombin generation assay.

Results: Analyse of *KANK3* transcript expression in both bulk and single cell RNAseq, and tissue cell line data bases shows that KANK3 is enriched in human EC. Cultivation under flow reveals that under shear stress KANK3 expression is elevated at RNA and protein level in EC, accompanied by a cellular re-localization. In scratch assay, *KANK3* siRNA EC had a faster gap closure rate, in both serum-starved and non-starved conditions, compared to controls. Tissue factor expression is increased at both the transcript and protein level at the cell surface in KANK3 depleted EC. Thrombin formation is enhanced with KANK3 depleted EC and suppressed by tissue factor blocking antibody.

Summary/Conclusion: In the present study, we verify that KANK3 is an EC-enriched protein. KANK3 expression is strongly induced in response to shear stress exposure with a corresponding colocalization to focal adhesions. In a wound healing model, depletion of KANK3 increases EC migratory capacity, but does not increase proliferative capacity. Therefore, KANK3 seems to have a protective role in EC against shear stress and increases adhesion to the extracellular matrix and basal membrane, allowing EC to withstand mechanical forces generated by bloodflow on the endothelial surface of the blood vessel. Furthermore, the increase in the expression of the pro-coagulant protein tissue factor on the cell surface in KANK3 depleted EC, suggests that KANK3 could have further regulatory roles in the coagulation pathway and be implicated in EC haemostasis function, beyond those associated with cytoskeletal modification and motility.

P128

AN ATLAS OF ENDOTHELIAL TRANSCRIPTOMIC ENRICHMENT AND SPECIFICITY ACROSS 20 HUMAN TISSUES

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Background: Endothelial cells (EC) line blood vessels and can regulate haemostasis, inflammation, coagulation and blood pressure. Genes with EC specific expression across vascular beds typically encode for proteins that have EC specific or critical functions, conversely genes with heterogenic EC specific expression in limited number of tissues may have an EC function limited to a specific EC type.

Aims: To produce a comprehensive atlas of human endothelial and immune cell enriched genes across the wide range of tissues in the human body, showcasing both tissue specific EC enriched genes and pan-tissue EC enriched genes using global, body-wide transcriptomics.

Methods: Using a bioinformatics-based analysis method we identified endothelial cell (EC) enriched genes across 20 separate tissues, using existing bulk tissue RNAseq data from the Genotype-Tissue Expression (GTEx) project, containing transcriptomic data from thousands of individuals.

For each analysed tissue, a panel of 3 highly cell type specific *´virtual reference transcripts´* (Ref.T) are selected as proxy markers for each constituent cell type. Other cell type-enriched genes were then identified based on their similarity with the expression profile of the Ref.T across the tissue sample set. Genes were classified as EC enriched if they had a correlation > 0.5 with the Ref.T, adjusted P-value <0.001, and a differential >0.15 to any other celltype.

Results: We identified 65 genes enriched in EC in at least half of the 20 tissues analysed, that we subsequently classified as the “core” EC transcriptome. Approximately 4000 genes were EC enriched in fewer than half of tissues analysed, revealing the high levels of EC heterogeneity between tissue types.

While many of the core EC enriched genes are well known, we also identified several novel EC enriched genes which are unstudied within endothelial cells. For example SHANK3 is well described as a neuronal protein, however we found that it was also EC enriched in 14/20 tissues analysed; a finding supported by tissue immunohistochemistry staining. KANK3 was also identified as an EC specific member of a larger family of proteins, the others of which have a known role in cell structural organisation and migration.

Summary/Conclusion: Here we present a tissue-centric, cell type gene enrichment atlas, generated from the analysis of thousands of biological replicates. We describe a widely applicable method to determine cell type specific transcriptome profiles both in individual tissues and throughout the human body. We identify novel endothelial biomarkers, and highlight candidates for functional studies to increase understanding of the endothelium in health and disease. Our study provides the only cell type gene enrichment atlas generated independently of scRNAseq, representing a new addition to our existing toolbox of resources for the understanding of gene expression across human tissues. Our transcriptomic atlas will be publicly available for all to use at www.proteinatlas.org/humanproteome/tissue+cell+type and identify celltype enriched genes within their tissue and celltype of interest

P129

SUPERFICIAL THROMBOPHLEBITIS OF THE THORACOEPIGASTRIC VEIN INDUCED BY TIGHT CLOTHING: A CASE OF MONDOR'S DISEASE

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Background: Mondor's disease (MD) is a rare and typically benign, self-limiting superficial thrombophlebitis affecting subcutaneous veins of the thoracic region, most commonly the thoracoepigastric vein. It is often underdiagnosed or misdiagnosed due to its subtle clinical presentation and self-resolving nature. Though idiopathic in many cases, MD has been associated with various underlying causes such as trauma, surgery, tight garments, malignancy and hormonal factors.

Aims: To describe a case of Mondor's disease in a middle-aged woman with no known thromboembolic or oncological history, likely caused by prolonged mechanical compression from tight-fitting undergarments, and to highlight the importance of clinical recognition to avoid unnecessary diagnostic procedures.

Methods: This case study presents a 43-year-old female physician who developed chest wall pain and a palpable subcutaneous cord-like lesion extending from beneath the right breast to the upper abdomen. The patient associated symptom onset with wearing a tight bra and high summer temperatures. Initial symptoms included burning pain and discomfort, especially in recumbent and sedentary positions. Physical examination revealed a palpable, tender cord consistent with thrombophlebitis of the thoracoepigastric vein. A clinical diagnosis of MD was made based on characteristic findings and reviewed literature. Symptomatic therapy included topical NSAIDs and heparin ointment, while the patient discontinued bra usage and declined immediate imaging or invasive diagnostics.

Results: Symptoms initially improved within one week but the palpable lesion remained for several weeks. Recurrence of burning pain occurred during the next menstrual cycle, prompting Doppler ultrasound imaging which confirmed a non-compressible tubular structure with partial recanalization, consistent with thrombosed thoracoepigastric vein. Hemostasis labs, including D-dimer, were within normal range. Follow-up ultrasound and MRI performed three months later showed resolution of the thrombosis. The case resolved fully without complications, highlighting the typical self-limiting course of MD.

Summary/Conclusion: Mondor's disease should be considered in the differential diagnosis of chest wall pain, particularly in women with localized tenderness and subcutaneous cord-like lesions. Awareness of this condition can prevent unnecessary imaging, biopsies, or oncological evaluation. This case illustrates a likely underreported mechanical etiology—tight clothing and positional pressure—as a trigger. Clinicians should rely on history and physical examination for diagnosis, and conservative management is typically sufficient. Ultrasound may assist in confirming diagnosis and monitoring resolution, especially when symptoms persist or recur. Raising awareness through such case reports may improve recognition and reduce unnecessary healthcare utilization.

P130

NILOTINIB POTENTIATES TNF-A INDUCED ENDOTHELIAL CELL- DEPENDENT THROMBIN GENERATION

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Background: BCR-ABL tyrosine kinase inhibitors (TKIs) represent an effective treatment for chronic myelogenous leukemia (CML). However, clinical studies have shown that the TKIs may be associated with the development of thrombotic events. Endothelial cells play a crucial role in coagulation, and their abnormal phenotype may influence the occurrence of thrombotic events. One of the key mediators of endothelial dysfunction is tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine known to induce a procoagulant and proinflammatory state in endothelial cells. Recent studies have highlighted the involvement of non-receptor tyrosine kinases, such as proto-oncogene tyrosine-protein kinase Src **(**c-Src) in TNF-α signaling. TKIs may affect c-Src and thereby TNF-α signaling in endothelial cells.

Aims: We investigated whether the BCR-ABL TKIs, dasatinib, nilotinib and ponatinib enhances the effect of TNF-α on thrombin generation measured in the presence of endothelial cells.

Methods: Cultured human coronary artery endothelial cells (HCAEC) were pretreated with different concentrations of TNF-α (10, 1, 0.1 ng/ml) for 4 hours. Threreafter, cells were treated with clinically relevant concentrations of dasatinib (100 nM), nilotinib (1 μM) or ponatinib (150 nM) for an additional four hours. As a control group, we used cells that were treated only with TNF-α. Thrombin-generating potential of treated HCAECs was assessed in control plasma in the presence of 4 μM phospholipid by fluorimeter. Tissue factor (F3), thrombomodulin (THBD) and VCAM1 mRNAs were quantified by qPCR.

Results: In the thrombin generation assay (TGA), we observed significant shortening in the time parameters, lagtime, (p=0.0013) time to peak p=0.0010) and start tail p=0.0243) already at 1 ng/ml of TNF-α. Furthermore, TNF-α treatment caused significant increase in the quantitative parameter, such as the endogenous thrombin potential (ETP) at 10 ng/ml concentration of TNF-α (p=0.0434). Dasatinib and ponatinib treatment did not influence the effect of TNF-α in TGA. However, nilotinib significantly increased the ETP (p=0.0408) at 10 ng/ml of TNF-α. These changes in TGA were associated by a 2.6 fold increase in F3 and a 1.8 fold increase in VCAM1 mRNA at a TNF concentration of 1 ng/ml.

Summary/Conclusion: Nilotinib enhanced the endothelial cell-dependent thrombin generation potential of TNF-α, which may contribute to the development of nilotinib-induced thrombotic processes in inflammatory conditions.

P131

INVESTIGATING HOW GLOBAL DNA HYPOMETHYLATION IMPACTS ENDOTHELIUM DRIVEN THROMBOSIS

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Background: DNA hypomethylation is increasingly recognized as a modulator of thrombosis and haemostasis, key processes involved in cardiovascular disease (CVD) pathogenesis. Thrombosis, driven by dysregulated coagulation and endothelial dysfunction, underlies acute events including myocardial infarction and stroke. Multiple Epigenome-Wide Association Studies (EWAS) have identified that the epigenetic regulation of the putative transcription factor ZFHX3 is significantly associated with a range of cardiovascular diseases associated with thrombosis. DNA hypomethylation of the EWAS site, cg07786668, is significantly associated with an increased risk of ischemic stroke and is located proximally in the first intron of the ZFHX3-203 transcript.

Aims: This study set out to investigate how 5-azacytidine-induced global DNA hypomethylation alters thrombotic endothelial phenotypes in human coronary artery endothelial cells (HCAECs) and aims to define the impact of methylation on the expression of ZFHX3-203.

Methods: HCAECs were treated with 5-Azacytidine and RT-qPCR, western blotting and flow cytometry were carried out to determine changes in endothelial cell thrombotic phenotypes. The functional effects of global DNA hypomethylation was determined using endothelial integrity and thrombus formation assays.

Results: HCAECs treated with 5-Azacytidine show no alterations in cell viability but do demonstrate a reduction in cell proliferation. 5-Azacytidine treatment was also observed to lead to a significant increase in expression of total ZFHX3 and the ZFHX3-203 transcript.

Summary/Conclusion: This will allow the functional consequences of DNA hypomethylation to be determined contributing to the understanding of its impact on endothelial driven thrombosis.

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