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ABSTRACT BOOK

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Poster Session

Science, Fast and Furious I

SFF01-01

A SIGNIFICANT NUMBER OF CASES OF ANTITHROMBIN DEFICIENCY DIAGNOSED WITHOUT SERPINC1 DEFECTS ARE DUE TO CONGENITAL DISORDERS OF GLYCOSYLATION

M. Llamas López [1,*], C. Bravo-Pérez [1], A. Miñano [1], E. Wypasek [2], B. Signes-Fort [3], E. García [4], B. De la Morena-Barrio [1], P. Garrido-Rodríguez [1], V. Vicente [1], A. Undas [2], M. L. Lozano [1], J. Corral [1], M. E. De la Morena-Barrio [1]

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Background: Antithrombin (AT) is a glycoprotein with a key anticoagulant function. Thus, congenital AT deficiency (ATD), which is considered a monogenic dominant disorder caused by defects in the *SERPINC1* gene, significantly increases the risk of thrombosis. However, up to 25% of ATD cases do not have *SERPINC1* defects. N-glycosylation plays a critical role in AT function which explains why patients with congenital disorders of glycosylation (CDG), a very rare and severe multisystem disorder, usually diagnosed in infancy, with inefficient N-glycosylation, also had ATD.

Aims: To identify and characterize CDGs from the analysis of patients with ATD, especially in those without SERPINC1 defects.

Methods: The study included 409 unrelated patients with suspected ATD, recruited over 27 years. *SERPINC1* defects were evaluated by sequencing and MLPA. Hypoglycosylation of AT was evaluated by Western blot under 2 conditions (native and SDS) and validated with PNGaseF treatment. Hypoglycosylated forms of other proteins (*FXI*, α1-antitrypsin, and transferrin -Tf-) were assessed by western blot and/or HPLC. NGS of a panel of 74 genes involved in N-glycosylation, 25 associated with CDGs, was performed in cases with hypoglycosylation.

Results: *SERPINC1* defects were identified only in 324 cases. Increased hypoglycosylated forms of AT and other hepaticproteins were observed in 33 of the 85 patients without *SERPINC1* defects (38.8%). Six cases had classical CDGs as biallelic pathogenic mutations were identified in genes involved in this disorder: *PMM2*; *MPI*; *ALG12*, *ALG13* and *GALT*. These patients have little or no psychomotor delay, ATD (anti-FXa: 55.7%) and high levels of hypoglycosylated Tf (AsialoTf: 9.5%; DisialoTf: 18.8%). Mannose overload in the MPI-CDG patient improved AT levels, allowing withdrawal of anticoagulation and resolution of other clinical signs. Lactose removal in GALT-CDG patients also brought AT to normal ranges. Interestingly, a 58-year-old patient with multiple dialysis-related thromboses, low anti-FXa activity (60%) and high levels of hypoglycosylated Tf (AsialoTf: 6.2%; DisialoTf: 16.4%) carried biallelic rare missense mutations in HK3 (p.Glu635Lys and p.Gly281Arg). In addition, 19 cases had only one pathogenic mutation in a CDG-realated gene, but they also reported mild to moderate alcohol consumption. These patients had higher AT activity (74.8%) and lower levels of hypoglycosylated Tf (AsialoTf: 2.9%; DisialoTf: 8.1%). Remarkably, in two of these cases, cessation of alcohol intake restored AT to normal levels and reduced hypoglycosylation. Finally, the remaining 7 cases had very mild CDG parameters (Anti-FXa: 75.2%; AsialoTf: 1.0%; DisialoTf: 4.1%), no pathogenic mutation was observed, and only alcohol intake was reported.

Summary/Conclusion: The results of one of the largest cohorts of patients with ATD show that CDG underlies up to 8% of all cases with ATD, 38.8% of cases without *SERPINC1* defects. Classical CDGs with atypical clinical signs can be diagnosed, allowing for appropriate treatment and clinical management. In addition, our study identified a potential new CDG: HK3-CDG, with ATD and risk of thrombosis but no psychomotor delay. Finally, we demonstrated the additive effects on N-glycosylation and ATD of two defects affecting N-glycosylation: one acquired (alcohol intake) and one genetic (mutation in a gene involved in the N-glycosylation pathway).

SFF01-02

TISSUE FACTOR PATHWAY INHIBITOR ATTENUATES CALCIUM-INDUCED VASCULAR SMOOTH MUSCLE CELL CALCIFICATION

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Background: Tissue factor pathway inhibitor (TFPI) acts as a constitutive inhibitor of the extrinsic coagulation pathway by inhibition of the tissue factor/FVIIa/FXa complex. Next to that, TFPI also exhibits a protective role in thrombo-inflammation and vascular remodelling. The role of TFPI in vascular calcification has not been studied so far.

Aims: The aim of this project was to identify the role of TFPI in vascular remodelling and vascular calcification.

Methods: Here, we used CRISPR-Cas9 technology to generate human induced pluripotent stem cells (iPSC) deficient in TFPI. Next, iPSC were differentiated into vascular smooth muscle cells (iVSMC) and transcriptomic differences in wild-type and TFPI-KO cells were elucidated using bulk RNAseq. For further analyses, iVSMC were driven into contractile and synthetic phenotypes to study the expression of VSMC marker, inflammatory, and calcification-related genes. Ultimately, cells were treated with calcification-inducing medium to assess the calcifying potential in wild-type vs. TFPI-KO cells.

Results: CRISPR/Cas9 modification resulted in cells synthesizing a partly non-functional truncated (Δ4 AA in K2 domain) TFPI (TFPI-K2-KO). *In silico* decomposition energy studies revealed that the shortened protein lacks binding to FXa. Functional analysis showed that the other functional domains of TFPI were still intact. RNAseq in wild-type vs. TFPI-K2-KO iVSMC resulted in 815 differentially expressed genes including modulators of VSMC migration, differentiation, and calcium metabolism. Comparing gene expression in phenotypically switched TFPI-K2-KO and wild-type, a significant decrease in the contractile VSMC markers αSMA and CNN1 was observed. While αSMA expression was reduced in both contractile and synthetic iVSMC, a decline of CNN1 was seen in the contractile phenotype only. Furthermore, expression of the inflammatory chemokine MCP-1 was considerably reduced in contractile TFPI-K2-KO iVSMC. There was no differential gene expression seen for the synthetic marker S100A4, the inflammatory cytokine IL-6, and the osteogenic marker BMP4. Ultimately, increasing calcium concentrations resulted in significantly increased calcification of TFPI-K2-KO iVSMC compared to wild-type iVSMC. Extracellular addition of recombinant TFPI reversed this effect.

Summary/Conclusion: We show that functional TFPI plays a protective role in the development of vascular calcification. Calcium regulation is highly dependent on the VSMC phenotype. Enhanced calcification in TFPI-K2-KO iVSMC might therefore derive from a FXa-dependent phenotypic switch into a less contractile state, as suggested by the downregulation of contractile markers in TFPI-K2-KO cells. Future studies including proteomics and pathway analyses will elucidate mechanisms involved in this process.

SFF01-03

DECREASING MORTALITY RISK IN CANCER-RELATED VENOUS THROMBOEMBOLISM OVER THE LAST THREE DECADES

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Background: Cancer is a well-established and major risk factor for venous thromboembolism (VTE). The development of VTE in cancer patients is associated with more advanced malignancies and decreased survival. The diagnostic and therapeutic strategies of both cancer and VTE have substantially improved during the last three decades. Whether the risk of mortality after cancer-related VTE has decreased during the same time period remains unclear.

Aims: To investigate the risk of mortality in cancer-related VTE over the last three decades in a population-based cohort.

Methods: Participants (n= 35,476) without cancer and VTE at inclusion in Tromsø 4-7 surveys (1994-2016) were included and followed through 2020. All first lifetime cancer and VTE events occurring during follow-up among participants were recorded. A VTE event was regarded as cancer-related when occurring within one year before or up to two years after the date of a cancer diagnosis. Participants neither exposed to cancer nor VTE (disease-free) served as the reference category. Cox regression models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for all-cause mortality, with cancer and VTE modelled as time-varying exposures. Analyses were conducted according to predefined time periods. i.e., 1994-2002, 2003-2011 and 2012-2020. Ethical approval and written informed consent from all participants were obtained.

Results: During a median follow-up of 19.4 years, 5,675 (16.0%) participants developed cancer, 1,007 (2.8%) developed VTE, and 208 (0.6%) experienced cancer-related VTE. The risk of mortality in cancer-related VTE decreased over the decades in age- and sex-adjusted analyses. The HRs of mortality in cancer-related VTE versus disease-free decreased from 41.3 (95% CI 28.7-59.4) in 1994-2002 to 23.7 (95% CI 18.4-30.7) in 2003-2011, and further to 14.5 (95% CI 10.9-19.4) in 2012-2020. Additional adjustments for body mass index, smoking, hypertension, diabetes, and arterial cardiovascular diseases had minor impact on risk estimates. Analyzes restricted to participants with non-metastatic cancer-related VTE also yielded a substantial decreasing trend in mortality.

Summary/Conclusion: Our results indicate a decrease in the risk of mortality in cancer-related VTE over the last three decades, a period in which substantial advances have also occurred in the management of cancer and VTE.

SFF01-04

LLAMA-DERIVED SINGLE-DOMAIN ANTIBODIES BLOCKING TISSUE FACTOR PATHWAY INHIBITOR AND INCREASING THROMBIN GENERATION IN FVIII- AND FIX-DEFICIENT PLASMA

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Background: Tissue factor pathway inhibitor (TFPI) is a promising therapeutic target in hemophilia. Concizumab, a blocking monoclonal antibody binding with very high affinity (25 pM) to the Kunitz-2 (K2) domain of TFPI, is currently being evaluated in clinical trials. However, its pharmacokinetics/pharmacodynamics (PK/PD) is complex, which results in an inconvenient daily mode of administration in patients.

Aims: To generate novel llama-derived single-domain antibodies (sdAbs) directed against TFPIα that block its anticoagulant activities and increase thrombin generation in the plasma of hemophilic patients.

Methods: A large synthetic library of llama-derived sdAbs was generated and selected by phage-display with three rounds of biopanning on *E. coli*-derived recombinant human TFPIα (rhTFPIα) immobilized onto ELISA wells. The sdAbs specifically binding to rhTFPIα in ELISA were produced in the periplasm of *E. coli* WK6 cells, and purified sdAbs were functionally screened in a FXa inhibition assay by rhTFPIα in a purified system. Blocking sdAbs were further characterized by testing their binding to a form of rhTFPIα expressed in FreeStyle 293-F cells (293F-rhTFPIα). Their ability to increase thrombin generation, as compared to concizumab, was evaluated by calibrated automated thrombography (CAT) in commercial pooled plasmas of patients with severe (< 1%) deficiency in FVIII and FIX. For localizing the epitopes of our sdAbs, we tested their binding to maltose-binding protein (MBP)-fusion proteins with the C-terminal part or the individual Kunitz (K1, K2 and K3) domains of TFPIα.

Results: The periplasmic extracts of 188 clones were screened and 21 of them were found to specifically bind to rhTFPIα. Two sdAbs (TFPI-26E5 and TFPI-26E8) abolished the ability of rhTFPIα to inhibit the amidolytic activity of FXa. Whereas TFPI-26E8 bound to none of the MBP fusion proteins, TFPI-26E5 specifically bound to MBP-K1 and not to MBP. This suggested that FXa inhibition by TFPIα could be impaired by blocking its K1 domain. As TFPI-26E5 and TFPI-26E8 were screened on *E. coli*-derived rhTFPIα, their binding to a more physiological form of rhTFPIα (293F-rhTFPIα) was evaluated. In ELISA, TFPI-26E5 and TFPI-26E8 bound to immobilized 293F-rhTFPIα with $K_{d\ app}$ values of 8.0±1.5 and 12.3±2.3 nM, respectively. Interestingly, TFPI-26E5 and TFPI-26E8 were found to increase thrombin generation in both FVIII- and FIX-deficient plasmas. A more detailed analysis in FIX-deficient plasma indicated that TFPI-26E5, TFPI-26E8 and concizumab dose-dependently increased thrombin generation. For TFPI-26E5, TFPI-26E8 and concizumab : maximal ETP values of 900.1±31.3, 811.6±27.1 and 1007±15.7 nM.min^{-1} were reached with EC50 values of 268±17, 217±18 and 1.6±0.2 nM, respectively ; maximal peak values of 67.4.1±1.9, 46.4±1.3 and 74.8±1.1 nM were reached with EC50 values of 295±13, 224±12 and 2.1±0.1 nM, respectively ; and maximal velocity indexes of 13.4.1±0.5, 6.8±0.3 and 11.0±0.2 nM.min^{-1} were reached with EC50 values of 312±17, 23±16 and 2.0±0.2 nM, respectively.

Summary/Conclusion: This study is a proof of concept that llama-derived monovalent sdAbs might constitute novel pharmacological agents blocking TFPI that could be used for the treatment of hemophilia. They are currently being engineered for ultimately generating versions of these sdAbs with higher potencies, and with advantageous PK/PD profiles as compared to concizumab.

Science, Fast and Furious II

SFF02-01

RISK OF BLEEDING AND VENOUS THROMBOEMBOLISM IN PATIENTS UNDERGOING TOTAL HIP OR TOTAL KNEE ARTHROPLASTY USING THERAPEUTIC DOSAGES OF DOACS

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Background: About 1.5% of patients undergoing total hip (THA) or total knee arthroplasty (TKA) still develop postoperative venous thromboembolism (VTE), indicating that current thromboprophylaxis, including extension of prophylaxis, is insufficient. Hence, other prophylactic strategies, such as increased dosages, need to be explored. However, before such approaches can be experimentally studied, approximations of the induced risks (i.e., bleeding) should be obtained from existing, real-world, data.

Aims: To estimate the feasibility of direct oral anticoagulants (DOAC)s, in therapeutic dosages, as thromboprophylaxis for high VTE risk patients, we determined the risks of bleeding and VTE in patients who underwent THA/TKA and were treated with DOACs in therapeutic dosages for atrial fibrillation (AF).

Methods: We conducted a registry-based cohort study from 2010 through 2018 in Denmark by linkage of multiple national registries. Because currently therapeutic dosages of DOACs are not prescribed as thromboprophylaxis following THA/TKA procedures, patients with AF, who have a life-long indication for therapeutic anticoagulation therapy, were used as proxy. Hence the main cohort consisted of AF patients on therapeutic dosage of DOACs who underwent THA/TKA. To put findings into perspective, we also included a cohort of non-AF patients undergoing THA/TKA on standard thromboprophylaxis (reference cohort 1), and a cohort of AF patients on therapeutic dosages of DOACs, not undergoing THA/TKA (reference cohort 2).

The primary outcome was the 49-days cumulative incidence (with death as competing risk) of bleeding. Included bleedings were surgical site bleedings, intracranial bleedings, gastrointestinal bleedings, urinary bleedings and bleedings of the airways. Secondary outcomes were the cumulative incidences of VTE (consisting of deep vein thrombosis [DVT] and pulmonary embolism [PE] at 49- and 90-days.

Results: 1,354, 96,751 and 13,516 procedures were included in the main, reference 1 and reference 2 cohorts, respectively. The 49-days cumulative incidence of bleeding in the main cohort was 1.40% (95%Confidence Interval[CI] 0.88-2.14%) and consisted for 68% of surgical site bleedings. In cohort 1 and 2, the bleeding risks were 0.69% (95%CI 0.64-0.74%) and 0.48% (95%CI 0.38-0.61%), respectively.

For the main cohort, risks of VTE, at 49 and 90-days, were 0.74% (95%CI 0.38-1.32%) and 0.59% (95%CI 0.28-1.13%). In reference cohort 1, incidence of VTE was 0.87% (95%CI 0.81- 0.93%) at 49-days and 1.03% (95%CI 0.97-1.10%) at 90-days. Lastly, for reference cohort 2, these were 0.07% (95%CI 0.03-0.13%) and 0.10% (95%CI 0.06-0.17%).

Summary/Conclusion: In summary, the difference in bleeding risk in the main cohort, relative to reference cohort 1 and 2, was limited, especially considering that the events were mainly surgical site bleedings. Additionally, the incidence of VTE was lower in the main cohort. Hence, these data provide a solid basis for the design of randomized controlled trials into establishing the safety and efficacy of therapeutic dosages of DOACs to prevent VTE in high-risk patients.

SFF02-02

TICAGRELOR PREVENTS STAPHYLOCOCCUS AUREUS INFECTIVE ENDOCARDITIS BY MITIGATING BACTERIAL VIRULENCE

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Background: Infective endocarditis is a deadly disease mainly caused by Gram-positive and highly virulent *Staphylococcus aureus*. Current therapy for these patients consists of antimicrobial therapy, having a low efficacy. Therefore, there is an urgent need to discover new strategies that could prevent this disease.

Aims: Assessing the ability of the antiplatelet drug ticagrelor, having antibacterial activity against Gram-positive bacteria, to prevent *Staphylococcus aureus* Infective endocarditis.

Methods: We used a mouse model of infective endocarditis induced by a *Staphylococcus aureus* infective endocarditis clinical isolate. Ticagrelor and clopidogrel were administered prior to local histamine infusion on the aortic valve, and infection. The presence of infected vegetations was determined by a Gram staining on heart sections after three days. The antibacterial effect of ticagrelor on key mechanisms of infective endocarditis was assessed *in vitro*.

Results: A single administration of ticagrelor at conventional antiplatelet dosage (3mg/kg) prior to infection significantly prevented the formation of infected vegetations, with infective endocarditis in 14.3% of ticagrelor-treated mice, compared to 55 % of vehicle-treated mice.
Interestingly, clopidogrel treatment failed to prevent disease development with infective endocarditis in 61.1% of mice which made it unlikely that solely the antiplatelet effect would explain infective endocarditis prevention. Ticagrelor dosed at plasma levels achieved in patients (0.75ug/ml-1.25ug/ml) did not affect *Staphylococcus aureus* growth in liquid cultures but caused drastic adherence defects on activated endothelial cells and extracellular components. We found that growing *Staphylococcus aureus* in the presence of ticagrelor altered the *Staphylococcus aureus* accessory gene regulator system, leading to reduced toxin production and toxin-induced platelet aggregation while preserving the ability of platelets to kill bacteria.

Summary/Conclusion: Our study demonstrates unprecedented ability of ticagrelor to prevent infective endocarditis by directly mitigating bacterial virulence. Hence, clinical trials using ticagrelor as adjunct therapy to antibiotics in patients at risk for infective endocarditis are warranted.

SFF02-03

ANALYSIS OF THE TFPIA - PROTEIN S ANTICOAGULANT PATHWAY IN VIVO

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Background: The initiation of coagulation is regulated by tissue factor pathway inhibitor (TFPI). In humans, TFPIα is the most effective plasma isoform. TFPIα consists of three Kunitz (K) domains that, together, exert its anticoagulant function. K1 and K2 directly inhibit TF-FVIIa and FXa, respectively, whereas K3 interacts with its cofactor, protein S (PS). Although TFPIα function has been well-characterised in vitro, the role/importance of the TFPIα anticoagulant pathway and its dependency on PS *in vivo* is less well understood due to the embryonic lethality of both *Tfpi*- and *Pros1*-deficient mice, the lack of appropriate models and due to differences in TFPI between humans and mice.

Aims: To define the role/importance of TFPIα anticoagulant function *in vivo* and to explore the contribution of PS enhancement of TFPIα in the regulation of thrombus formation.

Methods: Endogenous TFPI anticoagulant function in mice was blocked using an in-house generated monoclonal antibody (14D1 mAb) that blocked the murine TFPI (mTFPI) K2 domain, but not human TFPI (hTFPI). To explore the role of plasma TFPIα in regulating thrombus formation, hTFPIα was co-injected with the 14D1 mAb. We previously tested cross-species compatibility between hTFPIα, murine PS (mPS), and the murine coagulation factors using thrombin generation assays and FXa inhibition assays. The influence of injecting 14D1 mAb with increasing concentrations of hTFPIα on laser-induced thrombus formation was measured. We specifically blocked the mPS cofactor enhancement of hTFPIα *in vivo*, by co-injecting the recombinant C4BP β-chain CCP1-CCP2 domains, which binds/inhibits mPS cofactor function with high affinity, to assess the contribution of PS co-factor function in the laser-induced thrombosis model.

Results: *In vitro* studies: The 14D1 mAb bound to recombinant mTFPIα with high affinity (K_D=0.40±0.07nM) and blocked its inhibition of FXa (IC_{50}=0.75±0.10nM). In FXa inhibition and thrombin generation assays, hTFPIα was enhanced by mPS, indistinguishably from hPS, suggesting cross-species compatibility. The C4BP β-chain bound to mPS with high affinity (K_D=0.21±0.04nM) and completely reversed the mPS enhancement of hTFPIα in thrombin generation assays, highlighting the potential of C4BP β-chain as a blocker of the mPS cofactor function.

In vivo studies: 14D1 mAb injection inhibited the endogenous mTFPI in mice as seen by a profound increase in fibrin deposition at the site of laser injury. In mice where endogenous mTFPI was blocked by the 14D1 mAb, co-injection of recombinant hTFPIα dose-dependently reduced fibrin deposition, demonstrating the profound anticoagulant effect of plasma TFPIα *in vivo*. Co-injection of C4BP β-chain in these mice markedly reduced the anticoagulant effect of hTFPIα controlling fibrin deposition, revealing the critical importance of PS in augmenting the hTFPIα anticoagulant pathway *in vivo*.

Summary/Conclusion: We have developed the first *in vivo* model in mice that is sensitive to the anticoagulant properties of the TFPIα-PS pathway in plasma. We show for the first time the involvement of PS as a cofactor in the anticoagulant function of hTFPIα *in vivo.* Our *in vivo* mouse model will pave the way in assessing the involvement of TFPIα-PS anticoagulant pathway in the physiology/pathophysiology of

thrombosis, its therapeutic potential, as well as the contribution of other cofactors like FV-short in this pathway.

SFF02-04

FLI-1 AS A MAJOR REGULATOR OF TALIN (TLN1) EXPRESSION IN MEGAKARYOCYTE AND PLATELETS

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Background: The ETS transcription factor, FLI-1, plays a fundamental role in megakaryopoiesis. Germline variations in FLI-1 have been associated with bleeding, moderate thrombocytopenia, granules and platelet aggregation defects. The mechanisms by which mutated FLI-1 promotes platelet abnormalities remain unclear.

Aims: Here, we performed a comparative analysis of megakaryocyte transcriptomes to identify which molecular mechanisms are involved in platelet defects in patients carrying FLI-1 variants.

Methods: Four pathogenic Fli-1 variants were studied (Arg337Gln, Lys345Asp already described and Gly307Arg and Arg340Cys, never reported). High MYH10 levels and large α -granules were evidenced in all patients confirming variant pathogenicity. The transcriptomic profile of $CD34^+$-derived megakaryocytes (MKs), assessed by single cell RNAseq, allows the identification of differentially expressed genes. Gene ontology and KEGG pathway analysis were used to determine FLI-1 related pathways. Validation was performed using genetically modified cell lines (Meg01 and HEL), patients' platelets and luciferase reporter systems.

Results: We detected 626 significantly differentially expressed genes (log fold change > 0.25, adjusted p-value < 0.05) between the controls and patient (Arg337Gln) including downregulation of genes related to "degranulation" and "aggregation" pathways. A key gene, TLN1 encoding talin involved in integrin activation was among the most downregulated. Western-Blot evidenced strong reduction in talin expression in FLI-1 deficient compared to control platelets (88% decrease, n=5 vs 9, p<0.001). FLI1-siRNA clearly decreased talin expression in Meg01 cell line (72% decrease). ChIP-Seq data in human MKs [1] revealed 4 FLI-1 binding regions, located in promoter and in the first intron of the TLN1 gene . In addition, i-cistarget analysis uncovered 3 canonical binding sites (BS) one of them being highly conserved across species. Luciferase reporter assays showed the functional role of two BS of which one works in cooperation with GATA1.

Summary/Conclusion: Downregulation of Talin in FLI-1 deficient platelets may participate to the platelet dysfunction described in the patients and may represent a biomarker to improve the diagnosis of FLI-1 related thrombocytopenia and the classification of FLI-variant pathogenicity.

1- Tijssen MR, Cvejic A, Joshi A, Hannah RL, Ferreira R, Forrai A, Bellissimo DC, Oram SH, Smethurst PA, Wilson NK, Wang X, Ottersbach K, Stemple DL, Green AR, Ouwehand WH, Göttgens B. Genome-wide analysis of simultaneous GATA1/2, RUNX1, FLI1, and SCL binding in megakaryocytes identifies hematopoietic regulators. *Dev Cell* 2011; **20**: 597–609.

Focus Symposium - Antithrombin

OR05-02

FUNCTIONAL, BIOCHEMICAL, AND CLINICAL DISSECTION OF GENETIC VARIANTS AFFECTING ARGININES OF THE HEPARIN-BINDING SITE IN ANTITHROMBIN

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Background: Arginine (R) is a positively charged residue that plays key functional roles in antithrombin (AT). It may be involved in the interaction with heparin, an electronegative cofactor which increases AT activity up to 1000-fold. Indeed, five Rs have been proposed to directly interact with heparin: R45, R56, R79, R16 and R177, and natural mutations affecting all except R177 have been described to cause type II AT deficiency with heparin binding defect (HBS).

Aims: To dissect the impact of natural missense variants affecting Rs of AT trying to get a genotype-phenotype-clinical correlation.

Methods: The study was done in 2 cohorts of patients in whom *SERPINC1*, the gene encoding AT, was sequenced: 1) 409 unrelated Caucasian patients with suspicion of AT deficiency; 2) 75 COVID patients in whom we performed whole exome sequencing (WES). Clinical data of these patients were recorded. Anticoagulant activities were evaluated by chromogenic assays (anti-FXa, anti-FIIa) or by evaluating the formation of covalent complexes by Western blot after adding target proteases to the plasma (FXa, FIIa and FVIIa). Identification of AT forms with low heparin affinity was done by crossed immunoelectrophoresis (CIE). Secretion and functional features of all variants were also evaluated in a recombinant model of expression in HEK-EBNA cells.

Results: We identified 9 *SERPINC1* variants causing missense mutations affecting 8 Rs of AT located relatively close to the heparin binding site. Five were previously described (R45W; R56C; R79C; R79H; and R161Q), but 4 were new: R78Q, R89S, R177C and R291H. The presence of a variant AT in plasma was detected by native PAGE in all carriers except R89S, which caused a type I deficiency by introducing a new N-glycan leading to intracellular retention, as demonstrated in the recombinant model. One variant, R291H, identified by WES in a COVID patient, had no functional, biochemical, or clinical effect. Similarly, R177C had no functional or clinical impact. This mutation was identified in a 30-year-old woman with no history of thrombosis who had low AT activity (60%) in a first measure as part of a thrombophilia screening before fertilization, but normal values in all further studies. Low heparin affinity forms were not increased, and the recombinant variant had normal activity and heparin affinity. We validated the role of R45; R56; R79; and R161 in the interaction with heparin, as these mutations caused type II HBS defects with mild risk of thrombosis. Interestingly, we identified a new R directly involved in the interaction with heparin: R78. The R78Q substitution generated a form with low heparin affinity, which was of difficult identification by functional assays. Clinical consequences of the variant were mild, unless combined with other type II defect, as happened in the case included in our study: a 4-year-old girl with perinatal ischemic stroke who was compound heterozygous for R78Q and L131F.

Summary/Conclusion: We found a significant biochemical, functional, and clinical heterogeneity of genetic variants affecting Rs of AT. Two, R291H and R177C, should be considered benign; most of them (R45W; R56C; R79C; R79H; R161Q, and the new one R78Q) caused type II HBS with mild thrombotic risk except if they are in compound heterozygosis, which are viable but very severe; and one, R89S causes a severe type I deficiency because it affects N-glycosylation.

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OR05-03

RECURRENT MUTATIONS IN ANTITHROMBIN DEFICIENCY: ORIGIN AND EXPANSION; FOUNDER MUTATIONS AND HOTSPOTS

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Background: Antithrombin deficiency (ATD), a rare disease that increases the risk of thrombosis, is mainly caused by single nucleotide variants affecting SERPINC1. Two types of ATD have been described: type I, with no AT variant in plasma, and type II, with a circulating variant with impaired or no anticoagulant activity. Type II deficiencies usually have a milder risk of thrombosis and a higher prevalence in the general population than type I. Most mutations causing ATD are privative of a single family, but there are also mutations that are common in certain populations, such as Hungary (L131F), Finland (P73L), or Africa (T147A), all of which cause type II deficiencies. The founder effect has only been described for 2 SERPINC1 mutations: L131F and T147A. Identification and characterization of recurrent mutations in SERPINC1 could provide information about hotspots in this gene and and how these mutations have spread.

Aims: To characterize recurrent mutations in *SERPINC1*.

Methods: The study was performed in 324 unrelated patients with congenital ATD caused by SERPINC defects, consecutively recruited from a reference center over 27 years. We selected the mutations identified in more than 5 unrelated patients. The whole gene (13 Kb) was sequenced by NGS. In addition, a long PCR (6-7 Kb) covering the mutation was sequenced using a long-read-based method (nanopore sequencing) in a MinION device. Haplotype analysis was performed using an in-house pipeline.

Results: Nine recurrent variants were identified in 128 unrelated carriers (39.5% of ATD). Five missense changes caused type II deficiencies, 3 affected heparin affinity (R79C, P73L, and L131F), and 2 caused reactive defects (A416S, R425H). One missense change in the signal peptide caused a transient ATD (V30E). We also identified 3 recurrent mutations leading to type I deficiencies by aberrant splicing (c.1154-14 C>T), by generation of a premature codon (R161Stop), or by a missense mutation (I386T). The 4 more frequent mutations were type II: L131F (AT Budapest 3; N=36), A416S (AT Cambridge II, N=23), R79C (AT Toyama, N=20), or transient V30E (AT Dublin; N=17). Sequencing results strongly suggested a founder effect for 3 mutations: L131F, found in subjects from the Roma population; I386T, found only in Polish patients, and A416S. At least two different alleles were identified in carriers of c.1154-14 C>T; V30E, P73L, R79C, and R425H, with R161Stop being the mutation with the highest number of different alleles (N=4).

Summary/Conclusion: Recurrent mutations are common in ATD. Most recurrent mutations caused transient or type II deficiencies with low thrombotic risk. However, we also identified 3 recurrent mutations causing severe type I deficiencies. A potential founder effect was identified in two population-specific mutations, AT Budapest 3 and I386T. The most recurrent mutations (AT Budapest 3 and AT Cambridge II) seem to have a founder effect. A potential selective advantage, similar to that suggested for other minor prothrombotic polymorphisms such as Factor V Leiden, could be proposed to explain the expansion of recurrent SERPINC1 mutations. Also, spreading in isolated or highly consanguineous populations could be

the mechanism to explain the expansion of founder mutations. In contrast, at least four origins can be proposed for the type I deficiency caused by the nonsense R161Stop mutation, suggesting that this is a mutational hot spot of *SERPINC1*.

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Focus Symposium - Inflammation and NETs

OR06-02

THE IMPACT OF HYPOXIC PROFILES AND INFLAMMATION ON PRIMARY MONOCYTE RESPONSES

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Background: Venous thromboembolism (VTE) is a common disease with severe complications including death. Limited knowledge of disease mechanisms hampers development of targeted prevention. Venous thrombi mostly originate in the valve pocket of the deep veins where a local combination of cycling (intermittent) hypoxia and inflammatory mediators activate endothelial cells, platelets and monocytes and thereby trigger immunothrombotic processes. Monocytes have a central role in the pathophysiology of VTE, bridging innate immune activation and hemostatic pathways via the production of inflammatory cytokines and chemokines, and tissue factor (TF)-bearing extracellular vesicles. It is however unclear if the suspected VTE-triggers intermittent hypoxia and inflammation act in synergy to promote pro-thrombotic responses in monocytes.

Aims: To investigate how primary monocytes respond to sustained and intermitted hypoxia alone, as well as in combination with inflammatory cytokines.

Methods: Primary human monocytes were subjected to short-term (4.5 hours) sustained or intermittent hypoxia alone, or in combination with IL-1b to mimic the conditions in valvular pockets. Following stimulation, monocytic TF gene expressions and TF activities were measured, and the cell transcriptome was analyzed by RNA sequencing. Cytokine release was quantified by ELISA.

Results: Stimulation of monocytes with IL-1b was accompanied by elevated levels of TF expression and TF activity. Interestingly, the stimulatory effect of IL-1b was attenuated by sustained hypoxia, but not by intermittent hypoxia. Our transcriptome analysis confirmed the potent pro-inflammatory capacity of IL-1b. Sustained hypoxia alone promoted a metabolic shift in the monocyte transcriptome and attenuated many of the pro-inflammatory effects induced by IL-1b. In contrary, intermittent hypoxia alone had a modest effect, but when combined with IL-1b it induced a substantial effect on the monocyte transcriptome with more than 2200 differentially expressed genes. More specifically, intermittent hypoxia increased the IL-1b-induced expression of several chemokine and interleukin genes (e.g., IL-19, IL-24, IL-32, MIF), as well as genes that modulate coagulation (thrombomodulin) and fibrinolysis (VEGFA, MMP9, MMP14 and PAI-1). Increased production of CCL2, IL-6 and TNF following stimulation with intermittent hypoxia and IL-1b was confirmed by ELISA.

Summary/Conclusion: Short-term stimulation of monocytes with IL-1b under intermitted hypoxia promoted a vigorous pro-inflammatory response, with additive effects on several pro-inflammatory cytokines. In addition, the combination of either intermittent or sustained hypoxia and IL-1b modulated components of the fibrinolytic system. Our findings suggest that the hypoxic profile is pivotal for the immunothrombotic

response in monocytes secondary to inflammatory stimulation and shed new light on the early events in the pathogenesis of venous thrombosis.

OR06-03

REGULATION OF NETS BY PEPTIDIC INHIBITORS TARGETING EXTRACELLULAR HISTONES

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Background: Several studies have revealed that extracellular histones released from NETs (Neutrophil Extracellular Traps) exhibit cytotoxicity and consequently can induce cell death. Currently, only a few histone inhibitors are available and none of them are being used for clinical treatment. Thus, novel and potent histone inhibitors are needed.

Aims: We aim to develop peptidic inhibitors to neutralize the cytotoxicity of extracellular histone H2A/H4. To accelerate the discovery process, we have used structure-based method to design and develop bioactive compounds targeting histone H2A/H4.

Methods: We have utilized three-dimensional (3D) structures of histone H2A/H4 in complex with their binding partners as the structural basis for peptide design. Then, we have applied various computational approaches such as molecular docking, molecular dynamics (MD) simulations and binding free energy (BFE) calculations to investigate and identify the key interacting residues/areas of the binding partners with histone H2A/H4. These essential interacting regions of the binding partners were used as a starting template peptide which was then subjected for rational design to improve binding interactions with histone H2A/H4. Finally, the designed peptides were prioritized by their BFE with histone H2A/H4 and the top candidates were selected for further synthesis and functional characterization (Wichapong K. et al., *Comput. Struct. Biotechnol. J., 2021).*

Results: We have developed the first peptidic histone H4 inhibitor, called HIPe (**H**istone **I**nhibitory **Pe**ptide). Structurally, molecular model revealed that HIPe stably bound to the N-terminal tail of histone H4. Experimentally, HIPe not only prevented histone H4-mediated cell death (*in vitro* cytotoxicity assay) but this peptide also exhibited therapeutic benefits in mouse model of atherosclerosis (Silvestre-Roig C. et al., *Nature*, 2019). Recently, we have in silico designed peptidic inhibitors targeting histone H2A and experimentally tested their inhibitory activity in different experimental setting. The most potent inhibitor (**CHIP** - **C**yclical **H**istone H2A **I**nterference **P**eptide) exhibited high potential to prevent monocyte adhesion by targeting NET-resident H2A and reduced arterial adhesion in a model of LPS-accelerated atherosclerosis (Schumski A. et al., *Circulation*, 2021).

Summary/Conclusion: In this work, we have applied structure-based and computational methods to successfully to develop peptidic inhibitors targeting the disordered N-terminal tails of histone H2A/H4. The represented approaches are fast and generic which can also be applied to rationally design and develop novel bioactive compounds to target various drug targets including histone H3 and protein-protein complexes. Moreover, the developed peptidic histone H2A/H4 (CHIP and HIPe, respectively) can also be utilized for further improvement and development into drugs to treat histone- and/or NETs-mediated diseases e.g., sepsis, atherosclerosis, and acute lung injury.

Focus Symposium - Bleeding in Women

OR07-02

FAMILY HISTORY OF POSTPARTUM HEMORRHAGE IS A RISK FACTOR FOR POSTPARTUM HEMORRHAGE AFTER VAGINAL DELIVERY: RESULTS FROM THE FRENCH PROSPECTIVE MULTICENTER HEMOTHEPP COHORT STUDY

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Background: Postpartum hemorrhage (PPH) is a major component of perinatal morbidity and mortality affecting young women worldwide, and is still often unpredictable. Reducing the incidence of PPH is a major health issue, and identifying women at risk for PPH is a key element in preventing this complication.

Aims: To estimate PPH prevalence after vaginal delivery and to identify PPH risk factors.

Methods: Unselected pregnant women ≥ 16 years attending one of six maternity wards in Brittany (France) for vaginal birth after 15 weeks of gestation were recruited in this prospective multicenter cohort study between June 1, 2015 and January 31, 2019, after information and collection of their non opposition. PPH was defined as a blood loss ≥ 500 mL in the 24 hours following delivery, and measurement of blood loss was systematically performed with a graduated collector bag. Independent risk factors for PPH were determined by logistic regression. Missing data were compensated by multiple imputation (MICE method).

Results: Among 16,382 included women, PPH prevalence was 5.37%. A first-degree family history of PPH (aOR = 1.63, 95%CI 1.24-2.14) and a personal transfusion history (aOR = 1.90, 95%CI 1.23-2.92) were significantly associated with PPH. The use of oxytocin during labor was also a risk factor for PPH (aOR = 1.24, 95%CI 1.06-1.44), as well as PPH personal history (aOR = 2.51, 95%CI 1.83-3.45), multiple pregnancy (aOR = 2.10, 95%CI 1.34-3.30), pre-eclampsia (aOR = 2.43, 95%CI 1.46-4.03) and macrosomia (aOR = 1.55, 95%CI 1.22-1.96). Inversely, smoking during pregnancy and intrauterine growth restriction were associated with a reduced risk of PPH (aOR = 0.76, 95%CI 0.63-0.91, and 0.34, 95%CI 0.13-0.87, respectively).

Summary/Conclusion: In addition to classical risk factors, this study identified PPH family history and personal transfusion history as new characteristics associated with PPH after vaginal delivery. The association of PPH with a family history of PPH suggests a hereditary hemorrhagic phenotype and calls for genetic studies.

OR07-03

EFFECTS OF ANTIPLATELET THERAPY ON MENSTRUAL BLOOD LOSS IN PREMENOPAUSAL WOMEN: A SYSTEMATIC REVIEW

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Background: There is a growing burden of cardiovascular disease among women of reproductive age, which is associated with an increased use of antiplatelet therapy for the purpose of primary or secondary prevention. While several studies have reported that anticoagulant therapy in premenopausal women is associated with heavy menstrual bleeding (HMB), the effects of antiplatelet therapy on menstrual bleeding are less clear.

Aims: To systematically review the effects of antiplatelet therapy on menstrual blood loss in premenopausal women.

Methods: We searched Ovid MEDLINE, EMBASE, Web of Science, Cochrane Central Register of Controlled Trials, and Google Scholar from inception until the 28th of November 2022. Studies of women who had an active menstrual cycle and were treated with antiplatelet agents were included. Menstrual blood loss should be assessed either by pictorial blood loss assessment chart, menstrual pictogram, alkaline hematin technique, menstrual fluid loss, counts of sanitary items, measurement of iron or labeled red blood cells in pads, duration of menstruation, questionnaires, or self-perception methods. Studies were excluded if all participants had a bleeding disorder or if antiplatelet agents were combined with anticoagulants. Measures of menstrual blood loss both before and after initiation of antiplatelet therapy and from comparison groups were collected. Two reviewers independently assessed the risk of bias of individual studies with the Risk of Bias in Non-randomised Studies- of Interventions tool. This study is registered on PROSPERO (CRD42023388166).

Results: Of the 742 records identified from the databases, 13 studies with in total 611 women who used some type of antiplatelet therapy were included. Types of antiplatelet therapy used were aspirin only (N=8), aspirin and/or clopidogrel (N=2), prasugrel (N=1) and not specified (N=2). Contraceptive use was reported by eight studies: no contraceptives (N=3), intra-uterine device (N=2), oral contraceptives (N=2), oral contraceptives or intra-uterine device (N=1). Four studies reported on changes in menstrual blood loss volume. Two of these studies showed no increase in menstrual blood loss during antiplatelet therapy compared with the use of paracetamol or placebo during menstruation or the amount of menstrual blood loss before taking antiplatelet drugs. The other two studies reported a higher amount of menstrual blood loss among antiplatelet users compared with non-users or the pre-antiplatelet therapy control cycle. Three studies assessed the duration of menstrual bleeding. Of these, one study reported a longer duration of menstruation among aspirin users (mean 7.9 days; 2.4SD) compared with non-users (mean 6.9 days; 2.2SD). In the other two studies up to 12% of women reported an increased duration of menstruation. Four studies reported the intensity of menstrual flow: 13-38% of women reported an increase in intensity of flow compared with before the use of antiplatelet therapy. However, increases in intensity of flow were comparable to placebo and ibuprofen treatment. Seven studies reported the prevalence of HMB in women treated with antiplatelet drugs, with estimates ranging from 7% to 90%.

Summary/Conclusion: Conflicting results have been reported on the effects of antiplatelet therapy on menstrual blood loss. Some studies suggest that antiplatelet therapy may increase menstrual blood loss, whereas others showed no effect.

Focus Symposium - Platelet Receptors & Mechanisms

OR08-02

THE CLEARANCE RECEPTOR CLEC4M REGULATES PLASMA FACTOR V LEVELS THROUGH FACTOR V BINDING AND INTERNALIZATION

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Background: Coagulation factor V (FV) is a large plasma glycoprotein homologous to factor VIII (FVIII). FV displays both pro- and anticoagulant properties, and FV plasma levels have been associated with both venous thrombosis and bleeding. A genome-wide association study of FV levels in 4373 participants of European descent identified a significant association ($p = 2.22 \times 10^{-8}$) with a SNP (rs594793) in the *CLEC4M* gene, suggesting that CLEC4M may contribute to the inter-individual variation of plasma FV levels (Trégouët et al. ISTH 2022). CLEC4M is an adhesive receptor for pathogens and has been previously shown to bind and internalize von Willebrand factor and FVIII. Therefore, we hypothesized that CLEC4M may also contribute to the clearance of FV.

Aims: To test the hypothesis that CLEC4M binds FV and mediates its internalization by cells.

Methods: Direct interaction and binding affinity of FV to CLEC4M was probed using antigen-based solid-phase assays. HEK293 cells stably expressing CLEC4M were generated, and cultured in the presence or absence of FV. FV binding to CLEC4M on the cell surface was determined by flow cytometry. FV internalization was analyzed by immunoblot on cell lysates. Endocytosis and degradation of FV in CLEC4M-expressing cells were investigated by treating cells with clathrin mediated endocytic and lysosomal inhibitors respectively, and FV content was analyzed by immunoblot and ELISA. Non-transfected HEK293 cells, which do not express CLEC4M, were used as a control in all cell-based experiments.

Results: FV in both buffer and plasma environment bound to immobilized CLEC4M in a concentration-dependent manner (K_d ~ 0.95 nM). Activated FV, which lacks the heavily glycosylated B domain, also bound to CLEC4M, but with lower affinity as compared to full-length FV. The interaction of FV and CLEC4M was calcium-dependent. When HEK293 cells stably expressing CLEC4M were cultured in the presence of FV, FV was detected both on the cell surface (by flow cytometry) and inside the cells (by immunoblot on the cell lysates) in a dose-dependent manner. Although CLEC4M-negative HEK293 cells also bound and internalized FV, both phenomena were more pronounced in CLEC4M-positive cells. Our investigation also revealed an increased FV detection, when cells were treated with lysosomal inhibitors, suggesting that FV might be degraded through a lysosome-based clearance mechanism. Treatment of cells with clathrin mediated endocytic inhibitor, resulted in decreased FV detection as compared to untreated, infering the possibility of FV to be internalized through clathrin coated-pits.

Summary/Conclusion: FV binds to CLEC4M in a calcium-dependent manner. The K_d estimated from the solid-phase binding assay suggests that this interaction may occur at the physiological concentration of FV in plasma. The presence of CLEC4M receptors on cells facilitates enhanced binding and internalization of

FV, which is trafficked through clathrin-coated pits and then degraded by the lysosomes. Taken together, these findings support a role of CLEC4M in the clearance of FV.

OR08-03

IDENTIFICATION OF POLO-LIKE KINASE 3 IN PLATELETS AND ITS ROLE IN THE REGULATION OF HEMOSTASIS AND THROMBOSIS

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Background: Polo-like kinase 3 (Plk3) is a serine/threonine kinase involved in cell cycle regulation. Its expression and function in platelet are not known.

Aims: To elucidate the role of Plk3 in platelet activation and function.

Methods: Plk3 expression in platelets was evaluated by western blot and immunofluorescence imaging. Platelet aggregation was performed using lumi-aggregometer. Dense-granule secretion was measured using ^{14}C serotonin. Alpha-granule release and alphaIIbeta3 activation was assessed using flowcytometry. Age-matched congenic *Plk3* knockout and C57BL/6 mice of both genders were used for *in-vivo* studies. To evaluate hemostasis, tail-bleeding and liver bleeding assays were performed. To determine thrombosis $FeCl_3$-induced carotid artery injury and collagen/epinephrine-induced pulmonary thromboembolism assays were performed. *Ex-vivo* thrombus formation was performed under arterial flow on collagen surface.

Results: We found that Plk3 is expressed in platelet and localizes to the filopodia. Deletion of *Plk3* in mice (*Plk3*$^{-/-}$) showed a significantly (*P<0.05*) delayed tail bleeding time (325 s), compared to WT mice (130 s) suggesting a role for Plk3 in hemostasis. *In-vivo* thrombosis was also significantly affected in *Plk3*$^{-/-}$ mice (vessel occlusion time ~14 min), compared to WT (7-9 min; *P<0.001*). Furthermore, *Plk3*$^{-/-}$ mice were protected from thromboembolism compared to WT mice (*P<0.001*). Moreover, we found significantly reduced *in-vitro* thrombus formation under arterial flow ($800s^{-1}$) in the absence of *Plk3*. Thrombin-induced platelet aggregation (*P<0.0001)*, TxA_2 generation (*P<0.05*), secretion of both alpha- and dense- granules was significantly reduced (*P<0.01*) in *Plk3*$^{-/-}$ mouse platelets compared to WT, consistent with the observed anti-thrombotic phenotype *in-vivo*. Furthermore, *Plk3*$^{-/-}$ null platelets failed to retract fibrin clot. Interestingly, thrombin induced intracellular Ca^{2+} rise was unaffected, suggesting that Plk3 operates downstream of Ca^{2+} mobilization.

Summary/Conclusion: These results suggest that Plk3, plays a significant role downstream of agonist induced Ca^{2+} rise to regulate platelet activation thus affecting the process of hemostasis and thrombosis.

Focus Symposium - Coagulation Genetics

OR13-01

SHEDDING LIGHT ON NON-CODING 5'UTR VARIANTS ALTERING UPSTREAM OPEN READING FRAMES IN MAIN NATURAL COAGULATION INHIBITOR GENES

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Background: Genetic variations located in the 5'UTR of coding genes and altering upstream open reading frames (upORFs) are still a neglected class of non-coding variations that are not optimally looked for in clinical practice. This can be explained by the lack, until recently, of easy-to-use bioinformatics tools that can automatically read VCF files and detect/annotate variants that could alter upORFs. In absence of automated methods, predicting which 5'UTR variant could be an upORF-affecting variant is very time-consuming and not adapted neither to the huge number of variants identified in high throughput sequencing analysis nor to the large variety of upORFs. Besides, claiming for the pathogenicity of such variants requires characterizing how they could affect gene expression and how the resulting dysregulations could lead to disease. Recently, we identified a pathogenic mutation in the 5'UTR of *PROS1* in a family affected with Protein S deficiency and inherited thrombophilia (Labrouche *et al*., 2020). The identified variant was a never reported C>T substitution at c.-39 position creating an upstream AUG at the origin of an overlapping ORF (uoORF) of 156 nucleotides ending at a stop codon located at position c.111 within the coding sequence.

Aims: To identify all possible upORF-altering single nucleotide variants (SNVs) in the 5'UTR of the three main natural inhibitors of the coagulation, *PROC*, *PROS1* and *SERPINC1*.

Methods: In this work, we conducted an *in silico* mutational saturation analysis of all possible 5'UTR SNVs in *PROC*, *PROS1* and *SERPINC1*, and used an updated version of the MORFEE bioinformatics tool in order to detect any variant that could create or alter upORFs.

Results: The number of possible elongated CDS or fully upstream ORF generated by SNVs in *PROC*, *PROS1* and *SERPINC1* was 28, 94 and 47, respectively. Specific numbers for SNV-generated uoORFs were 83, 55 and 44, respectively. Interestingly, we identified 3 SNVs predicted to generate additional *PROS1* uoORFs also ending at c.111. These were rs1009672929, rs767438604 and rs370938580, the latter being classified as Variant of Unknown Significance (VUS) in ClinVar. Besides, 2 additional uoORF-creating variants (*PROC* rs886054845, *PROS1* rs368555701) were reported as VUS in ClinVar. Moreover, 5 additional rare SNVs in the 5'UTR of *PROS1*, 4 in the 5'UTR of *PROC* and 1 in the 5'UTR of *SERPINC1* predicted to generate uoORFs have also been reported in databases but absent in ClinVar. Whether all these variants alter the protein levels still need to be experimentally tested.

Summary/Conclusion: By providing a catalog of all possible variants at the origin of upORFs in *PROC*, *PROS1* and *SERPINC1*, this work provides new clues about which variants could be prioritized for experimental investigations and new knowledge guiding the interpretation of variants identified in the context of clinical diagnosis. Similar *in silico* investigations for all human genes are ongoing.

OR13-02

GENOME-WIDE IDENTIFICATION OF LOCI CONTROLLING PLASMA COAGULATION FACTOR IX ACTIVITY

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Background: Elevated coagulation factor (F) IX activity is associated with cardiometabolic diseases including venous thrombosis. Factor IX activity may be affected by environmental and genetic factors. However, genetic factors affecting FIX activity remain elusive.

Aims: We aimed to identify novel genetic loci affecting plasma FIX activity.

Methods: We performed a genome-wide association study (GWAS) using the Netherlands Epidemiology of Obesity study, including 6,671 individuals. We excluded participants who used anticoagulants (n=130), had missing information for any needed variables (n=163), or who did not pass individual level quality control for GWAS (n=927). Genotype data were imputed to the 1000 Genomes reference panel phase III. FIX activity was measured with a coagulometric clot detection method. We performed linear regression analysis for FIX activity in the original unit using an additive genetic model adjusted for age, sex, hormone therapy, menopausal status, and the first 5 principal components. The genome-wide significance threshold was defined as 5×10^{-8}. An additional GWAS and meta-analysis of the results are planned based on the German population-based Gutenberg Health Study.

Results: In total, 5,581 participants were included with a mean (SD) plasma FIX activity of 123.4 % (20.2). We identified 6 novel genetic loci associated with plasma FIX activity, i.e. *GCKR* (rs1260326), *NRP2* (rs143629900), *KNG1* (rs698078), *F12* (rs2731674), *ABO* (rs597974) and *CDH4* (rs145541636). The identified associations ranged between -3.28 and 20.70%. Of the associated genetic loci, *ABO* (rs597974), located in chromosome 9, showed the most significant association with plasma FIX activity (β-estimate = 4.39 %, *p*-value = 4.01E-25).

Summary/Conclusion: We identified novel genetic loci affecting plasma FIX activity. Our results aid in a better understanding of the biological mechanism regulating plasma FIX activity and shed light on potential preventive and therapeutic targets of cardiometabolic diseases associated with FIX activity.

OR13-03

GENOMIC LONG-READ SEQUENCING IDENTIFIES THE MOLECULAR BASIS OF UNDIAGNOSED PATIENTS WITH HEMOSTATIC DISORDERS: UNEXPECTED COMPLEX STRUCTURAL VARIANTS AND RETROTRANSPOSON INSERTIONS

B. De La Morena-Barrio [1,*], A. Zamora [1], J. Padilla-Ruiz [1], P. Garrido-Rodríguez [1], A. Marín-Quilez [1], M. E. de la Morena-Barrio [1], C. Bravo-Pérez [1], R. Cifuentes-Riquelme [1], M. Llamas [1], P. Gómez-González [1], J. M. Bastida [2 3], M. L. Lozano [1], J. Rivera [1 3], J. Corral [1]

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Background: Next-generation sequencing (NGS) has significantly improved the genetic diagnosis of human diseases, including congenital thrombophilias and inherited platelet disorders (IPD). However, in many cases with clear clinical and intermediate phenotypes, such as thrombosis and antithrombin deficiency (ATD), bleeding and absence of platelet αIIb/β3 integrin (Glanzmann thrombasthenia -GT-), or dense platelet granules and oculocutaneous albinism (Hermansky-Pudlak syndrome -HPS-), conventional molecular analysis of candidate genes (*SERPINC1*; *ITGA2B* or *ITGB3*; and *HPS1-11*) provides a negative or incomplete genetic diagnosis.

Third generation sequencing methods based on unmanipulated genomic long reads (up to 2.5 Mb) facilitate the identification and characterization of gross genetic structural variants (SV) and may be a suitable alternative method to search for hidden gene defects in these patients.

Aims: To investigate the diagnostic potential of nanopore sequencing, a third-generation sequencing method, in patients with distinct haemostatic disorders but with no genetic diagnosis by conventional methods.

Methods: The molecular bases of patients with ATD (N= 350) and IPD (N= 400) were evaluated after candidate gene sequencing by NGS and/or Sanger. Genomic nanopore sequencing was performed in the following patients: 15 ATD cases, all negative for MLPA and glycosylation defects; 2 GT patients, one without a previously identified molecular defect in *ITGA2B* and *ITGB3,* and one patient heterozygous for a novel splice site mutation in *ITGB3*; and 2 HPS patients one without a previously identified molecular defect and other with a single missense mutation in *HSP3*. Whole genome sequencing was performed in 10 cases with ATD on a PromethION device and the remaining 9 cases on an affordable MinION device by enrichment of regions of interest using *adaptive sampling*. Validation of genetic defects and family segregation analysis were performed using specific PCR amplification and sequencing.

Results: Nanopore sequencing identified 4 ATD patients carrying a large insertion in *SERPINC1* introns that corresponded to two retrotransposons according to *de novo* assembly: an SVA-E insertion in intron 6 shared by three patients and an SVA-F1 insertion in intron 3, both SVs potentially affect splicing according to in silico prediction. Moreover, both GT patients shared complex SV affecting coding regions of *ITGB3* (deletion [788 bp], inversion (1.5kb) and duplication [174 bp]). This SV was homozygous in one patient and compound heterozygous with c.2301+1G>C in the second GT patient. Finally, 2 deletions were detected in

the two HPS patients; one carries a homozygous 10.2 kb deletion in *HPS5* and the other a heterozygous 3 kb deletion in *HPS3* in compound heterozygosis with the nonsense variant c.2464C>T [p.R822*].

In all cases, Alu elements flanked the breakpoints of the genetic SVs.

Summary/Conclusion: Nanopore sequencing, even using the affordable MinION device and *adaptive sampling* allowing up to 15-fold coverage of selected regions, has revealed the molecular abnormalities in patients with clear clinical and laboratory diagnosis but lacking genetic diagnosis, in some cases for more than 30 years, despite previous molecular studies. Our results also demonstrate the relevance of SVA insertions in ATD (4/350 cases: 1%), and strongly encourage the analysis of SVs in IPD.

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Focus Symposium - Aspects of von Willebrand Factor

OR14-03

THERAPEUTIC EFFICACY OF A NOVEL HUMANIZED ANTI-ADAMTS13 ANTIBODY TO TREAT LEFT VENTRICULAR ASSIST DEVICE-INDUCED ACQUIRED VON WILLEBRAND SYNDROME

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Background: Bleeding is a frequent complication in left ventricular assist device (LVAD) patients and is linked to the occurrence of acquired von Willebrand syndrome (aVWS). LVADs cause an increased shear-induced proteolysis of von Willebrand factor (VWF) mediated by ADAMTS13, leading to aVWS. We developed a murine anti-ADAMTS13 monoclonal antibody (mAb) 17C7 as a novel therapeutic agent and showed that this mAb efficiently treated LVAD-induced aVWS in a preclinical LVAD calf model. Generation of humanized mAb 17C7 and testing its therapeutic efficacy is needed to advance our drug towards the clinic.

Aims: To generate humanized 17C7 and study its therapeutic efficacy in our validated LVAD-induced aVWS calf model.

Methods: Nine different variants (combination of 2 heavy and 5 light chain modifications) were generated by grafting the murine CDRs on a human acceptor framework. The binding affinity (Kd) of all variants was determined via ELISA and the inhibitory activity was analyzed using the FRETS-VWF73 assay. Next, the optimal variant was selected and tested in our preclinical LVAD calf model (n=4).

Results: All humanized variants could efficiently bind to human ADAMTS13 with comparable binding affinities as the murine mAb. Moreover, humanization did not impact the inhibitory potential of all variants. The most human-like variant with the highest binding affinity (Kd= 0.4 µg/mL, IC50= 0.6 µg/mL) was selected. Similar to our previous results with the murine mAb, the loss of HMW VWF multimers after pump implantation could be rescued upon injection of the humanized mAb 17C7. As expected, ADAMTS13 activity was completely blocked after injecting one dose (600 µg/kg) of the humanized mAb. Importantly, no severe thrombocytopenia nor hemolytic anemia was observed.

Summary/Conclusion: We confirmed that humanization did not impact the therapeutic efficacy of our drug. Hence, our humanized anti-ADAMTS13 mAb could become a promising therapy to treat aVWS-induced bleeding in LVAD patients.

Focus Symposium - Novel Laboratory Tests

OR15-01A

REPRODUCIBILITY STUDY OF A FAST AND FULLY AUTOMATED FVIII FUNCTIONAL INHIBITOR TEST

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Background: Laboratory detection of Factor (F)VIII inhibitors is preferentially performed with the Nijmegen assay. It requires a 2-hours incubation of test sample with the FVIII-source mixture because of slow FVIII inactivation, due to its reversible binding to von Willebrand Factor (VWF) delaying inhibitor action. Extended incubation time results in non-specific FVIII inactivation, that, together with complicated liquid handling, may contribute to the considerable variability (CV: 30–40%) seen in inter-laboratory surveys. We hypothesize that testing in a VWF-free assay matrix using recombinant (r)FVIII can dramatically lower incubation time that, together with full automation, will substantially improve standardisation.

Aims: Test reproducibility of a fast, fully automated FVIII inhibitor test using VWF-free rFVIII and a dedicated analyser.

Methods: As in the original Nijmegen assay, test samples are heated for at least 30 minutes at 58°C and centrifuged for 10 minutes to destroy residual FVIII.
The coagulation analyser employed must provide on board ability of three subsequent sample dilution steps and three reagent additions. An application was defined on a Ceveron s100 (Technoclone), as below.
After loading the heat inactivated samples, sequential automated analytical steps occur as follows:

- Predilution with heat inactivated FVIII/VWF deficient plasma (if needed)
- Mixing with rFVIII (1.0 IU/mL)
- Incubation for 20 minutes at 37°C
- Analysis for residual rFVIII activity

Results: Five samples and two lyophilized controls, whose inhibitor activities with the original Nijmegen assay were between 0 and 40 BU, were analysed. In a reproducibility study with three laboratories, the samples and controls were assayed twice a day on five independent days. For all samples and controls, precision was analysed exhibiting a coefficient of variation of less than 15% for all samples.

Summary/Conclusion: Rapid, fully automated FVIII-inhibitor testing can be performed with a dedicated coagulation analyser using rFVIII in a VWF-free matrix. Automation and reduced assay time improve viability and potentially the availability of a normally protracted assay, permitting a more rapid and informed clinical response.

OR15-01B

FULLY AUTOMATED DETERMINATION OF APIXABAN, EDOXABAN, RIVAROXABAN LEVELS USING A STANDARD HEPARIN REAGENT ON COBAS T COAGULATION ANALYZER

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Background: Measurement of direct oral anticoagulants such as apixaban, rivaroxaban and edoxaban, show suitability in patient samples and are used more and more often. As DOAC sample numbers are increasing and the determination of the respective levels is recommended in case of urgent surgery or bleeding, laboratories benefit from the possibility of fully automated testing using their standard heparin reagent.

Aims: The aim of this study was to adapt the test procedure of a standard heparin assay on a fully automated cobas t instrument for the measurement of apixaban, rivaroxaban and edoxaban, by additionally using in house produced calibrators and controls for each of the respective DOAC. Sample results were compared to levels determined with mass spectrometry in these samples.

Methods: Using a standard heparin reagent, the corresponding test setting was modified separately for the measurement of apixaban, rivaroxaban as well as edoxaben levels in samples. For establishment of reference curves DOAC specific calibrators were produced in house and levels were determined by mass spectrometry (LC-MS/MS) prior use. For each of the respective DOAC, two different test settings were created to reliably measure in the low as well as in the high range. For testing the performance of each of the assays in house produced lyophilized controls and in vitro spiked plasma samples were used. Patient samples were used for the comparison with the HPLC/MS-MS reference method to evaluate the assay correlation.

Results: Sensitivity of the assay was tested with different lots of reagent and calibrators. The limit of blank (LoB) was found below 5ng/mL. The limit of detection (LoD) was found below the limit of quantification (LoQ), which was found below 10ng/mL with an imprecision of SD ≤3.5ng/mL for all of the three different DOACs with the adapted test settings. Linearity was shown between 0 and 500ng/mL for all three DOAC settings using both applications each. Accuracy was shown with a repeatability and reproducibility below 10%CV. For a metod comparison patient samples from a different study with assigned mass values were used and very good correlation with a Passing-Bablok regression of 1.0±0.1 and a Pearson correlation >0.9 was found.

Summary/Conclusion: In this study we could demonstrate that by adapting test settings, DOACs levels can reliably be determined when using a well established fully automated cobas t analyser. Specific calibrators and controls and high and low applications have to be used.

OR15-02

PERFORMANCE OF THE LUMINESCENT-BASED ADAMTS13 ACTIVITY ASSAY

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Background: ADAMTS13, a metalloprotease that cleaves VWF in the A2 domain modulates the activity of Von Willebrand Factor (VWF). Thrombotic thrombocytopenia purpura (TTP) is caused by a congenital or acquired deficiency in ADAMTS13. The first-tier assay for initial diagnosis of TTP recommended by the ISTH in 2020 is ADAMTS13 activity analysis. A near patient ADAMTS13 assay is supposed to be a welcome addition to the current available test panel for measuring ADAMTS13 activity as it may support therapeutic decision-making process by limiting the laboratory turnaround time.

Aims: To develop a near patient luminescent-based ADAMTS13 activity assay with equivalent performance as the current available assays.

Methods: The basis of our chemiluminescent platform is the detection of photons generated based on enzymatic activity. For the ADAMTS13$_{lum}$ assay, a chimeric protein construct was expressed in BL21 *E. coli* containing in frame an N-terminal NanoLuc® Luciferase followed by the VWF-A2 domain (with the ADAMTS13 cleavage site Y1605-M1606), a C-terminal His-tag, and a free cysteine at the C-terminus to enable purification and immobilization, respectively. This expressed and purified construct (N-NLuc-VWFA2-C) showed susceptibility to ADAMTS13 by SDS-PAGE. Immobilized N-NLuc-VWFA2-C, via its free-cysteine to commercially available maleimide-coated plates, was also cleaved by ADAMTS13 resulting in the release of NanoLuc. The amount of liberated NanoLuc in the supernatant resulted in the generation of photons after transfer of the supernatant to wells with the furimazine substrate (the substrate for NanoLuc). The performance of the newly designed assay was tested with recombinant ADAMTS13 (TAK-755, Takeda) titrated in ADAMTS13-deficient plasma according to the CLSI protocol EP10-A3-AMD.

Results: The designed ADAMTS13$_{lum}$ assay with an immobilized construct showed excellent concentration dependent luminescence with samples consisting of rADAMTS13 titrated in ADAMTS13 over the range of 0-2 IU/ml (R^2 0.997). Based on the EP10 protocol an inter-assay variation was observed of <18%. A direct comparison between the predicate TECHNOZYM® ADAMTS13 activity ELISA from Technoclone and the ADAMTS13$_{lum}$ assay demonstrated a slope of 0.708 and r2 of 0.907.

Summary/Conclusion: A luminescent-based assay specific for ADAMTS13 activity was developed with equal performance as current ADAMTS assay. The next step is to incorporate the assay in our EnzySystem platform to allow near patient testing.

OR15-03

MONITORING DIRECT ORAL ANTI COAGULANTS (DOAC) IN A CHEMILUMINESCENT THROMBIN GENERATION ASSAY

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Background: Bleeding remains a clinical concern for patients at risk for thrombosis treated with traditional and direct oral anticoagulants (DOACs). In general DOACs do not need any monitoring, but the following patient groups may experience an unmet medical need for DOAC therapy monitoring: acute trauma, advanced age, severe kidney disease, or other chronic medical conditions. DOAC plasma levels display a high inter- and intra-individual variation and do not necessarily reflect the coagulation status of the patient. Thrombin generation is a global hemostatic assay with the capacity to overcome this limitation. We analyzed whether our chemiluminescence-based thrombin generation assay (TGA_{lum}), that is assembled to develop a point of care use handheld system, is susceptible for anticoagulant DOAC therapy monitoring.

Aims: Evaluate the ability for our TGA_{lum} to monitor DOAC therapy.

Methods: The chemiluminescence-based thrombin generation assay was performed in a semi-automated fashion in 60 µL volume, using 40 µl 0.32% citrated plasma, and 20 µL buffer containing calcium, 1.0 pM Tissue Factor, phospholipids, a thrombin-specific aminoluciferin substrate and luciferase. The effect of Apixaban, Dabigatran and Edoxaban were assessed at concentrations of 0, 50, 250, 500 and 1000 ng/mL spiked in normal pooled plasma.

Results: All DOACs significantly affected the chemiluminescent thrombin generation assay in the therapeutic range of 50 to 250 ng/mL. Apixaban had no effect at 50 ng/mL and only a marginal effect at 250 ng/mL (Apixaban 250 ng/mL: +11% lagtime, -13% peak). Dabigatran and Edoxaban had more pronounced effects on lagtime and peak height in the therapeutic range (Dabigatran 50 ng/mL: +100% lagtime, -23% peak; 250 ng/mL: +200% lagtime, -57% peak. Edoxaban 50 ng/mL: +23% lagtime, -15% peak; 250 ng/mL: +54% lagtime, -53% peak). Effects of Apixaban and Edoxaban increased concentration dependent at 500 and 1000 ng/mL. Dabigatran completely inhibited thrombin generation at a concentration of 500 ng/mL and higher.

Summary/Conclusion: Our TGA_{lum} thrombin generation assay is a versatile tool for monitoring of DOACs. Ongoing studies are aimed at controlling/standardizing the variables associated with the assay to monitor DOAC levels in acute or home settings using a prototype device that enables nanoscale-volume chemiluminescent thrombin generation assays.

Focus Symposium - Inflammation and Haemostasis

OR16-02

DEVELOPMENT OF A MICROFLUIDIC MODEL TO EVALUATE THE EFFICACY OF COMBINATION THERAPIES AGAINST THROMBOINFLAMMATION IN THE MICROCIRCULATION

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Background: In ischemic stroke, reperfusion of the ischemic area is dependent on collateral anastomoses' function to redirect the blood flow from neighboring arteries to the site of ischemia. Efficient collateral function is curbed by thromboinflammation, which is initiated immediately after ischemia and consists of activation, recruitment and interactions between platelets and neutrophils. This leads to thrombosis and alteration of the brain-blood barrier function in the microvasculature downstream of the thrombus.

Drugs that target actors of thromboinflammation such as platelets, neutrophils extracellular traps (NETs), Von Willebrand factor, and the contact activation pathway, have emerged as potential therapies for stroke treatment. Previous studies have shown these drugs are safe and efficacious, but they have never been tested in combination.

Microfluidic flow chambers allow the study of platelet aggregation in real time as well as enabling the modulation of parameters such as coating, rate of shear stress, and choice of perfusate. Microfluidics have been shown as suitable for different models in coagulation and thrombosis, and could thus potentially be useful tools to study the mechanics of thromboinflammation in small vessels.

Aims: The aim of this study is therefore to develop a microfluidic model for the evaluation of combination therapies to prevent thromboinflammation in the microcirculation.

Methods: In order to determine the best coating to mimic thromboinflammation, microfluidic channels (width: 400 µm x depth: 100 µm x length: 28 mm, volume: 1.12 µL) were coated with collagen, neutrophils previously isolated from human blood and stimulated overnight with phorbol myristate acetate (PMA) to produce NETs, or both. To visualize the mechanisms of thromboinflammation, citrated human blood was stained with immunofluorescent labels for DNA, platelets, fibrin and Von Willebrand factor. To determine the best concentration of calcium for fibrin fibers formation without occlusion of the channel, the blood was recalcified at increasing concentrations ranging 2-5 mM $CaCl_2$. It was subsequently perfused at arterial (60 µL/min) or venous (8 µL/min) shear rates for up to 20 minutes.

Results: Preliminary results indicate that both platelet aggregates and fibrin formation were both increased at venous shear compared to arterial shear rate. Coating with collagen and neutrophils also led to an increase in platelet aggregates number and fibrin formation, with the NETs strands guiding fibrin fibers

development. Recalcification also caused an increase in fibrin formation, with an optimal concentration of 4.25mM leading to consequent fibrin fibers synthesis without causing occlusion of the channel.

Summary/Conclusion: The microfluidic model using a collagen with stimulated neutrophils at venous shear rate and recalcified at 4.25 mM $CaCl_2$ appears to be suitable for the study of thromboinflammation. Future experiments will evaluate the efficacy of combination therapies against different actors of thromboinflammation using this model.

OR16-03

POLYPHENOLS ATTENUATE INCREASED PLATELET-DERIVED REACTIVE OXYGEN SPECIES (ROS) AND PLATELET PROCOAGULANT ACTIVITY IN HYPERGLYCAEMIA

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Background: Plant-derived compounds (polyphenols) have been shown to modulate post-prandial glycaemic response and reduce oxidative stress. These, amongst other, are reported observations associated with the potential beneficial effect of polyphenols at decreasing cardiovascular risk in patients with diabetes. Whether polyphenols can contribute to decreased thrombosis risk by attenuating platelet-derived reactive oxygen species (ROS) and platelet procoagulant activity remains to be explored.

Aims: This study aims to determine the effect of polyphenols on platelet ROS, platelet mitochondrial density, procoagulant platelet development and fibrin clot structure in normo- and hyperglycaemia.

Methods: Four polyphenols shown to modulate glycaemic response *in vitro* (resveratrol, hesperetin, epigallocatechin gallate (EGCG) and quercetin) were selected for analyses. Platelet ROS was measured in isolated platelets from healthy volunteers (in isolation buffer containing 5 or 25 mM glucose) following exposure to polyphenols (20 μM; 1 hr incubation) using commercially available DCFDA cellular ROS assay kit. Citrate synthase assay was used to determine platelet mitochondria density following exposure to polyphenols (20 μM; 1 hr incubation) in platelet lysates from healthy volunteers. Procoagulant platelets (annexin-V) and fibrin fiber density (AlexaFluor 488 fibrinogen) were analysed in platelet-rich plasma (PRP) clots by laser scanning confocal microscopy in normo- (5 mM glucose; 20 mins incubation) and hyperglycaemic (25 mM glucose; 20 mins incubation) conditions. Clot pore size was measured by permeation analysis in PRP and platelet-poor plasma (PPP) in normo- and hyperglycaemic conditions, as above. Tyrosine kinase inhibitors (namely, PRT-060318, ibrutinib and dasatinib) shown to impact clot structure and procoagulant platelet development were used in bioenergetic analysis as a comparator.

Results: Platelet ROS and platelet procoagulant number were increased in hyperglycaemic conditions. Resveratrol, quercetin and EGCG reduced platelet ROS in normo- (43±15%, 60±9% and 49±15%, respectively) and hyperglycaemic conditions (72±7%, 71±15% and 57±12%, respectively). Platelet mitochondrial density was decreased by 36% by quercetin and EGCG (±11 and 3%, respectively) compared to control. PRT-060318 and dasatinib significantly decreased ROS in normoglycaemia (38±8% and 34±19%, respectively). Tyrosine kinase inhibitors had no effect on ROS in hyperglycaemia or mitochondrial density in either condition. Procoagulant platelet number was reduced following resveratrol treatment in normo- (22±3 and 39±7 for resveratrol treated and control, respectively; $p<0.05$) and hyperglycaemic conditions (32±5 and 48±5 for resveratrol treated and control, respectively; $p<0.05$). No significant effects on fibrin fiber clot density or clot pore size were observed following polyphenol treatment.

Summary/Conclusion: Our data show that polyphenols modulate platelet bioenergetics and procoagulant activity. Furthermore, our results indicate that specific polyphenolic compounds may counteract hyperglycaemia-induced increases in cellular ROS and platelet procoagulant activity. These findings may have important implications for treatments aimed at reducing thrombosis risk in patients with diabetes.

Oral Communication – Thrombotic Risk Factors

OR01-01

GENETIC ASSOCIATION OF TISSUE FACTOR PATHWAY INHIBITOR (TFPI: RS7586970 VARIANT) WITH CIRCULATING TISSUE FACTOR PATHWAY INHIBITOR LEVELS AND ISCHEMIC CORONARY ARTERY DISEASE

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Background: Regulation of the tissue factor pathway inhibitor (TFPI) plasma levels is a complex array of processes including genetic and non-genetic factors. The identification of the contribution of the TFPI-rs7586970 genetic variant to the ischemic coronary artery disease (ICAD) which constitutes a multifactorial, pathologic process is challenging.

Aims: To assess the TFPI plasma level and the TFPI-rs7586970 genetic variant as risk factors in patients with ischemic coronary artery disease (ICAD).

Methods: A descriptive case control study in the Premises of the department of Clinical Pathology and Cardiology in Suez Canal University Hospitals, Ismailia, Egypt. The study population included 156 individuals; 78 ICAD patients & 78 apparently healthy individuals as a control group. All participants were subjected to interview questionnaire, clinical and laboratory assessment, quantitative TFPI plasma level using sandwich ELISA and molecular genotyping of TFPIrs7586970 genetic variant using Real-time PCR Eva-Green-based high resolution melting curve (HRM) analysis. An informed consent was taken from all participants, the study protocol was approved by the Ethics Committee, Faculty of Medicine, Suez Canal University. Significant difference between two groups was calculated using t-test, Mann-Whitney U test, chi-squared-test ($\chi 2$), one-way ANOVA (analysis of variance), Kruskal-Wallis test and binary logistic regression model.

Results: A statistically significant increase in the mean TFPI plasma level in the CAD patients group as opposed to control group (124.85 ± 69.51 ng/mL, 40.11 ± 10.46 ng/mL) which could be attributed to TFPI increased release from injured vascular endothelium, and might be a compensatory reaction to increased (TF) levels in myocardial infarction, as increased levels of TF can overwhelm the inhibitory capacity of TFPI, resulting in thrombosis. The ROC for the plasma TFPI level showed that the TFPI plasma level of more than 60.4 ng/ml as a cut-off value, can detect the occurrence of MI [95% confidence interval (CI): 0.918 to 0.986, with a sensitivity of 80.77% and a specificity of 98.72%. Positive predictive value and negative predictive value of the TFPI were 98.40% and 83.70% respectively, in diagnosing MI.

The ICAD group recorded 26 allele copies of the TFPI-rs7586970 genetic variant whereas the control group recorded 6 copies. The binary logistic regression model for ICAD occurrence showed that the TFPI-rs7586970 genetic variant polymorphism is a significant predictor for the development of ICAD.

Summary/Conclusion: Our results indicate that the TFPI-rs7586970 variant is associated with the susceptibility of ICAD and can be used for predicting the risk of its development. TFPI plasma level was higher in ICAD patients; TFPI is a good diagnostic biomarker in ICAD and an independent risk factor of this

disease. Our findings could contribute to the elucidation of the TFPI-rs7586970 variant genetic basis, role and biological pathways responsible for circulating TFPI levels and the development of ICAD.

OR01-02

HISTO-BLOOD GROUP ABO SYSTEM TRANSFERASE LEVELS AND RISK OF VENOUS THROMBOEMBOLISM

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Background: Histo-blood group ABO haplotypes A1 and B are established genetic risk factors for venous thromboembolism (VTE). Although the ABO locus influences haemostasis, the biological mechanism(s) through which ABO determines thrombotic risk remains poorly understood. The glycosyltransferase histo-blood group ABO system transferase (BGAT) is the gene product of the ABO gene, and this transferase catalyses the addition of specific sugar residues that affect the quantity and function of several proteins involved in haemostasis.

Aims: To investigate whether plasma BGAT levels are associated with risk of future first-time VTE.

Methods: We established a case-cohort study with 294 first-time VTE cases occurring within 5 years of blood sampling and a randomly sampled age-weighted sub-cohort of 1066 derived from a population-based cohort – The HUNT3 Survey. Blood samples (EDTA plasma) were collected at inclusion (2006-08), stored at -80°C, and subjected to aptamer-based proteomics (SomaScan® Assay v4.1) in 2022. Proteome-wide analyses revealed a significant association between plasma BGAT levels and VTE risk after adjustment for multiple testing using Bonferroni correction (significance threshold: $p < 6.9\times10^{-6}$). To further explore the association between BGAT and VTE, weighted Cox-regression models adjusted for age, sex, and body mass index were used to calculate hazard ratios (HR) with 95% confidence intervals (CI) according to quartiles of BGAT levels (Q1-4). Q1, the lowest BGAT levels, was used as reference. Ethical approval and informed consent were obtained for all study participants.

Results: We found a threshold effect between plasma BGAT levels and VTE risk. Compared to individuals in Q1, those with plasma BGAT levels in Q2 had a HR of 2.02 (95% CI 1.33-3.04), those in Q3 had a HR of 2.53 (95% CI 1.68-3.81), and those in Q4 had a HR of 2.22 (95% CI 1.47-3.35) for first-time VTE.

Summary/Conclusion: Our results indicate that individuals with low plasma BGAT levels are protected against the risk of future VTE. Even though BGAT levels are strictly regulated by ABO haplotypes, future studies on the relationships between ABO haplotypes, BGAT levels, and other ABO-related proteins, may shed new light on the role of ABO haplotypes in the pathogenesis of VTE.

OR01-03

THE OCCURRENCE OF THROMBOPHILIC RISK FACTORS IN PATIENTS WITH CENTRAL RETINAL ARTERY OCCLUSION AND ITS RELATION TO ULTRASOUND PARAMETERS

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Background: Central retinal artery occlusion (CRAO) is a common cause of blindness and visual morbidity. In the majority of cases, it is related to thrombotic embolism. Nevertheless, the role of inherited or acquired thrombophilic risk factors in CRAO pathogenesis has not been comprehensively studied.

Aims: The aim of this study was to analyse the thrombophilic risk factors in a group of patients with CRAO and evaluate the relationship between these factors and carotid intima-media thickness (IMT) and transthoracic echocardiography (TTE) parameters.

Methods: In 126 CRAO patients (66 [52.4%] men, median age 55 [range: 18–80] years) and 107 matched controls (56 [52.3%] men, median age 53 [range: 34–78] years) we evaluated classical atherosclerotic risk factors, including serum lipid profile and glucose level, analyzed IMT of external carotid arteries and performed TTE. Furthermore, we established the prevalence of inherited and acquired thrombophilic risk factors, such as factor V Leiden (FVL) and prothrombin 20210 G/A genetic variants, plasma activity of factor (F) VIII, protein C, and antithrombin and free protein S levels. We also assessed the presence of antiphospholipid antibodies (APLA) and evaluated blood homocysteine in all enrolled subjects. Moreover, we estimated the occurrence of Val34Leu polymorphism of the A subunit of coagulation factor XIII (FXIII-A) in both groups as a potential thrombosis-protecting factor.

Results: Among traditional atherosclerotic risk components, obesity/overweight and hypercholesterolemia were the most common in the CRAO group and occurred in 103 (81.7 %) and 85 (67.5 %) patients, respectively. CRAO patients also had elevated IMT and altered echocardiographic parameters, indicating diastolic cardiac dysfunction. In thrombophilia investigations, at least one laboratory risk factor occurred in 73.0 % (n = 92) of CRAO patients, with APLA as the most frequent, detected in 38.1 % (n = 48) of them (almost seven times more frequent than in controls, $p<0.001$). Deficiencies in protein C activity and free protein S levels were also common in the CRAO group, reported in 17.5 % (n = 22) and 19.8 % (n = 25) of patients, respectively. Interestingly, among two analyzed prothrombotic genetic variants, only the FVL was related to CRAO, with the allelic frequency 2.4 times more prevalent than in controls ($p = 0.044$). Finally, the CRAO group was characterized by hyperhomocysteinemia, almost twice as common as in controls ($p = 0.026$). Antithrombin deficiency, elevated FVIII, and FXIII-A Val34Leu polymorphism were not associated with CRAO. In a multiple regression model, among thrombophilic risk factors, elevated FVIII, anticardiolipin antibodies in IgG class, free protein S level, protein C activity, and homocysteine predicted greater IMT values, explaining 27% of the IMT variability. Moreover, TTE parameters describing thicker heart walls correlated positively with several thrombophilic risk factors, including free protein S, ACL antibodies in IgM class and FVIII activity.

Summary/Conclusion: Our findings suggest that thrombophilia plays a vital role in the pathogenesis of CRAO. Furthermore, several thrombophilic risk factors showed a relationship with intima-media thickness, a prognostic marker of atherosclerosis, and TTE parameters, routinely used in cardiovascular system evaluation. Thus, proper laboratory screening should be considered in the primary and secondary prevention of those episodes, with implementing appropriate therapy as needed.

OR01-04

CORRELATION BETWEEN ETP-BASED APC RESISTANCE AND THE RELATIVE RISK OF VENOUS THROMBOEMBOLISM IN WOMEN USING COMBINED ORAL CONTRACEPTIVES

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Background: The International Society on Thrombosis and Hemostasis (ISTH) Scientific and Standardization Committee (SSC) supports the potential use of the endogenous thrombin potential-based activated protein C resistance (ETP-based APC-R) to assess acquired APC resistance. The implementation of this assay into routine clinical practice requires this test to be recognized as a surrogate biomarker for venous thromboembolism (VTE) risk assessment.

Aims: This study aims to evaluate acquired APC resistance induced by combined oral contraceptives (COCs), using the ETP-based APC-R assay and to refine an existing VTE prediction model.

Methods: ETP-based APC-R was assessed on 197 plasma samples. Values from non-COC (n=56) and COC users (ethinylestradiol (EE) with levonorgestrel (n=23 for EE 20µg, n=34 for EE 30µg), desogestrel (n=25 for EE 20µg, n=6 for EE 30µg) or cyproterone acetate (n=6)) were used to build the VTE prediction model. Relative risks (RR) of VTE associated with these COCs were extracted from published epidemiological studies. The model performance was challenged by estimating VTE RR of 3 other COCs, ethinylestradiol/dienogest (n=11), estradiol/nomegestrol acetate (n=5) and estetrol/drospirenone (n=34), based on their ETP-based APC-R values.

Results: The model showed a Spearman's rank correlation of 0.94. Based on this model, RR estimates were 3.55 for ethinylestradiol/dienogest, 1.66 for estradiol/nomegestrol acetate and 1.59 for estetrol/drospirenone versus non-COC users

Summary/Conclusion: The model predicted RR estimates concordant with recently published post marketing surveillance data comparing COC VTE risk versus non-COC users. The lower predicted risk for estetrol/drospirenone fits with results from clinical studies showing a low impact of this new combination on hemostasis parameters. These findings support that ETP-based APC-R could become a surrogate biomarker for estimating the VTE risk of a particular COC, which represents the main cause of acquired APC resistance in a young population.

OR01-05

TARGETED PANEL GENE SEQUENCING FOR IDENTIFYING RARE VARIANTS ASSOCIATED WITH VENOUS THROMBOEMBOLISM (VTE): APPLICATION TO 139 PATIENTS WITH UNPROVOKED VTE AND NEGATIVE THROMBOPHILIA SCREENING

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Background: The current clinical assessment of inherited thrombophilia only explains a small portion of episodes of venous thromboembolism (VTE). However, this assessment is limited to the search for 5 biological defects (Antithrombin/Protein C/ Protein S deficiencies, and factor V Leiden and prothrombin G20210A variations).

Aims: The objective of our study is to evaluate the contribution of high-throughput sequencing for the identification of potential variations responsible for VTE in patients with a current negative thrombophilia screening.

Methods: 139 VTE patients were selected for next generation sequencing of a panel of 24 genes previously described as associated with the risk of VTE: *F2, F3, F5, F7, F8, F9, F10, F11, F12, F13A1, F13B, FGA, FGB, FGG, KNG1, PROC, PROCR, PROS1, SERPINC1, SERPINE1, SLC44A2, TFPI, THBD* and *VWF*. Any variation identified in coding/regulatory regions and presenting an allelic frequency in the general population lower than 0.1% was classified according to the recommendations of the American College of Medical Genetics. Only variations of class 3 (uncertain significance), 4 (potentially deleterious) and 5 (pathogenic) were recorded as candidates.

Results: In the studied population, the mean age at the first episode of VTE was 35 years; 68% of patients had a first-degree family history of VTE. And the observed sex ratio was 0.99. 56 candidate variations were identified in 51 patients, most of these being of class 3 (n = 35; 62%). 22 variations were located in the *SERPINC1*, *PROC*, and *PROS1* genes, encoding for antithrombin, protein C, and protein S. Among them, we observed 3 recurrent variations: 3 patients had the Dublin variation (p.V30E) and 2 patients had the Cambridge II variation (p.A416S) located on *SERPINC1*; 5 patients carried the Heerlen variation (p.S501P) located on *PROS1*. Of these 22 variations, 19 were identified in patients with normal plasma levels for the corresponding protein. The other 3 variations were detected in patients on anticoagulant therapy. Among the other genes evaluated, *F2* had the largest number of class 4 variations (n = 4). Three of them were not present in general population databases. The first one, p.R541W, has already been described in the literature. We found it in 2 unrelated patients suggesting that it could be a recurrent variation in symptomatic patients. The second variation, p.R425L, is not reported in the literature. It was also identified in 2 unrelated patients. The 2 identified variations segregate with the thrombotic phenotype in the explored families. The last variation, p.R596Q, has been described in the literature as the prothrombin Belgrade.

Summary/Conclusion: This study allowed the identification of variations potentially responsible for episodes of venous thromboembolism (VTE) despite a negative standard thrombophilia screening. The rate of identification of potentially pathogenic variations was 14%. When considering class 3 variations, this rate was 37%. Sequencing of coagulation inhibitor genes could identify potentially deleterious variations in the absence of plasma deficiency. Functional studies are mandatory to validate the role of newly identified variations.

OR01-06

NET CLINICAL BENEFIT AND COST-EFFECTIVENESS OF INDEFINITE ANTICOAGULATION AMONG CLINICALLY RELEVANT SUBGROUPS OF PATIENTS WITH FIRST UNPROVOKED VENOUS THROMBOEMBOLISM

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Background: Clinical practice guidelines recommend continuing anticoagulation indefinitely over discontinuing anticoagulation after completing 3 to 6 months of initial treatment for a first unprovoked venous thromboembolism (VTE), yet the benefit-harm tradeoffs and cost-effectiveness of indefinite treatment, which likely vary between patient subgroups, have not been formally assessed. While a randomized controlled trial (RCT) would be the optimal study design to provide evidence for or against continuing anticoagulation indefinitely in patients with a first unprovoked VTE, it is unlikely to be conducted due to the lifelong (i.e. until death) follow-up and extremely large sample size that would be required. Decision-analytic methematical modelling can provide evidence to inform guidelines under circumstances in which RCTs are unfeasible.

Aims: To examine the benefit-harm tradeoffs of indefinite anticoagulation and determine its cost-effectiveness in clinically relevant subgroups of patients with first unprovoked VTE.

Methods: Using best available evidence from the published literature, we conducted a decision-analytic modeling study to simulate lifetime outcomes (recurrent VTE, major bleeding, costs, and quality-adjusted life years [QALYs]) for two hypothetical cohorts of patients with a first unprovoked VTE that had completed 3 to 6 months of initial anticoagulant treatment – one cohort intended to continue anticoagulation indefinitely and another intended to discontinue anticoagulation. We created a probabilistic Markov model adopting a 1-month cycle length and a third-party payer perspective pertaining to the Canadian publicly-funded healthcare system. We examined the following patient subgroups: men, women, patients with an initial isolated deep vein thrombosis (DVT), patients initial isolated pulmonary embolism (PE), patients aged 35 years, and patients aged 75 years. Future costs and QALYs were discounted at an annual rate of 1.5%. The model structure, input parameters, and assumptions were validated by clinical experts to ensure that they coincided with current clinical practice.

Results: In patients with an initial PE, continuing anticoagulation indefinitely cost $12 014 more and added 0.123 QALYs (or 45 days of perfect helath) per person, with a 80% probability of providing an incremental benefit in QALYs and a 24% probability of being cost-effective. In all other patient subgroups, indefinite anticoagulation was dominated (i.e., higher costs, no improvement in QALYs) with a low (range 0% to 31%) probability of an incremental benefit in QALYs and a 0% chance of being cost-effective. In a two-way sensitivity analysis, an annual risk for major bleeding during extended anticoagulation below 0.67% was required for indefinite anticoagulation to provide an incremental benefit in QALYs, regardless of the risk for recurrent VTE after discontinuing anticoagulation.

Summary/Conclusion: Continuing vs. discontinuing anticoagulation indefinitely might provide a mortality benefit in certain subgroups including patients with an initial PE or those at a very low risk for major bleeding. However, indefinite anticoagulation is unlikely to ever be cost-effective. With the tightly balanced mortality tradeoffs, incorporating patient preferences and values in shared decision-making and using validated clinical prediction tools to allow individualized assessment of the long-term risks for both recurrent VTE and major bleeding should be emphasized in deciding treatment duration for unprovoked VTE.

Oral Communication – Vessel Wall Biology

OR02-01

NATIVE VASCULAR EXTRACELLULAR MATRIX COATING FOR IN VITRO CARDIOVASCULAR MODELS

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Background: While vascular cell types and their activities have been studied extensively in the past, components of the extracellular matrix often are neglected in developing suitable *in vitro* models for studying cardiovascular diseases. The crosstalk between cells and matrix is crucial for vessel stability, as well as cellular migration, cytoskeleton organization, and cellular signalling. Current *in vitro* models frequently utilize singular types of matrix proteins or mouse tumour-derived Matrigel® to coat culture plates, which do not accurately represent the *in vivo* situation.

Aims: The aim of our study was to extract and characterize native human and bovine extracellular matrix from blood vessels to improve translatability of *in vitro* models for cardiovascular diseases.

Methods: Matrix coatings were produced from vascular tissue by cryogenic grinding, decellularization steps in concentrated NaCl, solubilization with urea buffer, and sterilization of the material. Characterization was done by protein electrophoresis and mass spectrometry. Cellular apoptosis and proliferation were measured using HUVECS and induced pluripotent stem cell-derived smooth muscle cells. Platelet reactivity was assessed using light transmission aggregometry and flowcytometry.

Results: A differential abundance in proteins was found in vascular matrix coating compared to Matrigel® in mass spectrometry. Processes related to cellular motility, adhesion, and endocytosis were shown to be significantly overrepresented in Gene Ontology enrichment analysis. The matrix coating supported proliferation and cell survival of HUVECs and induced pluripotent stem cell-derived smooth muscle cells similar to Matrigel®, collagen, and fibronectin. Despite the abundance of collagens in the coating, limited platelet reactivity to the matrix coating was observed.

Summary/Conclusion: In the future we aim to investigate extracellular matrix from distinct vascular beds for differential effects on cellular phenotype and function in combination with the application of flow. Our preliminary results highlight the potential for our matrix coating as an alternative *in vitro* cell culture coating which more accurately depict the *in vivo* situation.

OR02-02

ENDOTHELIAL P2X7 PROMOTES VENOUS THROMBOEMBOLISM

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Background: Venous thromboembolism (VTE) affects 117 people per 100,000 each year and is an important cause of morbidity and mortality. VTE can lead to 1) death through pulmonary embolism, 2) the post-thrombotic syndrome, or 3) pulmonary sequelae that dramatically impair quality of life. Thrombosis has long been characterized by the Virchow's triad encompassing hypercoagulability, venous stasis and vascular wall damage. However, inflammatory pathways are now well recognized mechanisms involved in the physiopathology of venous thromboembolism. Endothelial cells play critical roles in regulating immune functions in response to PAMPs and DAMPs. During inflammation, adenosine triphosphate (ATP) is released in the extracellular compartment and is recognized as a danger signal by endothelial cells. ATP can interact with the CD39/CD73 system involved in the metabolism of ATP into AMP. Recent data indicated that the CD39/CD73 system might protect against venous thrombosis by downregulating the NLRP3 inflammasome. ATP can also interact with the P2X7 receptor involved in a wide range of responses including NLRP3 inflammasome activation leading to IL1β production. However, the role of P2X7 receptor in VTE is unknown.

Aims: To determine how the endothelial P2X7 receptor contributes to venous thromboembolism.

Methods: After pretreatment with TNFα, HUVECs were incubated with BzATP alone or with thrombin. Immunofluorescence, western blot and real-time quantitative PCR analyses were used to study P2X7 expression in endothelial cells, activation of p38 and NFκB signaling pathways and gene expression, respectively. The P2X7 expression was also study in in vivo model.

Results: We confirmed that HUVECs expressed P2X7 receptor in vitro and in vivo after induction of venous thrombosis in an experimental model. BzATP and thrombin induced the activation of p38 and NFκB signaling pathways. Interestingly, the TNFα priming is associated with an increase of NLRP3 and IL1β expression. There expressions are observed in ARN and in protein. In addition, ICAM-1 and VCAM-1 expression was increased, while thrombomodulin expression was decreased. We also discusses of the P2X7 impact on the thrombus composition and size in P2X7 -/- mouse.

Summary/Conclusion: Our data suggest that ATP released in the extracellular space during venous thrombosis induced inflammasome activation in endothelial cell through P2X7 activation. P2X7 might have a pro-thrombotic role exacerbating venous thromboembolism.

OR02-03

PAR1 KNOCK OUT IPSCS-DERIVED VASCULAR SMOOTH MUSCLE CELLS DISPLAY HIGHER TISSUE FACTOR-DEPENDENT THROMBIN GENERATION COMPARED TO WILD TYPE

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Background: Vascular smooth muscle cells (VSMCs) undergo phenotypic switching that influences vascular remodeling. Activation of protease-activated receptors (PARs) may influence phenotypic switching of VSMCs**.** While absence of the thrombin receptor (PAR1) in mouse embryos results in partial lethality with cardiovascular failure, the precise role of PAR1 in vascular development and vascular remodeling is yet to be fully understood.

Aims: Primary aims of this study are to assess the effect of PAR1 deficiency on VSMCs differentiation as well as the effect of PAR1 on thrombogenicity and phenotype switching of VSMCs.

Methods: PAR1 was knocked out in human induced pluripotent stem cells (iPSCs) using CRISPR/Cas9 technology. PAR1 KO and control iPSCs were differentiated into induced VSMCs (iVSMCs). Differentiated iVSMCs were characterized by immunocytochemistry of canonical VSMC markers. Calibrated Automated Thrombogram (CAT) was used to assess thrombin generation of PAR1 KO and control iVSMCs. Additionally, PAR1 KO and control iVSMCs were induced to phenotypic switching by platelet-derived growth factor-BB (PDGF-BB) whereafter VSMCs marker expression was measured by immunocytochemistry and western blotting.

Results: PAR1 KO-iVSMCs displayed a fully mature phenotype comparable to control iVSMCs. However, PAR1 KO iVSMCs showed higher Tissue Factor (TF)-dependent thrombin generation compared to control. Furthermore, TF expression was significantly increased in PAR1 KO iVSMCs compared to control. While both control and PAR1 KO VSMC marker expression significantly decreased in response to PDGF-BB, TF expression only increased in control iVSMCs treated with PDGF-BB.

Summary/Conclusion: PAR1 neither has an effect on canonical iVSMC markers in differentiation from iPSCs, nor affect expression of VSMC markers during phenotype switching. However, increased TF expression in the PAR1 KO iVSMCs suggests that the absence of PAR1 may result in increased thrombogenicity.

OR02-04

SUSTAINABLE SYNTHETIC POLYMER FOR A MORE HEMOCOMPATIBLE AND DURABLE PROSTHETIC AORTIC VALVE

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Background: Valvular heart diseases affect about 13% of the elder population (over 75 years old), which represents more than 100 million people worldwide. Heart valve replacement is the only available treatment nowadays, using either mechanical or biological prostheses. Mechanical valves provide long-term durability, but bring along high risk of thromboembolism. Biological prostheses provide better hemocompatibility, but they are prone to early degeneration. Considering the pressing need for a biomaterial that meets both requirements, hemocompatibility and durability, our team currently assesses new synthetic polymers with potential use for prosthetic valves. Among the polymers which are popular in the implantation field, polyurethane (PU) is one of the preferred ones. However, health and safety issues are associated with the production of toxic intermediate reagents (isocyanates) alongside with long-term implantation complications such as thrombotic events, calcification or infection.

Aims: Alternatively, our team focuses on the development of isocyanate-free polyhydroxyurethanes (PHUs), which represent the “green” alternative to PU. Our main goal is to synthesize and test innovative PHUs, particularly derived from polypropylene glycol (PPG) or polytetrahydrofuran (PTHF), aiming at improved properties when compared to medical grade PU.

Methods: PPG and PTHF-based bis(cyclic carbonate)s were synthesized and mixed with polyamines, and PHU networks were obtained by thermal-crosslinking. The materials were evaluated regarding their hemo/biocompatibility by incubating PU and PHUs with different blood components and human cells. After hemo/biocompatibility verification, tri-leaflet prosthetic heart valves (diameter of 19 mm) were produced by injection molding, acquiring the 3D structure previously designed by computational fluid dynamic simulation (ANSYS software). The design of the valves was defined to comply with strict hydrodynamic regulatory requirements. The produced valves were tested in a pulse duplicator (*ViVitro* labs).

Results: PPG-PHU and PTHF-PHU did not show hemolytic effects, both inducing less than 2% of hemolysis, similarly to PU. The PHUs did not activate the contact phase of coagulation, in contrast to PU, that shortened the clotting time. Interestingly, platelet adhesion on the surface of the PHUs was drastically reduced when compared to PU surfaces. Altogether, data confirm low thrombogenicity and improved hemocompatibility of PHU, which outperforms PU. Upon contact with PHUs, human fibroblasts kept their normal growth and shape. Considering these promising *in vitro* results, PPG-PHU and PTHF-PHU aortic valves were produced and tested, showing hydrodynamic performance comparable to the bioprostheses used in clinics (*Mosaic* and *Trifecta*). PHU valves experienced low regurgitation, well below the threshold (10%), and a normal transaortic flow. The effective orifice areas of PHU valves were also regulation-compliant (>0.85 cm^2).

Summary/Conclusion: In conclusion, the work developed by our team sets up the basis for a sustainable, hemocompatible and hydrodynamic-competent prosthetic heart valve made of PHU.

OR02-05

APPLICATION OF METABOLOMICS IN THE STUDY OF NEW MARKERS OF ATHEROMA PLAQUE INSTABILITY

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Background: Carotid artery stenosis caused by a burden of atherosclerotic plaque located at vascular wall is one of the major causes of stroke in general population. Atheroma plaques can be classified as stables or unstable. Unstable plaques are more prone to the rupture and can, eventually, cause neurologic symptomatology. Circulating biomarkers of vulnerability have been previously proposed without usefulness in clinical practice.

Aims: The aim of this study is the search of molecules involved in metabolic pathway related with plaque vulnerability and, eventually, with the risk of stroke in patients with carotid stenosis. With this purpose, we have performed a metabolomic analysis of atheroma plaque and serum from patients with symptomatic and asymptomatic carotid stenosis, using Nuclear Magnetic Resonance (NMR) spectroscopy.

Methods: Fragments of carotid plaque and serum samples were obtained from asymptomatic and recently symptomatic patients during endarterectomy surgery. Symptomatology was defined as transient ischaemic attack, *amaurosis fugax*, and stroke occurred at most 21 days before surgery.

Plaque samples (23 asymptomatic and 17 symptomatic) were analyzed by using HRMAS-NMR spectroscopy. Serum samples (25 asymptomatic and 19 symptomatic) were analyzed by using high resolution NMR spectroscopy. Analysis were carried out in a 600mHz spectrometer. Univariate and multivariate analysis (Principal component analysis [PCA] and Partial least squares-discriminant analysis [PLS-DA]) were performed to identify discriminant metabolites between groups using the SPSS and SOLO software based on PLS-tools. A P-value <0.05 was considered significative

Results: The PLS-DA model calculated to discriminate plaque metabolites from symptomatic and asymptomatic patients showed a sensitivity of 81.8% and a specificity of 83.3%. N-acetylglucosamine [1][2] mioinositol and some lipids were found among the metabolites differentially expressed in plaque samples. The model performed for serum samples showed sensitivity of 92 % and a specificity of 78 %. Relative concentration of circulating isoleucine, an essential amino acid, was found different in symptomatic and asymptomatic patients. Circulating levels of Isoleucine have been previously associated with increased risk of cardiovascular disease [3].

Summary/Conclusion: We have demonstrated the feasibility of metabolomics on the study of atheroma plaque instability. We have successfully applied HRMAS-NMR and high resolution NMR spectroscopy for the detection of differentially expressed metabolites in both, plaques and serum samples, respectively. Our

results confirm the importance of circulating isoleucine in cardiovascular diseases. Plaque analysis could help to find new therapeutic targets of plaque stabilization. Identification of circulating metabolites is a useful method to deepen in metabolic pathways and to find new biomarkers that would be complementary to current image techniques helping on the stratification of high risk stroke patients. ISCIII-FEDER (PI20/01171, PI20/00075, FI21/00171)

[1] Konopka JB *et al*. N-acetylglucosamine (GlcNAc) functions in cell signaling. (2012) Scientifica 1;2012:489208.

[2] Zachara NE *et al*. The roles of O-linked -N-acetylglucosamine in cardiovascular physiology and disease (2012). Am J Physiol Heart Circ Physiol 302: H1905–H1918.

[3] Wang Y *et al.* Association of circulating branched-chain amino acids with risk of cardiovascular disease: A systematic review and meta-analysis (2022). Atherosclerosis. 350:90-96.

OR02-06

ACTIVATED PROTEIN C REGULATES ABERRANT THROMBO-INFLAMMATORY T-CELL ACTIVITY IN IMMUNOTHROMBOTIC DISEASE

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Background: Inflammatory bowel disease (IBD) patients experience a 3- to 6-fold increased risk of venous thromboembolism (VTE) compared to the general population, although the mechanistic basis for this increased risk is unknown. Pre-clinical studies indicate a crucial role for diminished anticoagulant and anti-inflammatory protein C (PC) pathway activity in IBD pathophysiology.

Aims: This study aims to evaluate the procoagulant activity of colitogenic T cells and assess the impact of APC signalling in regulating T cell pro-inflammatory and prothrombotic activity.

Methods: The pro-thrombotic potential of T cells was determined using bespoke T cell-dependent calibrated automated thrombinography assays and flow cytometry. Meta-transcriptomic and gene expression analysis were used to assess the dysregulated expression of coagulation and T cell-associated genes in IBD patient gut biopsies. To elucidate the potential anti-inflammatory effect of activated protein C (APC) on T cells, *ex vivo* functional assays were established using donor PBMC-derived T cells treated with either wild-type or recombinant APC variants. Flow cytometry and ELISAs were used to measure signature cytokine outputs.

Results: Notably, pro-inflammatory $CD4^+$ T cells were procoagulant in plasma. Following activation, Th0 and Th1 cells promoted rapid plasma thrombin generation, which depended on upregulated TF gene expression (*F3*) and procoagulant activity. TF is typically expressed in an encrypted state to prevent aberrant thrombin generation and requires decryption to be fully procoagulant. To evaluate TF decryption on the $CD4^+$ T cell surface following activation, we measured the expression of acid sphingomyelinase (ASMase), which has been demonstrated to confer TF decryption. ASMase expression was significantly increased in activated inflammatory Th0 and Th1 cells, indicating pro-inflammatory T cell activation such as that observed in IBD patients may contribute to their increased VTE risk.

Using meta-transcriptomic and gene expression analysis, we next determined that 20 genes involved in the regulation of coagulation, including PC (*PROC)* and its receptor (EPCR; *PROCR),* are dysregulated in IBD and revealed an environment permissive to, but deficient in, PC-pathway anti-inflammatory activity in the gut of IBD patients. Functional studies revealed that APC regulates $CD4^+$ T-helper (Th) cell inflammatory responses, inhibiting colitogenic Th1 and Th17 activity. Moreover, APC enhanced the generation of tolerogenic $FOXP3^+$ regulatory T-cells. To evaluate the receptor requirements for APC-mediated signalling on T cells, we generated novel recombinant APC variants with a spectrum of EPCR binding affinities and assessed their ability to modulate colitogenic T-cell activity. Interestingly, APC variants deficient in EPCR binding mediated similar capacity to restrict Th1 and Th17 generation and inflammatory responses as wild-type APC, suggesting EPCR is dispensable for APC anti-inflammatory activity in Th1 and Th17 cells. These data highlight a non-canonical, EPCR-independent APC signalling mechanism on the T cell surface.

Summary/Conclusion: Collectively, these data provide novel insights into the potential thrombogenicity of activated T cells. Furthermore, these results indicate a prominent role for APC in regulating colitogenic T-

cell thrombo-inflammatory activity, underscoring the potential utility of therapeutically targeting this pathway to treat thromboinflammatory disease.

Oral Communication – Neutrophil Extracellular Traps

OR03-01

PLASMA BIOMARKERS OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS) LEVELS

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Background: Neutrophils Extracellular Traps (NETs) is an emerging biomarker hypothesized to associate with many human diseases including cancer, Alzheimer and various cardiovascular disease including venous thrombosis (VT). While NETs plasma levels have been observed to correlate with many inflammatory markers (eg interleukin, TNF-α, CRP), the exact molecular mechanisms underlying their inter-individual variability remain to be elucidated.

Aims: In this work, we aimed to identify new molecular components that could be participate to NETs regulation.

Methods: A targeted plasma proteomic profiling was deployed in the FARIVE case-control study for VT where NETs were quantified by measuring myeloperoxidase (MPO)-DNA complexes using an in-house capture ELISA in a subsample of 410 VT patients and 327 controls. FARIVE plasma samples were profiled for 376 Human Protein Antibodies (HPAs) designed to target 285 independent proteins selected for being involved in endothelial and platelets functions as well as in various cardiovascular traits. Associations of each HPA with NETs were tested using a log-link based Compound Poisson-Gamma model to handle the semicontinuous distribution of the trait and were adjusted for age, sex, smoking, case-control status and internal controls.

Results: Among the 376 tested HPAs, 2 correlated (ρ=0.48) ones passed the study-wide statistical threshold of $p=2.8x10^{-4}$ for declaring significance: One standard deviation (SD) increase of CLEC3B HPA034793 was associated with a 60% ($p=5.6x10^{-6}$) decreased expression of NETs. Similarly, one SD

increase of GPR183 HPA013784 was associated ($p=1.5x10^{-4}$) with a 69% decrease NETs expression. Altogether these 2 HPAs explained 4.1% of NETs variability. Of note, in this study, the association of CRP levels and NETs was close to significance ($p=3.7x10^{-3}$).

Interestingly, *CLEC3B* has recently been shown to be involved in inflammatory mechanisms, particularly through the alteration of platelet and neutrophil degranulation. In addition, *CLEC3B* is associated with plasma levels of tetranectin, a regulator of plasminogen activation, and studies have shown that NETs contribute to the resistance of plasminogen activation. The *GPR183* gene is anti-sense to UBAC2 gene, an ubiquitin associated protein that has been also shown experimentally to participate to NETs regulation. Whether the GPR183 association we observed here is indirectly related to the association known at UBAC2 remains to be elucidated.

Summary/Conclusion: This work provides new insights on the molecular players participating to the regulation of NETs plasma levels and new support from human epidemiological data for a role of CLEC3B in NETs biology. How these data translate to human diseases deserves additional extensive investigations.

OR03-02

POTENTIAL ROLE OF NETOSIS BIOMARKERS IN DISEASE DIAGNOSIS AND MANAGEMENT IN HIGH-GRADE SEROUS OVARIAN CANCER

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Background: Neutrophils are involved in cancer progression by releasing neutrophil extracellular traps (NETs). NETosis seems a pre-requisite for the establishment of omental metastases in early stages of high-grade serous ovarian cancer (HGSOC), the most lethal subtype. However, its role in advanced stages remains unknown.

Aims: To characterize a NETosis biomarker profile in biofluids from patients with advanced HGSOC.

Methods: Five biomarkers of NETosis were quantified in plasma and paired peritoneal fluid (PF) of 45 patients and 40 control women: cell-free DNA (cfDNA), nucleosomes, citrullinated histone 3 (citH3), calprotectin and myeloperoxidase (MPO) with specific kits. DNaseI activity was measured with the SRED. Finally, we created a NETosis score to assess the discriminative capacity of NETosis biomarkers between clinical groups.

Results: HGSOC patients presented a higher concentration of cfDNA, citH3 and calprotectin in plasma, and of all NETosis biomarkers in PF, than controls (Figure 1). DNaseI was not responsible for the cfDNA increase. These biomarkers showed a strong ability to differentiate both groups, mainly in PF, by means of ROC curves (Figure 2). Interestingly, neoadjuvant treatment (NT) seemed to reduce NETosis biomarkers mainly systemically (plasma) compared to the tumor environment (PF). Our NETosis score allowed to clearly differentiate HGSOC from control women with the levels of the NETosis biomarkers in PF employing a cut-off value of 23% (NETosis score >23 corresponds to HGSOC patients).

Summary/Conclusion: NETosis biomarkers are present in the tumor environment of advanced HGSOC patients, which might contribute to disease progression. Plasma cfDNA and calprotectin could represent minimally invasive surrogate biomarkers for HGSOC. NT modifies NETosis biomarkers mainly at the systemic level. We have developed a NETosis score that allows the discrimination of HGSOC patients from controls. ISCIII-FEDER (PI20/00075, FI21/00171, PI22/01872), GVA (ACIF/2020/216), FIHGUV Awards (2020, 2021), SETH (2020, 2021), AECC.

OR03-03

IMPAIRED BALANCE BETWEEN FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS AND THEIR DEGRADATION BY DNASES IN COVID-19 PATIENTS

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Background: Immunothrombosis and in particular Neutrophil Extracellular Traps (NET) formation are key players in host defense against bacteria, viruses and fungi. But it is also a double-edged sword as excessive NETosis leads to vascular occlusion. Increased NET formation is a key feature of SARS-Cov2 infection, especially in severe forms of the disease. It is highly suspected that NETs participate in the formation of microthrombis, especially in alveolocapillaries, contributing to Acute Respiratory Distress Syndrome (ARDS). The reason for excessive NET formation during severe COVID-19 is still an open question. Some authors reported the presence of anti-NETs antibodies that may stabilize NETs and impair their degradation. NETs are physiologically degraded by DNases1 and 1L3. Lack of DNase1 and/or 1L3 leads to vascular occlusion in a sepsis mouse model, reinforcing the idea that the balance between NET and DNases is of major importance. The balance between NETs and DNase in patients with various COVID-19 severity has not previously been explored.

Aims: Our objectives were to compare the balance between NET biomarkers and DNase activity according to disease severity and study the mechanisms responsible for a possible imbalance.

Methods: One hundred and forty-five (145) COVID patients were included in the study, 93 in the non-severe outpatients group, and 52 in the inpatients group, of whom 15 had a severe disease and 37 had critical disease on admission. NETs markers (MPO-DNA, H3Cit, H3cit-DNA), total DNase activity, DNase 1 & 1L3 proteins and plasmacytoid dendritic cell & dendritic cells were evaluated. We sequenced *DNase1* and *DNase1L3* genes and analyzed scRNAseq public data from COVID-19 patients (PMID: 33879890).

Results: DNase activity was lower in the most severe patients together with impaired balance between NET markers and DNase activity. Whereas the amount of DNase1 increased in outpatients, it decreased in the most severe ones. DNase1L3 levels were similar in all subgroups and did not increase together with NET markers. *DNASE1* polymorphisms were associated with decreased amount of circulating DNase1 while a quantitative defect of plasmacytoid dendritic cells (pDC), the main cells expressing DNase1L3, was observed in critical patients. Besides, analysis of public single cell RNAseq data revealed that pDC from COVID-19 patients express less DNase1L3.

Summary/Conclusion: Severe and critical COVID-19 were associated with an imbalance between NETs and the amount and activity of DNases. Our results suggest that non severe patients with an unbalanced NET/DNase ratio could benefit most from early inhaled DNase1 administration. Besides, our results encourage the development of systemic administration of DNase, either subcutaneously, as has been very recently shown in a mouse model of COVID-19 or intravenously, as is often done in mouse models in which increased NETosis is involved.

OR03-04

PROTEOLYTIC NEUTRALIZATION OF EXTRACELLULAR HISTONES BY NEUTROPHIL ELASTASE IS ENHANCED BY HEPARIN

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Background: The release of extracellular histones during cell death or neutrophil extracellular trap (NET) formation is linked to the initiation and progression of several acute inflammatory disease like sepsis and COVID-19. As the presence and proteolysis status of extracellular histones is associated with disease severity and the risk of thromboembolic events, targeting of histones by proteolysis or complexation constitutes a potential therapeutic option. We have shown earlier that circulating levels of the serine protease neutrophil elastase (NE), released during neutrophil activation or during NET formation, associate with extracellular histone plasma levels in severe ICU COVID-19 patients. The mechanism and requirements for NE-catalyzed histone cleavage are however unclear.

Aims: We aimed to investigate the ability of NE to proteolyze extracellular histones in plasma and characterize the effect of NE-catalyzed proteolysis on histone-mediated inflammation and cytotoxicity and explored the influence of heparin on this proteolysis.

Methods: We used human activated neutrophil elastase in the presence or absence of heparin to proteolyze several classes of extracellular histones in plasma. The presence or effects of full size and fragmented histones were analyzed by Western blot analysis, mass spectrometry, and *in vitro* cell-based systems to assess TLR activation with HEK-blue TLR4 cells and cell cytotoxicity with EA.hy926 cells.

Results: All classes of extracellular histones (H1, H2A, H2B, H3 and H4) can be cleaved by NE in plasma, which resulted in multiple histone fragments reminiscent of the histone fragments observed in the plasma of COVID-19 patients. For histone H3 and H4, proteolysis significantly reduced TLR4 activation and reduced their cytotoxic potential. Addition of heparin significantly increased the rate of proteolysis of histones by NE and significantly reduced histone-mediated cytotoxicity.

Summary/Conclusion: NE is able to proteolyze extracellular histones in plasma, which results in reduced cytotoxicity and inflammation *in vitro*. Histone proteolysis can be enhanced by addition of heparins, indicating the potential beneficial combinatory treatment of heparins and NE in acute inflammatory diseases.

OR03-05

PATIENTS WITH PROTEIN C DEFICIENCY HAVE INCREASED NETOSIS, POSSIBLY MEDIATED BY REDUCED LEVELS OF CIRCULATING ACTIVATED PROTEIN C

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Background: Neutrophil extracellular traps (NETs) are highly prothrombotic networks that activate the extrinsic and intrinsic coagulation pathways. NETs also inhibit natural anticoagulants like activated protein C (APC), which also neutralizes their anti-inflammatory and cytoprotective functions. In turn, APC inhibits NETosis, which would reduce thrombotic risk and inflammation, although its relevance *in vivo* remains unexplored.

Aims: To analyze the possible regulation of NETosis exerted by APC in families with protein C deficiency (PCdef).

Methods: We obtained citrated plasma from a total of 84 individuals, 23 probands with PCdef and at least one thrombotic episode, 25 relatives with PCdef and 36 relatives without. We quantified circulating APC levels using by ELISA [1]; PCag, PCamid and PCcoag (Stago) levels; and NETosis markers cell-free DNA (cfDNA; PicoGreen, Life Technologies), citrullinated histone 3 (citH3; Cell Death Detection ELISA^{Plus} Roche, modified), DNA-histone complexes (Cell Death Detection ELISA^{Plus}) and calprotectin (Human Calprotectin ELISA Kit, Hycult Biotech). We performed the statistical analysis with SPSS v21.

Results: PCdef patients have lower levels of circulating APC than their relatives without PCdef (0.87 *vs.* 0.92 ng/ml, P=0.002), as well as PCag (53.3 *vs.* 84.2 ng/mL, P<0.001). In addition, patients with PCdef have increased NETosis: H3cit markers (0.27 *vs.* 0.15 U.A., P=0.009) and DNA-histone complexes (0.16 *vs.* 0.12 U.A., P=0.031). H3cit correlated with DNA-histone complexes (0.409, P<0.0001) and PCag (-0.244, P<0.045), and PCag correlated with APC (0.347, P<0.011).

Summary/Conclusion: The reduction in APC levels in patients with PC deficiency seems to favor the increase in NETosis, as evidenced in previous *in vitro* studies. This combination of reduced anticoagulant activity and hypercoagulability induced by NETs could favor thrombotic episodes, which should be verified in large families with PC deficiency and members with and without thrombosis. ISCIII-FEDER (PI20/00075, PI20/01171, FI21/00171), GVA-CIACIF/2021/192, López Borrasca Award 2020 SETH and Grants to SETH Working Groups 2019 & 2022.

[1] Martos L et al. A simplified assay for the quantification of circulating activated protein C (2016). Clin Chim Acta 1;459:101-104.

OR03-06

EXCESSIVE INTRAHEPATIC NEUTROPHIL ACCUMULATION AND EXTRACELLULAR TRAP FORMATION IMPEDES LIVER REGENERATION AFTER PARTIAL HEPATECTOMY IN MICE AND HUMANS

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Background: The liver is the only organ that can regenerate itself; however underlying liver disease can complicate these processes. While inflammation is essential in initiating hepatic regeneration, the immunothrombotic effects that can accompany it may have harmful consequences. Hence, neutrophils play a crucial and dynamic role during regenerative processes, but their specific contribution to human liver regeneration remains unclear. Particularly as underlying liver disease, which is present in the majority of patients, significantly impacts hepatic regeneration, potentially leading to adverse post-surgical outcomes, such as post hepatectomy liver failure (PHLF).

Aims: Our aim is to investigate the dynamics of neutrophils and neutrophil extracellular traps (NETs), and their consequent role in immunothrombosis, in patients with and without PHLF. We also aim to validate the significance of these factors in a mouse model of partial hepatectomy (PHx).

Methods: We investigated the influx of neutrophils, macrophages, eosinophils, and mast cells, and the presence of their respective extracellular traps, including immunothrombosis, in liver biopsies of 35 patients undergoing hepatectomy (10 patients with PHLF), prior to and after the initiation of liver regeneration, using fluorescence microscopy. Additionally, NET formation and neutrophil activation was confirmed by plasma analysis of 99 patients (24 patients with PHLF) prior to and up to five days post-surgery. In a murine PHx model we inhibited NET formation (via DNase and protein-arginine deaminase 4 (PAD4) inhibition) and evaluated the regeneratory capacity

Results: We detected rapid intrahepatic neutrophil accumulation, elevated levels of myeloperoxidase release, NET formation and immunothrombosis in regenerating human livers, with a significantly higher increase of infiltrating neutrophils and NETs in PHLF patients. Circulating markers of neutrophil activation, including elastase, myeloperoxidase and citrullinated histone H3, correlated with markers of liver injury. In a murine PHx model, DNase treatment and PAD4 inhibition accelerated hepatocyte proliferation and liver regeneration.

Summary/Conclusion: PHLF patients show accelerated intrahepatic neutrophil infiltration, NET formation and immunothrombosis, which were associated with liver damage. Pharmacological inhibition of NETs were beneficial in a mouse model of PHx, supporting NET blockage as a potential therapeutic target in appropriately selected patients undergoing liver resection.

Oral Communication – Platelet Biology

OR04-01

PROTEIN DISULFIDE ISOMERASE 1 AND 3 (PDIA1, PDAI3) REGULATE PLATELET-DERIVED EXTRACELLULAR VESICLE (PEV) FORMATION

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Background: Protein disulfide isomerases 1 and 3 (PDIA1, PDIA3) regulate platelet activation and thrombus formation, however, their role in the formation of platelet-derived extracellular vesicles (PEVs) is still unknown.

Aims: This study aimed to evaluate the effects of inhibition of PDIA1 and PDIA3 on PEV formation in murine platelets.

Methods: Washed Platelets (WP) were obtained from C57BL/6 mice. PEV formation in murine WP was induced by convulxin or ionophore A23187 and analyzed by dedicated nano flow cytometry (A50-Micro, Apogee Flow Systems, Hertfordshire, UK). Platelet aggregate formation was evaluated by both FC and 96-well plate-based aggregation assay. PDIA1 or PDIA3 were blocked by bepristat 2a, C-3389, and C-3399, respectively. For comparison, the effects of reference antiplatelet drugs (tirofiban - antagonist of active αIIbβ3 integrin, acetylsalicylic acid - COX-1 inhibitor, and cangrelor - P2Y12 receptor antagonist) on PEV formation were evaluated.

Results: Convulxin in a concentration-dependent manner induced platelet aggregation followed by PEV formation, while calcium ionophore A23187 also promoted a burst of platelet vesiculation, but without inducing platelet aggregation. Bepristat and C-3389 dampened PEV release induced by either convulxin or calcium ionophore A23187, while C-3399 inhibitor diminished PEV formation only in response to convulxin. In contrast to C-3389, bepristat, and C-3399 reduced convulxin-induced platelet aggregation. Cangrelor and tirofiban also reduced platelet aggregation, and similarly to C-3399 inhibitor, PEV formation induced by convulxin, while these drugs did not affect PEV release stimulated with ionophore A23187.

Summary/Conclusion: Our results demonstrated the involvement of PDIA1 and PDIA3 in the regulation of PEV release and revealed their distinct role in PEV formation. PDIA3 supported PEV release associated with platelet aggregation, while the effect of PDIA1 on PEV release was aggregation-independent.

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OR04-02

PLATELET AGGREGATION AND PLASMA COAGULATION IN FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS REQUIRING SPECIFIC HYPOLIPIDEMIC TREATMENT

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Background: Elevated serum cholesterol levels lead to atherosclerosis and are associated with enhanced platelet activity. For this reason, patients suffering from familial hypercholesterolemia (FH) have a high risk of thrombotic cardiovascular events. Normalization of cholesterol levels is not an easy task and frequently requires specific treatment, e.g. proprotein convertase subtilisin kexin 9 monoclonal antibodies (PCSK9Ab) and lipid apheresis. There are currently no data on the effect of PCSK9ab on platelet reactivity. In addition, plasma coagulation is another process which can be conditioned by this disease.

Aims: The aim of the study was to analyse the differences in blood coagulation and platelet aggregation between FH patients and age-matched healthy controls as well as investigate the impact of PCSK9AB on both processes.

Methods: This study enrolled all 15 patients treated in University Hospital Hradec Králové for FH and 15 age-matched healthy volunteers. Twelve out of the 15 patients were being treated with PCSK9ab, and 8 of them were also undergoing lipid apheresis. Samples from all patients including pre- and post-apheresis period were tested for platelet aggregation and plasma coagulation. Platelet aggregation was triggered with 7 inducers (collagen, ADP, AA, U46619, TRAP, ristocetin and PAF), and analysed by impedance aggregation. In addition, the effect of 3 antiplatelet drugs (acetyl salicylic acid, ticagrelor and vorapaxar) was compared. In coagulation experiments, PT and aPTT methods were used and the effects of 4 clinically used anticoagulants (dabigatran, argatroban, rivaroxaban and apixaban) at equimolar concentrations was studied.

Results: Platelet reactivity was decreased after apheresis in general, but platelet responses were not different between non-apheresis patients treated with PCKS9ab and post-apheresis patients except for ristocetin-triggered aggregation. When compared to age-matched healthy population, treatment of FH resulted in significantly lower platelet aggregation responses to 4 out of 7 used inducers and improved the effect of 2 out of 3 used antiplatelet drugs. Similarly, the response to all tested anticoagulants was stronger in healthy individuals compared to that of FH patients. There were no differences between apheresis-treated patients and patients treated with PCKS9Ab.

Summary/Conclusion: In this study, we showed for the first time the suitability of PCKS9ab treatment on reduction of platelet reactivity and blood coagulation. An interesting finding is also higher effect of antiplatelet and anticoagulant drugs in our FH patients compared to control group.

This work was possible thanks to the finantial support from the Czech Health Research Council (NU21J-02-00021).

OR04-03

PLATELET AGGREGATION IN COLORECTAL CANCER PATIENTS UNDERGOING SURGERY

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Background: Surgical treatment of colorectal cancer (CRC) carries a risk of venous thromboembolism. Minimally invasive surgery is considered low risk compared with open surgery, but still extended thromboprophylaxis of 28 days is offered after both procedures. As an initial step, platelet aggregation was investigated to examine perioperative changes in hemostasis induced by the different surgical procedures.

Aims: The aim was to investigate perioperative changes in platelet aggregation in patients undergoing open cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) for peritoneal metastases from colorectal cancer compared with patients undergoing minimally invasive resection for localized rectal cancer.

Methods: Informed consent is obtained from all included patients. Blood samples are obtained after induction of anesthesia (baseline) and after wound closure for platelet count and platelet aggregation. Platelet aggregation is measured with Multiplate® Analyzer using ADPtest®, ASPItest®, and TRAPtest® as agonists. Results are expressed as area under the curve (AUC, AU*min) and reported as mean difference (Δ) with 95% confidence interval (CI).

Results: To date, 37 CRC patients treated with CRS+HIPEC and 33 patients undergoing minimally invasive rectal cancer resection were included. The difference in platelet count from baseline to after wound closure was increased in patients undergoing minimally invasive rectal resection and decreased in patients undergoing CRS+HIPEC (Δ 5 (-3:13) vs. -24 (-40:-8), P=0.002) (*10^9/L). Platelet aggregation was increased after minimally invasive rectal resection than following CRS+HIPEC ((ADP: Δ 250 (163:338) vs. 98 (3:193), P=0.02), (ASPI: Δ 195 (61:328) vs. 83 (4:163), P=0.14), TRAP: Δ 205 (104:307) vs. 227 (132:322), P=0.75).

Summary/Conclusion: Decreased platelet count was found after CRS+HIPEC. The platelet aggregation was increased after both surgical procedures but to a lesser extent following CRS+HIPEC. Patient inclusion will continue until fall 2023 also investigating perioperative changes in secondary hemostasis and fibrinolysis.

OR04-04

SOLUBLE AND EXTRACELLULAR VESICLE-BOUND PLASMA P-SELECTIN AS A DIAGNOSTIC BIOMARKER FOR ACUTE DEEP VEIN THROMBOSIS

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Background: P-selectin is a transmembrane protein expressed on the surface of activated platelets and endothelial cells. In plasma, P-selectin exists in a soluble (monomer) or dimer form when bound to extracellular vesicles (EVs). Elevated levels of P-selectin have been found in plasma following acute deep vein thrombosis (DVT) and reported to predict recurrent and cancer-associated VTE. Commonly used immunoassays measuring the levels of P-selectin in plasma (e.g., ELISA) cannot distinguish soluble from EV-bound P-selectin. It is therefore unknown whether specific detection of EV-bound P-selectin would improve the diagnostic performance compared to detection of total concentration in plasma (soluble and EV-bound) for VTE.

Aims: To investigate the correlation between total and EV-bound P-selectin in plasma and to assess the use of the two as a diagnostic biomarker for acute DVT.

Methods: Plasma samples were collected from a cohort of individuals admitted to Akerhus Hospital in Oslo with suspected DVT, and in whom DVT diagnosis was confirmed (n=67) or rejected (n=118). Total P-selectin was measured using a commercial ELISA kit. An in-house bead-based assay was developed to specifically detect EVs displaying P-selectin on their surface. P-selectin positive EVs were captured by beads conjugated with anti-P-selectin antibodies and detected with either a cocktail of PE-labeled antibodies against tetraspanin proteins commonly expressed on most EVs (CD9, CD81, CD63), or anti-CD41a to identify platelet-derived EVs. Samples were analyzed using flow cytometry and the results were presented as the mean fluorescence intensity (MFI) minus background (isotype control). Logistic regression models were used to estimate odds ratios (OR) for VTE across quartiles of total and EV-bound plasma P-selectin.

Results: A high correlation was found between EV-bound P-selectin detected either by tetraspanin antibodies or anti-CD41a (Pearson r=0.85, P<0.0001), whereas the correlation between total and EV-bound P-selectin levels in plasma was moderate (r=0.34, p< 0.0001). Total P-selectin levels, measured by ELISA, were a strong diagnostic biomarker of acute DVT. Patients admitted to hospital with suspicion of DVT and total plasma P-selectin levels in the highest quartile (>43.3 ng/mL) displayed a 24-fold higher risk of the diagnosis (OR 24.42, 95% CI: 5.17-115.41) compared to those admitted with plasma levels in the lowest (reference) quartile. No significant association was found between plasma levels of EV-bound P-selectin and the diagnosis of acute DVT.

Summary/Conclusion: Our findings indicate that EV-bound P-select in plasma was mostly derived from platelets and was only moderately correlated with the total level of P-selectin in plasma. Further, high levels of total P-selectin, but not EV-bound P-selectin, predicted high risk of acute DVT in patients admitted to hospital with the suspicion.

OR04-05

PLATELETS PROMOTE PERI-INFARCT CELL PROLIFERATION AND VASCULAR INTEGRITY TWO DAYS FOLLOWING FOCAL CEREBRAL ISCHEMIA

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Background: Stroke preclinical studies report that platelets play an ambivalent role at the acute phase of ischemic stroke. Through their participation in microvascular thromboinflammation following ischemia, they worsen the stroke outcome by recruiting neutrophils and propagating thrombosis. On the other hand, they also play a beneficial role by continuously preventing hemorrhagic transformation. Whether platelets intervene after the acute phase of ischemic stroke remains however unknown. Because platelets participate in healing processes, a feature that has long been taken advantage of in regenerative medicine, we postulate that platelets promote post-stroke cerebral remodeling and repair. .

Aims: The aim of this study is thus to investigate the contribution of platelets to post-stroke repair processes

Methods: Focal cerebral infarction was induced in C57Bl/6J mice by permanent occlusion of the middle cerebral artery (pMCAo, thermocoagulation). Two days after pMCAO induction, mice were treated with either a control or a platelet-depleting antibody, R300. Because platelet depletion led to an increased mortality in association with severe intracranial hemorrhage in the 1st week following pMCAO, mice were sacrificed at day 3 for assessment of infarct size, platelet and leukocyte recruitment, vascular leaks, vascular density and cell proliferation.

Results: Cresyl Violet staining showed that platelet depletion 2 days after pMCAO led to a significant increase in cerebral injury area. In addition, it caused severe haemorraghes leading to increased mortality in the thrombocytopenic mice. Immunostaining showed that platelets accumulated in the peri-infarct vasculature of mice treated with the control antibody, with no signs of vascular leaks, as assessed by the absence of extravascular IgG. In contrast, infarct enlargement in platelet-depleted mice occurred in association with massive peri-infarct vascular leaks from peri-infarct microvessels, indicated by the presence of extravascular IgG in leukocyte-rich areas. In addition, platelet depletion was associated with a reduction in cell proliferation, as estimated by quantification of KI67 positive staining and reduced vascular peri-infarct area.

Summary/Conclusion: Our work indicates that platelet depletion has a deleterious effect on brain vascular integrity and peri-infarct cell-proliferation. Moreover, platelets seem to participate to the initiation of post-stroke remodeling by preventing brain hemorrhages and leukocyte infiltration resulting in increased mortality.

OR04-06

PLATELET-EDUCATED CANCER CELLS OVEREXPRESS FIBRONECTIN AS WELL AS DIFFERENT GLYCOSYLTRANSFERASES

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Background: Cancer-associated thrombosis, also known as Trousseau's syndrome, is the second most common cause of death among cancer patients, after the disease itself. Indeed, venous thrombosis is 4 to 7 times more common in these patients. Many factors contribute to this hypercoagulable state, but one of them is the ability of tumor cells to activate platelets and induce their aggregation (TCIPA). This phenomenon of cancer cell-educated platelets is associated with higher metastatic potential. Nevertheless, our team demonstrated the opposite concept in 2019 in which platelets educate cancer cells by transferring material to them.

Aims: In this study, we aim to better understand how platelets facilitate metastasis formation during their interaction with tumor cells.

Methods: Changes in the expression of 43 glycosyltransferases (GT) families as well as 184 genes involved in inflammation and metastasis were analyzed by RT-qPCR in digestive cancer cell lines following their interaction with platelets. Functional tests (transwell migration and invasion assay, wound healing assay, ...) and holo-tomographic microscopy were used to understand the role of the identified genes of interest in glycosylation and formation of metastasis.

Results: As platelets have been previously shown to initiate and extend glycosylation of extracellular molecules, we thought they could modify the glycosylation of cancer-cell expressed proteins during their interaction. We first demonstrated, however, that platelets by themselves express very few glycosyltransferases with limited functional activities. Using holo-tomographic microscopy we observed that tumor cells that interacted with platelets have a higher dry mass than before their interaction. This may reflect a transfer of material in a process described as platelet-educated cancer cells. Platelet-educated cancer cells are also able to proliferate significantly more than cancer cells, indicating a selective advantage in the transfer of material from platelets to the cancer cells. We screened 43 different GT families in tumor cells educated or not by platelets. Three families of GT were overexpressed (B3GNT, GCNT, C1GALTC1) respectively by 200, 100 and 60% in tumor cells after their interaction with platelets. These GT significantly increased the potential glycosylation of proteins in cancer cells. Interestingly, we also found that out of 184 genes involved in inflammation and metastasis, mRNA coding for the glycoprotein fibronectin (FN1) was overexpressed by 160% in platelet-educated tumor cells. In tumor cells, knocking down FN1 significantly affects the migration and proliferation of the cells.

Summary/Conclusion: We concluded that the interaction between tumor cells and platelets can induce the overexpression of different glycosyltransferases and FN1 in tumor cells. This education may have an impact on cancer development and metastasis.

Oral Communication – COVID-19 and Thrombosis

OR09-01

EFFECT OF SARS-COV-2 MRNA VACCINATION ON THROMBIN GENERATION AND DISEASE ACTIVITY IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE

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Background: Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC), are associated with higher thrombotic risk and enhanced thrombin generation (TG) in adults. IBD patients were underrepresented in SARS-CoV-2 mRNA vaccine trials. Case reports indicated that adverse events post-vaccination, including IBD flare, were more common among children, and those with prior COVID-19.

Aims: To find out whether TG is increased in children with IBD as compared to healthy controls and whether TG parameters show significant changes following SARS-CoV-2 mRNA vaccination.

Methods: In this observational case-control study, 37 children with IBD (CD:16, UC: 21) aged 12-18 years and 55healthy age-matched children were enrolled. Blood was collected before and 2-4 weeks after the second dose of BNT162b2 (Pfizer-BioNTech) vaccine dose. Whole blood count, fibrinogen, inflammatory markers (CRP, ferritin), anti-SARS-CoV-2 antibody levels were investigated, TG assay was carried-out using platelet-poor plasma. Lag time, endogen thrombin potential (ETP), peak thrombin, time-to-peak were calculated. Detailed clinical parameters including post-vaccination symptoms, COVID-19 history, disease activity scores (PUCAI, Mayo score, PCDAI) were registered.

Results: CRP was significantly elevated in children with IBD and showed a positive correlation with ETP (CD: r=0.700; p=0.003 and CU: r=0.501; p=0.020). TG parameters did not differ between patients and controls pre- or post-vaccination. TG parameters remained unaltered post-vaccination in both groups. IBD disease flare was not observed post-vaccination, but reduced anti-SARS-CoV-2 antibody titers were found in 4 patients receiving immunosuppressive therapies. Previous COVID-19 infection had no effect on TG levels.

Summary/Conclusion: Although TG parameters correlated with the level of inflammation in children with IBD, the extent of TG was not significantly different from healthy controls. TG parameters and IBD disease activity scores did not increase significantly following mRNA vaccination. Our results support the safety of SARS-CoV-2 mRNA vaccination in children with IBD, highlighting observations of lower antibody levels in immunosuppressed children.

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OR09-02

TRANSIENT AUTOREACTIVE ANTIBODIES IN PROSPECTIVELY SAMPLED COVID-19 VACCINE RECIPIENTS

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Background: Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare autoimmune thrombophilic disorder linked to recombinant adenovirus (rAV)-based coronavirus disease 2019 (COVID-19) vaccines. It is believed that VITT is caused by pathogenic autoantibodies against platelet factor 4 (aPF4), induced by vaccine-induced inflammation and the formation of neo-antigenic complexes between platelet factor 4 (PF4) and the rAV vector. Alternatively, the rAV vector may induce a generalized production of polyreactive autoantibodies, including aPF4, as part of a normal antiviral response. This polyreactive response may lead to a first manifestation of overt autoimmune disease in individuals predisposed to it. Understanding the relative importance of these mechanisms may be relevant for the development of further rAV-based vaccines.

Aims: We aimed to determine whether rAV-based vaccines specifically induce aPF4.

Methods: We analysed sera from age, sex and prior COVID-19 infection status matched health-care workers (HCW) vaccinated with rAV-based (AZD1222, AD26.COV2.S) or messenger RNA (mRNA)-based (mRNA-1273, BNT162b2) COVID-19 vaccines between January and June 2021. The primary endpoint was the antibody fold change (FC) for aPF4 over baseline of the rAV-based and mRNA-based vaccines. We compared this FC to the FC for antiphospholipid antibodies (aAPL), including anti-beta2-Glycoprotein I (aβ2GP) and anti-cardiolipin (aCL), which are often detected as part of a polyreactive antiviral response. The secondary endpoint was seroconversion defined as a 2-fold increase after first and second vaccine compared to baseline. Ethical approval was obtained from the institutes' ethics committees.

Results: A total of 145 HCWs vaccinated with AZD1222 (n = 37), Ad26.COV2.S (n = 35), mRNA-1273 (n = 47) and BNT162b2 (n = 26) were analysed. We did not observe any significant differences in the mean FCs for aPF4 after the first (ADZ1222 vs mRNA: 0.99 vs 1.08) and second dose of ADZ1222 (ADZ1222 vs mRNA: 0.99 vs 1.10) and after a single dose of Ad26.COV2.S (Ad26.COV2.S vs mRNA: 1.01 vs 0.99) compared to mRNA-based vaccines. Similarly, the mean FCs for aβ2GP and aCL in the rAV-based vaccines were not different from the mRNA-based vaccines. Seroconversion for aPF4 occured in a small number of HCWs (Ad26.COV2.S (n=1), AZD1222 (n=1) and mRNA-1273 (n=1)), while aAPL seroconversion was more frequently observed (AZD1222 (n=7), Ad26.COV2.S (n=2), BNT-162b2 (n=1), mRNA-1273 (n=1)). HCWs vaccinated with rAV-based vaccines more often seroconverted for any of the tested autoantibodies compared to those vaccinated with mRNA-based vaccines (10/145 (6.9%) vs 3/145 (2.1%)).

Summary/Conclusion: Our findings suggest that rAV-based COVID-19 vaccines do not specifically induce PF4 antibodies, but rather elicit a transient polyreactive autoantibody response. This response occurred more frequently in HCWs vaccinated with rAV-vectored vaccines compared to mRNA-based vaccines, likely reflecting a normal physiological antiviral response. Future research is needed to determine whether VITT is

a rare complication associated with rAV-based COVID-19 vaccines, or an unrecognized autoimmune syndrome with various predisposing factors, including the rAV-based vaccines.

OR09-03

SARS-COV-2 VACCINATION AND RISK OF VENOUS THROMBOSIS – CASE CONTROL STUDY ON ROUTINE CARE DATA FROM DUTCH GENERAL PRACTITIONERS

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Background: Conflicting results have been reported regarding the risk of a venous thrombotic event (VTE) after SARS-CoV-2 vaccination. Little is known about possible risk factors for vaccine-related VTE.

Aims: To determine the risk of VTE after SARS-CoV-2 vaccination and to determine whether there are subgroups of individuals who are particularly at increased risk of VTE after vaccination.

Methods: We performed a case-control study using routine care data. We selected, based on ICPC coding, free text and medication use, patients who had a first VTE in 2021 from general practitioners (GP) in the region of Leiden, the Netherlands. Controls were selected from the same GP database, with at least one contact with a GP in 2021, but without a VTE in 2021. Both cases and controls were aged 18 years and older.

Data on demographics, history of VTE, vaccination details (type and date of vaccination) and other VTE risk factor were extracted. After matching on calendar date (ratio cases: controls 1:3), odds ratios (OR) were estimated to assess the risk of VTE associated with SARS-CoV-2 vaccination, adjusted for age and sex. Vaccination was checked in the 28 days prior to the date of VTE or the matched date for their controls. A stratified analysis was performed for the risk of VTE.

Results: A total of 346 first VTE cases and 1038 controls were selected. Overall, SARS-CoV-2 vaccination was not associated with VTE risk (mRNA OR 0.78 95%CI 0.5 – 1.2; Vector OR 1.6 95%CI 0.7-3.9). However, after stratification for vaccine type, vector-based vaccines were associated with an increased risk, particularly for participants with high risk for VTE (OR: 2.8; 95%CI 1.0-7.7).

Summary/Conclusion: While overall no increased VTE risk was observed in 28 days after SARS-CoV-2 vaccination, results indicate that vector-based vaccines do increase the risk, particularly in the presence of other VTE risk factors.

OR09-04

THE MAIN PROTEASE (MPRO) FROM SARS-COV-2 TRIGGERS PLASMA COAGULATION BY ACTIVATING FVII AND FXII

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Background: Up to one third of patients with COVID-19, caused by SARS-CoV-2, are affected by severe/fatal thrombotic complications. However, the link between infection and thrombosis is not yet fully understood. Since the cleavage of a protein can result in loss or gain of function, we hypothesised that the Main protease (M^{pro}) of SARS-CoV-2 could trigger coagulation by proteolytically activating coagulation factors at specific Arginine residues. M^{pro} is necessary for viral duplication, as it cleaves the viral polyprotein at Glutamine-X bonds, to release the capsid-forming subunits.

Aims:

- Investigate whether M^{pro} can trigger plasma coagulation by activating one or more coagulation factors and unveil the mechanism of activation;

- Verify whether products of M^{pro} activity toward coagulation can be detected in plasma.

Methods: The ability of M^{pro} to clot plasma *in vitro* was evaluated by turbidimetric assays followed by Survival Analysis using Kaplan-Meier curves [10.1016/J.THROMRES.2017.09.011]. The screening of coagulation factors activation was performed by turbidimetric assays and enzymatic assays. M^{pro} cleavages on coagulation factors were identified using the Terminal Amine Isotopic Labeling of Substrates (TAILS) protocol coupled to Mass Spectrometry (MS) [10.1038/NPROT.2011.382]. M^{pro} substrate specificity was determined using the High-Throughput Protease Screen (HTPS) method [10.1038/S41467-021-21754-8]. To verify whether the activation of coagulation factors by M^{pro} can also be detected in plasma, we incubated human plasma with or without M^{pro}, digested it with Glu-C and identified the resulting fragments by label-free MS. We then performed a differential analysis at the peptide level to detect direct M^{pro} cleavages or differences in accessibility to Glu-C.

Results:

- The survival analysis of plasma form 20 healthy donors treated or un-treated with M^{pro} (50-100nM), showed a 2.8-fold increase of the clotting probability for the M^{pro}-treated group compared to the control group within 60 min of assay (p-value= 0.022).

- Enzymatic assays showed that M^{pro} can activate FVII and FXII. The TAILS experiment indicated that M^{pro} activates FVII in a canonical-way, by cleaving at the R212-I213 bond. TAILS also showed that M^{pro} cleaves the FXII heavy-chain in multiple sites, suggesting that M^{pro} leads to FXII activation by proteolytically removing the steric hindrance of the heavy-chain to the activation site, mimicking the effect induced by the binding of FXII to negative surfaces.

- HTPS analysis confirmed that M^{pro} has a secondary substrate specificity for Arginine residues.

- The label-free analysis at the peptide-level of M^{pro}-treated plasma showed an up-regulation of a peptide originating from the FXII heavy chain, suggesting that this region becomes more accessible in the

presence of M^{pro}. This is compatible with a conformational shift form a FXII inactive close-form to an active-open form.

Summary/Conclusion: We demonstrate that M^{pro} has an intrinsic prothrombotic activity by activating the two initiators of the coagulation system, FVII and FXII, hence representing a potential co-factor to the thrombotic complications in COVID-19. Furthermore, since it activates FXII, which is key to the contact system activation, M^{pro} could also constitute a triggering agent for inflammation, explaining the cytokine and bradykinin storm, typical of COVID-19. Lastly, we report a secondary substrate specificity of M^{pro}, that must be considered when performing target prediction studies.

Oral Communication – Risk Factors and Biomarkers for Thrombosis

OR10-01

HIGH PLASMA EXPRESSION LEVELS OF MICRORNA-145 ARE ASSOCIATED WITH REDUCED RISK OF FUTURE VENOUS THROMBOEMBOLISM

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Background: MicroRNAs (miRs) are non-coding RNAs that execute their function by targeted downregulation of gene expressions. Growing evidence indicates that miRs are involved in the pathophysiology of several human diseases. miR-145 is well-known to act as a tumor suppressor. Recently this miR has been shown to also regulate the expression levels of tissue factor and factor XI *in vitro,* and to decrease venous thrombus formation in animal models. However, the role of miR-145 for the risk of future venous thromboembolism (VTE) remains unknown.

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

Methods: Cases with incident VTE (n= 510) and a subcohort (n= 1890) were derived from the third survey of the Trøndelag Health Study (HUNT3), a Norwegian population-based cohort study (inclusion 2006-08, follow-up on VTE to 2015). The expression levels of miR-145 were determined in plasma samples obtained at cohort baseline using commercially available assays from QIAGEN (Hilden, Germany). The study population was divided into quartiles based on normalized, log-transformed expression levels of miR-145 in the subcohort participants, and weighted Cox regression models were used to estimate hazard ratios (HRs) for VTE with 95% confidence intervals (CIs). Ethical approval and informed consent were obtained for all study participants.

Results: High levels of miR-145 expression in plasma were associated with decreased risk of VTE. Participants with miR-145 levels in the highest quartile had a 49% lower risk of VTE (HR 0.51, 95% CI 0.38-0.68) compared with those with miR-145 levels in the lowest quartile in age- and sex-adjusted analysis. Risk estimates remained virtually the same after further adjustment for body mass index, cancer, and arterial cardiovascular diseases at baseline (HR 0.52, 95% CI 0.39-0.69). When miR-145 was modelled as a continuous variable using a restricted cubic spline function, there was a clear inverse dose-response relationship between miR-145 levels and VTE risk.

Summary/Conclusion: Our results indicate that expression levels of miR-145 are inversely associated with VTE risk. Our finding of a protective role of miR-145 against VTE is consistent with previous experimental data and suggests that miR-145 may not only serve as a biomarker for assessing the risk of future VTE but could also be involved in VTE pathogenesis. Future studies are needed to explore the potential of miR-145 as a therapeutic target for VTE.

OR10-02

IDENTIFICATION OF A TARGETED PLASMA PROTEIN SIGNATURE TO PREDICT RECURRENT VENOUS THROMBOEMBOLISM AND DEATH

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Background: Venous thromboembolism (VTE) is one the largest sources of morbidity and mortality. Anticoagulation remains the cornerstone of VTE treatment, which is associated with increased risk of bleeding. Hence, new tools are required to stratify patients who are at risk of recurrence and who benefit from extended anticoagulant therapy.

Aims: The aim of this study is two-fold: (1) to identify biomarkers that provide insight into the pathophysiology of recurrent VTE; (2) to assess the prognostic ability of these.

Methods: Data from 461 patients diagnosed with acute VTE without malignancy, from a prospective, multi-center cohort from Germany, the GMP-VTE project, were used. The relative concentration of 444 circulating proteins was measured by immuno-qPCR (Olink Proteomics, Uppsala, Sweden). Elastic net-penalized Cox regression models were used to identify laboratory and clinical variables that predict recurrent VTE or death.

Results: Patients were followed for a total of six years, where 140 recurrent VTE or death were recorded. The penalized elastic-net Cox regression model identified a best predictor set of 60 variables. The levels of 51 plasma proteins, and 9 clinical or stablished laboratory markers were included in the model (Figure 1). The model showed a good prognostic value (C-Index: 0.81; CV C-index=0.60). Age, D-dimer, the use of antihypertensive medication, chronic pulmonary disease, diabetes and C-reactive protein were associated with an increased risk of recurrent VTE or death. Conversely, higher estimated glomerular filtration rate, female sex, and surgery were associated with a decreased risk. The identified proteins were enriched in pathways associated with leukocyte chemotaxis, regulation of IL-6, cellular response to interferon-γ, and respond to hypoxia. The derived protein score was able to stratify the incidence of recurrence or death. Individuals in the highest third of the score had the highest incidence of recurrent VTE or death (HR: 4.97; 95% CI: 2.96-8.35) compared to individuals in the lowest third, and a 2.5-fold increase for individuals in the middle third (HR: 2.53, 95% CI: 1.46-4.37).

Summary/Conclusion: The plasma protein biomarkers discovered in this investigation could be used for the identification of mechanisms associated with a worst clinical course following VTE, aiding in risk stratification and prevention of VTE sequelae.

OR10-03

PREDICTIVE RISK FACTORS FOR POST THROMBOTIC SYNDROME IN INFANTS AND CHILDREN

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Background: Post thrombotic syndrome (PTS) is a severe chronic condition following deep vein thrombosis (DVT), caused by venous insufficiency of the veins and is associated with pain, swelling and restricted use of the affected limb. Despite lack of curative treatment for PTS, it is important to identify patients at increased risk to evaluate the efficacy of preventive measures.

Aims: The primary aim of this study was to identify risk factors to predict PTS development in pediatric DVT patients. The secondary aims were to determine the incidence of PTS and to determine the median time to PTS development.

Methods: This retrospective cohort study included children (0-18 years) with a DVT treated at Emma Children's Hospital, Amsterdam UMC between 2000 and 2021. The Modified Villalta Scale (MVS) was used to diagnose PTS. PTS was defined as mild PTS (1-3 points), moderate (4-8 points) and severe (≥9 points). The outcome consisted of the incidence of PTS, PTS-free survival and risk factors for PTS. As the incidence of DVT has a bimodal distribution, we divided the children in two groups: < 1 year of age and ≥1 year of age.

Results: PTS occurred in 106/323 patients (32.6%. ♂ 51.4%). PTS free survival was 83.0%, 73.7% and 67.2% after respectively 1, 2 and 5 years in all patients. In infants < 1 year the incidence of PTS was 19.8% (23/116 patients). All infants, but one, had mild PTS (95.7%). Median time to PTS diagnosis was 13.4 months (range 6-60). In children ≥ 1 year, 83/ 207 (40.1%) experienced PTS. In this group the median time to PTS was 12.3 months (range 6-24). Most patients were diagnosed with mild PTS (n=54, 66.7%), followed with moderate PTS (n=22, 27.1%), and severe PTS (n=5, 6.2%).

In infants <1 year no complete thrombus resolution (OR 2.8; 95% CI 1.0-7.9; p=0.047) was the only significant risk factor for PTS. In children ≥ 1 year, ≥ 2 vessels involved (OR 23.5, 95% CI 6.0-92.6; p < 0.001), unprovoked VTE (OR 14.8, 95% CI 3.2-69.1, p < 0.001), oral contraceptives use at moment of DVT (OR 5.6; 95% CI 1.2-27.2; p=0.032) and age per year increase (OR 1.2; 95% CI 1.0 – 1.3, p = 0.009) were statistically significant risk factors. The only protective factor was exercise ≥ 3 times/week (OR 0.22; 95% CI 0.07-0.72; p = 0.013).

Summary/Conclusion: This study demonstrated a high incidence of PTS, especially in the older children. In infants < 1 year a lack thrombus resolution was the only significant factor for PTS. In children ≥1 year expanded vessel involvement, unprovoked VTE, oral contraceptives use and age independently increase the risk of PTS in pediatric patients. The only protective factor was exercise ≥ 3 times/week. These findings could lay the foundation for more preventive treatment strategies in the future.

OR10-04

METABOLIC AND EPIGENETIC PERTURBATIONS UNDERLIE MALADAPTIVE TRAINED IMMUNITY-ASSOCIATED MYELOID CELL HYPERCOAGULABILITY

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Background: Individuals with chronic inflammatory disease have an increased risk of venous thromboembolism (VTE), although the molecular basis for this phenomenon remains poorly understood. We hypothesised that disease-associated myeloid cell 'trained immunity', which describes long-term epigenetic and metabolic modifications that occur in response to specific inflammatory stimuli that increase responsiveness to subsequent non-specific inflammatory events, may contribute to hypercoagulability associated with chronic inflammatory disease.

Aims: To examine how trained immunity enhances myeloid cell procoagulant and antifibrinolytic activity.

Methods: Murine bone marrow-derived macrophages were trained with β-glucan, washed, then left for 7 days before lipopolysaccharide (LPS) re-stimulation. Macrophage gene expression and function were analysed by ELISA, RNA-seq and cell-based calibrated automated thrombinography. In vivo trained immunity was achieved by i.p. β-glucan injection or β-glucan-rich diet.

Results: Surprisingly, re-stimulated β-glucan-trained macrophages exhibited significantly enhanced procoagulant and antifibrinolytic gene expression compared to macrophages stimulated with LPS alone, suggesting the induction of 'trained hypercoagulability'. Moreover, trained macrophage-dependent thrombin generation was associated with significantly shortened lag-time compared to LPS-stimulated macrophages, which was dependent upon increased tissue factor (TF) activity via enhanced TF gene expression and increased acid sphingomyelinase-mediated TF decryption. b-glucan-trained macrophages also inhibited fibrinolytic activity through increased plasminogen activator inhibitor 1 (PAI-1) secretion. Early growth response-1 (EGR-1), a master transcription factor that controls both TF and PAI-1 expression, was significantly upregulated in b-glucan-trained macrophages compared to LPS treatment alone and EGR-1 gene silencing dampened procoagulant activity in 'trained' macrophages.

Mechanistically, myeloid cell trained immunity depends on long-term epigenetic and metabolic cellular adaptions. Pharmacological inhibition of histone methylation and acetylation significantly attenuated trained immunity-associated myeloid cell hypercoagulability. Moreover, inhibition of glycolytic metabolic reprogramming was also sufficient to block procoagulant and antifibrinolytic activity in β-glucan-trained macrophages.

To assess trained immunity-associated myeloid cell hypercoagulability in vivo, we performed transcriptomic analysis of splenic monocytes isolated from mice trained with β-glucan 3 weeks prior to sacrifice, which identified up-regulation of genes associated with both trained immunity and hypercoagulability. Furthermore, splenic monocytes isolated from b-glucan-trained mice exhibited enhanced procoagulant activity compared to control mice monocytes. Remarkably, monocyte procoagulant activity increased in parallel with the time period since β-glucan training, consistent with the induction of a training-dependent hypercoagulable state, whereas no change in procoagulant activity was observed in splenic monocytes isolated from mice fed a β-glucan-rich diet.

Summary/Conclusion: This study demonstrates that a lowered threshold for myeloid cell-dependent hypercoagulability is a maladaptive consequence of innate immune cell memory. Furthermore, these data suggest that epigenetic and metabolic perturbations associated with trained immunity represent novel therapeutic vulnerabilities in immunothrombotic disease.

Oral Communication – Etiology of Thrombosis

OR11-01

ADP-DEPENDENT PLATELET ACTIVATION IS IMPORTANT IN THE DEVELOPMENT OF VENOUS THROMBOSIS DURING A LONG-DISTANCE FLIGHT

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Background: The incidence of high altitude in venous thrombosis embolism (VTE) has been intensively documented. Prolonged exposure to hypobaric hypoxia triggers several mechanisms including coagulation disorders. Clinical studies show a correlation between long-distance flight and the development of deep vein thrombosis (DVT). Aircraft manufacturers create pressurized environments for the cabin with atmospheric gas partial pressures equivalent to altitudes of 1981 m to 2440 m.

Aims: The objective of this project is to investigate the effect of a long-distance flight in VTE

Methods: For this, we performed 24 hours of DVT in mice exposed to 6 hours of hypobaric hypoxia (“flight”) or normobaric normoxia (control) conditions. These “flight” conditions simulate a 6 hours airline cabin environment.

Results: We observed that thrombi were significantly bigger and heavier in mice exposed to flight conditions than the control group. The composition of the thrombi in the two groups was also different. Thrombi from the flight group of mice contained significantly more neutrophils and fibrin fibers than the control ones. Consistently, a significant decrease in the concentration of circulating leukocytes -and neutrophils - was observed in mice exposed to hypobaric hypoxia conditions. Interestingly, no difference was observed in the quantity of NETs, CitH3 positive, NE-positive and Fibrin positive neutrophils in the thrombi in the two groups of mice, indicating that neutrophils/NETs may not be involved in the development of venous thrombosis during a flight. ADP has been previously described as a key mediator produced during hypobaric and hypoxia. We then compared the development of venous thrombosis in wild-type and P_2RY_{12} deficient mice. Although no difference was observed in the “control” conditions between the two group of mice, the development of thrombi obtained in the $P_2RY_{12}^{-/-}$ mice were not affected by the changes in the atmospheric gas partial pressures to mimic the flight conditions.

Summary/Conclusion: Taken together, these data indicate that ADP-dependent activation of platelets is playing an important role in the development of DVT under hypobaric hypoxia conditions. Targeting activation of platelets rather than the coagulation factors may thus constitute a new strategy to prevent DVT during long flight.

OR11-02

LENGTH OF ISCHAEMIA DURATION IS ASSOCIATED WITH FIBRIN FILM COVERAGE OF ACUTE MYOCARDIAL INFARCTION THROMBI

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Background: We have shown that fibrin forms a film on the surface of blood clots. Fibrin films have also been reported on thrombi extracted from patients with acute myocardial infarction and ischaemic stroke, although these observations were retrospective and descriptive. Clinical and biochemical factors affecting fibrin film formation within the vasculature are unknown.

Aims: The aims of this study were to quantify the presence of fibrin films on thrombi from patients with acute myocardial infarction (MI), and to determine which clinical and laboratory parameters influence film coverage.

Methods: Patients (n=51) with acute ST-elevation MI were recruited after admission to Leeds Teaching Hospitals NHS Trust. Thrombi were obtained through aspiration thrombectomy, immediately washed in saline, fixed in 2% glutaraldehyde, dehydrated and subjected to critical point drying, prior to imaging by scanning electron microscopy. Peripheral blood samples were taken from the antecubital vein and used for plasma turbidimetric analysis of clotting and lysis, analysis of clot fibre density by confocal microscopy, and determination of clot pore size by permeation. Data were expressed as median (range) and correlation was assessed by Spearman rank analysis.

Results: The majority (84.6%) of patients were male. Median age was 61 (31-88) and BMI was 27.1 (21-49). Median ischemia time, from call for help to thrombus extraction was 2.2hrs (1.0-7.3). Film coverage ranged from 0.6-60.8% on all thrombi, with a median of 5.5%. Correlation of clinical data and film coverage showed a significant positive correlation of film coverage with ischaemia duration (r=0.4346, p=0.0014). For the laboratory data, film coverage significantly correlated with lag-phase (r=0.489, p=0.0016), time to 25% (r=0.432, p=0.014), 50% (r=0.456, p=0.0035), 75% (r=0.340, p=0.0341), 100% (r=0.312, p=0.0432) MaxOD, and with time to reach Vmax (r=0.444, p=0.0047). There was no effect of anticoagulation and antiplatelets therapies with film coverage.

Summary/Conclusion: These data show the presence of variable coverage with fibrin film on all acute MI thrombi. Film coverage increased with ischaemia duration and slower *ex-vivo* clotting.

OR11-03

HUMAN TRANSTHYRETIN AMYLOID FIBRILS INDUCE PLASMA CLOTTING BY ACTIVATING THE INTRINSIC PATHWAY OF BLOOD COAGULATION: IMPLICATIONS IN THE PATHOGENESIS OF HTTR INTRACARDIAC THROMBOSIS

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Background: hTTR amyloidosis, from both wild-type TTR and pathogenic variants (e.g.S52P), is characterized by amyloid deposition in several organs, especially in heart chambers. It is associated with cardiomyopathy (1-3), intracardiac thrombosis [6,7] and cerebral amyloid angiopathy. Cardiomyopathy and heart failure, associated to acquired wild-type hTTR amyloidosis, have a prevalence of 1–3% in elderly people >75 years of age, while intracardiac thrombosis has a higher prevalence in patients treated with antiarrhythmic drugs, if compared with arrhythmic patients without amyloidosis. The composition of amyloid deposits is a mixture of intact hTTR and its most abundant proteolytic fragment hTTR(49-127) [4,5]. However, the biochemical pathways leading to intracardiac thrombosis are unknown.

References:

1. Galant NJ et al. (2017) CLINICAL SCIENCE 131, 395–409. doi:10.1042/CS20160413
2. Witteles RM et al. (2019) HEART FALURE 7, 709–716. doi:10.1016/j.jchf.2019.04.010
3. Ruberg FL et al. (2019) J. AMER. COLL. CARDIOL. 73, 2872–2891. doi:10.1016/j.jacc.2019.04.003
4. Mangione PP et al. (2018) J. BIOL. CHEM. 293, 14192–14199. doi:10.1074/jbc.RA118.003990
5. Peterle D et al. (2020) COMMUN. BIOL. 3, 764. doi: 10.1038/s42003-020-01493-0

Aims: Demonstrate the ability of hTTR fibrils to induce plasma clotting, and establish the molecular mechanisms resulting in the activation of blood coagulation.

Methods: Natural fibrils were isolated from autopsies of patients with SSA. Wild-type hTTR and hTTR(49-127) were obtained as recombinant species in E.coli, and allowed to fibrillate for 72h at 900rpm. Fibrils were characterized by thioflavin-T binding, DLS, and TEM, and added to plasma or whole blood (WB), monitoring fibrin generation by turbidimetry and thrombin generation (plasma), or by thromboelastometry (WB). The role of each coagulation factor was established by incubating fibrils either with factor-depleted plasma samples, or with isolated zymogens and the corresponding chromogenic substrates. Binding of coagulation factors to hTTR fibrils was quantified by ELISA. Histopathological images of cardiac amyloid infiltrations were from endomyocardial biopsies of patients with thrombosis in ATTR-CM.

Results: Histopathological investigation reveals exposure of amyloid fibrils in the heart chambers and localization of a small intracardiac thrombus nearby. Data obtained by assays on plasma and WB indicate

that both natural and artificial hTTR fibrils similarly induce clotting, with a clotting time of 20-30 min. Addition of either hTTR in a non-fibrillar state or of the amorphous precipitate of ß-casein did not trigger clotting. Furthermore, amyloid fibrils induce autoactivation of the coagulation factor XII, at the initial step of the intrinsic pathway of blood coagulation cascade, and increase the efficiency of prothrombin activation by FXa of 40 times. Binding assays confirmed the strong binding ability of FXII, FII, FVa and FXa to fibrils.

Summary/Conclusion: Amyloid fibrils could represent a negatively charged and ordered surface that mimics the surface of activated platelets, on which coagulation factors, especially FXII, FII, FX and FV, can anchor and become activated. These mechanisms can explain the hypercoagulable state that can lead to the formation of thrombi observed in patients affected by cardiac amyloidosis and cerebral amyloid angiopathy.

OR11-04

GARDOS CHANNEL INHIBITOR PREVENTS AN ENHANCED EXPERIMENTAL VENOUS THROMBOSIS IN SICKLE TRAIT MICE

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Background: Individuals with sickle cell trait (SCT) are heterozygous carriers of the sickle beta-globin gene. Systematic analyses of large cohort studies over the past decade have established that SCT is a risk factor for a limited number of complications, most notably venous thrombosis (VT). Although the odds ratio for VT in SCT is only 1.5-2.0, the high prevalence of SCT in the African American population and in African countries implies that the attributable risk for VT conferred by this mutation exceeds that of the prothrombin G20210A mutation in the white population. SCT red blood cells (RBCs) can undergo sickling, although the degree and duration of hypoxia required to produce sickling are much greater than in sickle cell disease due to the lower intra-erythrocyte sickle hemoglobin beta concentration of 25-45%.

Aims: SCT is a known risk factor for venous thrombosis (VT), but the mechanism accounting for this complication is poorly understood. We tested whether sickling of SCT red blood cells (RBCs), triggered by exposure to prolonged severe hypoxia within venous thrombi, induces RBC-dependent changes that contribute to VT.

Methods: VT was induced by complete occlusion (stasis) of the inferior vena cava (IVC) in humanized, control mice expressing two copies of normal hemoglobin beta globin (AA) or heterozygous for mutated sickle hemoglobin beta globin (AS). Senicapoc, an oral Gardos channel inhibitor, was administered twice daily (20mg/kg) for 2 weeks to prevent RBC dehydration and subsequent sickling in AS mice. Sickling of RBCs was determined by histological and electron microscopy analysis.

Results: Human sickle hemoglobin beta accounts for 30.1% of total hemoglobin expression in AS mice. Consistent with the human SCT condition, AS mice do not develop anemia and their blood cell counts did not differ from AA controls. Basal plasma thrombin-antithrombin complex levels were also not different between AA and AS mice. The weight of thrombi formed 24 hours after IVC stasis was significantly increased ($p<0.01$) in AS mice (mean+/-SEM; 29.0 mg +/- 1.7; n=8) compared to AA controls (20.0 mg +/- 1.7; n=7). Histologic and electron microscopy evaluation demonstrated that severe hypoxia ($pO_2 \approx 10$ mm Hg) present in the nidus of venous thrombi was associated with extensive sickling of RBCs in AS mice. To determine if the enhancement of VT observed in AS mice was mediated by RBC sickling, mice were pretreated with Senicapoc or vehicle. Compared to vehicle-treated AS mice (27.3 mg +/- 2.2 n=9) pre-treatment with Senicapoc significantly reduced ($p<0.05$) clot weight (18.1mg +/- 1.1; n=7) to that observed in untreated AA controls.

Summary/Conclusion: We have established a mouse model of the venous thrombotic complications associated with SCT and have demonstrated that hypoxia-induced sickling of RBCs contributes to enhanced VT observed in AS mice. We are now investigating if the protective effects of Senicapoc are mediated by changing procoagulant and/or physical properties of sickled AS RBCs.

Oral Communication – Platelet Aggregation

OR12-01

EFFECTS OF RED BLOOD CELL HEMOLYSATE, UNCONJUGATED BILIRUBIN AND FAT EMULSION ON LIGHT TRANSMISSION PLATELET AGGREGATION USING TA-8V INSTRUMENT

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Background: International guidelines recommend to reject any abnormal samples for platelet aggregation studies: i.e., those appearing hemolyzed, icteric or lipemic (HIL). Other scientific societies suggest rejecting only hemolytic specimens and reporting other conditions. These recommendations are also based mainly on the HIL interferences on the detection limits of most available aggregometers that measure at wavelengths < 750 nm. Nevertheless, the effects of red blood cells-hemolysate (RBCH), icteric (bilirubin) and lipemic (fat emulsion) on platelet aggregation is poorly documented.

Aims: To reveal the *in vitro* effects of a RBCH, bilirubin and fat emulsion on the aggregation of platelet-rich plasma (PRP) from healthy volunteers using a new instrument (TA-8V) detecting platelet aggregates in infrared wavelength.

Methods: Platelet aggregation assay was performed on PRP in presence of increasing doses of either a RBCH (0, 0.3, 0.6, 2, 5, 10, 20 g/L of hemoglobin), bilirubin (0,15, 60, 100, 180, 300, 400 mg/L), or fat emulsion (intralipid 20%®) (0, 0.5, 1, 1.5, 3, 5 g/L). Spontaneous aggregation as well as aggregation induced by 5µM ADP or 2 µg/mL collagen were studied. The maximum intensity (MI) and the velocity (VEL) of the platelet aggregation were documented. When necessary, the added RBCH solution was previously treated with 0.1 U/ml of apyrase for 30 minutes at 37°C. The aggregation study was performed using reagents (ADP, Collagen, Apyrase) on the TA-8V aggregometer (Diagnostica Stago, Asnière sur Seine, France).

Results: A spontaneous aggregation occurred with the RBCH (starting at 2g/L, min-max: IM= 14-31%; VEL=5-25%). This effect disappeared in presence of 0.1 U/ml of apyrase (min-max: IM= 1.6-6%; VEL=4-6%). MI and VEL induced by ADP and collagen (n= 5 or 11 depending on the RBCH concentration) were not significantly affected regardless of the RBCH concentration and/or the use of apyrase in comparison to control PRP (RBCH 5mg/ml with vs. without apyrase 0.1U/ml : ADP (n=11) IM = 72±12% vs. 78±12%; collagen (n=11) IM = 86±22% vs. 76±20%, ns.). Addition of different bilirubin concentrations (15 to 400 mg/L) to PRP did not induce any significant spontaneous aggregation and did not affect the response to ADP or collagen in comparison to control PRP without bilirubin (bilirubin at 180g/L vs. control, ADP n=11: IM= 82±11 vs. 81±7%; collagen n=12: IM= 84±10% vs. 78±8%, ns.). Different doses of fat emulsion did not either affect the aggregation results in response to ADP or collagen (fat 1 g/L vs. control, ADP n=12, IM= 95±12 vs. 83±6%; collagen n=13, IM= 96±13 vs. 87±6%, ns.), but fat concentration above 3g/L results in an increased sample inter-variability suggesting technical limitation.

Summary/Conclusion: Discarding any residual ADP or ATP resulting from the RBC hemolysis process with apyrase prevents any spontaneous platelet aggregation, nevertheless the analysis of the platelet response induced by the different agonists (ADP, Collagen) remains exploitable until 20 g/L of RBCH.The addition of bilirubin to PRP did not affect the LTA, indicating that icteric PRP (up to 400 mg/L of bilirubin) can be acceptable for testing on the TA-8V. Lipaemic samples with less than 3g/L of fat can be acceptable for

LTA. The overall results obtained on TA-8V aggregometer should make it possible to better specify the criteria for the acceptability of an HIL sample, allowing not to reject systematically any HIL sample.

OR12-02

PLATELET ACTIVATION INDUCED BY CPG-RICH OLIGODEOXYNUCLEOTIDES: THE INVOLVEMENT OF THE C-TYPE LECTIN RECEPTOR CD93

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Background: CpG-oligodeoxynucleotides (CpG-ODNs) are short single-stranded synthetic DNA molecules containing unmethylated CpG motifs, which mimic bacterial DNA and act as pathogen associated molecular patterns (PAMPs). CpG-ODNs exhibit potent immunostimulatory properties in vertebrates and are extensively exploited in clinical trials as adjuvants for new anticancer therapies and vaccines.

As other PAMPs, CpG-ODNs induce platelet activation, but very little is known on the molecular mechanisms responsible for this process. Platelets are emerging players in innate immunity and express several pathogen recognition receptors (PRRs) involved in pathogen sensing and immune response. The C-type lectin (CTL) receptor CD93 is a type I transmembrane glycoprotein which plays a prominent role in inflammation, angiogenesis, and cancer. In nucleated cells, the CTL domain of CD93 has recently been shown to bind CpG-ODNs, suggesting a possible function for CD93 as a novel unidentified PRR.

Aims: In this study we have investigated the role of CD93 in platelet activation induced by CpG-ODNs.

Methods: Type C CpG-ODNs (ODN2395) were selected for this study and their effect on control wild type (WT) and CD93-knockout (KO) murine platelets was investigated. Integrin αIIbβ3 activation and granule secretion were analysed in whole blood by flow cytometry. Platelet aggregation and protein phosphorylation were analysed on purified platelets by light transmission aggregometry and immunoblotting, respectively.

Results: CpG-ODNs induced aggregation of WT platelets, accompanied by integrin αIIbβ3 activation and α-granule release. Platelets lacking CD93 exhibit a significant impairment in α-granule secretion, whereas integrin activation and platelet aggregation were only marginally affected. Immunoblotting analysis revealed that the CpG-ODNs stimulated the tyrosine phosphorylation of different signalling proteins in WT platelets, including PLCγ2 and Pyk2. These responses were significantly defective in the absence of CD93. Moreover, activation of the AKT/GSK3 axis and of the MAP kinases cascade by CpG-ODNs were markedly dependent on CD93 expression.

Summary/Conclusion: CD93 is involved in platelet stimulation induced by CpG-ODNs by regulating the activation of protein kinase-dependent pathways. Further studies are required to clarify the relevance of CD93 in functional platelet responses to CpG-ODNs in thrombosis and immunity.

OR12-03

APPLICATION OF THE MACHINE LEARNING-BASED IMAGE ANALYSIS IN THE QUANTIFICATION OF THROMBI IN FLOW CHAMBER ASSAYS

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Background: Thrombus formation in vitro under flow conditions is one of the most widely used methods to study haemostasis and to evaluate the activity of potential antithrombotic compounds. Assessment of the results of these experiments is often based on quantification of microscopic images of thrombi. There are however certain imperfections in this approach. One such example is differentiation between area covered by single layer of platelets versus multilayer platelet aggregates. This difference is not relevantly mirrored by such parameter as total area covered by platelets. In presented work we show how the problem can be solved with the use of machine learning-based image analysis.

Aims: The aim of the presented work was to create a new method of quantification of images of thrombi generated in flow chamber experiments. The method should be capable of differentiating between single layer of platelets versus multilayer platelet aggregates, so that the two classes could be quantified separately.

Methods: Blood samples were collected from healthy volunteers and perfused through the channels coated with type I collagen. In some experiments, abciximab (10 µg/ml) was added prior to perfusion. Adherent platelets were stained with platelet-specific anti-CD41 PE-conjugated antibodies. Imaging was performed using a wide-field epifluorescence microscope.

A public domain software Ilastik was used to train two machine learning models: one of them segmented all CD-41 positive objects while the second was capable of differentiating between single layer of platelets versus multilayer platelet aggregates. Training was performed by two independent analysts experienced in analysis of images acquired in flow chamber experiments. A set of images of thrombi generated in the presence of abciximab was then quantified with the two models.

The study was approved by the Medical University of Lodz committee on the Ethics of Research in Human Experimentation (number of the consent RNN/323/20/KE).

Results: The pattern of platelet aggregates formed in the presence of a used concentration of abciximab differed significantly from that formed under control conditions. Blood platelets treated with abciximab formed less multilayer thrombi, but the surface coverage with single layered platelets was not affected. Quantification of the images with the use of a model which segmented all of the CD41-positive objects (total coverage area) did not reveal significant differences between control and abciximab-treated platelets ($342x10^3$ [$287x10^3$;$378x10^3$] μm^2 vs $445x10^3$ [$324x10^3$;$575x10^3$] μm^2 (median with IQR); n=6, n.s. Implementation of the model selective towards multilayer objects, however, resulted in a significantly decreased values of the area covered by abciximab-treated platelets ($218x10^3$ [$136x10^3$;$262x10^3$] μm^2 vs 119 $x10^3$ [$16x10^3$;$151x10^3$] μm^2 (median with IQR); n=6, p=0.0001.

Summary/Conclusion: Classification of thrombi based on their morphology and quantifying them with respect to these classes could provide more information than a simple assessment of a total area covered by platelets.

Machine learning based image analysis is an effective tool to fulfil this task.

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OR12-04

DIFFERENTIAL ANTIPLATELET EFFECTS OF A NEW INHIBITOR OF PDI ISOMERASES - C-3399

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Background: Platelet disulfide isomerases (PDIA) regulate platelet activation, including αIIbβ3 integrin activation and aggregation. As reported previously, selective PDIA1 blockade by bepristat 2a was related to impairment of GPVI receptor-induced platelet activation due to the inhibition of reactive oxygen species (ROS) and tromboxane A2 (TXA_2) production in platelets.

Aims: We investigated a newly in-house synthesized inhibitor, C-3399, blocking the PDI isoforms PDIA1, PDIA3 and PDIA6.

Methods: To show antiplatelet effects of C-3399 compared to PDIA1 selective inhibitor, bepristat 2a, and reference PDIA1/3/6 inhibitor, PACMA-31, platelet aggregation and platelet surface activation markers in response to the GPVI receptor agonist convulxin versus TXA_2-mimetic, U46619, were evaluated using light-transmission aggregometry and flow cytometry, respectively. TXA_2 production was measured by ELISA. ROS were determined using dichlorodihydrofluorescein-based fluorescent assay. To test effects of PDI inhibitors on platelet signaling pathways, phosphorylation of Syk (Y525/526), ERK1/2 (T202/Y204), p-38 (T180/Y182) and Akt (S473) was evaluated using standard SDS-PAGE/Western blot analysis.

Results: C-3399 significantly inhibited dose dependently the activation of platelet αIIbβ3 integrin induced by convulxin and partially diminished CD62P or CD63 surface expression in diluted platelet-rich plasma (PRP). The effects of PACMA-31 were similar to those observed for C-3399, while bepristat displayed the strongest inhibitory effect on αIIbβ3 integrin activation and nearly completely abolished surface expression of CD62P or CD63. C-3399 and bepristat partially reduced platelet aggregation in PRP induced by high convulxin concentration, whereas PACMA-31 had no effect. In line with the aggregation results, C-3399 and bepristat reduced convulxin-induced ROS generation and downregulated p-Akt (S473) and p-ERK1/2 (T202/Y204) with no effect on p-38 (T180/Y182). PACMA-31 increased generation of ROS and activation of p38 (T180/Y182) associated with only partial downregulation of p-Akt (S473) and no effect on p-ERK1/2 (T202/Y204). Interestingly, the blockade of PDIA1, PDIA3 and PDIA6 by C-3399 or PACMA-31 nearly completely inhibited U46619-induced activation of αIIbβ3 integrin, CD63 surface expression and platelet aggregation (in our experimental setting, U46619 did not induce ROS generation), while the selective blockade of PDIA1 by bepristat resulted in significantly weaker inhibitory effects.

Summary/Conclusion: C-3399 shows differential inhibitory effects on GPVI-mediated platelet function compared to bepristat and PACMA-31. In contrast to bepristat, C-3399 blocks not only GPVI-mediated TXA_2 synthesis but also TXA_2-mediated activation of human platelets. Our results suggest that PDIA1 plays a major role in GPVI-mediated activation of platelet αIIbβ3 integrin activation and granule exocytosis in diluted PRP (less ROS production compared to aggregation condition), while additional PDIs, i.e. PDIA3 and/or PDIA6 predominantly regulate these platelet responses induced by TXA_2 with a minor role of ROS. However, further studies are required to explore mechanisms behind.

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Oral Communication – Clotting Structure & Function

OR17-01

DETECTION OF TFPI CITRULLINATION IN BLOOD AFTER NEUTROPHIL ACTIVATION BY PMA

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Background: Neutrophils are known to undergo NETosis, the externalization of their DNA and other intracellular contents to entrap and kill invading pathogens. NETosis has been shown to enhance blood coagulation during inflammation. Previously, it was shown that TPFI, an important anticoagulant, also binds to neutrophil extracellular traps (NETs), and is inactivated through cleavage by elastase. PAD4 is a crucial enzyme during the NETosis process, which is localized intracellularly but expelled from the cell upon neutrophil activation. In this research, we investigate the effect of PAD4 on TFPI and probe the changes to TFPI activity in full blood and plasma.

Aims: The aim of this work is to elucidate effects of neutrophil activation and subsequent consequences for TFPI activity induced by PAD4 measured via thrombin generation .

Methods: Neutrophils in whole blood were either stimulated to undergo NETosis using phorbol 12-myristate 13-acetate (PMA) or suppressed using metoprolol. Plasma derived from this blood was subsequently analyzed for presence of citrullinated TFPI by western-blotting. Effects on coagulation were measured by thrombin generation.

Results: Activation of neutrophils in whole blood resulted in a substantial increase in both peak-height and endogenous thrombin potential. Western blotting enabled the detection of the presence of citrullinated TFPI while suppression of neutrophil activation by metoprolol showed no detectable levels of citrullinated TFPI. Solid-phase competitive interaction studies revealed that the C-terminus of full-length TFPI dose-dependently binds to PAD4, thereby facilitating citrullination of crucial arginine-residues.

Summary/Conclusion: The findings indicate that TFPI is citrullinated after neutrophil activation in whole blood. Since FXa is the main target of TFPI, it is speculated that this explains the substantial increase in thrombin generation. This prompts for further investigation of inflammation-related diseases for citrullination of TFPI and possibly other coagulation factors. It can be hypothesized that citrullination of TFPI contributes to the risk of thrombosis in patients with thrombophilia or inflammation.

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OR17-02

STRUCTURAL REARRANGEMENTS IN THE ADAMTS13 CUB DOMAINS PERTURB THE ENZYME GLOBAL LATENCY

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Background: The mouse monoclonal antibody 17G2 disrupts the ADAMTS13 spacer-CUB interaction and induces conformational changes that allosterically activate the metalloprotease domain. Docking models identified interaction sites of CUB1 and CUB2 with the spacer domain, but the mechanism for antibody-mediated spacer-CUB interdomain disruption remains elusive.

Aims: Explore the molecular dynamics in the ADAMTS13 CUB1 and CUB2 domains upon 17G2 activation using hydrogen/deuterium exchange mass spectrometry (HDX-MS).

Methods: HDX-MS analysis was performed in triplicate using a fusion protein of the truncated ADAMTS13 C-terminal tail: Albumin domain 1 (AD1)-T2C2. In absence of 17G2, AD1-T2C2 was analyzed undeuterated as well as after 10, 100, 500 and 1000 seconds of deuterium exchange. An equimolar complex of AD1-T2C2 and 17G2 was evaluated at identical deuterium exchange timepoints.

Results: With an average residue redundancy of 9.52, the ADAMTS13 CUB domains were fully covered by 202 peptides in presence as well as in absence of 17G2. Relative fractional deuterium uptake was mapped onto the CUBs crystal structure (PDB ID: 7B01), which identified 2 loops in the CUB1 domain to contribute to the conformational epitope of 17G2. HDX rate differences revealed structural dynamics on CUB1, CUB2 and the CUB1-CUB2 interface, whereas antibody binding showed minimal HDX difference at the suggested spacer-CUB interaction site.

Summary/Conclusion: HDX-MS localized the 17G2 binding epitope in 2 loops of the CUB1 domain. HDX rates of the CUB domains at the suggested spacer-CUB interaction site remained unaffected upon binding of 17G2, illustrating marginal effect of 17G2 binding to this interface. Interestingly, 17G2 induced HDX rate differences at various CUB1 and CUB2 regions and at the CUB1-CUB2 interface. These data suggest that structural flexibility of the CUB1-CUB2 complex could explain spacer-CUB disruption upon antibody-mediated activation. Mutagenesis studies are ongoing to further support these data.

OR17-03

FIBRIN'S ALPHAC-REGION ACTS AS A SECURITY LATCH AT HIGH STRAINS BUT DOESN'T LEAD TO MAJOR ALTERATIONS IN OVERALL MECHANICAL BEHAVIOUR OF FIBRES, AS INDICATED BY ALPHA-TRUNCATED FIBRIN VARIANTS.

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Background: High extensibility and relatively low stiffness of fibrin fibres are both essential properties to fulfil their mechanical role as scaffold of blood clots. It is currently unknown how the inner structure of fibrin fibres underpins mechanical load-bearing elements.

Aims: To elucidate the load-bearing role of the protofibril backbone and the fibrin αC-region and develop a structural model underpinning mechanical behaviour.

Methods: Recombinant human fibrinogen variants were created by selectively truncating either the αC-domain (a390) or the complete αC-region (a220). Clots of wild type (WT), a390 and a220 fibrinogen were made on striated surface. Individual fibrin fibres were stretched using fluorescent microscopy-combined lateral force sensing atomic force microscopy and the measured stress-strain behaviour was analysed.

Results: At strains <~1.5, fibre stiffness was similar in all variants ($p>0.9999$, figure) indicating that low-strain response is independent of the αC-region. At intermediate strains (~1.8-2.8), fibre stiffness was lower in a390 and a220 clots compared with WT, suggesting some load bearing function of the globular αC-domain at these strains. Fibre rupture strain only decreased when the complete αC-region, but not αC-domain alone was truncated (0.885-fold, $p=0.007$ and 0.999-fold, $p=0.8384$ respectively), showing a role of the flexible αC-region in fibre integrity. Rupture stress and fibre toughness decreased gradually with gradual removal of the αC-region. Double exponential decay fitted well the relaxation periods of incremental fibre pulls and the value of stress relaxation constants t1 and t2 remained the same at all strains. This indicates that two distinct structures unfold, at small and large strains likewise.

Summary/Conclusion: Even though absence of the αC-region leads to apparent differences in network structure, it does not lead to major alterations in the low-strain mechanical behaviour of fibrin fibres. Consequently, other structural elements are load bearing at low strain. We propose a novel model where the protofibril backbone is the main load bearing element yet branching of protofibrils enables their selective loading. Meanwhile the αC-region acts as a security latch at high strains thus contributing to fibrin's high extensibility.

OR17-04

DESIGN AND CHARACTERIZATION OF NOVEL ACTIVATED PROTEIN C (APC) VARIANTS FOR ENHANCED PROTEOLYSIS OF HISTONE H3

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Background: Extracellular histone H3 can induce pathological effects *in vivo* including inflammation, cell death and immunothrombosis. Neutralization of histone H3 may therefore be beneficial in various diseases. Activated protein C (APC) can cleave histone H3, which reduces its cytotoxicity. However, due to the anticoagulant properties of APC, use of wildtype APC is not optimized for the treatment of histone-mediated cytotoxicity.

Aims: We aimed to investigate the molecular interactions between human APC and histone H3 and subsequently rationally designed and generated novel APC variants. The variants are predicted to have reduced anticoagulant activity, whilst also possessing an enhanced ability to bind and cleave histones, as compare to wildtype APC.

Methods: We used computational design (molecular docking and molecular dynamics methods) to identify key residues that mediate the interactions between APC and histone H3. By computational design we explored *in silico* APC variants, to obtain variants with optimized functional characteristics. These variants were expressed and purified and their ability to function as an anticoagulant, bind or cleave histones, and their cytoprotective properties were tested quantitated.

Results: Analysis of APC-mediated histone H3 proteolysis and structural modelling identified the cleavage sites in histone H3 and the APC residues that are important for the interaction with histone H3. APC variants were engineered and generated by mutation of the identified key cationic residues to neutral or anionic residues, resulting in novel APC-H3-1 and APC-H3-2 variants. Compared to wildtype APC, both the APC-H3-1/2 variants showed a significantly decreased anticoagulant activity and increased binding to histone H3, whilst they showed similar ability to proteolyze histone H3.

Summary/Conclusion: It is possible to rationally design novel optimized APC variants that can be further developed and utilized to treat histone-mediated disease, by reduction of histone-associated cytotoxic properties, whilst not inducing an increased bleeding risk.

Oral Communication – Venous & Arterial Thrombosis Aspects and Risks

OR18-01

PLASMA LEVEL OF HUMAN EPIDIDYMIS PROTEIN 4 IS ASSOCIATED WITH RISK OF FUTURE VENOUS THROMBOEMBOLISM

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Background: Human epididymis protein 4 (HE4) is a glycoprotein secreted mainly by epithelial cells. HE4 is upregulated in many cancer types and serum HE4 levels improve the performance of risk assessment models (RAMs) for ovarian cancer and is strongly associated with heart failure severity and prognosis. To the best of our knowledge, no previous study has investigated whether plasma HE4 levels are associated with risk of future venous thromboembolism (VTE).

Aims: To investigate whether plasma HE4 levels are associated with risk of future incident VTE.

Methods: A case-cohort study including 294 VTE cases and 1,066 participants randomly sampled into a sub-cohort was derived from a population-based cohort – the Trøndelag Health Study (HUNT3). Baseline information was collected at inclusion (2006-08) by physical examination, self-administered questionnaires, and blood samples (EDTA plasma). Study participants were followed-up for five years after inclusion. Plasma samples were stored at -80°C and subjected to aptamer-based proteomics (The 7k SomaScan® v4.1 aptamer-based platform) in 2022. Plasma HE4 levels turned out to be significantly associated with VTE risk in the proteome-wide analyses when multiple testing was accounted for using the Bonferroni method (significance threshold: $p<6.9x10-6$). To further explore the association between HE4 and VTE, weighted Cox-regression models adjusted for age, sex, and body mass index were used to calculate hazard ratios (HR) with 95% confidence intervals (CI) according to quartiles of HE4-levels (Q1-4) with the lowest HE4-levels (Q1) used as reference. Ethical approval and informed consent was obtained for all study participants.

Results: We found a dose-response relationship between plasma HE4 levels and VTE risk. Participants with plasma HE4 levels in the highest quartile (Q4) had a HR for incident VTE of 2.45 (95% CI 1.55-3.87) compared to those with HE4 levels in the lowest quartile (Q1) in analysis adjusted for age and sex. Further adjustment for body mass index had minor impact on the risk estimate (HR 2.81, 95% CI 1.77-4.48).

Summary/Conclusion: Our results indicate that those with increased plasma HE4 levels are at greater risk for future VTE. In future studies we will perform cause-specific analyses to determine whether the relationship is driven by cancer-related VTE and apply experimental studies and Mendelian randomization analyses to assess a potential causal relationship.

OR18-02

RISK OF CANCER AFTER VENOUS THROMBOEMBOLISM

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Background: Patients with venous thromboembolism (VTE) are at increased short-term risk of cancer (i.e., cancer occurring <1 yr after the VTE event). However, diverging results have been reported regarding long-term risk (>3 yrs after the VTE event) of cancer after VTE.

Aims: To investigate the risk of incident cancer after a first-lifetime VTE.

Methods: Participants (n=35045) without VTE or cancer at inclusion were enrolled in the Tromsø 4-7 surveys (1994-2016), a population-based cohort study, and followed through 2020. All first-time VTE and cancer events were validated and recorded during follow-up, and VTE was modeled as a time-varying exposure. Hazard ratios (HRs) for cancer with 95% confidence intervals (CIs) were calculated according to categories of the time interval since VTE (< 1 yr, 1-3 yrs, > 3 yrs after VTE). HRs were adjusted for age (as a time-scale), sex, body mass index, smoking, hypertension, diabetes, and arterial cardiovascular diseases (stroke, MI, and angina). Sub-distribution hazard ratios (SHRs) were used to evaluate the influence of competing risk by death. Ethical approval and written informed consent were obtained.

Results: In total, 700 participants developed VTE, of whom 137 (19.6%) experienced a subsequent cancer diagnosis during a median follow-up of 16.9 years. The multivariable-adjusted HRs for cancer after VTE were 5.43 (95% CI, 4.14-7.12) at <1 yr, 1.41 (95% CI 0.94-2.13) at 1-3 yrs, and 1.13 (95% CI 0.88-1.46) at >3 yrs when compared to individuals without VTE. Competing risk by death influenced the relative risk at <1yr (SHR 4.01, 95% CI, 2.94-5.47), while the estimates from 1-3 yrs and >3 yrs were unaffected.

Summary/Conclusion: Our findings demonstrate that incident VTE is accompanied by by a transient increased risk of cancer during the first 3 years after the VTE event.

OR18-03

GLUCOCORTICOID TREATMENT AND COAGULATION PARAMETERS IN PATIENTS WITH A FIRST VENOUS THROMBOSIS: DATA FROM THE MEGA STUDY

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Background: Glucocorticoid (GC) treatment increases the risk of venous thromboembolism(VTE) through unclear mechanisms. Particularly, it is unclear to what extent GCs influence coagulation and fibrinolysis directly. It is also unknown whether GC-induced coagulation changes mediate the risk of a recurrent VTE.

Aims: To assess whether GC treatment is associated with changes in coagulation and fibrinolysis in outpatients with a first VTE and their contribution in mediating the risk of a recurrent VTE.

Methods: The MEGA-study is a case-control study into causes of VTE, where clinical data were collected on 4956 patients and blood was sampled in a random 50%. Data on 2547 patients could be linked to the Dutch Pharmacies Register to obtain information on GC-prescriptions, out of whom a blood sample was available in 1155 patients. To disentangle the contribution of the GC to the coagulation changes from that of the underlying disease, patients were classified as: 1) treated with GC if the date of blood sampling was within a period of GC-use, 2) untreated if they never had a GC prescription, 3) later GC-users if they had a prescription within 90 days of blood sampling (e.g. excluding later GC-user). Fibrinogen, factor(F)V, FVIII, FX, von Willebrand factor (VWF), protein(P)C, PS, thrombin activatable fibrinolysis inhibitor, plasminogen activator inhibitor-1, clot lysis time(CLT) and endogenous thrombin potential(ETP) were measured in each group. Mean differences and 95% confidence intervals(CI) were estimated with linear regression adjusted for possible confounders. For the mediation analysis, hazard ratios and 95%CI of recurrent VTE adjusted for possible confounders and procoagulant factors were estimated through Cox regression.

Results: Of 1155 patients, 31 were using GC at the time of blood sampling, 890 never were and 40 used a GC within 90 days of blood sampling. Patients treated with a GC were slightly older compared to never users and had instead a similar age to later GC-users (mean age was 57 [interquartile range 42-64], 50 [39-48] and 58 [47-66] respectively). Mean levels of all pro- and anti-coagulant parameters, except for FX, were higher in the patients treated with GC compared to the untreated. In particular, both FVIII activity and levels as well as VWF levels were markedly increased in GC-treated patients (mean difference of 65.1% [34.7 - 95.4], 89.1 UI/dL [51.6 - 126.7] and 55.6 UI/dL [17.5 - 93.6] respectively). Moreover, all parameters were similarly increased in patients treated with a GC when compared to later GC-users. Instead, ETP and CLT were not different between the three groups. Patients treated with a GC during blood sample had an increased risk of a recurrent VTE compared to never-treated (adjusted HR of 2.4 [95%CI 1.2-4.8]), which decreased to a HR of 1.7 (95%CI 0.8-3.6) when further adjusted for procoagulant factors.

Summary/Conclusion: Mean levels of all anti- and pro-coagulant proteins were higher in patients treated with GC compared to the untreated, with a similar ETP and CLT. These findings are in line with the literature

on patients with Cushing's disease. The increase in procoagulant factors partially mediated the risk of a recurrent VTE in GC users, although replication in larger studies is warranted.

OR18-04

DNASE-I OVERCOMES RT-PA RESISTANCE OF PLATELET-RICH ISCHEMIC STROKE THROMBI

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Background: Recombinant tissue plasminogen activator (rt-PA) is the only approved drug that can be used to pharmacologically lyse the occluding thrombus in ischemic stroke patients. Due to several contraindications and therapy resistance, the recanalization is achieved in only a small number of patients. The exact nature of this rt-PA resistance is not fully understood but it is thought that thrombus composition plays an important role. We previously showed that, besides fibrin, extracellular DNA and neutrophil extracellular traps (NETs) are a structural feature of ischemic stroke thrombi which could be important for thrombus stability, especially in platelet-rich thrombi.

Aims: To investigate the rt-PA resistance in ischemic stroke thrombi and to explore the additional thrombolytic effect of an adjuvant therapy of the DNA-degrading enzyme DNase-1 in combination with rt-PA.

Methods: A total of 63 ischemic stroke thrombi were divided into three treatment groups (1µg/mL rt-PA, combination of 1µg/mL rt-PA and 100U/mL DNase-1 or nothing). For each thrombus, one part was used for detailed histological analysis of red blood cells (RBC), fibrin, platelets, von Willebrand factor (VWF), leukocytes, intra- and extracellular DNA and NETs. The other part was subjected to lysis for 180 minutes, after which lysis efficiency was calculated as the percentage of residual thrombus weight compared to original weight.

Results: We observed that RBC-rich thrombi (>50% RBC) were significantly more susceptible to rt-PA-mediated lysis compared to platelet-rich thrombi (<50% RBC) (residual weight of 24.9%±18.6% and 59.6%±25.1% respectively). A strong positive correlation between the percentage of RBC and the lysis rate of the thrombus and a negative correlation with the percentage of VWF was found. Next, we wanted to test if addition of DNase-1 could increase lysis of ischemic stroke thrombi and more specifically the rt-PA resistant platelet-rich thrombi. Strikingly, combined use of rt-PA and DNase-1 significantly improved thrombolysis of platelet-rich thrombi (residual weight of 27.4%±18.8%), but had no additional effect on the rt-pa mediated lysis of RBC-rich thrombi (residual weight of 24.9%±7.7%).

Summary/Conclusion: This study showed that RBC-rich ischemic stroke thrombi are more susceptible to lysis using standard rt-PA treatment and that combined use of rt-PA and DNase-1 can improve the lysis of rt-PA resistant platelet-rich thrombi. These insights open new perspectives for the treatment of ischemic stroke patients and could potentially improve the thrombolysis of rt-PA resistant ischemic stroke thrombi.

Oral Communication – Regulation of Protein Function in Hemostasis

OR19-01

SENSITIVE MEASUREMENT OF CLINICALLY RELEVANT FACTOR VIII LEVELS IN THROMBIN GENERATION ASSAYS REQUIRES PRESENCE OF FACTOR XIA

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Background: Hemophilia A (HA) is a bleeding disorder characterized by decreased or absent FVIII. Clinical analysis of coagulation potential in this patient population is classically based on APTT based FVIII assays. Although both the onestage FVIII assay and the chromogenic FVIII assay can measure FVIII concentrations reliably these types of assays only give insight on the initiation of coagulation. Global coagulation assays, like thrombin generation (TG), can be used to measure the full coagulation spectrum of initiation, amplification and propagation. However the frequently used, commercially available, TG kits lack sensitivity for measurements of hemophilia plasma within the lower FVIII ranges (below 20%).

Aims: We aim to optimize the sensitivity of thrombin generation for measurements in severe hemophilia A patients, especially at lower FVIII levels.

Methods: The efficiency of several coagulation triggers of the intrinsic, extrinsic, common pathway and combinations thereof were investigated in thrombin generation on severe hemophilia A plasma with and without the addition of additional FVIII. Subsequently the chosen trigger, TF/FXIa, was used in further optimization of the assay based on assay temperature, phospholipid use and contact activation inhibitor use.

Results: Combinations of TF/FXIa, TF/Xa and (to lesser extend) TF/IIa were superior in thrombin generation measurements of severe hemophilia A plasma at through levels. TF-only, FIXa and TF/IXa failed to provide reliable measurements at these through levels. TF/FXIa initiated thrombin generation showed a greatly increased sensitivity of thrombin generation in hemophilia A plasma at through levels compared to commonly used TF-only trigger. TF/FXIa was able to provide a solid thrombin generation curve at these through levels where TF-only did not. Measurements of several individual severe hemophilia patients at through levels showed high sensitivity for thrombin generation at through and higher levels of FVIII.

Summary/Conclusion: TF/FXIa dual activation thrombin generation shows markedly increased FVIII sensitivity in severe hemophilia plasma when compared to classic tissue factor triggered TG. This allows for dose-dependent measurements in low FVIII ranges and provides the means for a solid baseline curve that can be used for better clinical evaluation of coagulation potential and therapeutic monitoring in hemophilia A.

OR19-02

IMPLEMENTING ANDEXANET ALFA IN THE HOSPITAL – PRACTICAL EXPERIENCE AND LEARNING POINTS

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Background: Since 2019 andexanet alpha is approved in the EU for the reversal of life-threatening or uncontrollable bleeding in patients taking apixaban or rivaroxaban. In case of a life-threatening or uncontrollable bleeding while using apixaban or rivaroxaban the ESH/EHRA guideline first recommendation is the use of andexanet alpha. Implementing a new antidote in a hospital is challenging in terms of the correct treatment indication, the way of administration, the role of laboratory tests and multidisciplinary cooperation.

Aims: To provide practical learning points of implementing andexanet alfa in the hospital based on a case series of patients with a life-threating bleeding.

Methods: All patients treated with andexanet alfa between January 2020 and October 2022 in the Maastricht University Medical Center+ were included. Date, indication and dosing strategy were prospectively registered for safety evaluation and critical assessment of the local implementation strategy. Additional data as demographics, laboratory results and complications were retrospectively collected from the electronic patient files.

Results: In total, 26 patients with a life-threatening bleeding were treated with andexanet alfa. Indications for administration were mostly intracerebral bleeding (65.4%), subdural hematoma (11.5%) and subarachnoid bleeding (7.7%). In 84.6% a low dose andexanet alfa was indicated. In two thirds of the patients a DOAC plasma concentration was measured before administration of andexanet alfa, which was in one third of these patients higher than the expected value based on dosing and timing of DOAC intake. In 4 patients (15.4%) a bleeding complication occurred during admission, while in 5 patients (19.2%) a thrombotic event occurred in the days after administration of andexanet alfa. Learning points following this case series were to 1) always consult a coagulation expert about the correct indication and dosage, 2) provide clear instructions due to the urgent situation and a lack of experience, 3) provide necessary materials with the medication, 4) consider measurement of the DOAC plasma concentration before administration of andexanet alfa, 5) avoid sequential administration of andexanet alfa and prothrombin complex concentrate, 6) avoid using andexanet alfa prior to heparinization.

Summary/Conclusion: It is vital to know how to act in case of a life-threating bleeding while using apixaban and rivaroxaban. Up-to-date protocols and proper instructions to involved healthcare professionals concerning the use of andexanet alfa are crucial.

OR19-03

PATIENTS WITH HEMOPHILIA A HAVE INCREASED LEVELS OF CIRCULATING ACTIVATED PROTEIN C: POTENTIAL CONTRIBUTION TO THEIR HEMOSTATIC STATUS

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Background: The hemorrhagic phenotype of hemophilia A (HA) differs among patients, even with apparently identical clinical forms. One hypothesis is an imbalance in the natural anticoagulants.

Aims: To analyze the levels of activated protein C (APC) in plasma of HA patients and carriers and to correlate their levels with the hemostatic status, hitherto unknown.

Methods: We recruited 100 HA patients (59 severe, 11 moderate, 30 mild) in a stable clinical situation and 48–72 h after factor administration and 21 HA carriers. We quantified the levels of circulating APC, coagulation factors (FVIII, FV, fibrinogen, FIX, FXI, FXII, FX, FII, FVII, FVW), PC, protein S (PS), antithrombin (AT) and the thrombin generation test (TGT) with the BleedScreen ST Genesia (Stago).

Results: All HA patients had higher circulating APC levels than carriers (7.5 *vs.* 5.1 ng/mL, P=0.012) and its cofactor PS (115.6 *vs.* 94.3%, P<0.001), while they had similar PC levels. AT levels were similar in both groups. They had lower levels of FVIII, FXII, and of the TGT peak, time to peak, ETP, velindex and start tail (P<0.001). When patients were analyzed by severity (severe, moderate, and mild), APC levels increased with HA severity, and TGT slightly decreased. Finally, HA severity correlated with APC (0.196, P=0.037), peak (-0.595), ETP (-0.460), velindex (-0.604) and start tail (0.672) (P<0.0001 in all cases), as well APC levels and peak (-0.242, P=0.016) and ETP (-0.272, P=0.006).

Summary/Conclusion: HA patients have higher circulating APC levels than carriers, which increases with severity despite having similar levels of PC. Other anticoagulants like AT are unaltered and HA patients have a lower capacity to generate thrombin. Furthermore, APC levels correlate with HA severity and TGT. Thus, APC seems to modulate the hemostatic status of HA patients and could perhaps modulate their bleeding phenotype. SOBI, ISCIII-FEDER (PI20/00075, FI21/00171), GVA-CIACIF/2021/192 and SETH.

OR19-04

IDENTIFICATION OF FXI MONOMERS IN PLASMA: FUNCTION, CLEARANCE AND RELEVANCE OF CYS339

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Background: Factor XI (FXI) is a procoagulant molecule that is activated by both FXIIa and thrombin and is mainly inhibited by C1 inhibitor, but also by antithrombin (AT). It has been suggested that dimerization of FXI monomers by a disulfide bridge at Cys339 is required for secretion from hepatocytes, as only FXI dimers are detected in plasma. FXI deficiency, a probably underestimated disorder, moderately increases the risk of bleeding but strongly protects against venous thrombosis.

Aims: To identify FXI monomers in plasma; to know when and why they are produced; and to understand the physiological and clinical implications of circulating FXI monomers.

Methods: We studied plasma FXI by coagulation and chromogenic assays, ELISA, and Western blot under non-reducing conditions in 3 cohorts of unrelated individuals: 110 with congenital FXI deficiency, 250 blood donors and 350 with AT deficiency (ATD) to find a negative modulator of thrombotic risk. *F11* p.Cys339Phe was genotyped using a Taqman assay. Expression of FXI wild-type and variant was evaluated in transiently transfected HEK cells and in HepG2, a human hepatic cell line.

Results: Evaluation of FXI in plasma by Western blot identified only dimers in all subjects except in 3 patients,all with ATD caused by different *SERPINC1* mutations. These 3 individuals had monomers at a similar rate as dimers; the latter were reduced compared to healthy controls. These 3 cases were heterozygous for the *F11* p.Cys339Phe variant (GnomAD MAF: 2.00e-3). Genotyping of this mutation in all 710 subjects confirmed that these were the only 3 carriers. Mutant FXI monomers were fully activated by FXIIa when the contact pathway was exposed to silica. Accordingly, the functional activity of carriers by FXI:C and chromogenic assay was in the low range of normality, 90.5%±3.5 and 82.5%±7.7%, respectively, and the antigen levels were normal, 110.5%±10.6%. From a clinical point of view, carriers had no signs of bleeding and no history of venous thrombosis despite all having ATD.

HEK cells expressing the FXI Phe339 variant secreted high levels of monomer but no dimers. Interestingly, HEK cells transfected with wild-type FXI secreted both dimers and monomers into the conditioned medium, but dimers at a higher rate (x1.5). Similarly, HepG2 also secreted FXI dimers and monomers into the conditioned medium. Supplementation of whole blood, platelet-rich plasma (PRP), and platelet-poor plasma (PPP) with conditioned medium from HEK cells transfected with wild-type and mutant FXI and ulterior centrifugation cleared wild-type monomer but not mutant monomer or wild-type dimer in whole blood, but not in PPP or PRP.

Summary/Conclusion: We first identified monomers of FXI in the plasma of 3 heterozygous carriers of p.Cys339Phe. The variant monomer is activated by FXIIa and only a moderate reduction of FXI activity was associated with this variant, which would not be detected by coagulation assays. Interestingly, all carriers of this mutation also had ATD caused by various *SERPINC1* mutations, suggesting an association of AT with FXI and an antithrombotic protection that needs to be further investigated. We confirmed that wild-type FXI

monomers are secreted by hepatic cells but are cleared in the circulation by binding to blood cell component in a process that requires Cys339. Further studies are needed to identify the mechanism of this clearance.

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Oral Communication – Regulation of Coagulation and Inflammation

OR20-01

A SINGLE-DOMAIN ANTIBODY ENHANCING PROTEIN S ACTIVITY REDUCES VASO-OCCLUSION IN TWO MURINE MODELS OF SICKLE CELL DISEASE

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Background: Ongoing coagulation activation is involved in sickle cell disease (SCD). Moreover, SCD patients have lower Protein S (PS) levels than healthy volunteers, with a further reduction during vaso-occlusive crises (VOC). PS003biv is the bivalent form of a recently described anti-PS single-domain antibody (sdAb) enhancing the activated protein C-cofactor activity of PS and exerting an antithrombotic effect in vivo, which makes it potentially relevant in SCD.

Aims: To assess if PS003biv can enhance PS activity in SCD patients and limit VOC in SCD mice.

Methods: PS003biv's effect on PS activity in SCD patients (n=8) was assessed with a clotting-based assay in plasma. We tested PS003biv effect on VOC in SCD Townes mice by intravenously injecting PS003biv at 10 mg/kg or the vehicle before triggering VOC (n=4 in each group). Two VOC models were tested: hypoxia/reoxygenation (H/R) injury or hemin injection (50 µmol/kg). Vaso-occlusion was quantified by Ter-119 immunofluorescence staining of red blood cells (RBC) congesting vessels in the liver and lungs, expressed as a percentage of the field of view (%FOV). Bilirubin, free hemin (H/R) or free hemoglobin (hemin injection model), and thrombin-antithrombin complexes (TAT) were measured in plasma.

Results: PS003biv increased PS activity in SCD patients (control sdAb 67.9±6.3% versus PS003biv 97.2±11.8%, p<0.0001). PS003biv decreased RBC accumulation and vaso-occlusion in both VOC models. In the H/R injury model, Ter-119 staining was reduced in the liver and lungs (liver control: 13.5±0.7 versus PS003biv 6.9±0.4 %FOV p<0.05; lung control 7.6±0.8 versus PS003biv 3.5±0.9 %FOV, p<0.05). PS003biv-treated mice displayed lower levels of hemolysis biomarkers: (bilirubin control 6.9±0.2 versus PS003biv 4.9±0.6 mg/dL p<0.05; heme control 133.0±10.4 versus PS003biv 77.5±5.3 µM p<0.05); and reduced coagulation activation (TAT control: 10.1±0.7 versus PS003biv 7.5±0.3 ng/mL, p<0.05). In the hemin injection model, Ter119-staining was also decreased in the liver and lungs of PS003biv-treated mice (liver control: 22.0±2.4 versus PS003biv 13.0±4.2 %FOV p<0.05; lungs control 27.8±1.6 versus PS003biv 15.4±3.2 %FOV, p<0.05). Hemolysis biomarkers levels were reduced (bilirubin control 11.4±2.1 versus PS003biv 8.3±0.7 mg/dL p<0.05; free hemoglobin control 7.6±1.3 versus PS003biv 4.6±0.4 mg/mL p<0.05) as well as coagulation activation, although not significantly (TAT control: 12.2±8.9 versus PS003biv 4.4±0.2 ng/mL).

Summary/Conclusion: PS003biv significantly enhanced the activated protein C-cofactor activity of PS in SCD patients and reduced vaso-occlusion, hemolysis, as well as coagulation activation in two models of VOC in SCD mice. The precise mechanism of action of PS003biv remains to be determined.

OR20-02

IMPACT OF MIR-15A-5P ON GENE REGULATION IN PRIMARY HUMAN MONOCYTES AND ENDOTHELIAL CELLS AND MOUSE LIVER CELLS

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Background: MicroRNAs (miRs) are small noncoding RNAs that post-transcriptionally regulate their target genes. MiR-15a-5p is abundantly found in multiple cell types, as well as in plasma. Dysregulation of miR-15a-5p, and closely related miRNAs with a homologous seed sequence (i.e., miR-15b, miR-16, miR-195 and miR-497), has been reported in cancer as well as in several cardiovascular diseases such as myocardial infarction and stroke. Although miR-15a is associated with several diseases, its regulatory effects and direct gene targets have not been systematically investigated.

Aims: To explore how miR-15a influence gene expressions in primary human monocytes and endothelial cells *in vitro* and mouse liver cell *in vivo*.

Methods: To identify possible targets of miR-15a in humans, primary human monocytes and endothelial cells were transfected with miR-15a mimic (LNA miR mimic, Qiagen) or its respective scramble control. RNA was purified from the cells and in-depth RNA sequencing was performed. In addition, RNA was purified from the liver tissue of control mice or mice treated with miR-15a mimic. Gene expressions were analyzed by RNA sequencing. Genes with a significantly reduced expression level and log fold change < 0.6 were considered potential direct targets of miR-15a. Gene ontology (GO) was performed to understand the possible effects of miR-15a on human and mouse cells.

Results: Transfected monocytes and endothelial cells with miR-15a-5p mimic resulted in a significant increase in miR-15a expressions. Elevated levels of miR-15a in monocytes were accompanied by upregulation of 202 genes and downregulation of 84. In endothelial cells, 39 genes were upregulated and 24 downregulated. Following administration of miR-15a to mice, 191 genes were upregulated and 183 were downregulated in liver cells. Few genes were commonly upregulated in the different cell type: DNLZ was upregulated in both monocytes and endothelial cells, LOXL, KCNK5 and PLK3 were upregulated both in monocytes and mouse liver cells. None of the downregulated genes, however, were found in two independent cell types. The most effectively downregulated genes in monocytes were involved in regulation of actin filament (*PDXP* and *EPS8*) and cell signaling (*PTH2R, SPOCK1, KLHL13*). In endothelial cells elevated levels of miR-15a downregulated genes involved in transport (*SLC22A15*), cell differentiation (*ITGA8*), RNA regulation, transcription and cellular response to cytokines (*EFHB, SATB2* and *IL1RAPL1,* respectively). In mouse liver cells, three pseudogenes were downregulated (*Gm6505*, *Gm2026*, and *Gm49519*) by miR-15a treatment. Other genes effectively downregulated by miR-15a were *Bcl6* (regulator of cell apoptosis) and *Fgf21 (*growth factor highly produced by liver cells).

Summary/Conclusion: MiR-15a regulates multiple genes, either directly or indirectly, but no common genes across various cells. Specific interaction between miR-15a and its targets (mRNA) will be further investigated by luciferase reporter gene assay.

OR20-04

AN ANTICOAGULANT TICK SALIVARY PROTEIN INHIBITS PLATELET ACTIVATION AND IMPAIRS IN VITRO THROMBUS FORMATION

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Background: As several physiological processes such as coagulation, platelet aggregation, complement activation, and inflammation are driven by proteolysis and proteolytic enzymes, protease inhibitors identified in tick saliva have attracted particular research attention. Multiple transcriptomic and proteomic analyses of tick salivary gland secretions have revealed high expression of several protease inhibitors while the *in vitro* and *in vivo* assays have shown that these inhibitors have specific targets in host cells. The identification and functional characterization of molecules that interfere with physiological pathways and networks on the vertebrate host may provide the basis for developing new biotechnological tools for vector control in addition to antithrobotics

Aims: To functionally characterize a tick salivary protein (Iripin-3) and investigate its effects on coagulation, platelets, and *in vitro* thrombus formation.

Methods: Recombinant Iripin-3 was expressed in *E. coli*, purified and LPS decontaminated. To assess the anticoagulant properties, plasma thrombin generation was measured in the absence and presence of Iripin-3 using calibrated automated thrombinography. The effect of Iripin-3 on different individual coagulation factors was determined using chromogenic assays with the appropriate substrates. To investigate potential anti-platelet effects, isolated platelets from healthy donors were treated with Iripin-3 and subsequently stimulated with collagen-related peptide (CRP), thrombin or ADP. Integrin $a_{IIb}b_3$ activation, P-selectin expression and phosphatidyl serine (PS) exposure were measured using flow cytometry. Finally, thrombus and fibrin formation under flow using the Maastricht flow chamber were measured to determine the overall antithrombotic potential in a whole blood environment. To this end, citrate anticoagulated whole blood from healthy donors was treated with Iripin-3, recalcified and co-perfused with tissue factor over a collagen surface at 1000 s^{-1} for ten minutes.

Results: Iripin-3 had a dose-dependent effect on both extrinsic and intrinsic pathways of coagulation. The effect was explained by the inhibition of FXIIa and FXIa through the classic serpin inhibition mode and the interaction with FXa through exosite binding. Regarding the effects on platelet activation, incubation of washed platelets with Iripin-3 resulted in a minor, but significant inhibition of CRP-mediated integrin $a_{IIb}b_3$ activation (% positive platelets, 86.88±2.893 *vs.* 60.21±23.5; $P = 0.0323$). Further, Iripin-3 partially inhibited thrombin-mediated platelet integrin $a_{IIb}b_3$ activation (87.97±3.335 *vs.* 50.97±1.50; $P = 0.0022$), as well as decreased PS exposure upon dual CRP/thrombin stimulation (49.62±10.59 *vs.* 26.64±8.40; $P = 0.0203$). Whether this is the result of direct inhibitory effects on platelets or indirect platelet inhibition via modulation of thrombin activity, remains to be shown. In a whole blood environment under flow conditions, incubation of healthy blood samples with Iripin-3 led to a delay in thrombus growth as well as a reduction in fibrin formation.

Summary/Conclusion: Collectively, our findings confirm the pleotropic properties of tick salivary compounds and constitutes a proof of their applicability as antithrombotics.

Poster Session – Bleeding

P-001

THE AXIS NETOSIS-ACTIVATED PROTEIN C MAY MODULATE THE HEMOSTATIC STATUS OF PATIENTS WITH HEMOPHILIA A

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Background: Neutrophil extracellular traps (NETs) are highly prothrombotic networks that activate the extrinsic and intrinsic coagulation pathways. NETs inhibit activated protein C (APC) and APC inhibits NETosis. Whether the axis NETosis-APC modulates the hemostatic status of hemophilia A (HA) patients is unknown.

Aims: To analyze the dysregulation of NETosis and APC levels in plasma of HA patients and to correlate their levels with their hemostatic status.

Methods: We recruited 100 HA patients (59 severe, 11 moderate, 30 mild) in a stable clinical situation and 48–72 h after factor administration and 53 male healthy controls. We quantified NETs markers (cell-free DNA, cfDNA; DNA-histones complexes and calprotectin) with specific assays, circulating APC [1] and DNaseI activity with the SRED.

Results: APC levels increased with HA severity, having severe patients higher APC levels (8.2 ng/mL) than moderate (6.1) or mild (6.6) (P<0.05). In line, NETosis decreased with HA severity, having severe patients lower levels of cfDNA (994 ng/mL) than moderate (1239) or mild (1053) (P=0.02). Plasma DNaseI also decreased in severe HA (0.35 cm^2) compared to moderate (0.43) or mild (0.42) (P<0.05), thus DNase I is not responsible for the decrease in plasma cfDNA with HA severity. Compared with controls, HA patients had slightly lower APC levels (8.2 *vs.* 9.8 ng/ml) but a significantly lower cfDNA (1059 *vs.* 1132) and DNaseI (0.389 *vs*. 0.46) (P<0.03). HA severity correlated with APC (0.299), cfDNA (-0.359) and DNaseI (-0.316) (P<0.0001 in all cases).

Summary/Conclusion: Circulating APC levels increase with HA severity, while NETosis decreases. The decrease in cfDNA is not caused by an increase in plasma DNaseI. Thus, the combination of increased anticoagulant APC and decreased procoagulant NETosis may modulate the hemostatic status of HA patients and their bleeding phenotype. SOBI, ISCIII-FEDER (PI20/00075, FI21/00171), GVA-CIACIF/2021/192 and SETH.

[1] Martos L et al. A simplified assay for the quantification of circulating activated protein C (2016). Clin Chim Acta 1;459:101-104.

P-002

TWO RHEOLOGICAL MODES IN THE INJURY SITE

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Background: Rheological conditions profoundly affect adhesion, activation and aggregation of platelets in vivo, and also affect local concentrations of coagulation factors inside and out of thrombus. Any pathological condition, be it stenosis or traumatic puncture of vessel wall, creates novel rheological conditions at the injury site. However, there is little information on these rheological conditions, in particular, for the acute conditions of partial vessel occlusion and puncture.

Aims: Our aim was to investigate rheological conditions that arise at the injury site and in the intact part of circulatory system.

Methods: A computer simulation of blood circulation in the human arm was carried out to model rheological changes at the micro- and macro-scales. The model assumed that intact vessels have rigid walls and blood is Newtonian fluid. Flow in intact vessels was considered in one dimensional space (1D) and in 3D when propagating through the injury region. Based on Hagen-Poiseuille's law, Navier-Stokes and continuity equations and experimental knowledge of sizes of vessels, a system of corresponding algebraic and differential equations was solved for flux, pressure and velocity fields.

Results: When median cubital vein was punctured, the pressures at the ends of injured vessel decreased with increase of the radius of the wound (up to 42% compared with intact system). Pressures in other nodes decreased by less than 1%. The blood flow from the supply vessels increased (up to 74%), and the outflow from the discharge vessels decreased. In basilic vein it stopped completely (at radius of wound about 700 um) and changed its direction (to 18% of the value in intact vessel). The flow changes did not extend (a change less than 0,6%) to the deep veins. Flow lines near the wall opposing injury did contain a curved section suggesting that blood cells circulate in the injury area before passing through the wound near the damaged wall. For realistic macroscopic injuries (more than 300 um), the rate of the blood loss was determined by resistances of the supply vessels and the shear rate decreased with increasing wound size. For small injuries, it mostly depended on the resistance of the wound. Shear rate increased (up to 10^5 c^{-1}) with the wound size (less than 300 um) in this mode.

Summary/Conclusion: These data provide insight into how traumatic puncture affects flow redistribution in a circulatory system, and how hydrodynamic resistances of the vessels and of the wound determine blood loss and conditions for the hemostatic system functioning.

P-004

PREDICTORS OF HEALTH-RELATED QUALITY OF LIFE IN ADOLESCENT GIRLS WITH HEREDITARY BLEEDING DISORDERS

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Background: In developing countries, health-related quality of life (HRQOoL) is not very well investigated among adolescent girls with Hereditary Bleeding Disorders (HBDs). After the age of menarche, this age group might present with heavy menstrual bleeding (HMB), which poses a significant impact on HRQoL.

Aims: The aim of this work is to assess HRQoL in adolescent girls with HBDs and explore their potential predictors.

Methods: A cross-sectional study done on all girls aged 10-16 years attending the hematology clinic in our center throughout a duration of 1 year (Jan-December 2022). 36-Item Short Form Survey (SF-36) was utilized to assess HRQol. Informed consent was obtained from patients and their guardians, and ethical approval was obtained from the research committee in our institution.

Results: Out of 155 patients recruited, 141 completed the questionnaire. Known cases of von Willebrand disease constitute the majority (112/141 = 79.5%). HRQoL score was significantly lower in studied patients, compared to controls (61.1 ± 12.9 vs. 72.4 ± 15.5) (p-value < 0.0001). Multiple regression analysis revealed that the most significant predictor of total HRQoL score was adherence to hormonal therapy (B = 0.59, 95% confidence interval [CI] 0.12 - 1.04, P = 0.008), outweighing other parameters. In addition, proximity to a specialized healthcare center, maintaining a hemoglobin level above 11 g/dL and higher socioeconomic status were significantly correlated with better HRQoL (P-values: 0.02, 0.04 & 0.05 respectively). On the other hand, adherence to tranexamic acid, bleeding phenotype in childhood and family history of bleeding diathesis were not significantly correlated to HRQoL.

Summary/Conclusion: Overall, HRQoL is significantly lower in teenager girls with HBD. Factors related to optimum control of HMB, such as adherence to hormonal therapy, easier access to healthcare & normal Hb for age/gender, together with higher socioeconomic status are significantly correlated with better HRQoL. Recognition of these factors could help healthcare professionals to develop effective management strategies of HBD in these patients. Establishing a National bleeding Registry is mandatory to offer better care.

P-005

ACQUIRED VON WILLEBRAND SYNDROME IN THE SETTING OF UNEXPECTED MAJOR BLEEDING AFTER INVASIVE PROCEDURE: A DELAYED DIAGNOSIS

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Background: Acquired von Willebrand syndrome (avWs) is an acquired bleeding disorder which may suddenly manifest in individuals without personal or family history of bleeding. AvWs is characterized by the deficiency or dysfunction of von Willebrand factor and can be caused by several underlying medical conditions, such as lymphoproliferative disorders. It is much less common than the congenital form and the diagnosis is not often suspected since many cases may be clinically silent or mild.

Aims: We herein report a case of avWs diagnosed in the setting of unexpected major bleeding during a liver biopsy.

Methods: A 78-year-old man, with previous medical history of chronic lymphocytic leukemia, was admitted to our hospital for pulmonary aspergillosis. We would like to note that the patient was chronically medicated with prednisolone.

At admission, coagulation study was normal and the patient didn't present any bleeding symptoms. At the 21st day of admission, he started to present a slight activated partial thromboplastin time (aPTT) prolongation and some bruises in the arms. These symptoms and aPTT prolongation were not valorized because of concomitant therapy with enoxaparin (for a newly diagnosed flutter) and frequent phlebotomies.

A liver biopsy was performed, 55 days after admission, with adequate anticoagulation suspension, and after the procedure the patient complicated with major bleeding and was admitted in the intensive care unit. Surgical hemostasis, red blood cell and plasma transfusion were required to manage and successfully control the bleeding. 4 days after the procedure (D65) this case was reported to our departments. At the time, aPTT was still prolonged. A mixture test was requested and was suggestive of factor inhibitor. At that time, the bleeding symptoms were controlled and hemoglobin was stable. Specific factor determination was conducted in and the results are shown then.

The patient was discharged after 12 days without any new bleeding, maintaining the same prednisolone dose. He will be reevaluated in outpatient appointment after discharge.

Results: The results are:

- TP (sec): Admission 14.0; D21 13.3; D55 12.0; D59 12.6; D62 12.4; D63 13; D64 11.9.

- aPTT (sec): Admission 25.6; D21 48; D55 37.3; D59 45.9; D62 39.9; D63 47.1; D64 35.7

- Fibrinogen (mg/dL): D55 422; D64 319

- mixing studies (Rosner index): D59 17

- FVIII (%): D62 31; D63 61.9; D64 63

- FIX (%): D62 101; D63 98.9

- FXI (%): D63 95.5

- FXII (%): D63 69.9

- FvW: Ag (%): D63 53.6; D64 59

- FvW: Rco (%): D63 21; D64 41

- AL screening (SCT/dR VVT) D62 0.98/1.43 AL POSITIVE

Summary/Conclusion: Bleeding symptoms in an individual with isolated unexplained prolonged aPTT and any diseases that can cause avWs should raise suspicion and prompt an evaluation. Evaluation and management may be complex, especially in those waiting an invasive procedure or that require antithrombotic therapy.

P-006

EVALUATION OF FVIII PK PROFILE IN KOREAN HEMOPHILIA A PATIENTS ASSESSED WITH MYPKFIT: A RETROSPECTIVE CHART REVIEW

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Background: Prophylaxis, which is regular replacement therapy with factor concentrates to prevent bleeding and its long-term consequences, is the standard of care for people with severe hemophilia and for those associated with a severe bleeding phenotype. Substantial inter-patient variation in pharmacokinetics results in significant variation in the amount of factor VIII (FVIII) required to sustain the desired trough level. But there is limited information on FVIII pharmacokinetics among Korean hemophilia patients, and the reimbursement restriction on factor concentrates makes it difficult to apply personalized treatment.

Aims: This retrospective study is to analyze the real-
world pharmacokinetics of FVIII in Korean hemophilia A patients assessed with myPKFiT.

Methods:
Severe to moderate hemophilia A patient's pharmacokinetics (PK) result assessed with myPKFiT, which is t he most widely used population PK tool in Korea, was collected through retrospective chart review in 5 hem ophilia treatment centers. The correlation between the PK profile and prophylaxis regimen was analyzed for the octocog alfa and rurioctocog alfa pegol in severe patients. And the PK profile differences of each produ ct by age, blood type O or non-
O, body mass index (BMI), and von Willebrand factor antigen (vWF:Ag) were evaluated.

Results:
PK data from 48 patients aged 5 to 52 years and 81 patients aged 14 to 64 years were collected for octoco g alfa and rurioctocog alfa pegol, respectively. The median half-lives were 9.90 (range: 6.30–
15.20) hours and 15.30 (range: 10.40–
23.90) hours, and the ranges of the time to 1% for each product were 27-88 hours and 63-
150 hours, respectively, and they also showed 2- to 3-fold inter-individual variation. When the effects of half-life and time to 1% on the FVIII dose and frequency were analyzed, only frequency in the patients using ruri octocog alfa pegol showed a statistically significant effect. The half-
life, time to 1%, and clearance for each product did not show the difference by age groups, but blood type O patients had a significantly shorter half-
life and time to 1% while having a greater clearance and volume in steady state compared to the non-type O patients. No difference in half-
life, time to 1%, or clearance was found for each product, but volume in steady state showed a negative effe ct according to BMI groups. The vWF:Ag on the half-
life and time to 1% showed a statistically significant positive effect for rurioctocog alfa pegol.

Summary/Conclusion: Through this study, we were able to confirm a significant inter-
patient variation in the pharmacokinetics of factor VIII concentrates in Korean hemophilia patients. For an o ptimized prophylactic regimen, we need to personalize the dose of factor VIII concentrates according to the individualized pharmacokinetic profile. This study has limitations inherent to any retrospective study, but we expect that this study result will be used as a basis for expanding reimbursement for FVIII concentrates and contributing to providing personalized treatment in Korea.

P-007

COMPARATIVE DIAGNOSTIC STUDY EVALUATING CLOT FORMATION BY ROTATIONAL THROMBOELASTOMETRY IN 63 PATIENTS WITH CONGENITAL DYSFIBRINOGENEMIA

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Background: Rotational thromboelastometry (ROTEM) is a global hemostasis assay increasingly used in the perioperative and emergency assessment of bleeding diathesis. The diagnosis added value of ROTEM in congenital dysfibrinogenemia remains to be established.

Aims: To analyze clot formation by ROTEM in a cohort of patients with dysfibrinogenemia and to establish correlations with genotype, clinical features and coagulation parameters.

Methods: The study included genetically confirmed congenital dysfibrinogenemia (CD) cases (n=63) and healthy controls (n=50). EXTEM, INTEM, and FIBTEM tests were used to measure selected ROTEM parameters, including clotting time (CT), clot formation time (CFT), maximal clot firmness (MCF) and amplitude 10 min after CT (A10).

Results: Bleeding phenotypes with at least one bleeding complication were observed in 23 (36.5%) patients. Thrombotic and pregnancy-related complications were observed in 6 (9.5%) patients. CT in INTEM was statistically significantly shorter in CD patients compared to controls while CFT in EXTEM was prolonged. MCF in FIBTEM, EXTEM and INTEM were similar in both CD patients and controls while A10 in FIBTEM was statistically significantly lower in CD. Fibrinogen activity was positively correlated with fibrinogen antigen, A10 and MCF in all three assays. CT in EXTEM was slightly prolonged and A10 in FIBTEM was reduced between carriers of the *FGA* mutation p.Arg35His compared to controls.

Summary/Conclusion: We observed that several ROTEM parameters are not able to distinguish patients with dysfunctional fibrinogen from controls and none were correlated with the clinical manifestations, as has been previously documented. Although this study has a large patient group, additional multicentric studies are needed to evaluate the added value of ROTEM analysis for the clinical management of CD patients.

P-008

VALIDATION OF A FREEZING PROCEDURE FOR CHROMOGENIC FACTOR VIII REAGENTS

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Background: One stage assay (OSA) , two stage assay and Chromogenic assays (CSA) are used in the laboratory diagnostics of hemophilia A and B. OSA is the most common used among others due to perception that CSA is more expensive. CSA is used in Sweden for almost forty years even in 24/7 laboratory. Although freezing of reagents has been used in our laboratory, validation of the freezing procedure on reagent stability is lacking.

Aims: To study the effect of freezing on the stability of FVIII chromogenic assay reagents.

Methods: 20 unidentified samples previously tested for FVIII concentration (0.003–2.50 KIU/L) were collected in our routine laboratory work. No ethical permission was needed. Analysis was performed using Coamatic Factor VIII reagent (Chromogenix) in Sysmex CS2500. The chromogenic substrate reagent and the factor reagent had been aliquoted and at the time of analysis they were frozen for two weeks, one and two months.

Results: No differences were observed between the results obtained with fresh reagents and those frozen for two weeks, one-and- two months. The correlation coefficient was 0.99 ($p<0.001$) for all comparisons between fresh and frozen reagents. Bland Altman testing showed bias of 9%, 16% and 16% between fresh reagents and reagents frozen for two weeks, one and two months respectively. These results are predominantly a consequence of a higher bias around the lower limit of detection at very low values (<0.01 KIU/L).

Summary/Conclusion: Our results indicate that chromogenic reagents (both substrate reagent and factor reagent) for the determination of FVIII can be stable under freezing up to two months without any significant influence on the reliability of the tests and accuracy of the results.

P-009

HEMOPHILIA A USE CASE SCENARIOS FOR A PORTABLE TESTING DEVICE IN DIFFERENT REGIONS OF THE WORLD

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Background: A portable multi-parameter blood coagulation analysis system (EnzySystem) is being developed to improve the diagnosis and management of patients with hemophilia A. It simultaneously measures Factor VIII activity, thrombin generation and internal controls in 100 µl of capillary whole blood within one hour.

Aims: Survey use case scenarios for the EnzySystem in different regions of the world. The use cases of interest are divided into (1) the main application: diagnosis or management of hemophilia A, and (2) the use case environment: in clinic, near-patient or home-use.

Methods: Use-case analysis was performed with healthcare professionals and patients with hemophilia A to determine their use preferences regarding the EnzySystem. Data was acquired using (online) interviews with patients, healthcare professionals, and patient organizations. If personal identifiable information was provided an informed consent was obtained.

Results: The results show that the suitable use cases for hemophilia A differ per region. For Europe the most apparent use case is the management of hemophilia A with a near-patient (38%) or home-use (46%) device. In Europe, diagnosis and in-clinic testing are considered in place. Hemophilia treatment centers are accessible to all patients (although in some Northern and Eastern European countries they can be a great distance away). Patients would use home-testing for hemophilia A management in a (severe) bleeding situation or in unstable times. Professionals would prefer getting used to understanding/interpreting results in a near-patient environment before advising patients to use the device at home.

For developing countries in Asia (and some Eastern European countries) the most promising use case is the diagnosis of hemophilia A with an in-clinic (40%) or near-patient (60%) device. In these regions, not all hemophilia A patients have been diagnosed. Hemophilia treatment centers are not accessible to all patients. Moreover, transportation of blood samples to a laboratory is expensive (and not covered by insurance) and test reagents are not (always) available and affordable.

So far, limited data has been obtained for the Northern America, which is insufficient to draw any conclusions.

Summary/Conclusion: From the survey it can be concluded that the recommended use case scenario for European countries is the management of Hemophilia A with a home-use device. Yet, it was suggested to first raise awareness using the near-patient device.

For developing countries in Asia, the recommended use case scenario is for diagnosis with a near-patient device to catch up with the diagnosis of hemophilia A patients.

The input is still limited and not equally distributed over the regions of interest. Most respondents (79%) were from European countries. To draw conclusions for Northern America, more information is needed from

professionals and patients located in this region. Therefore, we will continue collecting information with a focus on the USA.

P-010

ROTATIONAL THROMBELASTOMETRY IN PERIOPERATIVE MANAGEMENT OF PATIENTS WITH INHERITED BLEEDING DISORDERS

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Background: Severe joint degeneration is a common complication in patients suffering from inherited bleeding disorders. Rotational thromboelastometry (ROTEM) allows to monitor hemostatic treatment with the possibility of specific treatment intervention.

Aims: To show the value of rotational thrombelastometry in perioperative management of patients with inherited coagulopathy during surgery.

Methods: We are reporting the perioperative management in 21- and 22-year old patients with von Willebrand disease type 3 (vWD) and severe hemophilia A (HemA) during ankle arthrodesis. Standard laboratory tests and ROTEM-guided therapy were used.

Results: To the patient with vWD there was preoperatively administered 30 IU/kg FVIII/vWF concentrate. The basal levels before substitution were: FVIII: 34%, vWF activity: 16%, vWF antigen: 30%, aPTT: 32.4s and clotting time (CT) of INTEM: 178s. After the surgery these levels were normalized: FVIII: 103.5%, vWF activity: 84%, VWF antigen: 138% and aPTT: 29.2s. During the first 48h after the surgery, the patient was administered 20I U/kg FVIII/vWF every 8h. The patient was discharged home after 96h with aPTT 26.8s and control CT of INTEM 148s.

To the patient with severe HemA there was preoperatively administered rFVIII 50 IU/kg. After the surgery, FVIII level was 78% and CT of INTEM was 255s. During the first 24h after the surgery, the patient was administered rFVIII 30 IU/kg every 8h. The FVIII level was maintained 89±1% and CT of INTEM 200±5s. On the 1st postoperative day, the rFVIII dose was changed to 35 IU/kg every 12h. FVIII level achieved 76-66% and CT of INTEM 174-181s. Based on the CT of INTEM and FVIII level, the rFVIII dose was reduced to 20 IU/kg every 12h. On the 7th postoperative day, the patient was discharged home with a FVIII level 52% and CT of INTEM 82s. Bleeding symptoms were not observed in both patients.

Summary/Conclusion: Combination of standard coagulation testing and ROTEM represents adequate method for designing perioperative management in patients with inherited bleeding disorders.

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P-011

WHEN THERE IS NO AGE LIMIT: AN ELDERLY PATIENT WITH MELAENA, SEVERE ANEMIA AND EXTREME HEMOLYSIS

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Background: Thalassemia syndromes are a heterogeneous group of inherited disorders caused by genetic lesions leading to decreased synthesis of one or more of the globin. The normal ratio of alpha globin to beta globin production is disrupted due to a genetic variant in one or more alpha or beta globin genes. This abnormal chain ratio causes the unpaired chains to precipitate, leading to destruction of red blood cell precursors in the bone marrow (ineffective erythropoiesis) and in the circulation (hemolysis). Patients with thalassemia have variable degrees of anemia and extramedullary hematopoiesis, which in turn can cause bone abnormalities, impaired growth, and iron overload. It is estimated that ~10% of the Greek population are carriers of some type of thalassemia. Delta beta (δβ)-thalassemia especially results from a deletion in both the delta and beta genes on chromosome 11. The gamma genes on the affected chromosome increase their production of gamma globin, thereby increasing the amount of hemoglobin F (HbF). δβ-Thalassemia heterozygotes clinically show characteristics of thalassemia minor, while homozygous δβ-thalassemia may give a clinical picture of thalassemia intermedia with a mild anemia at first.

Aims: Aim of the present is to highlight an atypical diagnosis of δβ thalassemia in an elderly patient with delayed diagnosis already facing multiorgan permanent damage. At least nine mutations can result in δβ-thalassemia. This type of thalassemia is observed in many ethnic groups, including Mediterranean populations like the Italians, Greeks and Turks. Although the exact diagnosis of δβ-thalassemia requires genetic analysis for mutations, Hb electrophoresis with markedly elevated HbF may be suggestive. The incidence of δβ-thalassemia in different parts of the world cannot be estimated owing to the rarity of this Hb variant, since only a handful of case reports were identified from across the world.

Methods: A 76–year-old, Greek woman, was transferred to our Clinic due to melena with significant anemia (Hb: 6.39 g/dL) and incompatible units of packed red blood cells. She suffered from pulmonary hypertension, atrial fibrillation, and chronic kidney disease, a month ago she was also hospitalized due to decompensated heart failure.

Results: The patient was hemodynamically unstable, with type I respiratory failure, palpable hepatosplenomegaly, as well as severe anemia (further deterioration of Hb up to 5.7 g/dl, MCV 65, positive direct Coombs) and acute renal injury. Vasoconstrictors, somatostatin IV, PPI infusion and corticosteroids/folic acid were administered along with multiple transfusions. Endoscopy revealed no cause of bleeding. Full-body CTs noted hepatosplenomegaly and a thoracic spinal mass(T9-T10 level) with soft tissue density. She underwent bone marrow biopsy and hemoglobin electrophoresis, which revealed homozygous δβ thalassemia. The spinal mass was then identified as extramedullary hematopoiesis focus. Despite the multifactorial support, the patient died due to fever/sepsis from multi-resistant nosocomial microorganisms and multiorgan failure.

Summary/Conclusion: The diagnosis of δβ thalassemia at an advanced age is indeed unusual and extremely rare, hemoglobin electrophoresis is fundamental though; since the delay in diagnosis is accompanied by potentially fatal complications High index of suspicion is needed by clinicians to diagnose the entity, in order to prevent further multiorgan damage.

P-012

THE CHALLENGE OF COAGULATION INHIBITOR THERAPY IN DENTISTRY - A COMMUNITY INTERVENTION PROJECT

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Background: Coagulation inhibitor therapy is becoming increasingly common in the chronic patients. Given the dual nature of this therapy in terms of thrombotic and hemorrhagic risk, its management is essential to avoid complications during all types of invasive procedures. In our geographical area, patients are mainly treated in private institutions of the community, which do not have support from other medical specialties. Therefore, investing in training of dentistry's is of utmost importance to ensure greater agility and safety during procedures.

Aims: The aim of this project is to invest in management training of the dentists in anti-aggregant and/or anti-coagulant therapy areas, and to improve their performance in order to contribute to more safety in procedures.

Methods: A comprehensive review of national and international guidelines on the subject was carried out. Clinical guidelines on the management of these drugs were then developed, taking into account the hemorrhagic risk of dental procedures versus the thrombotic risk of patients. These guidelines were presented in the form of a clinical session and made available in virtual and physical formats for quick consultation. After three months, a form was sent by email to evaluate the applicability and satisfaction of the participants.

Results: Statistical analysis of the answers to the questionnaire showed a high applicability of the guidelines during the three months following their presentation. The results also revealed greater safety in the management of this pharmacology by the dentists.

Summary/Conclusion: The results demonstrate that with the applicability of those recommendations, it was possible to improve practices in dentistry and, therefore, increase the safety of patients treated at the community level. The use of algorithms for quick and objective consultation systematizes good practices in clinical activity for patients with complex pathology, where it is necessary to consider the hemorrhagic and thrombotic risk before procedures.

P-013

CARDIOMETABOLIC RISK LEVEL OF PATIENT WITH HEMOPHILIA IN KOREA

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Background: Cardiometabolic syndrome associated with obesity and physical inactivity is common comorbidity in patients with hemophilia (PwH), as the number of patients with hemophilia who are overweight is increasing. Evidence suggests that excess weight has significant impact on lower extremity joint range of motion, joint range of motion and functional ability, joint pain, and on overall quality of life in PwH.

World Hemophilia Foundation, therefore, recommends that PwH should have regular monitoring of body mass index (BMI), and obese PwH should be referred for dietary advice and/or weight management including regular physical activity to promote normal neuromuscular development and physical fitness.

To the authors' knowledge, no data has been published on the cardiometabolic health status of Korean or Asian PwH.

Aims: This single-center, prospective study aims to assess the cardiometabolic health status of Korean PwH.

Methods: Korea Hemophilia Foundation (KHF) collected data on cardiometabolic risk factors from PwH who visited the KHF clinics over a 3 year-perod from 2020 to 2022. To evaluate cardiometabolic risk level, height, weight, BMI, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG) were measured.

Results: Data from 111 PwH including 91 patients with hemophilia A (PwHA) and 20 patients with hemophilia B (PwHB) were collected and evaluated. 37% (41/111) of the subjects were overweight, associated with the BMI score of 25.0 kg/m^2 or higher; 7% (8/111) of the subjects were found obese, associated with the BMI score of 30.0 kg/m^2 or higher. The median BMI was highest among the severe PwHA at 24.70±3.68 SD (range 18.00-43.50) kg/m^2. The rate of obesity among PwH at KHF was similar to the non-hemophilic population.

However, HDL, LDL, and TG results were high across both PwHA and PwHB. HDL was measured among 40 subjects. While HDL cholesterol of 40 mg/dL or less is considered normal range, the median HDL cholesterol among PwHA and PwHB was 45.00±9.81 SD (20.40-63.00) and 48.00±5.50 SD (38.00-51.00) respectively. The median TG was high in both PwHA and PwHB compared to the normal range of ≤130 mg/dL at 188.00±283.99 SD (range 77.00-1590.00) and 214.50±67.53 SD (range 132.00-302.00) respectively. 13 of 45 subjects who were evaluated in terms of total cholesterol, HDL, LDL, and TG were associated 2 or more risk factors on high blood cholesterol or dyslipidemia.

Summary/Conclusion: Although the rate of obesity among PwH at KHF was similar to the non-hemophilia general population in Korea, PwH at KHF were also associated with high risk of dyslipidemia when evaluated in terms of HDL, LDL, and TG.

A well-designed, controlled study to evaluate the clinical benefits of weight management program recommended by WFH, coupled with PK-based individualized prophylaxis tailored to the physical activity plan, is warranted.

Poster Session – Clotting

P-014

THE PLASMA PROTEOME AND RISK OF VENOUS THROMBOEMBOLISM (VTE)

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Background: Proteome-wide analyses in plasma have the potential to reveal predictive biomarkers of venous thromboembolism (VTE) and provide new insight into molecular pathways involved in the pathogenesis of the disease.

Aims: To identify plasma proteins associated with 5-year risk of first-lifetime VTE.

Methods: We performed a case-cohort with incident VTE-cases (n=294), and a randomly sampled age-and sex-weighted subcohort (n=1066) from the Trøndelag Health Study cohort (HUNT3, n=50800). The study was approved by the Regional Committee for Health and Research Ethics and informed consent for participation was obtained from all participants. Blood samples (EDTA plasma) were collected at baseline inclusion (2006/08), and all participants were followed-up for a maximum of 5 years. An aptamer-based platform was used for semi-quantitative measurement of >7K proteins. For proteome-wide analyses, weighted Cox-regression models adjusted for age and sex were used to estimate the association between each protein and VTE, with the Bonferroni method applied to account for multiple testing (significance threshold: $p<6.9x10^{-6}$). For proteins reaching proteome-wide significance, the association to VTE was further explored by estimating hazard ratios (HR) with 95% confidence intervals (CI) according to increasing quartiles (Q) with Q1 as reference group.

Results: In total, 7288 human proteins were measured. The proteome-wide analyses identified seven proteins significantly associated with increased VTE risk. The majority of these proteins displayed a dose-response relationship to VTE risk, and included coagulation factor VIII (FVIII) (procoagulant function) and collagen alpha-3(VI):BPTI/Kunitz inhibitor (antifibrinolytic function) where individuals with plasma concentrations in Q4 had a 5-fold (HR 4.98, 95% CI 3.220-7.70) and 2.5-fold (HR 2.52, 95 CI: 1.64-3.88) higher VTE risk, respectively, compared to those with plasma concentration in Q1. The remaining five proteins were considered novel biomarker candidates, as they all had plausible functions in the pathophysiology of VTE.

Summary/Conclusion: In a proteome-wide analysis, we identified seven proteins that were differentially expressed in individuals who developed VTE within five years compared with those who did not develop VTE. Of these, five were considered novel candidates with plausible function in VTE pathogenesis. As the next step, we will validate our findings with conventional protein quantification assays in an external population.

P-015

THE ASSOCIATION BETWEEN VENOUS THROMBOSIS-ASSOCIATED GENETIC VARIANTS AND COAGULATION FACTOR LEVELS AND THROMBIN GENERATION POTENTIAL

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Background: Recent three large meta-analyses of genome-wide association studies for venous thromboembolism (VTE) identified over 130 genetic variants associated with VTE. However, the mechanisms by which recently described and therefore less explored VTE-associated genetic variants increase the risk of VTE remain unclear.

Aims: We investigated the association between 61 recently described VTE-associated genetic variants, and the levels of coagulation factor (F) VIII, FIX, FXI, and fibrinogen as well as thrombin generation parameters (lag time, peak, endogenous thrombin potential, time-to-peak, and velocity), which were considered intermediate phenotypes for VTE.

Methods: This study was conducted on 5341 participants with European ancestry of the Netherlands Epidemiology of Obesity study. The associations between VTE-associated genetic variants and the levels of coagulation factors and thrombin generation parameters were examined using linear regression analyses, adjusting for age, sex, oral contraceptive use, hormone replacement therapy, and menopausal status. The Medical Ethical Committee of the Leiden University Medical Center, Leiden, The Netherlands approved the study. All participants gave their written informed consent.

Results: The mean (SD) age of participants was 56 (6) years and 56% were women. Of the 61 genetic variants, 19 were negatively associated with one or more of coagulation factor levels and thrombin generation parameters, of which *MAP1A* rs55707100 was most strongly associated with each thrombin generation parameter as well as FXI levels (-5.43 % per allele, 95% CI: -8.61, -2.26). Among the 14 genetic variants associated with increased coagulation, *ST3GAL4* rs35257264 was the most strongly associated with each thrombin generation parameter as well as FVIII levels (8.30 % per allele, 95% CI: 2.29, 14.31).

Summary/Conclusion: These results suggest that the genetic variants likely increase the risk of VTE by affecting the coagulation pathway. Our findings contribute to a better understanding of biological mechanisms by which genetic variants increase the risk of VTE.

P-016

BLADDER CANCER PATIENTS HAVE INCREASED NETOSIS AND IMPAIRED DNASEI-MEDIATED NET DEGRADATION THAT CAN BE THERAPEUTICALLY RESTORED IN VITRO

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Background: During tumor development there is a greater release of neutrophil extracellular traps (NET), in a process called NETosis. This process enhances tumor growth, metastasis, and cancer-associated thrombosis, which may be, in part, mediated by a decreased degradation of NETs. This mechanism has never been described in bladder cancer (BC).

Aims: To analyze the possible increase in NETosis in plasma of BC patients, to ascertain whether this process is mediated by alterations in the plasma DNaseI of BC patients, and to *in vitro* explore therapeutic interventions with Dornase Alfa (a recombinant human DNaseI used in clinical practice for management and treatment of cystic fibrosis) in NET degradation.

Methods: We quantified NET markers (cfDNA, nucleosomes, calprotectin and MPO) in plasma samples from 73 BC patients and 64 controls and DNaseI activity with the SRED assay. Next, we compared the ability of plasma from patients and controls to degrade NETs. To do this, we isolated neutrophils from the blood of a healthy volunteer by gradient centrifugation, seeded them, and induced NETosis with phorbol myristate acetate (PMA) for 4h. The next day, we incubated the NETs for 6h with 5 µl of plasma from each participant individually, and quantified the residual fluorescence after staining the NETs with SytoxGreen (Invitrogen). In parallel, to assess the ability of DNaseI to restore the DNaseI deficiency in patients, we supplemented the plasma of patients with BC with Dornase Alfa (Roche), a control pooled plasma or vehicle (PBS1X + 0.1%BSA). Likewise, we analyzed the presence of NETs in tumor tissue of the patients by immunofluorescence, staining for DNA, citrullinated H3 and elastase. Statistical analysis was performed with Graphpad (v.8.0.1).

Results: BC patients had significantly increased NETs markers in plasma compared to controls (P<0.0001) and lower DNaseI activity (P<0.0001). Likewise, the plasma of the BC patients presented a lower capacity to degrade NETs *in vitro* than that of controls (P<0.0001). The addition of DNaseI restored the ability to degrade NETs to the level of healthy controls (P<0.0001). We evidenced a greater presence of NETs in tumor tissue of patients with severe BC (muscle-invasive bladder cancer) compared to mild BC (non-muscle-invasive bladder cancer).

Summary/Conclusion: Patients with BC have an increased NETosis both in plasma and in the tumor microenvironment, which is more profuse in those with the most severe form. This is partly mediated by a reduced DNaseI activity in plasma. The *in vitro* addition of exogenous DNaseI, an approved treatment for cystic fibrosis, restores NET degradation in BC patients to the level of healthy controls, thus becoming a potential therapeutic tool to reduce the risk of cancer progression and cancer-associated thrombosis. ISCIII-FEDER (PI20/00075, FI21/00171), GVA-CIACIF/2021/192 and SETH.

P-017

AGE AND SEX SPECIFIC RISKS OF MAJOR CARDIOVASCULAR COMPLICATIONS AND DEATH FOLLOWING ELECTIVE HIP AND KNEE ARTHROPLASTY IN THE NETHERLANDS: A DUTCH HOSPITAL DATA REGISTRY STUDY

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Background: Total Hip Arthroplasty (THA) and Total Knee Arthroplasty (TKA) are associated with serious cardiovascular complications and mortality. With the increasingly aging population, numbers of THA and TKA procedures are expected to rise and absolute numbers of cardiovascular complications will rise likewise. Hence continuous efforts have to be made at optimizing prevention and for this, risk estimates need to be specified on the individual patient rather than the general population. Yet, up-to-date, age and sex stratified absolute risk estimates are lacking.

Aims: We aimed to 1) estimate the risk of cardiovascular complications and death over recent years (2015-2020) in the entire Dutch hospital population, 2) assess possible time-trends in these risks and 3) estimate age- and sex specific risks for a first primary THA or TKA procedure.

Methods: We conducted a nationwide registry-based cohort study, including primary THA and TKA procedures for osteo-arthritis performed between 2015 and 2020 in Dutch hospitals. For each person only their first procedure was included to avoid dependency between observations. Data on baseline characteristics, procedures and outcomes were obtained from multiple registries managed by Statistics Netherlands.

Overall risks for Venous Thromboembolism (VTE), Arterial Thromboembolism (ATE), bleeding and mortality were estimated at 90-days following surgery. For the time trend analysis, the 90-day risks were plotted by year and visually inspected. Lastly, the 90-day risks were stratified by sex and age-groups (18-49, 50-59, 60-69, 70-79 and 80+) for each of the outcomes.

Results: In total 224,974 procedures were included, of which 109,037 were THA procedures and 115,937 were TKA procedures. Mean age in both procedure groups was approximately 70 years and around 65% of patients was female.

The overall risk of VTE at 90-days was 0.38% (95%Confidence Interval[CI] 0.34-0.41%) and 0.34% (95%CI 0.31-0.38%) for THA and TKA, respectively. For ATE these risks were 0.34% (95%CI 0.31-0.38%) and 0.43% (95%CI 0.39-0.46%). Similarly, for bleeding they were 0.84% (95%CI 0.78-0.89%) and 0.80% (95%CI 0.75-0.86%). Lastly, mortality following THA was 0.19% (95%CI 0.16-0.22%) and 0.27% (95%CI 0.24-0.3) following TKA.

In the time-trend analysis, for both THA and TKA procedures, some fluctuation in the risks over the years was observed but no clear time-trend could be identified.

Stratification on sex and age-groups showed that the risks of ATE and mortality, following both THA and TKA, are generally higher for males and increase exponentially with age. The same was true for bleeding following TKA but in THA patients, although the risk of bleeding was higher in males than females, a V-shaped pattern was observed with the lowest risk in the 60-69 age-group. For VTE, the risk following THA was higher in females compared to males while these were similar following TKA. Lastly, the combined risk of

cardiovascular complications and mortality, for both THA and TKA, was approximately 3% for males in the highest age-group.

Summary/Conclusion: Generally the incidence of cardiovascular complications and mortality rises with age and is higher for males compared to females. With this knowledge, shared decision making can be enhanced and preventive measures can be targeted more precisely.

P-018

APPLICATION OF COMPUTER-BASED METHODS TO DISCOVER AND OPTIMIZE BIOACTIVE COMPOUNDS WITH ANTI-INFLAMMATORY AND ANTI-ATHEROSCLEROTIC ACTIVITIES: FROM IN SILICO TO IN VIVO

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Background: The drug discovery process, from the identification of drug targets to the launch of new drugs to market, requires enormous investments in time and cost. Nowadays, computational methods such as structure-based virtual screening (SBVS) and computer-aided molecular design (CAMD) offer solutions to both academic and industrial research to accelerate the hit identification and drug discovery process.

Aims: We demonstrate case studies in which we have applied various in silico methods to successfully develop bioactive compounds (from small molecules, peptides, to engineered proteins) to regulate the function of drug targets that play crucial roles in inflammatory responses.

Methods: To discover small-molecule inhibitors, we have conducted a SBVS approach to hierarchically filter compounds by first selection of compounds with drug-like properties and then utilization of multi-step molecular docking. Also, we have applied structure-based methods to develop peptidic inhibitors and therapeutic proteins. Complexes between drug targets and their binding partners were used as starting structures to investigate key interactions and build a peptide/protein template. These complex structures can be obtained either from the Protein Data Bank (PDB) or can be *de novo* computationally modelled. Subsequently, rational *in silico* design was employed to optimize newly discovered small molecules, peptides, and therapeutic proteins to improve their binding strength with drug targets and their specificity.

Results: By utilization of computational methods, we are able to significantly reduce the number of compounds subjected to experimental testing. For example, after performing SBVS, we selected around 1000 compounds from more than a billion compounds available in chemical space to investigate their inhibitory activity against e.g. coagulation factors V and VIII, TRAF2 and TRAF6 and SMPD3. The identified small-molecule inhibitors showed IC_{50} at low micro- and nano-molar level. Of these, the TRAF6 inhibitors exhibited therapeutic benefits in case of atherosclerosis, peritonitis and sepsis as was tested in mouse models and in non-human primates. Likewise, we designed around 20-25 peptides (*in silico*), and from these we selected and tested only 5-10 candidates in *in vitro* and 1 compound for *in vivo* experiment. We have successfully developed peptidic inhibitors targeting CCL5-HNP1, GPIbα-VWF A1, and histone H2A/H4. The peptidic inhibitors of CCL5-HNP1 and histone H2A/H4 demonstrated their efficacy for myocardial infarction and atherosclerosis in mouse models. Lastly, we have employed various computational methods to rationally engineer proteins that are relevant for thrombosis and haemostasis, including Annexin A5, ADAMTS13, and APC. Following a computational rationale, we were able to efficiently design novel molecules that possess desired biological activities.

Summary/Conclusion: We here present computational methods utilized to discover and optimize novel inhibitors (i.e., small-molecule inhibitors, peptides, and therapeutic proteins) targeting a wide variety of proteins. Given the generic applicability of the methods, the described computational approaches and protocols can be applied to develop lead compounds to target virtually any type of drug targets, intracellular or extracellular, as demonstrated here, from enzymatic proteins (SMPD3), the membrane binding proteins FV/FVIII, protein-protein interactions (CCL5-HNP1, TRAF6-CD40 complex) to proteins containing disordered regions (extracellular histones).

P-019

THE INCREASED NETOSIS IN PLASMA OF PATIENTS WITH PERIPROSTHETIC JOINT INFECTION IS AGGRAVATED BY LOWER DNASEI ACTIVITY: POTENTIAL THERAPEUTIC INTERVENTION TO ELIMINATE THE BACTERIAL BIOFILM

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Background: Bacterial infection activates neutrophils through immunothrombosis, which release neutrophil extracellular traps (NETs) into bacterial biofilms of periprosthetic joint infections (PJI). In a pilot study, we observed an increase in NET markers in plasma of patients with PJI, and that levels of cell-free DNA (cfDNA) could improve PJI diagnosis. In disorders such as thrombotic microangiopathies and lupus nephritis, the increase of NETs in plasma is, in part, mediated by decreased plasma DNaseI activity.

Aims: To validate the increase in NETosis in plasma from patients with PJI, to ascertain if this process is mediated by plasma DNaseI and, if positive, to *in vitro* correct this deficiency with Dornase Alfa (Roche), a recombinant human DNaseI (rhDNaseI) used for the treatment of cystic fibrosis.

Methods: We obtained citrated plasma from 94 prospective patients before prosthetic surgery, in 49 of whom the diagnosis of PJI was confirmed. We quantified NETs markers (cfDNA, nucleosomes, calprotectin, myeloperoxidase, elastase, and α-defensins) and interleukin-6 with specific assays, and DNaseI activity with the SRED assay. We generated NETs from neutrophils isolated from a healthy donor by gradient centrifugation and stimulated with phorbol myristate acetate (PMA) for 4h. We analyzed the ability of plasma from patients with and without PJI to degrade these NETS for 6h in triplicate by measuring the residual fluorescence after staining the NETs with SytoxGreen (Invitrogen). In parallel, we supplemented the plasma of PJI patients with Dornase Alfa, a control pooled plasma or vehicle (1X PBS + 0.1% BSA) to assess the ability of rhDNaseI to restore the DNaseI-deficiency of the PJI plasma. We performed the statistical analysis with GraphPad (v.8.0.1).

Results: Patients with PJI had increased markers of NETs and IL-6 in plasma and lower DNaseI activity than patients without PJI (P<0.001). In addition, they had a lower ability to degrade NETs *in vitro* than patients without PJI (P<0.001). The addition of rhDNaseI restored the ability to degrade NETs to the level of controls.

Summary/Conclusion: Patients with PJI have increased plasma NETosis in part mediated by reduced DNaseI activity. The *in vitro* addition of an exogenous rhDNaseI, an approved treatment for cystic fibrosis, restores the ability to degrade NETs in patients with PJI. Future studies are needed to assess the ability of Dornase Alfa as therapeutic tool to degrade NETs and, with it, the biofilm of periprosthetic joint infections.
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P-020

COAGULATION EFFECTS OF INHIBITION OF FACTOR XA BY REVERSIBLE INHIBITORS ARE DIFFERENT FOR THE INTRINSIC AND EXTRINSIC ACTIVATION PATHWAYS OF FACTOR X

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Background: Recently, direct reversible inhibitors of factor Xa became available and are evaluated in clinical trials (Xabans; rivaroxaban, apixaban and edoxaban). The exact mechanisms in coagulation are not delineated yet.

Aims: We evaluated the effects of these inhibitors on plasma clotting times using various pathways of activation, notably the intrinsic and extrinsic routes.

Methods: Plasma clotting was studied considering three different pathways of activation. Next to a contact route (intrinsic route: starting in the test with factor XIa), two tissue factor routes (extrinsic routes) were evaluated. One of the extrinsic routes (The tissue factor / factor VII / factor X route) was specifically studied in factor VIII or factor IX deficient plasma.

Results: The contact route and tissue factor route including factor VII/ factor IX/factor VIII /factor X were poorly inhibited (IC50 > 2000 ng/ml). However, the tissue factor / factor VII / factor X route was inhibited at low Xaban concentrations (50-200 ng/ml). There was a small potency difference among rivaroxaban, apixaban and edoxaban only.

The specificity for the factor Xa formed by the extrinsic route was confirmed by addition of activated factor VII (Novoseven) to plasma, which formed factor Xa that was also inhibited by low concentrations of Xabans.

Summary/Conclusion: Xabans are not equally effective in inhibiting factor Xa formed in all pathways. The prime target at low concentrations concerns prothrombin activation by extrinsically formed factor Xa following factor VIIa.

It implies that Xabans are primarily targeted at coagulation activation originating from cellular tissue factor and tissue factor on microparticles in circulation.

P-021

SCREENING OF TRIPEPTIDE SUBSTRATES SPECIFIC TO BLOOD COAGULATION PROTEASES

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Background: The active enzymes in blood coagulation and fibrinolytic systems are mostly serine proteases, which cleave natural cleavage site preferably with lysine or arginine at P_1 position. The selective cleavage is also observed with synthetic tripeptide-functionalized substrates, for instance, luminescent substrates. Luciferase in combination with enzyme-specific cleaved chemiluminescent substrates will generate photons that can be determined by light-sensitive sensors. The screening of a tripeptide substrate library, with lysine or arginine in P_1 and the natural essential amino acids except cysteine in P_2 and P_3, in reaction with blood coagulation proteases gives the opportunity to identify the most specific ones. A target substrate should demonstrate a conversion rate that allows usability in global assays and also quantitative assays and preferably should have minimal cross-reactivity. The hits found are aimed to be used in further assays for the implementation on a point-of-care system, making use of a microfluidic platform with a multisensory readout.

Aims: Find chemiluminescent substrates with optimal affinity and specificity to blood coagulation and fibrinolytic proteases.

Methods: The enzymatic activity of the proteases was detected by a chemiluminescent-based luciferase-catalyzed assay *in vitro.* Aminoluciferin substrates for the assay were obtained by a click condensation reaction with D-cysteine to provide higher stability and synthetic feasibility of the substrates. The library containing 711 substrates (Symeres and Radboud University, Nijmegen, The Netherlands) was screened at a concentration of 400 µM with the following blood coagulation enzymes: thrombin, Factor VIIa, Factor IXa, Factor Xa, Factor XIa, Factor XIIa, activated protein C (APC), plasma kallikrein, and plasmin all at a concentration of 10 nM. To evaluate the replicability of the assay, the reactions were carried out in duplicate.

Results: Up to today, only substrates with lysine in P_1 have been screened. Relatively high affinity (luminescent signals > 2×10^4 RLU) was observed for thrombin with substrates with proline at P_2 (n = 11) and for FXa with aromatic amino acids (phenylalanine, tyrosine, tryptophan) at P_2 (n = 19). Higher signals (> 10^6 RLU) were measured for kallikrein (n = 42) and plasmin (n = 4) with aromatic amino acids at P_2. FXIa showed high signals with alanine, glutamic acid, histidine, and asparagine in P_2 (n = 18; > 10^6 RLU). For FVIIa, FIXa, FXIIa, and APC, low enzymatic activity was observed (< 5×10^3 RLU) indicating a low affinity for these enzymes under the given conditions.

Summary/Conclusion: A number of candidate lysine substrates for thrombin, FXa, FXIa, kallikrein, and plasmin were identified. A library of arginine substrates will be screened in the coming months. These findings allow further research on the miniaturization of the chemiluminescent assay with these substrates for the development of microfluidic coagulation factor assays on a point-of-care platform.

P-022

INCREASED LEVELS OF NETOSIS-RELATED CYTOKINES IN PERITONEAL FLUID FROM ADVANCED HIGH-GRADE SEROUS OVARIAN CANCER PATIENTS

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Background: High-grade serous ovarian cancer (HGSOC) is the most lethal gynecologic cancer and one of the most pro-thrombotic tumors. Peculiarly, this type of tumor spreads across the peritoneal cavity following peritoneal fluid (PF) dynamics, being the most representative biofluid of the tumor micro-environment (TME). It has been widely described that in the TME, neutrophils are able to participate in immunothrombosis and cancer progression through the release of neutrophil extracellular traps (NETs) in a process called NETosis. Previous evidence in murine OC models suggests that the primary ovarian tumor can secrete specific cytokines (G-CSF, GROα, IL-8, MCP-1) to the TME, triggering neutrophil recruitment and NETosis in the peritoneal cavity.

Aims: To identify whether the NETosis-related cytokines described in murine OC models are increased in the TME of HGSOC patients and to unravel association with NETosis biomarkers.

Methods: PF samples from advanced HGSOC patients without neoadjuvant treatment (NT) (n=36), with NT (n=13), and control women (n=21) were analyzed. For simultaneous quantification of 7 cytokines: G-CSF, GROα, IL-1β, IL-6, IL-8, MCP-1, and TNFα, the MILLIPLEX Human Cytokine/Chemokine/Growth Factor Panel A assay (Millipore, HCYTA-60K-10) was employed. Levels of NETosis biomarkers (cell-free DNA (cfDNA), nucleosomes, citrullinated histone 3, calprotectin, and myeloperoxidase) were quantified by fluorimetric detection assay (cfDNA) and ELISA (others). A NETosis score was created to consider the joint behavior of NETosis biomarkers levels according to published methods [1]. R (v.3.6.2) was used for statistical analyses.

Results: Compared to control women, HGSOC patients without NT presented a significantly higher concentration of GROα [(median 33.83 pg/mL; Q1-Q3, 14.54-79.93) *vs.* (14.79; 9.86-20.78), p=0.004], IL-6 [(2866.48; 537.47-7520.86) *vs.* (26.68; 7.39-44.46), p<0.001], IL-8 [(88.96; 42.48-191.56) *vs.* (5.56; 3.46-10.77), p<0.001] and TNFα [(60.12; 44.71-82.73) *vs.* (12.53; 10.45-22.29), p<0.001]. Considering HGSOC patients without NT and control women, a positive correlation was observed among the 4 cytokines (Spearman-ρ≥0.389; p≤0.011) and between the cytokines and all the measured NETosis biomarkers (Spearman-ρ≥0.361; p≤0.039). In these patients, cytokines levels behaved according to the 3 groups of the

NETosis score (low, intermediate and high) being inferior in the low group and gradually increasing in the intermediate and high groups, respectively ($p<0.001$).

Summary/Conclusion: The observed increase of GROα, IL-6, IL-8 and TNFα levels in advanced-HGSOC patients' PF demonstrate their presence in the HGSOC TME. Moreover, the positive correlation described between the 4 cytokines and NETosis biomarkers suggest their possible involvement in triggering NETosis in the peritoneal cavity. Altogether, we found that GROα, IL-6, IL-8 and TNFα are present in HGSOC TME and could be involved in neutrophil recruitment and induction of NETosis, thus favoring HGSOC progression by means of a local activation of immunothrombosis.

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References:

Voabil P et al. An ex vivo tumor fragment platform to dissect response to PD-1 blockade in cancer. Nat Med (2021) 27:1250–61. doi: 10.1038/s41591-021-01398-3

P-023

WHICH SCORE WILL PREDICT THE BEST THE RISK OF FIRST THROMBOTIC EVENT IN CANCER WITH A HIGH RISK OF BLEEDING?

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Background: Cancer associated thrombosis (CAT) is a frequent complication in cancer patients. Gastric cancer et colorectal cancer patients are at high risk of thrombosis. However, clinicians are reluctant for systematic thromboprophylaxis due to the high risk of thromboprophylaxis associated bleeding events. Several risk assessment models have been developed to propose score to help clinician to identify patient at high risk of CAT. We hypothesize that the scores peformances are specific digestive cancer sites and that a tailored site assessment is needed.

Aims: To compare the 3 existing scores (PROTECHT, KHORANA and CONKO) to predict the risk of CAT in gastric and colo-rectal cancer.

Methods: We used data on gastric and colo-rectal cancer from the ONCOCIP Study, a multicentric study of consecutive ambulatory adult patients requiring catheter implantation for chemotherapy. Primary outcome was symptomatic proximal pulmonary embolism or non-catheter related proximal deep venous thrombosis validated by a central adjudication committee. Three scores were selected: PROTECHT, KHORANA and CONKO. Cut- off value for high risk patient was fixed at 3 points for all the scores. Scores discriminative power was assessed by calculating area under the ROC curve (AUC).

Results: We included 474 colorectal cancer patients with an incidence of 40 CAT (8.7%), and 103 gastric cancer patients with 19 CAT (18.4%). Among patients with a score classifying into high risk of CAT, the scores performed differently by site. None of the AUCs exceeded 0.6.

Summary/Conclusion: In our cohort, scores work poorly and differently between the different digestive cancer site group and within the same cancer site group. The scores are based on RAMs build on hundreds of patient's cohorts which not allow the power to obtain a better discrimination. Data-sharing could potentially optimize the construction of site-tailored scores or site-driven improvements for existing scores.

P-024

ALTERATIONS IN THROMBIN GENERATION AFTER SARS-COV-2 VACCINATION AND THE RELATION WITH INFLAMMATION

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Background: In the first months in which SARS-CoV-2 vaccines were administered, thrombotic events following vaccination were reported. However, reports on the effect of SARS-CoV-2 vaccination various coagulation parameters have shown contradictory results.

Aims: To assess changes in coagulation parameters following intramuscular (IM) or intradermal (ID) SARS-CoV-2 primary immunization using the mRNA-1273 LPN (Moderna) vaccine and to assess the association between potential coagulation changes and the inflammatory response.

Methods: This study was embedded in a randomized trial studying the non-inferiority of ID fractional dose administration of the Moderna vaccine [Roozen, MedRxiv 2021]. Healthy unvaccinated participants, 18-30 years, with no history of COVID-19, were randomized between 1/5th fractional dose ID and standard dose IM. Informed consent was obtained and this study protocol was approved by the local medical ethical committee. Blood was drawn at baseline (prior to vaccination; Day(D)1) and one week after the second dose (D36). Changes in coagulation parameters (thrombin generation (TG); primary endpoint Peak height) and the inflammatory response (hsCRP) were assessed between both timepoints using linear regression analysis.

Results: The analyses included 123 participant. Peak height increased after vaccination, especially in the IM group (table 1). Other parameters of thrombin generation also indicated an increased coagulability after vaccination. HsCRP increased only after IM vaccination (table 2). There was no association between the change in peak height and the change in hsCRP (Beta 1.6; 95%CI -3.5 – 6.6). However, change in several secondary TG endpoints were associated with change in hsCRP (e.g., ETP: Beta 28; 95%CI 7.6-48.3). IgG binding antibodies against SARS-Cov-2 spike and RBD proteins were similar in ID and IM vaccinated participants.

Summary/Conclusion: These results indicate an increase in coagulability after SARS-CoV-2 vaccination, which was associated with the inflammatory response. While ID administration induced a similar efficacy as IM administration, ID administration was associated with less thrombin activation and inflammation.

P-025

CHANGES IN THE PLASMA PROTEOME INDUCED BY A STANDARDIZED AUTOLOGOUS ACUTE PULMONARY EMBOLISM IN A PORCINE MODEL

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Background: The recommended diagnostic algorithm for acute pulmonary embolism (PE), including pre-test probability assessment and D-dimer measurement, is associated with overuse of radiological procedures, most often computed tomography pulmonary angiography (CTPA). Overuse of CTPA increases patients' exposure to ionizing radiation, contrast-induced morbidity, and healthcare costs. Discovery of diagnostic blood biomarkers of acute PE with high specificity could potentially reduce the current overuse of CTPA.

Aims: To identify plasma proteins that are differentially expressed in pigs exposed to standardized acute PE compared to sham pigs.

Methods: We performed an exploratory study in a porcine model with ex vivo autologous standardized PE-formation. Twelve pigs were randomly allocated to induction of autologous PE (n=6) or sham (n=6). Blood samples and hemodynamic measurements were collected at baseline and at 30, 60, 90, 180, 360, and 720 minutes after PE induction or saline administration (sham). Proteome analyses were performed in EDTA plasma using the 5k SOMAscan® proteomics platform. Proteins which fulfilled the following pre-set criteria were considered to have potential as specific diagnostic biomarkers of acute PE: i) significant differential expression in the two groups, ii) relation to pathophysiological response to PE (ontology investigations) or anatomical specificity to pulmonary tissues.

Results: The PE-pigs experienced hemodynamic changes similar to intermediate-high risk acute PE in humans. Of the 5269 proteins detected, 270 were differentially expressed in the pigs subjected to autologous PE compared to sham. Ontology investigation identified chemotaxis as the most prominent pathway and five pathways were considered related to a pulmonary response of acute PE. Of the 66 proteins differently expressed and considered related to pathophysiological response to PE and/or anatomical specificity to pulmonary tissues, four proteins also fulfilled our additional criteria (effect size and early onset differences), namely RASA1; NME2; BNIP3L and ATG5.

Summary/Conclusion: We identified 270 proteins differentially expressed in pigs subjected to PE compared to sham, of which 4 proteins fulfilled criteria for having diagnostic potential. The diagnostic utility of these proteins should be explored in clinical studies of humans with suspected PE.

P-026

THE ONSET OF THROMBOSIS IN DEEP VEINS (DVT): THE UNEXPECTED ROLE OF STRETCH AND SHEAR STRESSES ON ENDOTHELIAL CELLS

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Background: DVT is a severe health issue due to the formation of a blood clot, predominantly in the sinus valves of the legs, and its potentially lethal complication: the pulmonary embolism (PE) when it breaks and gets lodged in the pulmonary arteries. Despite the severity of this acute condition, we still currently lack knowledge on the DVT causing events and on the cellular and molecular mechanisms involved in the development of the thrombus. DVT is often associated with stasis due to immobility i.e., after surgery, however, stasis alone is not a sufficient explanation for the propensity of thrombi to develop, since prolonged periods of stasis associated with sleep do not result in thrombus formation.We already know that the expression of the anticoagulant protein Thrombomodulin (TM) is upregulated and that of the procoagulant Von Willebrand factor (vWf) is downregulated in the endothelium of the valve sinus, compared to the endothelium of the venous lumen.The question naturally arises: Why does the onset of DVT occur precisely in the valve sinus, where endothelial cells primarily express antithrombotic proteins? The hemodynamic modulation of the endothelial response in the valves may play a decisive role on the activation of the coagulation cascade.

Aims: We present here the study of the onset of Thrombosis disease in deep veins, for the first time, from a cellular point of view, by deciphering the different roles played by the stresses existing in the valve sac: stretch, when the valve is closed and the pressure increases, and shear stress, when the valve opens and the blood flows back towards the heart.

Methods: In a first set of experiments, cyclic uniaxial stretching of human umbilical vein endothelial cells (HUVECs) is performed to understand the relationship between the mechanical constraints and the pro/anti-thrombotic proteins of the endothelial monolayer. In a second complementary approach, we develop a microfluidic device capable of performing uniaxial stretch while subjecting confluent HUVECs to constant or oscillating flow to couple stretch and shear stress through the inflation of a thin PDMS membrane.

Results: We demonstrate that HUVECs align orthogonally to the direction of stretch and 24h of 10% cyclic uniaxial elongation induces a thromboresistant phenotype on the surface of the ECs. TM expression increases as early as 6h after the start of stretching We also show that discontinuous stretching over a period of 18h followed by discharge, experimental conditions that mimics stasis in a pathological state, triggers the coagulation cascade, with increased expression of vWf on ECs. The new organ-on chip device can perform 10% of cyclic uniaxial stretch and shear stresses between 1-10 dyne/cm2, mimicking real valve conditions in vitro.

Summary/Conclusion: Here we propose innovative approaches to study the behavior of ECs in complex mechanical situations and highlight the molecular and cellular links between stretch, shear and Thrombosis. Our results show that uniaxial cyclic elongation induces a thromboresistant phenotype on ECs and that the dynamics of stretch plays a role in the genesis of the coagulation cascade, predominantly in a discontinuous manner. We ultimately developed an organ on chip device to couple stretch and shear.

P-027

RESTING HEART RATE AND RISK OF INCIDENT VENOUS THROMBOEMBOLISM

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Background: Resting heart rate (RHR) is associated with cardiovascular disease (CVD) and premature mortality. Few studies have investigated the association between RHR and venous thromboembolism (VTE) with somewhat diverging results.

Aims: To investigate the association between RHR and risk of future incident VTE in a population-based cohort.

Methods: Participants (n= 36,395) from the Tromsø 4-7 surveys (enrolled 1994-2016) were followed through 2020. All first-time VTEs among study participants were validated and recorded. RHR was measured, and the mean of the last two out of three measures (consecutively assessed in 1-minute intervals) was used in the analysis. Cox regression models were used to estimate hazard ratios (HR) for VTE with 95% confidence intervals (CIs) according to RHR categories (61-70bpm, 71-80bpm and >80bpm) with ≤60bpm as reference. RHR category was entered as a time-varying covariate in the analyses (i.e., RHR was updated during follow-up in those attending several surveys). Models were adjusted for age, sex, body mass index, CVD, cancer, physical activity and C-reactive protein (CRP). The study was approved by the Regional Committee for Medical and Health Research Ethics, and all participants provided written informed consent for participation in the study.

Results: During a median of 6.6 years of follow-up, 1072 participants experienced a first-time VTE-event. Fully adjusted HRs (95% CI) for overall VTE were 1.21 (0.97-1.51), 1.32 (1.04-1.66) and 1.17 (0.90-1.53) for RHR categories 61-70bpm, 71-80bpm and >80 bpm, respectively, when compared with the reference. The association appeared to be more pronounced for pulmonary embolism (61-70bpm: HR 1.41 (1.01-1.96); 71-80bpm: HR 1.53 (1.08-2.16), >80bpm: HR 1.41 (0.96-2.07)) and unprovoked VTE (61-70bpm: HR 1.55 (1.07-2.24), 71-80bpm: HR 1.54 (1.04-2.29), >80bpm: HR 1.56 (1.01-2.41). There was no clear association between RHR and risk of deep vein thrombosis or provoked VTE.

Summary/Conclusion: Elevated RHR was associated with a 32% elevated risk of overall VTE in those with RHR 71-80bpm, compared to those with RHR ≤60bpm. The association appeared to be driven by an increased risk of PE and unprovoked VTE among those with higher RHR.

P-028

INCREASED THROMBIN GENERATION IN PATIENTS WITH ANTIBODY-MEDIATED REJECTION (ABMR) AFTER KIDNEY TRANSPLANTATION

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Background: Antibody mediated rejection (ABMR) is a common cause of graft loss. The development of de novo anti-HLA donor specific antibodies (DSAs) is associated with poor outcomes in kidney transplant recipients. It is surmised that an interaction between DSAs and the graft endothelium cause tissue injury, however, the exact underlying pathomechanism and optimal management of patients with de novo DSAs remain undetermined.

Aims: We hypothesized that in kidney transplant recipients the presence of DSAs induce endothelial cell damage and may lead to hypercoagulability by increasing thrombin generation.

Methods: In this observational cohort study, 21 kidney transplant recipients with DSAs (DSA+ group) and 20 age- and sex-matched transplant recipients without DSAs (DSA- group) were enrolled. Venous blood samples were obtained at baseline and the following measurements were carried out: routine laboratory tests including CRP, von Willebrand factor antigen (VWF), soluble E selectin (sEsel), thrombin generation assay (TGA). Lag time, endogen thrombin potential (ETP), peak thrombin, time-to-peak were calculated. In order to correlate results with potential changes in DSA status over time, patients were followed and blood samples were taken again 6±1.5 months later.

Results: CRP, VWF and sEsel levels did not differ between groups. As compared to DSA- patients, peak thrombin was significantly increased in the DSA+ group at baseline (median: 350 [IQR: 276-386] vs. 379 [IQR: 343-437] nM, respectively, p=0.028). Similar results were found at follow-up measurements, moreover, ETP was also significantly increased in the DSA+ group (median: 1490 [IQR: 1134-1673] vs. 1646 [IQR: 1399-2057] nM*min, p=0.048). Upon follow-up, de novo DSA+ result was found in 2 cases, and in one patient it was associated with highly increased thrombin generation.

Summary/Conclusion: In patients with DSAs, the extent of endothelial damage was not significant in this cohort. On the other hand, thrombin generation was significantly increased as compared to DSA- transplant recipients, suggesting that the presence of antibodies may induce hypercoagulability, potentially influencing the extent of tissue injury and ABMR.

P-029

UROLOGICAL CANCER PATIENTS DISPLAY AN INCREASE IN MARKERS OF HYPERCOAGULABILITY AND INFLAMMATION

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Background: Not only cancer growth develops a subclinical hypercoagulable state, but also hypercoagulability is a key contributor in cancer progression beyond cancer-associated thrombosis. Increasing evidence shows that coagulation proteins have multiple functions in tumor progression and metastasis, although current knowledge is rather limited. Urological tumors are not an exception.

Aims: To analyze the dysregulation of an extensive profile of hemostatic proteins and analytical variables in urological cancer patients.

Methods: We recruited 70 cancer patients before surgery (38 bladder, 20 prostate, 12 renal) and 42 healthy controls. We quantified a profile of 81 variables: 25 blood biochemistry parameters, 24 parameters of full hemogram, 8 parameters of hemostasis and 24 markers of coagulation, natural anticoagulants and fibrinolysis (fibrinogen, D-dimer, FVIII, FVII, FXIII, FVW, protein C, protein S, antithrombin, plasminogen, PAI-1, α2-antiplasmin, ADAMTS13).

Results: Compared to controls, urological cancer patients showed a significant increase in C reactive protein (*P*=0.010), leucocytes (0.007), neutrophils (0.010), monocytes (0.039), eosinophils % (0.005), TTPA (0.0332), D-dimer (0.039), fibrinogen (0.010), creatinine (0.025), ApoB/ApoA1 (0.03904), uric acid (0.021) and triglycerides (0.031); and a decrease in and α2-antiplasmin (0.017), ApoA1 (0.0001) and CHCM (0.020). Unlike previous studies, no differences in FVIII levels were evidenced. Attending to the different cancer types studied, bladder cancer patients displayed the greatest differences in variables, whereas renal cancer patients had the highest PAI-1 levels and prostate cancer the highest FXIII. No differences were observed in natural anticoagulants protein C, protein S or antithrombin.

Summary/Conclusion: Active urological cancer patients display an increase in blood cell counts and in markers of hypercoagulability and inflammation. The activation of a subclinical hypercoagulable state differs among cancer types, probably contributing differently to tumorigenesis. These markers could be useful in follow-up. Further studies in larger cancer cohorts are needed to validate our findings. ISCIII-FEDER (PI20/00075, FI21/00171), GVA-CIACIF/2021/192 and SETH.

P-030

NO DETECTABLE COAGULATION ACTIVATION AFTER VITAMIN K (MK-7) SUPPLEMENTATION IN PATIENTS ON DIALYSIS WITH FUNCTIONAL VITAMIN K DEFICIENCY: A ONE-YEAR RANDOMIZED, PLACEBO-CONTROLLED STUDY

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Background: Patients on dialysis treatment have poor functional vitamin K status, and this may increase the risk of vascular calcification. Vitamin K supplementation may therefore be relevant in patients on dialysis, but the procoagulant effects have not been studied.

Aims: We evaluated effects of high-dose menaquinone-7 (MK-7) supplementation on biomarkers of coagulation in patients on dialysis.

Methods: Double-blinded, placebo-controlled study in 123 patients on dialysis randomized to 52 weeks of vitamin K (MK-7, 360 µg/daily, n=61) or placebo (n=62). We obtained informed consent from all the study participants. Measurements at baseline and after 52 weeks of intervention included thrombin generation (endogenous thrombin potential (ETP), peak thrombin concentration, time to peak, and lag time), clot activities of vitamin K-dependent coagulation factors (F) II, VII, IX, and X, prothrombin fragment 1+2 (F1+2), and proteins induced by vitamin K absence II (PIVKA-II). Between-group differences (vitamin K vs. placebo) at 52 weeks were determined with an analysis of covariance. Within-group changes in vitamin K and placebo groups were analyzed with a paired t-test.

Results: A between-group difference at 52 weeks was observed for PIVKA-II ($p<0.001$). PIVKA-II decreased significantly from baseline to 52 weeks in the vitamin K group, but not in the placebo group. We observed no other between-group differences or within-group changes, except for FVII clot activity which was reduced in the placebo group ($p=0.04$).

Summary/Conclusion: One year of high-dose vitamin K supplementation in patients on dialysis has no detectable effects on biomarkers of coagulation activation and clot activities of vitamin K-dependent coagulation factors, indicating no procoagulant effects of this treatment.

P-031

ADIPOCYTE FATTY ACID-BINDING PROTEIN AND RISK OF FUTURE INCIDENT VENOUS THROMBOEMBOLISM

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Background: Obesity is a major risk factor for venous thromboembolism (VTE) but the mediators of the VTE risk in obese people remain poorly understood. Adipocyte fatty acid-binding protein (AFABP) is a lipid chaperone expressed by adipocytes and macrophages reported to be elevated in obesity. AFABP induces proinflammatory pathways and could serve as a mediator for the link between obesity and VTE. However, whether AFABP is associated with VTE is unknown.

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

Methods: We established a case-cohort study with 294 incident VTE cases occurring within 5 years of blood sampling and a randomly sampled age-weighted sub-cohort of 1066 individuals derived from the third survey of the Trøndelag Health Study (HUNT3), a Norwegian population-based cohort study. Blood samples (EDTA plasma) were collected at inclusion (2006-08), stored at -80°C, and subjected to aptamer-based proteomics (SomaScan® Assay v4.1) in 2022. In a proteome-wide analysis, AFABP was moderately correlated with body mass index (BMI), with Spearman's coefficient= 0.38 ($p < 0.001$). To further investigate the association between AFABP and VTE, weighted Cox-regression models were used to calculate hazard ratios (HR) with 95% confidence intervals (CIs) per 1 standard deviation (SD) increment in log-transformed AFABP levels, and according to AFABP quartiles (Q1-4), with the lowest quartile (Q1) as the reference. Ethical approval and informed consent were obtained for all study participants.

Results: AFABP was linearly associated with VTE risk, with a HR of 1.37 (95% CI 1.15-1.63) per 1-SD increment in AFABP levels in age- and sex-adjusted analysis. Additional adjustment for BMI resulted in a moderate attenuation of the risk estimate (HR 1.29, 95% CI 1.07-1.56). Compared with participants in Q1, those with AFABP levels in Q4 had a HR for VTE of 1.80 (95% CI 1.12-2.90) in age- and sex-adjusted model, and a HR of 1.49 (95% CI 0.88-2.51) after further adjustment for BMI.

Summary/Conclusion: Our results suggest that higher AFABP levels are associated with increased risk of VTE, even though part of this association seems to be explained by confounding related to BMI. Future studies are needed to confirm our findings and to investigate whether AFABP is a mediator of VTE risk in obese people.

P-032

GENOME-WIDE GENETIC PREDICTORS OF PULMONARY EMBOLISM AMONG THOSE WITH A DEEP VEIN THROMBOSIS

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Background: Deep vein thrombosis (DVT) is a significant source of morbidity in adult populations. If untreated, the clot can dislodge and produce a pulmonary embolism (PE), which can be fatal. Little is known about determinants of PE among those with a DVT. The factor V Leiden genetic variant (rs6025) is known to be associated with a lower risk of PE among those with a DVT. A better understanding of other genetic contributors to clot stability may help us prevent PE in those with a DVT.

Aims: We conducted meta-analysis of DVT-PE genome-wide association studies (GWAS) to identify novel genetic variants associated with PE in those with a DVT.

Methods: We performed a case-only analysis of venous thromboembolism (VTE) and divided cases into those with a DVT alone (no clinical evidence of PE) and those with a PE (with or without clinical evidence of a DVT). The PE must have occurred at the time of diagnosis or within 14 days after the DVT diagnosis. Twenty-three studies performed GWAS using densely imputed genome-wide markers and a logistic mixed model that adjusted for age, sex, genetic principal components, any study-specific variables, and within-study genetic relatedness. PE (with or without DVT) was modeled as the outcome, and imputed genotype dosages were tested for association using an additive model. Summary results were meta-analyzed across studies. The threshold of genome-wide significance was set at $5x10^{-8}$. Data are currently limited to European-ancestry groups; African-ancestry meta-analyses are underway and cross-ancestry meta-analyses will be performed. Replication is also planned.

Results: The meta-analysis included 71,615 VTE cases among whom 39,388 had an DVT alone and 32,227 had a PE, with or without DVT. The Table shows the lead genetic variant at 4 loci that had p-values exceeding genome-wide significance: rs6025 in *F5* (FV Leiden variant); rs7681423 upstream from *FGG*; rs4253417 in *F11*, and rs3749748 in *SLC12A2-DT*.

Variants exceeding genome-wide significance from meta-analysis

rsID	CHR:POS:EA:NEA	EAF	OR	P	Locus

rs6025	chr1:169549811:T:C	0.054	0.66	3.10E-52	*F5* (Leiden variant)
rs7681423	chr4:154621096:T:C	0.307	1.08	2.98E-10	Upstream from *FGG*
rs4253417	chr4:186277851:C:T	0.452	1.07	4.55E-10	*F11* (intronic)
rs3749748	chr5:128014857:T:C	0.225	0.92	7.73E-09	*SLC12A2-DT* (intronic)

CHR:chromosome; POS:position (hg38); EA:effective alllele; NEA:non-EA; OR:odds ratio; P:p-value.

Summary/Conclusion: We identified 4 genetic signals associated with an increased or decreased risk of PE among those with a DVT alone; 3 were novel. Rs7681423-T (*FGG*) was associated with an increased risk of PE in our analyses and is in strong LD with rs2066864, a known VTE variant whose A allele (EAF=0.25) is associated with an increased risk of VTE (OR=1.23) relative to controls. Rs4253417-C (*F11*) was associated with an increased risk of PE in our analyses and is in strong LD with rs3756011, a known VTE variant whose A allele (EAF=0.39) is associated with an increased risk of VTE (OR=1.21) relative to controls. The rs3749748-T variant in *SLC12A2-DT* is strongly associated with red blood cell distribution width, possibly influencing clot characteristics. These variants may be markers or causes of clots propensity to break apart or to dislodge, leading to embolization in the lungs. The results are consistent with both shared and distinct mechanisms for formation of DVT clot formation and subsequent PE.

P-033

ELEVATED SERUM CONCENTRATION OF LIPOPROTEIN (A) IS ASSOCIATED WITH HYPOFIBRINOLYSIS IN PATIENTS WITH SEVERE AORTIC STENOSIS

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Background: In severe aortic stenosis (AS) serum level of lipoprotein a [Lp(a)] was a predictor of prolonged clot lysis time (CLT), however an involvement of Lp(a) in hypofibrinolysis and the contribution to AS development/progression has been poorly understood so far.

Aims: We investigated the impact of increased Lp(a) concentration on overall plasma fibrinolytic capacity and the level of proteins involved in fibrinolysis.

Methods: We recruited 75 patients with severe AS defined as aortic valve area (AVA) ≤ 1cm^2, peak transvalvular velocity (V_{max}) of ≥ 4.0m/s and mean transvalvular pressure gradient (PG_{mean}) ≥ 40mmHg. Patients were stratified into groups according to the level of Lp(a): 50 AS individuals with Lp(a) concentrations ≥ 50mg/dl and 25 with Lp(a) < 50mg/dl of similar age and sex. Routine laboratory assays were used to assessed Lp(a), glucose, creatinine, lipid profile, CRP and fibrinogen. Human plasminogen activator inhibitor type 1 (PAI-1) antigen, thrombin activatable fibrinolysis inhibitor (TAFI) and tissue plasminogen activator (tPA) levels were assayed using ELISA kits. CLT was performed to assess plasma fibrinolytic capacity and was determined turbidimetrically. All participants provided written informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethical Committee.

Results: The mean age of all AS patients was 66±9 years. For AS patients defined as high Lp(a) group the median Lp(a) concentrations was > 81mg/dl and > 7mg/dl for individuals defined as low Lp(a) group. AS patients with high Lp(a) were characterized by 54% higher PAI-1 and 14% higher TAFI levels, as well as 12% prolonged CLT compared to individuals with low Lp(a) (all p<0.01). There were no intergroup differences regarding other laboratory parameters. Solely in AS patients with high Lp(a) we observed associations between Lp(a) concentrations and PAI-1 (r=0.68, p<0.0001) and TAFI levels (r=0.46, p<0.001), but not with tPA (p=0.08). Elevated Lp(a) was associated with CLT (r=0.62, p<0.0001) and AS severity reflected by V_{max} (r=0.55, p<0.0001), PG_{max} (r=0.56, p<0.0001) and PG_{mean} (r=0.46, p=0.0007), but not AVA (p=0.07). Furthermore, elevated Lp(a) was weakly associated with fibrinogen (r=0.3, p=0.04). There were no associations of Lp(a) with other laboratory parameters in both groups.

Summary/Conclusion: Our study showed for the first time that in severe AS patients, elevated Lp(a) concentration is associated with increased levels of fibrinolysis inhibitors leading to hypofibrinolysis, which may drive the fibro-calcification remodeling of valvular leaflets.

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P-034

MOLECULAR CHARACTERIZATION OF ANTITHROMBIN IN SAMPLES OF PATIENTS WITH DEFICIENCIES: A MASS SPECTROMETRY-BASED NEXT-GENERATION TEST

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Background: Diagnosis of antithrombin (AT) deficiency currently relies on activity tests. These tests, originally developed over three decades ago, generate a single measure of the functionality of AT in an *in vitro* setting. While AT activity tests are generally accurate in healthy individuals, their performance is insufficient in patients with AT deficiency resulting in underdiagnosis. Specifically, AT deficiencies induced by type-II heparin-binding site or reactive site mutations as well as congenital disorders of glycosylation (CDG) may be missed. To enable studies into the effect of specific mutations in AT on clinical phenotypes, and eventually better diagnose AT deficiencies, a next-generation test for AT is required.

Aims: Molecular characterization of proteins through mass spectrometry (MS) provides in-depth information on the protein that is present in the blood of patients. We developed an MS-based test for molecular characterization of AT and analytically validated its quantitative performance (Kruijt et al., RPTH, 2023). Here, we aim to explore the potential of the test to discriminate qualitative defects in AT, specifically mutations or altered AT glycosylation.

Methods: In total 23 peptides originating from AT are analyzed, of which three quantitating peptides are used to define the concentration of AT. The test provides good imprecision (5.9 – 7.8 % for the quantitating peptides) and good correlation (r = 0.88) with the standard activity test in samples with transient quantitative deficiencies. To explore the qualitative aspect of the test, two cohorts were analyzed: a cohort of CDG patients (N = 45), and a cohort of patients with congenital AT deficiency (N = 87) with known activity and *SERPINC1* causative defect.

Results: Quantitative defects were observed in 34/45 CDG patients and 73/87 AT deficiency patients (mean concentration 0.82 and 0.91 µmol/L respectively, versus 1.28 µmol/L in healthy controls). In CDG-patients the % of beta-AT (a glycoform lacking glycosylation at site N^{167}) was significantly increased compared to healthy controls (28.9 % versus 5.4 %, respectively). Moreover, altered N-glycosylation was observed for a second N-glycosylation site (N^{224}). In patients with *SERPINC1* mutations, various mutated peptides could be identified, specific examples included p.Val30Glu (Dublin), p.Arg45Trp, p.Pro73Leu (Basel), p.Leu131Phe (Budapest3), p.Arg177Cys, p.Arg425Cys showing the potential of our next generation AT test. Interestingly, two patients with a qualitative defect had total AT concentrations above the reference intervals (1.90 and 2.01 µmol/L, ref int. 1.07 – 1.49 µmol/L), indicating a possible compensatory mechanism.

Summary/Conclusion: The results from the two cohorts demonstrate the ability of the test to discriminate CDG patients and AT deficiency patients with quantitative and qualitative AT deficiencies from healthy controls. The molecular information gained from this test enables exploration of the various subtypes of AT deficiency and the effects of specific mutations on the levels of wildtype and mutant AT. By combining this information with the clinical phenotype of patients, personalized and unequivocal diagnoses based on molecular AT-forms are eventually expected. Also, the down-stream consequences of this molecular test for patient management will be discussed.

P-035

IMPROVED SPECIFICITY OF ESTABLISHED ACTIVATED PARTIAL THROMBOPLASTIN TIME REAGENTS FOR LUPUS ANTICOAGULANT DETECTION

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Background: One of the recommended methods to screen for lupus anticoagulant (LA) is activated partial thromboplastin time (aPTT) testing. aPTT reagents are used in routine coagulation testing as well as LA detection, so false-positives occur in the presence of heparins and heparinoids, particularly unfractionated heparin (UFH).

Aims: To improve the specificity of an established LA-sensitive and LA-insensitive aPTT reagent pair used as LA screen and confirm reagents respectively by incorporating a heparin neutraliser.

Methods: Normal plasma samples were spiked with different concentrations of UFH and tested with LA sensitive and LA insensitive aPTT reagents containing different concentrations of polybrene to determine the optimal polybrene concentration where interference by UFH levels within the therapeutic range was successfully inhibited, but where polybrene itself did not interfere with the results. The reagents with the optimised polybrene concentration were then used to determine a reference range by testing 33 normal donors. They were also tested against the same LA sensitive and LA insensitive reagents without polybrene with a positive, negative and a non-LA abnormal control, 10 normal, and 10 LA-positive plasmas, as well as one strong positive neat and diluted 1:1 with normal plasma spiked with different UFH concentrations (0.1-1.0 IU/mL at 0.1 IU/mL increments). All tests were performed on a fully automated analyser.

Results: The heparin neutraliser showed no significant interference on the test results as the calculated reference ranges of the Normalised Screen/Confirm ratios with/without polybrene (0.88-1.12/0.89-1.13) as well as the means (1.00/1.01) and medians (1.00/1.00) were similar. Both populations (with/without polybrene) had Gaussian distributions (Kolmogorov Smirnoff p=0.99/0.99) and consequently, cutoffs were calculated as +2SD of the mean. Polybrene was shown to be effective at removing UFH interference as normal plasmas with/without UFH were in both cases read as negative (S/C ratio range 0.91-1.08/0.98-1.09, mean 0.98/1.03, median 0.96/1.03) and strong LA positives plasmas with/without UFH were in both cases read as positive and of comparable potency (S/C ratio range 1.56-2.39/1.67-1.91, mean 1.85/1.77, median 1.72/1.76). Paired t-test for all samples with and without polybrene was insignificant at p=0.9047. Polybrene was also effective in the weak LA positive plasma created by dilution, as the S/C ratio of the sample without UFH (1.33) was mirrored by the S/C ratio range of the aliquots spiked with different UFH concentrations (1.26-1.35, mean1.26, median 1.35). The determined optimal concentration of polybrene is 10µg/mL as it was shown to be effective in removing heparin interference while not affecting test results.

Summary/Conclusion: The use of routine aPTT reagents in LA detection is limited by heparin interference. In this study, we showed that the addition of polybrene to established aPTT reagents improved their specificity for LA detection, as normal and LA-positive plasmas gave the same interpretations and statistically significantly similar values with and without polybrene. Comparing LA-positive samples with different UFH concentrations using reagents with and without polybrene gave statistically significantly similar values and identical interpretations. The use of these improved aPTT reagents will therefore reduce false-positive results in LA testing.

P-036

ALTERATIONS OF FIBRINOLYSIS IN SARS-COV-2 INFECTED PREGNANT WOMEN: A PROSPECTIVE, CASE-CONTROL STUDY

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Background: Coronavirus disease-19 (COVID-19) is associated with disturbed coagulation and fibrinolysis balance. Only case reports are available on the degree of fibrinolysis and the development of some of its parameters in pregnant women infected with SARS-CoV-2.

Aims: We aimed to investigate COVID-19-associated fibrinolysis alterations in third trimester pregnancies and their associations with the clinical course and post-partum hemostasis events.

Methods: In this observational case-control study, 100 women with acute COVID-19 infection at 24-40 gestational weeks (COVID-19+ group) and 95 healthy age and gestational week matched pregnant women (COVID-19- group) were enrolled. All women were outpatients with mild/no symptoms at admission. Acute infection was confirmed/ruled out using SARS-CoV-2 RT-PCR and/or antigen test. In addition to screening tests of coagulation, a comprehensive set of fibrinolysis markers including D-dimer, plasminogen activity, α2-plasmin inhibitor (α2PI) activity, FXIII activity and FXIII-A_2B_2 antigen, plasminogen activator inhibitor-1 (PAI-1) activity and antigen levels, in vitro clot-lysis were measured. Detailed clinical parameters of pregnancy, labor and post-partum period were registered.

Results: Clot-lysis times (CLT) were significantly shorter in the COVID-19+ group as compared to controls (50%CLT median [IQR]: 25 [21-42] min vs. 46 [41-58] min, respectively, p<0,001). A significant decrease in plasminogen activity was observed in the COVID-19+ group compared to control pregnancies (COVID-19+: 162 [IQR:143-190] % and COVID-19-: 174 [IQR:164-197] %, p= 0.002). In case of more severe COVID-19 (stage 2 disease), FXIII levels and plasminogen activity showed a significant decrease as compared to mild cases (stage 1 disease). Fibrinogen, D-dimer, PAI-1 activity, and antigen levels did not differ between the groups. In the COVID-19+ group, postpartum hemorrhage (PPH) developed in 4 cases, associated with significantly reduced plasminogen and α2PI levels as compared to those without PPH. Thrombotic events did not occur in either group.

Summary/Conclusion: In this cohort, third-trimester COVID-19+ pregnancies were associated with marked hemostasis alterations and hyperfibrinolysis. In patients with PPH, reduced plasminogen and α2PI levels were observed.

P-037

ROTATIONAL THROMBOELASTOMETRY (ROTEM) MEASUREMENTS FOR THE PREDICTION OF THROMBOLYSIS SAFETY IN ACUTE ISCHEMIC STROKE PATIENTS

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Background: Intravenous thrombolysis using recombinant tissue-type plasminogen activator (rt-PA) is an effective treatment of acute ischemic stroke (AIS), however, 6-8% of patients develop intracranial hemorrhage (ICH) as side-effect. As of today, the occurence of therapy-associated ICH cannot be foreseen at the initiation of thrombolysis.

Aims: We aimed to investigate whether the rotational thromboelastometry (ROTEM) measurement, a point-of-care test (POCT) to study the viscoelastic properties of clot-formation and lysis could predict the safety of thrombolysis, and whether the predictive value of the test can be improved by modifying the assay.

Methods: In this prospective observational study, blood samples of 114 AIS patients, all receiving i.v. rt-PA were taken before and immediately after thrombolysis. ROTEM was performed at both time points using citrated whole blood in a ROTEM SIGMA device and the Cartridge Complete kit. In order to mimick the in vivo effect of rt-PA, the test was also performed in the presence of 140 ng/mL rt-PA (mROTEM-t-PA) added directly to pre-thrombolysis blood samples. Stroke severity was determined by NIHSS on admission. Therapy-associated ICH was classified according to ECASSII. Long-term outcomes were defined at 3 months post-event by the modified Rankin Scale.

Results: In pre-thrombolysis blood samples, clot firmness showed strong correlation between ROTEM and mROTEM-tPA measurements (A5: $r=0.7564$, $p<0.0001$). In case of more severe stroke (NIHSS>15), higher clot firmness was observed in pre-thrombolysis samples. Clot lysis index of mROTEM-tPA showed strong correlation with post-thrombolysis ROTEM results (LI45: $r=0.7192$, $p<0.0001$). Using the mROTEM-tPA test before thrombolysis, a LI60>0% parameter excluded the occurrence of post-lysis ICH with 100% sensitivity and 45% specificity (100% negative predictive value, $p=0.038$) in this cohort.

Summary/Conclusion: Based on our results, the modification of ROTEM is necessary to predict thrombolysis outcomes. The mROTEM-tPA might be used to exclude post-lysis ICH using pre-thrombolysis samples with high negative predictive value.

P-038

CIRCULATING ACTIVATED PROTEIN C LEVELS ARE REDUCED IN CANCER PATIENTS, A NOVEL CONTRIBUTION TO TUMORIGENESIS?

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Background: Cancer growth develops a subclinical hypercoagulable state and hypercoagulability is also a key contributor in cancer progression beyond cancer-associated thrombosis. Increasing evidence shows that coagulation proteins have multiple functions in tumor progression and metastasis, however the role of natural anticoagulants in cancer remains barely unexplored. Activated protein C (APC) displays anticoagulant and cytoprotective functions and its dysregulation may prompt cancer growth.

Aims: To ascertain whether circulating APC levels are dysregulated in cancer patients.

Methods: We obtained heparinized plasma from 88 cancer patients (45 bladder, 15 sarcoma, 9 renal and 19 prostate) and 14 controls, namely patients with a cancer suspicion anatomopathologically discarded. Circulating APC was quantified with an in-house sandwich ELISA, based on the interaction between APC and its main natural inhibitor PCI. Briefly, heparin forces APC:PCI complexation and these complexes are measured using an anti-PC primary antibody and an anti-PCI secondary antibody. Differences in circulating APC between groups was assessed with a Mann-Whitney test using GraphPad (v.8.0.1).

Results: Compared to controls, cancer patients showed a 16% reduction in circulating APC concentration (P=0.0210) (Figure 1). Attending to the different cancer types, bladder cancer patients showed a 23% APC decrease (P=0.0038) and sarcoma patients showed a 14% decrease (P=0.0116), whereas renal and prostate cancer patients did not show a significant decrease.

Summary/Conclusion: Circulating APC levels are significantly decreased in cancer patients. Differences among cancer types may be due to differential contribution of APC in tumorigenesis or by the regulation driven by cancer cells on APC levels. The reduction in circulating APC may further enhance hypercoagulability in cancer patients while reducing its cytoprotective functions, thus contributing to tumorigenesis. ISCIII-FEDER (PI17/00495, PI20/00075, FI21/00171), GVA (ACIF/2017/138) and SETH.

P-039

STAPHYLOCOCCAL BIOFILMS: MOLECULAR SCAFFOLDS FOR THE ACTIVATION OF BLOOD COAGULATION

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Background: Infective endocarditis (IE) is an infection of the cardiac endothelium. It has an annual incidence of 3–10/100,000 of the population with a mortality of up to 30%.[1] *Staphylococcus aureus* is the most prevalent cause of IE, accounting for approximately 27% of all cases.[2] After an endothelial injury, bacterial colonisation is facilitated, thus triggering additional endothelial injury and thrombus formation. Production of a biofilm, (defined as a multicellular community of microorganisms, enclosed in a self-produced extracellular polysaccharide matrix) assists bacterial persistence and contributes to antibiotic tolerance.[3]

Aims: Considered that thrombotic complications are often associated with IE, here we explore the possibility that biofilms produced by *Staphylococcus aureus* (coagulase +) and *Staphylococcus epidermidis* (coagulase -), could induce fibrin generation in human plasma and investigate their ability to convert directly fibrinogen into insoluble fibrin *in vitro*.

Methods: *S. aureus* and *S. epidermidis* were cultured each at 37°C in LB broth and after dilution, added to a 96-well microtiter plate and incubated at 37°C for 36h, leading to the formation of bacterial biofilms. To test fibrin generation in plasma, diluted (1:2) human plasma was added to biofilms of each bacterium, and to empty wells as a blank experiment. To test fibrin generation from purified fibrinogen, a solution of fibrinogen (0.15 mg/mL) was added to biofilms of each bacterial species, and to empty wells as a control. In both assays, fibrin generation was monitored by turbidimetry, along with SDS-PAGE analysis of the ensuing fibrin clot.

Results: The data obtained indicate that: i) both biofilms of *S. aureus* and *S. epidermidis* efficiently and similarly induce fibrin clotting in human plasma; ii) both biofilms of *S. aureus* and *S. epidermidis* do not convert isolated fibrinogen solutions into fibrin.

Summary/Conclusion: The results of this study point out that bacterial biofilms can trigger blood coagulation, thus providing the molecular basis for explaining the positive relationship IE, biofilm formation and increased thrombotic risk. Further studies are need to elucidate the biochemical mechanisms underlying biofilm-induced activation of blood coagulation.

References:

1. Fernández-Hidalgo et al. (2008) *Clin Infect Dis.* 47(10),1287-97. doi:10.1086/592576

2. Selton-Suty et al. (2012) *Clin Infect Dis.* 54(9):1230-9. doi: 10.1093/cid/cis199

3. Cahill et al. (2016) *Lancet* 387(10021):882-93. doi: 10.1016/S0140-6736(15)00067

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P-040

EKOSONIC ENDOVASCULAR SYSTEM PROMOTES THROMBOLYSIS BY ALTERING FIBRIN FIBRE THICKNESS AND CLOT PERMEABILITY

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Background: The EkoSonic Endovascular System (EKOS) simultaneously delivers tPA and ultrasound (US) and is used for the treatment of venous thromboembolism (VTE). Despite studies supporting the use of EKOS to treat VTE, the mechanisms underlying acceleration of thrombolysis by EKOS are not fully understood.

Aims: The aims of this study were to quantify the *ex-vivo* effects of EKOS on tPA-mediated fibrinolysis, and on fibrin clot formation and structure.

Methods: Clots were formed from normal pooled plasma with 0.5U/ml thrombin and 10mM $CaCl_2$. For turbidity/lysis experiments (-/+ 50ng/ml tPA added to the clotting mixture), clots were formed in cuvettes containing EKOS catheters and optical density was measured at 340nm every 12sec for 75min. For scanning electron microscopy (SEM), clots were formed in open-ended tubes, with the EKOS catheter placed in the middle of the tube, for 2hr in a humidity chamber. After ultrasound application, clots were immediately fixed with 2% glutaraldehyde and processed for SEM to analyse fibrin fibre thickness. For permeation experiments, clots were formed in cuvettes (with 4x1.5mm holes drilled at the bottom) containing EKOS catheters, and after washing, the volume of liquid flowing through the clot was measure every 10min for 30min. For all experiments, US were applied at 0, 8, 15, 30, or 47 Watts and coolant was applied to maintain a constant sample temperature.

Results: Application of US at 0, 9 and 15W did not alter clot formation or lysis in our turbidimetry set-up. However, lysis rates were significantly reduced at 30W (0.8-fold) and 47W (0.6-fold) US. Clot formation was unaffected by the application of US, but already formed clots exhibited a significant reduction in dOD (0.7-fold for 30W, and 0.6-fold for 47W) during the US application phase before returning to normal. SEM analysis indicated that the application of 47W US led to a 34% reduction in fibre thickness compared to no US. Average clot pore size was also increased by 26% when 47W US was applied.

Summary/Conclusion: Our study indicates that EKOS promotes thrombolysis by inducing a thinning of fibrin fibres, and also altering clot permeability. These findings provide a structural underpinning for the mechanisms by which US accelerates fibrinolysis.

P-041

THE INCREASE IN PLASMA CELL-FREE DNA IN PATIENTS WITH VENOUS THROMBOEMBOLISM IS INDEPENDENT OF DNASE I ACTIVITY

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Background: Plasma circulating cell-free DNA (cfDNA) is fragmented DNA found in the non-cellular fraction of liquid biopsies. We previously identified an increase in cfDNA in plasma of patients with venous thromboembolism (VTE), which was associated with an increased risk of VTE [1]. An increase of cfDNA may be caused by 3 mechanisms: cell death such as apoptosis or necrosis, normally associated with different pathologies; an increased NETosis, a defensive mechanism of neutrophils based on the release of neutrophil extracellular traps (NETs) whose main component is DNA; or a deficiency in plasma DNaseI activity, as occurs in disorders like thrombotic microangiopathies or lupus nephritis.

Aims: To ascertain whether the increase in plasma cfDNA levels of patients with VTE may be mediated by lower DNaseI activity, measured with two functional assays.

Methods: We obtained plasma samples from 126 VTE patients and 79 healthy volunteers (controls). In patients, plasma samples were obtained between 6 and 24 months after the thrombotic event. cfDNA was quantified with PicoGreen (Life Technologies) and DNaseI activity was measured with two different assays: the DNaseI assay Kit (Abcam) after optimization, and with the single radial enzyme-diffusion (SRED). Statistical analysis was performed with GraphPad (v.8.0.1).

Results: cfDNA levels were significantly increased in VTE patients (median 1799 ng/ml, 1st-3rd quartiles 1661-2088) than in controls (1632, 1185-1753) (P<0.0001). However, DNaseI activity was also increased in VTE patients (5.814 µU/ml, 5.139-6.870) compared to controls (5.188, 4.794-6.217) (P=0.0041) when measured with the DNaseI assay Kit (Abcam); while, the increase was more discrete when quantified with the SRED assay (0.49 cm^2, 0.39-0.61 and 0.45, 0.36-0.52, respectively) (P=0.0512). DNaseI activity (measured by either method) and cfDNA did not inversely correlate, suggesting an independent mechanism for the cfDNA increase.

Summary/Conclusion: Patients with VTE have long-lasting increased plasma levels of cfDNA and DNaseI activity. However, the increase in plasma cfDNA in VTE patients appears to be independent of DNaseI activity, evidenced by two different functional assays. We speculate that the increase in plasma DNaseI might be a protective mechanism to compensate the presence of high cfDNA levels. ISCIII-FEDER (PI20/00075, FI21/00171) and SETH.

[1] Martos L et al. Increase of Neutrophil Activation Markers in Venous Thrombosis—Contribution of Circulating Activated Protein C (2020). Int J Mol Sci. 21(16), 5651.

P-042

FAMILIAL SEVERE THROMBOPHILIA COMBINED WITH INFERIOR VENA CAVA ANOMALY: HETEROZYGOUS ANTITHROMBIN BUDAPEST 3 MUTATION

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Background: Congenital malformations of the inferior vena cava (IVC) is rare and associated with high risk of venous thrombosis (VT) with unknown etiology, but intrauterine IVC thrombosis may be involved. A high penetrance of IVC anomaly and severe thrombophilia have been reported in patients with homozygous antithrombin (AT) Budapest 3 variant, c.391C>T, in the SERPINC1 gene.

Aims: To identify a possible inherited genetic cause in a family where the father and two sons had severe thrombophilia at young age. One son had atresia and the other an abnormally gracile inferior vena cava.

Methods: The patients were subjected to thrombophilia screen investigation and genetic testing and for acquired and inherited deficiencies, including four commonly used commercial AT activity assays. Whole exome sequencing (WES) data from the two brothers were analysed with a dominant model. Sanger sequencing was used for verification of specific variants.

Results: Depending on the assay, AT activity varied from moderately reduced to borderline values, and the Berichrome reagent (Siemens Healthineers) AT activity assay was the least sensitive assay to detect the Budapest mutation in a heterozygous form. Furthermore, in all assays, the father had more reduced AT activity than in the two sons. The son with the most severe thrombophilia also had triple-positive antiphospholipid syndrome (APS). From WES analysis we found the AT Budapest 3 mutation c.391C>T, p.Leu131Phe in SERPINC1 in heterozygous form in both brothers. In addition, we identified some loss of function variants and missense variants with uncertain significance with low frequency in genes with hitherto unknown connection to the phenotype. From Sanger sequencing of SERPINC1 in the whole family, we found the AT Budapest 3 variant in addition to the c.1063T>G, p.Phe355Val variant, both in heterozygous form, in father, and we confirmed the heterozygous AT Budapest 3 variant in his sons.

Summary/Conclusion: AT Budapest 3 in heterozygous form may explain the combined IVC anomaly and severe thrombophilia in this family. We experienced that the AT activity assay using the Berichrome reagent did not detect heparin binding site defects like Budapest 3 in heterozygous form.

P-043

IMPROVED DETECTION OF LUPUS ANTICOAGULANTS WITH NEW TAIPAN SNAKE VENOM TIME AND ECARIN TIME REAGENTS INSENSITIVE TO ANTICOAGULATION WITH VITAMIN K ANTAGONISTS, DIRECT FXA INHIBITORS, AND HEPARINS

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Background: Lupus anticoagulant (LA) detection by coagulation assays is compromised by other causes of elevated clotting times. Preponderance of thrombosis in the population warranting LA analysis leads to many requests for testing after initiation of anticoagulation, increasing the likelihood of false positive and negative interpretations in commonly used assays. Taipan snake venom time (TSVT) screening and ecarin time (ET) confirmatory assays are insensitive to vitamin K antagonist and direct factor Xa inhibitor (DFXaI) anticoagulation, and all factor deficiencies except prothrombin. The new reagents additionally contain a heparin neutraliser.

Aims: Evaluate new formulation TSVT/ET reagents for LA detection

Methods: TSVT/ET assays were performed on an automated analyser, Ceveron s100 (Technoclone, Vienna, Austria). TSVT ratio, ET ratio, % correction, and normalised screen/confirm ratio (NSCR) reference intervals (RI) were derived from 43 normal donors and calculated as ±2 standard deviations of the mean. TSVT and ET ratios were derived using RI mean clotting time denominators. TSVT/ET analysis was undertaken on 16 plasmas from non-anticoagulated patients known to have LA, 1 normal and 1 positive control, 10 from warfarinised non-LA patients (INRs 1.90-4.69, mean/median 2.78/2.26), 1 warfarinised patient with an LA (INR 4.1), and six non-LA plasmas containing DFXaIs (apixaban 101/299 ng/mL, rivaroxaban 79/104 ng/mL, edoxaban 34/370 ng/mL).

Results: TSVT ratio, ET ratio, % correction and NSCR RIs all had Gaussian distributions (Kolmogorov Smirnoff p=0.87/0.46/0.76/0.69 respectively), and upper limits for cut-offs were 1.12/1.09/10.9/1.11 respectively. 14/16 (87.5%) non-anticoagulated LA-positive plasmas had elevated TSVT ratios (range 1.16-1.62, mean/median 1.35/1.30), all with confirmed phospholipid dependence via elevated NSCRs (range 1.12-1.53, mean/median 1.34/1.36). One of the normal TSVT ratio samples nonetheless returned an elevated NSCR of 1.14, indicating positivity with the integrated interpretive model. Normal control TSVT ratio was 1.06, and positive control TSVT ratio was 1.68 with NSCR of 1.31. All DFXaI plasmas had normal TSVT ratios (range 0.90-1.09, mean/median 1.00/1.00) and ET ratios (range 0.95-1.06, mean/median 1.00/0.99) and no elevated NSCRs. 6/10 of non-LA warfarinised plasmas (INRs 1.90-2.26) had normal TSVT and ET ratios and NSCRs, whilst 4/10 (INRs 3.32-4.69) had elevated TSVT ratios (range 1.25-1.35, mean/median 1.29/1.28) but correspondingly elevated ET ratios (range 1.28-1.39, mean/median 1.32/1.31), and consequently, no false-positive interpretations as NSCRs were normal. The plasma from a warfarinised patient with an LA returned a TSVT ratio of 1.80 and NSCR of 1.16.

Summary/Conclusion: Standard LA detection with dilute Russell's viper venom time and activated partial thromboplastin time is commonly compromised by anticoagulants, and affected by factor deficiencies to which each assay is sensitive. Although charcoal adsorbents can remove direct oral anticoagulants prior to analysis they have their own limitations, whilst TSVT/ET analysis offers a direct route to LA detection in many anticoagulated patients, and is affected by fewer factor deficiencies. TSVT screen has good LA-sensitivity, warranting consideration as first-line assay in appropriately anticoagulated patients before

initiating other, less reliable strategies, to reduce anticoagulant interference, and can also be used in non-anticoagulated patients.

P-044

COVID-19: CLOT PREVENTION IN HOSPITAL INPATIENTS

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Background: COVID-19 is characterised by a cytokine storm with hyperinflammation, hypercoagulable state, platelet activation, endothelial dysfunction and sepsis-induced coagulopathy. Severe COVID-19, complicated with coagulopathy, is associated with an increased risk of venous thromboembolism (VTE) events and mortality. Anticoagulation management in COVID-19 is of particular interest and debate.

Aims: To assess VTE prevention management in COVID-positive hospitalised inpatients, and compliance to guidance. Objectives were to establish:

- VTE risk assessment completion on admission

- Prescribing of appropriate pharmacological thromboprophylaxis during admission and on discharge, unless contraindicated

- If critically ill adult inpatients are prescribed appropriate mechanical thromboprophylaxis during admission, unless contraindicated

- Thrombotic (venous and/or arterial) event(s) during and/or within 30 days of recent admission

Methods: 66 COVID-19 positive inpatients across two hospital sites (inclusion and exclusion criteria applied) were included for retrospective data collection and analysis. Patient demographics, medical documentation, VTE risk assessment forms, pathology/radiology results, oxygen requirements, medication chart and discharge summaries were reviewed to assess performance against standards to evaluate VTE prevention management. Patients were followed up for 30 days to identify if any thrombotic (venous and/or arterial) event(s) occurred.

Results: 89% (n=59/66) of inpatients had a completed VTE risk assessment within 14 hours and 97% (n=64/66) within 24 hours from admission.

84% (n=51/61) of inpatients were prescribed appropriate pharmacological thromboprophylaxis during admission, unless contraindicated.

67% (n=4/6) of COVID-positive critically ill inpatients were prescribed appropriate mechanical thromboprophylaxis.

80% (n=6/10) of symptomatic COVID-positive inpatients established on anticoagulation therapy prior to hospital admission were not appropriately switched to alternative anticoagulation.

100% (n=6/6) of asymptomatic COVID-positive inpatients established on anticoagulation therapy prior to hospital admission continued on the established anticoagulant agent during admission as appropriate.

63% (n=33/52) of inpatients were prescribed appropriate thromboprophylaxis on discharge, unless contraindicated.

3 thrombotic (venous and/or arterial) events occurred during a 30-day follow-up.

Patient demographics:

- Gender: 59% male and 41% female
- Ethnic Group: 62% white, 6% asian, 3% black, 2% mixed, 27% other
- Age: 20% under 60 years old, 80% over 60 years old
- Body Mass Index: 17% underweight, 40% healthy weight, 20% overweight, 23% obese
- Mean Hospital Stay Duration: 9 days
- COVID Vaccination Status
- Co-morbidities:
  - Cardiovascular disease: 11%
  - Respiratory disease: 7%
  - Endocrine: 30%
  - Malignancy: 15%
  - Renal disease ≥ Stage 3: 12%

Summary/Conclusion: The risk of thrombosis is increased in COVID-19 patients. VTE risk assessment is important to identify thrombosis and/or bleeding risk factors that may be present particularly for COVID-19 patients. Appropriate pharmacological thromboprophylaxis should be offered during admission and on discharge to patients at increased risk of VTE, unless contraindicated. Patients established on anticoagulation therapy prior to hospital admission should have appropriate management during admission. Ongoing education and awareness is required so clinicians are aware of the latest guidance to apply to clinical practice to reduce the risk of hospital associated VTE event(s).

P-045

EVALUATION OF DIGITAL MEASURING DEVICE WITH AN ADAMTS13 ACTIVITY SCREENING TEST

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Background: The determination of von Willebrand Factor cleaving protease ADAMTS13 activity in plasma is an important diagnostic test to enable the differentiation of patients with thrombotic thrombocytopenic purpura (TTP) from those with other thrombotic microangiopathies (TMAs). It is critical to have a reliable ADAMTS13 Activity result within a quick reporting turn-around time to increase the possibility of a positive outcome of patient care, especially with TTP patients. The screening test (Technoscreen ADAMTS13 Activity) is based on a flow through assay principle. A semi quantitative result can be achieved within 25 minutes, requiring no special laboratory equipment nor a highly skilled technician to perform the test. The operator determines the ADAMTS13 activity level by comparing the colour (red) intensity of the 4 different levels (0, 0.1, 0.4 and 0.8 IU/mL) indicated on the colour card verses the test well colouration. However, on relying in human interpretation from the colour card can potentially lead to variations in reported results.

Aims: To improve consistency of result interpretation with the screening method a small mobile measuring device was evaluated to determine the colour intensity of the test wells.

Methods: To show variability with human interpretation with screening method results, the same set of sample cartridges were read by x9 in-experienced operators using the colour card and also the measuring device. The patient and normal samples were assayed in parallel in the FRET assay (Technofluor ADAMTS13 Activity).

To test the analytical performance of the measuring device in combination with the screening method, a three-way method comparison was performed. Samples were assayed in a FRET assay (reference method) and the Screening method with result interpretation using a colour card (x3 experienced operators) and the measuring device.

Results: With multiple operators, there was a 100% consensus with interpretation of all the TTP patients, which were all correctly read as 0 IU/mL. Other sample test results showed slight variations between operators. However, the digital results from the measuring device were in line with the FRET result.

Determining the colour intensity using the colour card versus the measuring device value gave very comparable results. The calculated correlation between Technofluor vs Screening method/measuring device was $r^2 = 0.83$. The analytical performance of the Technofluor vs Screening method/device was calculated using a cut off value of ≤0.1 IU/mL and an appropriate digital value, resulting in a specificity of 89% and sensitivity of 79%. This analytical performance data reflects previously published data for the screening method (J Thromb Haemost. 2020;18:1686-1694).

Summary/Conclusion: Human result interpretation of the screening method using the colour card shows variability between operators with non TTP patient samples. However using a measuring device in combination with the screening method gave consistent results, which showed excellent correlation with FRET assay. Therefore, a digital output value reduces variability in patient reporting in the screening method.

P-046

AN OPTIMIZED THROMBIN GENERATION REAGENT SIGNIFICANTLY INCREASES SENSITIVITY FOR DETECTION OF THROMBOGENIC ACTIVITY IN IMMUNOGLOBULIN CONCENTRATES

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Background: In the past, immunoglobulin concentrates were found to contain impurities with thrombogenic potential. In some cases, this thrombogenic activity was caused by contamination with clotting factor FXIa.

As a consequence, the first international standard for FXIa has been established and producers of immunoglobulin concentrates have adapted their production methods and quality control in order to exclude any possibility for FXIa or other clotting factors to be present in the final product.
It also has been shown that a thrombin generation assay (TGA) is superior to non-activated partial thromboplastin time (NAPTT) in detecting any procoagulant activity in those concentrates.

Aims: In this study we show that a modified thrombin generation reagent (trigger) increases sensitivity for detection of traces of FXIa in intravenous immunoglobulin (IVIG) products.

Methods: Samples are diluted in FXI deficient plasma as well as pooled normal plasma and measured in TGA using modified triggers for thrombin generation. Results are reported as nM peak thrombin when using normal plasma as diluent or could be calculated in mU FXIa/mL when using FXI deficient plasma as diluent.

Results: Dilutions of the international standard for FXIa exhibited a linear dependency over a wide a wide range of concentrations. Thus, a calibration curve could be established to correlate the amount of thrombin generation seen with some IVIG preparations to the amount of activated FXI. In reproducibility studies, IVIG samples with very low levels of FXIa could be reproducibly measured.

Summary/Conclusion: Taken together, TGA is a suitable tool to determine pro-coagulant activity of preparations, especially of which will be administered intravenously. In additions we could show that sensitivity towards FXIa in IVIG preparations could be increased, by using FXI deficient plasma as a dilution matrix. Thus, pro-coagulant activity can be measured with very high sensitivity when an optimized trigger reagent for thrombin generation is used.

P-047

POST COVID 19 CATASTROPHIC ANTIPHOSPHOLIPID SYNDROME (CAPS): CASE REPORT IN PEDIATRICS

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Background: Coronavirus 2019 (COVID 19) is an emerging disease that was declared pandemic since March 2020. Severe immune response seen in COVID-19 could lead to pathogenic immunological features such as pediatric multisystem inflammatory syndrome and systemic lupus erythematosus (SLE)

Aims: Describe a COVID- 19-associated catastrophic antiphospholipid syndrome in pediatrics at Hospital Benjamín Bloom, El Salvador

Methods: Review of a COVID 19 related catastrophic antiphospholipid syndrome case report at Hospital Benjamín Bloom, El Salvador

Results: A 7- years girl, at emergency room presented prolonged fever, diarrhea and abdominal pain, leukocytosis, and acute renal injury (elevation of creatinine 7.9 mg/dL, erithrocyturia, proteinuria, nephromegaly and inflammatory findings by US), elevated acute phase reactants. Three weeks ago, she experienced superior tract infection and in relation to the current COVID-19 pandemic, COVID -19 antibodies were sent and showed positive IgG (45 UI/ml). The day after, she experienced left hemiparesis, computed tomography (CT) was performed showing ischemic stroke secondary to middle cerebral artery thrombosis, abdominal CT showed partial luminal obstruction of inferior vena cava. Immunological studies were ruled out showing: positive lupus anticoagulant (LA), ratio 1.9; positive direct antiglobulin test (IgG/C3). An echocardiogram was realized showing pericarditis, myocarditis, pancarditis and Libman-Sacks endocarditis.

We found involvement of thrombosis in 3 different organs within one week period: CNS, cardiac and renal (probably microthrombosis); laboratory (LA) and imagine studies test results led us to COVID infection secondary CAPS diagnosis. Treatment consisted in anticoagulation with enoxaparin, acetylsalicylic acid, oral hydroxychloroquine and intravenous immunoglobulin.

Summary/Conclusion: COVID-19 infection led to multiple clinical findings of thrombosis, the presence of antiphospholipid antibodies in COVID patients is still controversial. The data provided by the literature show that there is a coagulopathy associated with COVID-19 that encompasses a spectrum of alterations that suggest an immune-mediated mechanism reminiscent of APS, and its most severe form, CAPS.

In this way, the early introduction of anticoagulant treatment meant an improvement in the prognosis of these patients.

P-048

BILATERAL RENAL ARTERY PARADOXICAL EMBOLISM IN A PATIENT WITH CONCURRENTLY REVEALED UNKNOWN PATENT FORAMEN OVALE AND ANTIPHOSPHOLIPID SYNDROME

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Background: Paradoxical embolism (PDE) is an uncommon cause for acute arterial occlusion, which occurs when a thrombus crosses an intracardiac defect into the systemic circulation. A patent foramen ovale (PFO) is a small flap-like opening between the atria acting as a pathway for a thrombus from the peripheral veins, bypassing the lungs and entering the systemic circulation. A cerebral event is the usual presenting symptom in patients with PFO. Systemic, non-cerebral, paradoxical embolisms occur less frequently, accounting for 5%–10% of all paradoxical embolisms.

Aims: Aim of the present is to highlight an interesting case of bilateral renal artery PDE in a patient, who was concurrently diagnosed with a patent foramen ovale (PFO) and Antiphospholipid Syndrome (APS), a rarely-described entity. PDE especially involving kidneys has been reported to commonly involve multiple organs, such as the lung, kidneys and brain.

Methods: A 46-year old Caucasian man, with no previous medical history, was admitted due to sudden onset of severe constant abdominal pain predominantly in the right flank, radiating to the groin along with nausea, vomiting and diaphoresis. No history of fever, infection or trauma was documented. He was afebrile and physical examination revealed sinus tachycardia, painful abdomen as noted, leukocytosis, elevated d-dimers and inflammation markers.

Results: He was initially supported with IV fluids and opioid analgesia. Contrast-enhanced abdominal CT scan revealed extensive thromboemboli in the right renal artery causing its occlusion. 4 hours after the pain onset, he underwent emergency catheter-directed thrombolysis with mechanical thrombectomy/aspiration of his right renal artery, with good angiographic restoration of perfusion afterwards. 24hours later the patient experienced fever up to 38.5°C, severe dyspnea and shock, along with elevated creatinine levels. He was transferred to our clinic for further investigation and treatment. Repeated CT scans detected acute pulmonary emboli in the left lower lobe pulmonary arteries, thromboemboli in his left renal artery and right renal cortical sign. No filling defects within the left heart were found to suggest a cardiac source of the emboli. An echocardiogram revealed a PFO with significant right-to-left shunting of saline bubble contrast and no intracardiac thrombus. His coagulation profile and blood testing for thrombophilia and connective tissue disorders were unremarkable apart from highly positive anti-cardiolipin/anti-β2 GPI IgM antibodies suggestive of APS. The patient was treated with low molecular weight heparin SC, acetylsalicylic acid 100mg/d, atorvastatin 40 mg/d and antibiotics. He was discharged with normal creatinine levels, no monoclonal protein or malignancy were noted, no atrial fibrillation in multiple holter monitor tests or cerebral infarcts in brain MRI/MRA, thrombophilia panel was repeated with no findings, while the APS-associated antibodies were found twice elevated. PFO closure is still under consideration by his cardiologist, while he is currently treated by rheumatologists as APS.

Summary/Conclusion: Renal infarction is a rare entity, needs immediate reperfusion, can be a vigorous acute abdomen mimic with a delayed diagnosis though up to several days. APS is a systemic inflammatory vascular disease with variable clinical manifestations and masquerades. High index of suspicion is needed by clinicians to diagnose both entities in order to prevent permanent kidney injury.

P-049

INTRACARDIAC THROMBUS, A RARELY CARDIAC MANIFESTATION OF ANTITHROMBIN DEFICIENCY. IS SCREENING FOR CONGENITAL THROMBOPHILIA USEFUL?

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Background: Antithrombin (AT) is one of the most important inhibitors of blood coagulation. The usually manifesting of AT deficiency is venous thromboembolism (VTE) with deep and superficial vein thrombosis, pulmonary embolism (PE), or both, but the intracardiac thrombus (ICT) is a relatively rare cardiac manifestation.

Aims: A rare cause of ICT is represented by congenital prothrombotic conditions. Some case reports dealing with ICT and single prothrombotic defects have been presented, the reported cases concerned mainly protein C and protein S deficiencies and rarely AT deficiency.

Methods: We report the observation of a 28-year-old Algerian female patient with a personal history of an atrial septal defect (ASD) and a cardiovascular risk factors, obesity and dyslipidemia. She was admitted to the cardiac surgery department for percutaneous closure of ASD. Physical examination showed palpitations and splitting of the second heart sound above the pulmonic area. During preoperative transesophageal echocardiography, an intra right atrium thrombus was discovered. A curative anticoagulant treatment by Unfractionated heparin (UFH) was set up during 5 days, then relayed by a prolonged treatment with vitamin K antagonists (VKAs) led to a progressive regression of the thrombus.

Results: In testing thrombophilia performed away off the acute phasis and after completion of anticoagulant therapy, the levels of PC and PS was in normal limits, the polymorphism screening of the FV Leiden, FII G20210A were negative and the screening of lupus anticoagulant was negative.

AT level measured by chromogenic and immunoturbidimetric assays was found abnormally low at 65% and 62% respectively, the genetic study showed a new mutation of PROC1 gene.

Summary/Conclusion: We suggest that congenital thrombophilia testing may be proposed in investigation of patients with ICT in young one. Our case illustrates also the need for a cardiac imaging evaluation in thrombophilic patients with others cardiovascular risk factors.

P-050

SINGLE-CENTER REAL-WORLD EXPERIENCE WITH IDARUCIZUMAB FOR DABIGATRAN REVERSAL

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Background: Idarucizumab reverses the anticoagulant effect of dabigatran by specifically binding to it. Since its approval in 2015, it has been indicated for patients with uncontrolled bleeding or in whom emergent invasive procedures were needed. Unfortunately, it's not yet widely available in Spain.

Aims: We aim to describe our clinical experience with idarucizumab to contribute to the available evidence and improve its accessibility.

Methods: We retrospectively analyzed a cohort of patients who received idarucizumab for dabigatran reversal in one center (La Paz University Hospital, Madrid, Spain), from June 2016 to October 2022. Clinical data were collected from electronic medical records. Patients were divided based on the idarucizumab indication (need of imminent surgery or major bleeding) for the analysis. The reversal was assessed according to the clinical outcome and the related adverse events (bleeding, thrombosis or death within 30 days).

Categorical variables were described as frequencies and percentages, and the continuous variables as mean ± standard deviation (SD) or median (range). For the categorical data, the chi-squared or Fisher's exact test were applied, as appropriate. Statistics were performed with IBM SPSS software version 21.

Results: A total of 22 patients treated with idarucizumab were included. The mean age was 74.7 ± 15.7 years, and half of the cohort were women. Only 27.3% presented with impaired renal function, and basal activated partial-thromboplastin time (aPTT) was prolongated in 59.1%. The majority were under dabigatran for stroke prevention in non-valvular atrial fibrillation (81.8%), and just 4 patients (18.2%) were receiving it for thromboembolic disease treatment. Idarucizumab was mainly indicated for emergent surgery (n=13, 59.1%), with 9 patients (40.9%) receiving it for major bleeding. Gastrointestinal bleeding (n=5, 55.6%) was the most common type of bleeding, followed by intracranial haemorrhage (n=2, 22.2%). Clinical haemostasis with cessation of bleeding within the first 12 hours was achieved in 7 of 9 patients (77.8%) with major bleeding. An impaired renal function might explain the prolonged time presented in the 2 patients in whom it was >12 hours. The median time for bleeding cessation was 8 hours (range 1-72), although it was available only in 7 of 9 patients. There were no reports of excessive bleeding during surgery in the 13 patients who underwent surgery. Two of the 13 patients from the surgical group died upon the 30-day follow-up, both because of haemorrhagic complications. No deaths were reported in the bleeding group. Reversal in all the 22 included patients occurred independently of age, sex, renal function or indication for anticoagulation (p>0.05). Among the whole cohort, none experienced thrombotic complications within this period. At 30-day follow-up, anticoagulation was restarted in 19 of the 20 remaining patients (95%), in 9 of them with dabigatran, after a median time of 4 days (range 1-30).

Summary/Conclusion: The availability of specific reversal agents is crucial for improving the safety profile of anticoagulants. Our experience confirms the benefit of idarucizumab for the reversal of dabigatran in the clinical setting and proves the need to ensure its access in all the centers.

P-051

THE USEFULNESS OF CAPILLARY BLOOD COLLECTION DEVICES FOR COAGULATION PARAMETERS DETECTION IN HEMOPHILIA

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Background: Monitoring of coagulation factor VIII (FVIII) and IX (FIX) activity level is required for patients with hemophilia both to adjust prophylaxis and to guide therapy during bleeding. The TAP-100 blood collection system allows the collection of 100 µL of capillary blood from the upper arm within ±3 minutes without special training.

Aims: Determine whether capillary plasma (CP) collected by a citrated version of the TAP-100 device can be used to accurately measure coagulation status in healthy volunteers.

Methods: In this study, the levels of several coagulation-related biomarkers were compared in plasma collected by phlebotomy (VP) and a capillary blood collection system called the citrated version of TAP-100. Two citrated versions of the investigational TAP-100 (YourBioHealth) were created. Version one with dried-in 15 mM trisodium citrate and version two with 15 mM trisodium citrate and 40 µg/mL Thermostable Inactivator of Contact Activation (TICA) both dried in. Citrated VP (BD vacutainer) and CP (investigational TAP-100) were collected from healthy volunteers (n=30) after signing informed consent, by a trained phlebotomist. FVIII- and FIX-activity levels were analyzed with the BIOPHEN FVIII:C and FIX:C (HYPHEN BioMed) chromogenic assays. Prothrombin activation was determined by Fragment 1+2 levels using the Enzygnost F1+2 (Siemens) kit. VWF antigen levels were determined using the Zymutest vWF kit (HYPHEN BioMed). Data were analyzed using Pearson's correlation coefficient. Data is shown as (mean ±SD).

In addition, questionnaires were utilized to interview caregivers (n=15), researchers (n=7), and patients (n=12) with respect to the usefulness of the home-use blood collection device.

Results: FIX-activity levels measured in CP (128 ±12.4%) and VP (121 ±22%) are within the same range. FVIII-activity levels were lower in the CP method (36.9 ±22.7%) compared to VP (133.7 ±40.6%) which corresponds to ± 30% recovery of FVIII activity. F1+2 levels in CP (44 ± 28.5 nmol/L) are increased compared to VP (0.3 ±0.1 nmol/L). A negative correlation (Pearson R=-0.67) was found between FVIII-activity levels and F1+2 expression in CP samples. Addition of TICA to the TAP-100 device resulted in significantly less F1+2 formation (24 ±24 nmol/L). VWF levels were also lower in the CP (23 ±14.3%) method compared to VP (78 ±36.5%) and these levels correlated with FVIII-activity levels (Pearson R=0.57).

The majority (17/23) of professional responders (physician, nurse, researcher) reacted positively on the use of a home-use blood collection device. Professionals also underline the value of a pain-free blood collection system for pediatric purposes.

Summary/Conclusion: The citrated TAP-100 collection system (with or without the addition of TICA) can be used for capillary blood collection and to accurately determine FIX-activity levels. Prothrombin activation during capillary blood draws correlated with reduced FVIII-activity levels potentially by dissociation of thrombin-activated FVIII. This effect is partially prevented by the addition of TICA to the collection system.

The positive reactions from potential users on the TAP-100 device shows the value of adapting this tool to monitor coagulation status.

Currently, the TAP-100 device is being optimized to prevent pre-activation of the coagulation cascade.

P-052

HOW CAN WE BEST INFORM PATIENTS OF THE RISK FOR HOSPITAL ACQUIRED VTE? A COMPARISON OF PATIENT INFORMATION LEAFLETS AND RESULTS FROM A PATIENT SURVEY

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Background: Hospital acquired venous thromboembolism (VTE) is a leading cause of preventable death with approximately 122 patients affected per hospital, per year, in the UK. The National Institute of Clinical Excellence (NICE) recommends that all patients admitted to hospital and who are at increased risk, are provided with a patient information leaflet (PIL) during their admission.

Two PILs were considered for use. These differed in design, price and content. Leaflet 1 was from Thrombosis UK. It is a colourful, Z-fold leaflet with 11 illustrations, 316 words and a SMOG (Simple Measure of Gobbledygook) index of 11.1 (17–18-year-old reading). Leaflet 2 was designed by the University Hospitals Birmingham (UHB) NHS trust and contained 4 pictures, 1421 words, a SMOG index of 9.5 (15–17-year-old reading) and covers all the information recommended by NICE. UHB treats 2.2 million patients per year and therefore the decision on which PIL to distribute will many patients and stakeholders, with significant cost implications.

Aims: Our primary aim was to assess patient preference and guide decision-making on which PIL to use in UHB trust. Patient opinions could also guide the design of future PILs.

Methods: Ten Likert items, each of five points (strongly disagree to strongly agree), were designed to assess the degree of preference towards PILs. The item design was informed by a literature review and patient interview.

Patients awaiting discharge from 4 medical wards (neurology, haematology and oncology) were then surveyed over a 2-week period and asked first to rate both leaflets independently and then to compare one to the other using these items. Patients were then asked 4 white space questions explain the rationale for their answers.

Likert scales were calculated, and a paired, two tailed Student's T test was used to compare the means. Cronbach's alpha was used to measure the internal consistency of the items. Qualitative answers were analysed for recurrent themes and to provide triangulation of the quantitative data.

Results: 21 patients completed the survey. Cronbach's alpha for item performance when used to compare between the leaflets was high at 0.83. There were no significant differences in leaflet preference. However, our sample showed more heterogeneity than expected, with equal numbers of patients expressing a preference for either leaflet, with strong preferences for either PIL expressed.

Patients were asked directly what factors made them prefer one leaflet over the other. 5 patients cited the extra information in PIL 2 as a positive, however, 3 found this to be a negative; 5 patients cited the diagrams as a positive for PIL 1, but 1 patient thought this was a negative. When asked what factors they found most helpful and informative, 5 patients cited the degree of detail in PIL 1 as a positive whilst 3 patients cited diagrams in PIL 2.

Summary/Conclusion: This study challenged our assumption that ‘one size fits all’. There were a variety of preferences expressed, and our data therefore support providing patients with more choice. A group of patients preferring informative leaflets may be enriched within our sample however, as haematology and oncology patients tend to be more affected by VTE. Both leaflets used language that was written at a higher level than recommended. Future PILs should have both superficial and in-depth options, using simple language and illustrations to maximise effectiveness.

P-053

REFINING THE DIAGNOSIS OF ANTITHROMBIN DEFICIENCY WITH NEXT-GENERATION PROTEIN DIAGNOSTICS AND MOLECULAR DEFINED (GLYCO)PROTEOFORMS

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Background: This study concerns the scientific validation of a next-generation mass spectrometry (MS)-based antithrombin (AT) test for the evaluation of AT deficiency in the context of hereditary thrombophilia. In this next-generation test, AT can be determined at the molecular level, enabling identification of specific *proteoforms*, including mutations and glycosylation status [1-3]. This is in contrast to the current chromogenic AT activity tests, which only determine the overall AT activity of a mixture in the presence of therapeutic heparin, and where no single assay from one supplier appears to be able to detect all possible protein defects [4]. Hence, we hypothesize that precision diagnostics based on AT glycoproteotyping has the potential to detect discordances between these AT functional tests and could contribute to refining the diagnosis of AT deficiency.

Aims: To what extent will AT glycoproteotyping with next-generation mass spectrometry instead of AT activity testing improve the detection and diagnosis of AT deficiency as compared to the gold standard (i.e. genetic testing)? Proof-of-principle: refining the diagnosis of AT deficiency with precision diagnostics.

Methods: The *discordance analysis* will be performed between the current AT activity assays from different IVD-manufacturers (Stago, Siemens, Werfen) and the next-generation MS-based AT test. Both methods will be compared to the gold standard (i.e. genetic testing). Documented clinical specimens will be analyzed and include an adequate number of AT type II deficiencies, as these have been shown to lead to the highest method discrepancies [4-9].

Results: The results of *the discordance analysis* will be shown and the potential of the next-generation AT molecular test as “add-on” test [10] for refining the diagnosis of AT-deficiency in the current clinical care pathway of hereditary thrombophilia will be presented.

Summary/Conclusion: A next-generation AT molecular test has been developed, which has the potential to improve the current clinical care pathway of hereditary thrombophilia. The clinical sensitivity of the novel diagnostic strategy will likely increase diagnostic power and establish unequivocal diagnosis of AT-deficiency. In that respect, AT molecular testing is part of a larger vision on precision diagnostics as fundament for personalized medicine.

References

1. Ruhaak, L.R., et al., *Clin Chem Lab Med*, 2018. **56**(10): p. 1704-1714.
2. Kruijt, M., et al., *J Mass Spectrom Adv Clin Lab*, 2023. **27**: p. 61-65.
3. Kruijt, M., et al., *Res pract thromb haemost*, 2023: p. 100079.
4. Orlando, C., et al., *Thromb Res*, 2015. **135**(6): p. 1179-1185.
5. Javela, K., et al., *Thromb Res*, 2013. **132**(1): p. 132-137.
6. de la Morena-Barrio, M.E., et al., *Blood*, 2022. **140**(2): p. 140-151.

7. Kruijt, M., et al., *J Thromb Haemost*, 2022. **20**(1): p. 145-148.

8. Ungerstedt, J.S., et al., *Blood*, 2002. **99**(6): p. 2271-2272.

9. Feddersen, S. and M. Nybo, *Clin Biochem*, 2014. **47**(13): p. 1337-1340.

10. Horvath, A.R., et al., *Clin Chim Acta*, 2014. **427**: p. 49-57.

P-054

SEM AND 3D RECONSTRUCTION OF EX-VIVO ARTERIAL THROMBI: A PILOT STUDY

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Background: Thromboembolism, including acute limb ischemia (ALI) and myocardial infarction (MI) is the leading cause of death and disability worldwide. On one side, arterial thrombosis can result from atherosclerotic plaque rupture that exposes sub-endothelial cells and procoagulant material, leading to platelet activation and aggregation in high shear rates and stress. On the other side, cardio-embolism can lead to an acute occlusion of the vessel. The primary aim of our study was to compare clot composition among ALI thrombi from plaque rupture, embolisms and MI thrombi. We present some preliminary results.

Aims: The primary aim of our study was to compare clot composition among ALI thrombi from plaque rupture, embolisms and MI thrombi. We present some preliminary results.

Methods: Ex-vivo thrombi were collected by thrombectomy (ALI n=1; MI n=1) and analysed by Scanning Electron Microscopy (SEM) and SEM 3D reconstruction and compared to whole blood thrombus obtained in vitro from a healthy subject (made with addition of thrombin and calcium) (n=1). The external and internal surfaces of the thrombi were investigated after fixation and dehydration with SEM. SEM/FIB system was used to assess the ultrastructural organization of the clots. Thermo Scientific Amira, Imaris and ImageJ 2.3.0./1.51v software were used for the reconstruction.

Results: In MI thrombi we observed a biconcave shape of RBCs under the surface with a polyhedral shape of RBCs and abundant WBCs in the middle of the thrombi. Heterogenous networks of fibrin on the external and internal surfaces were identified compared to healthy controls. We found larger spaces between cells in the ALI thrombi and denser spaces in the MI thrombi.

Summary/Conclusion: In our pilot study the thrombi from ALI, MI and controls showed differences in the fibrin network. Future studies will include analysing the nature and number of different cells (WBCs, platelets and microparticles) found in the thrombi, categorising the types of fibrin fibers and the different shapes of RBCs, in order to define how specific cells are involved in the thrombus generation.

P-055

LUPUS ANTICOAGULANT: DOES A NORMAL CONFIRMATORY TEST VALUE NECESSARILY MEAN TIME CORRECTION?

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Background: Lupus anticoagulant (LA) is an antibody that interferes with one or more *in vitro* phospholipid-dependent coagulation reactions, which are dependent on interactions with protein–phospholipid complexes. In 2020, the International Society on Haemostasis and Thrombosis (ISTH) updated its guidelines suggesting simultaneous performance of the mixing and confirmatory step, in each sample with a prolonged screening test.

Aims: The aim of this study was to investigate if a normal value of a confirmation test in samples with slightly prolonged screening test means time correction and presence of lupus anticoagulant.

Methods: In 49 patients with slightly increased value of screening test (LA1) for detection of LA and normal confirmation test (LA2) value, 1:1 mixing studies with normal plasma were performed and then the Rosner index was calculated, which allows the differentiation between factor deficiency and the presence of an inhibitor. For the detection LA, Russells Viper Venom Time (dRVVT) was performed on plasma samples with Siemens BCS XP Coagulation analyzer. The Normalize LA1 and LA2 Ratios (NR) and the LA1/LA2 Ratios were also calculated.

Results: The Median values were as follows: LA1-NR: 1.26 (range: 1.18-1.45), LA2-NR: 1.09 (range: 0.9-1.2), LA1/LA2: 1.24 (range: 1.07-1.47) and Rosner index was 9.05 (range: -1.08 - 14.4). From the Rosner index values it seems that there are factor deficiencies and not presence of an inhibitor as Lupus Anticoagulant. Slight factor deficiency prolongated the clotting time of LA1 but not of LA2, due to its own excess of phospholipids.

Summary/Conclusion: Normal value of confirmation tests in samples with slightly prolonged screening test does not always means time correction and presence of lupus anticoagulant. As per ISTH guidelines, mixing and confirmation steps must be performed at the same time in samples with a prolonged screening test.

P-057

THE FIRST ADMINISTRATION OF CAPLACIZUMAB IN THE FIRST LINE OF TREATMENT FOR ACQUIRED ACUTE THROMBOTIC THROMBOCYTOPENIC PURPURA IN SLOVAKIA

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Background: Thrombotic thrombocytopenic purpura (TTP) is a very rare life-threatening disease caused by congenital or acquired deficiency of ADAMTS13 activity. Until recently, therapeutic plasma exchange (TPE) and immunosuppressive therapy were the only available treatment strategies. The current standard in the therapy of acquired TTP is a triple combination of treatment consisting of therapeutic exchange plasmapheresis, immunosuppressive treatment and the addition of the drug caplacizumab, thanks to which the morbidity and mortality of patients are significantly reduced. Caplacizumab is a humanized immunoglobulin anti-von Willebrand factor (vWF) capable of inhibiting the interaction between vWF multimers and platelets.

Aims: To describe the first efficacy of caplacizumab in a patient with newly diagnosed acute TTP in Slovakia.

Methods: Case Report

Results: A 60-year-old female patient with newly diagnosed acute renal insufficiency (ARI), severe anemia and thrombocytopenia with bleeding manifestations was acutely admitted to our clinic for suspected acute TTP. Initial investigations, in which ADAMTS13 values were less than 0.2%, with signs of hemolysis present in laboratory parameters, confirmed the diagnosis of TTP. The presence of the so-called "ultra large" HMW (high molecular weight) vWF multimers that we have investigated in our laboratory. The patient was urgently indicated for TPE and intensive immunosuppressive treatment with high-dose corticosteroids, and the next day the drug caplacizumab was added, always administered after the completion of TPE. After the 6th day from the start of TPE, the blood count stabilized, the platelet count was adjusted to the level of 247 x 109/l, and ADAMTS13 activity increased to 82%. The biochemical parameters were gradually adjusted. Due to the given condition, TPE was terminated, the immunosuppressive treatment with corticosteroids was gradually reduced, but the administration of caplacizumab in the standard dosage continued. Additionally, positive results of antibodies against ADAMTS13 (above 15 U/ml) were received. During treatment with caplacizumab, we noted a slight increase in bleeding manifestations and mild epistaxis, which subsided. On the 13th day of hospitalization, ADAMTS13 activity decreased again to 5%, but without significant decreases in the hemogram and other labs. parameters, and without the development of hemolysis. Due to the high risk of TTP relapse, the addition of the drug rituximab was indicated, 1x/week for a total of 4 submissions. Treatment with caplacizumab and corticosteroids was continued. We also received ADAMTS13 antibody results - neg. 5.10 U/ml (below 12 U/ml). ADAMTS13 activity gradually increased, the last value during hospitalization was 47.7%. On the 20th day of hospitalization, due to her good clinical condition, the patient was discharged to outpatient care and received further treatment on an outpatient basis. The total number of caplacizumab doses since the end of TPE was 38 administrations.

Summary/Conclusion: This case showed safety and effectiveness of Caplacizumab in acquired TTP poor responsive to standard therapy. ADAMTS-13 measurements supported diagnosis and therapy management.

P-062

HETEROZYGOUS FV LEIDEN MUTATION ASSOCIATED WITH MULTIPLE CEREBRAL VENOUS THROMBOSIS IN CHILDHOOD. A CASE REPORT

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Background: Protein C is at the center of a physiological system inhibiting coagulation : the protein C anticoagulant system. Protein C activated (PCa) in the presence of its cofactor, Protein S, cleave factors V activated and VIII activated, thus blocking the amplification loop of thrombin generation. Activated protein C Resistance (APCR) is a common cause of hereditary thrombophilia, which is often directly attributable to heritable mutations in coagulation factor V (FV). Venous thromboembolism is by far the most common clinical presentation of patients with APCR. Thrombosis in unusual sites (hepatic vein, sagittal sinus, retinal vein…) have been reported but they are rare even less in childhood. Precisely this is the theme of our case, which is a 22 monts old infant with APCR who presented multiple cerebral venous thrombosis.

Aims: The usually asymptomatic heterozygous FV Leiden mutaion is often diagnosed in adulthood where it is responsible of thromboembolic disease, especially when aassociated whith other contributing risk factors. We propose to report the case of a 22 month-old carrying this mutation who presented spontaneous multiple cerebral venous thrombosis to highlight the very heterogenous clinical expression of FVL which depends on many known or unknown risck factors.

Methods: Medical imaging by cerebral magnetic reasoning imaging and biological investigations including:
- Phenotypic screening for thrombophilia (AT, PC, PS, APCR).

- Screening for lupus anticoagulants (PTT-LA, drvv-S)

- Phenotypic APCR was confirmed by screening the c.1691G>A mutation (FVLeiden) using SSP-PCR

- Screening of the G20210A mutation of FII using SSP-PCR

- Family survey

Results: We report a clinical case of a 22-month-old girl referred to the laboratory for thrombophilia screening. In personal history, the patient was born premature at 33 weeks of amenorrhea, the onset of the disease was marked by an infectious syndrom whith diarrhea complicated by dehydration followed by neurological symptoms (drowsiness and walking disorders). Brain MRI showed massive venous thrombosis of the cortical veins, the sagittal sinus and the trocular of the right sinus **(Fig1)**. In family history, there aren't any venous or arterial thromboembolic disease, however, the mother presented 8 early abortions. screening for thrombophilia showed on the one hand, normal levels of plasma inhibitors, AT, PC, PS. The screening of antiphospholipid antibodies and lupus anticoagulants was negative. on the other hand, the sreening of the APCR was positif by phenotypic test confirmed by SSP-PCR (heterozygous FVL). The screening of the G20210A mutation of FII was negative.
Family investigation found the same mutation in her mother, the father and the two brothers were normal **(table1)**

Summary/Conclusion: This is an unusual observation, the patient was treated by oral anticoagulant VKA.The follow up during 4 years was favorable without any recurrence.
There is an interest in making wider screening of thrombophilia to detect possible associations.

P-063

EXPLORING TRANSCRIPTOME DIVERSITY OF HUMAN LIVER USING NANOPORE SEQUENCING

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Background: Short-read sequencing systems (NGS) are efficient, high-throughput methods routinely used for RNA-Seq. Nonetheless, these show certain drawbacks that could restrict the discovery of novel transcripts. Long-read sequencing (LRS) could improve transcript identification, providing further information regarding transcriptome diversity.

Aims: We explored the potential of nanopore LRS in RNA-Seq studies to define transcriptomic diversity of human liver, focusing on the consequences of this diversity in the hemostatic system, greatly dependent on hepatic synthesis.

Methods: We sequenced liver mRNA of 15 patients who underwent bariatric surgery using MinION with SQK-RNA002 kit. *De novo* assembly was perfomed with FLAIR v1.5.1 and GENCODE v39 as reference. Results were filtered with SQANTI v4.1.3. Hemostatic genes were selected according to ISTH recommendations (https://www.isth.org/page/GinTh_GeneLists). The study of these samples was approved by the Ethics Committee of Virgen de la Arrixaca Clinical Hospital and patients gave their informed consent.

Results: Bioinformatic analysis identified 183 transcripts for 63 hemostatic genes. Interestingly, 55% of these genes showed multiple transcripts, highlighting *FGC*, with 15 isoforms. Half of hemostatic transcripts (N=92) were novel. Most (N=91) arose from new combinations of canonical splice sites, 48 showed intron retention and 43 different combinations of exons. Only 20 would be degraded via Nonsense-Mediated Decay. 114 transcripts had low expression (TPM<10), while 69 (22 new) showed higher expression levels. We highlight a new fusion gene hovering neighboring genes *PRSS53* and *VKORC1*.

Summary/Conclusion: Nanopore mRNA sequencing doubles transcriptional diversity in the hemostatic system compared to conventional NGS methods as it allows identification of new alternative transcripts due to the direct RNA sequencing of long reads. These results strongly support an increased proteomic diversity whose physiological and pathological consequences in key biological systems must be explored.

Poster Session – Platelets

P-065

PLATELET F11R/JAM-A CONTRIBUTES TO PLATELET ADHESION UNDER FLOW CONDITIONS AND TO THROMBUS FORMATION

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Background: F11Receptor/Junctional Adhesion Molecule-A (F11R/JAM-A) is a transmembrane protein, which belongs to the immunoglobulin superfamily of cell adhesion molecules. F11R /JAM-A is expressed in endothelial cells, epithelial cells and in blood platelets. In the endothelium and epithelium it is involved in the formation of intercellular tight junctions. In blood platelets F11R /JAM-A participates in adhesion under static conditions and negatively regulates the activation of the platelet $\alpha_{IIb}\beta_3$ integrin.

Aims: The purpose of presented study was to evaluate whether F11R/ JAM-A is involved in platelet adhesion and thrombus formation under flow conditions.

Methods: Platelet adhesion to recombinant human F11R/JAM-A Fc chimera (dimeric F11R/JAM-A) or HisTag F11R/JAM-A (monomeric) alone and in a combination with fibrinogen was assessed under flow conditions using flow chamber assay. Monoclonal antibodies were used to assess the effects of F11R/JAM-A blockade on platelet aggregation and thrombus formation. *In vitro* thrombus formation and platelet aggregation were assessed using flow chamber assay and total thrombus formation analysis system. Effects of F11R/JAM-A blockade on thrombus formation in vivo was evaluated in two murine models of carotid artery injury: photochemically induced thrombosis, visualized and quantified by intravital microscopy, and in ferric chloride–induced thrombosis, quantified with the use of laser Doppler flowmetry.

Studies were approved by the respective local bioethical committees. Human participants signed informed consents.

Results: Neither dimeric nor monomeric forms of F11R/JAM-A supported human platelet adhesion under flow conditions when used alone. Dimeric F11R/JAM-A co-immobilised with fibrinogen increased the adhesion of blood platelets at 40 dynes/cm^2 (~890 s^{-1}), when compared to fibrinogen alone (943x10^3 [405x10^3 ; 825x10^3] µm^2 vs 502x10^3 [799 x10^3 ; 962 x10^3] µm^2)(median with IQR) (n=12; p<0.01), while monomeric F11R/JAM-A had no such an effect.

Fab fragments of J10.4 monoclonal antibodies, which block F11R/JAM-A cis-dimerisation, delayed (but not inhibited) the formation of occlusive thrombus *in vitro* in human whole blood on a surface coated with collagen and tissue factor (662 [427 ; 1126] s vs 601 [427 ; 698] s)(median with IQR) (n=11; p<0.01). However, they had no effect in primary a hemostasis assay, when thrombin inhibition was applied. Image analysis of thrombi formed in flow chamber system did not reveal significant effects of Fab fragments of blocking antibodies on the sizes of thrombi.

Laser Doppler flowmetry revealed delayed occlusion of carotid artery in ferric chloride–induced thrombosis model in mice treated with F11R/JAM-A blocking antibodies BV11 (30 [29 ; 30] min vs 18 [14 ; 26] min)(median with IQR) (n=7; p<0.01). In turn, in the model of photochemically induced thrombosis, BV11 antibodies did not alter thrombus size assessed by intravital microscopy.

Summary/Conclusion: Platelet F11R/JAM-A plays a role in a formation of occlusive thrombus, but its contribution is not related to platelet-platelet interactions in primary hemostasis. Based on the results, which show its ability to increase platelet adhesion to fibrinogen under flow conditions, it can be speculated that F11R/JAM-A plays a role in a stabilisation of platelet interactions with fibrin under shear stress conditions.

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P-067

SPONTANEOUS PLATELET AGGREGATION ASSESSED BY LIGHT TRANSMITTANCE AGGREGOMETRY: AN ATTEMPT TO ESTABLISH LABORATORY REFERENCE INTERVALS

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Background: Platelet aggregation without agonists is named spontaneous platelet aggregation (SPA). High platelet reactivity leading SPA is a hallmark of cardiovascular diseases. SPA measured in platelet-rich plasma (PRP) is enhanced in patients with diabetes, acute coronary syndrome, myocardial infarction, and cerebrovascular disease. It has also been reported to be a predictive risk marker of arterial occlusions in patients with diabetes and myocardial infarction.

Aims: Our aim in this study was to establish the reference intervals for SPA in our laboratory.

Methods: We used light transmittance aggregometry to measure SPA in two groups of participants. The first group consisted of 92 healthy subjects (53 female, mean age: 42±14 years). The second group consisted of 86 patients with stable coronary artery disease (38 female, mean age 63±17 years) under dual antiplatelet therapy with Clopidogrel 75 mg and Acetylsalicylic acid 100 mg.

The sample collection, the preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) was performed in accordance with the generally acknowledged international methods.

The calculation of a reference interval was done using the robust method (Horn & Pesce, 2005) which can be used as an alternative to the percentile method when sample size is less than 120.

Results: In the group of healthy subjects, mean SPA was 8.7±5%, 95% CI [7.1,10.2], with a minimum of 1% and a maximum of 17%. On the other hand, in the group of patients under dual antiplatelet therapy, mean SPS was 5.8±4.1%, 95% CI [4.6,7.0], with a minimum of 1% and a maximum of 15%.

Levene's test was used to test samples for equality of variances. Analysis (independent samples t-test) showed that SPA in the group of patients under dual antiplatelet therapy was significantly lower than SPA in the group of healthy subjects (p=0.004).

The calculated reference interval, using the robust method in the healthy group was 0-18% whilst in the group of patients under anti-PLT therapy 0-14%.

Summary/Conclusion:

1. SPA is significantly lower in patients receiving anti-PLT therapy.
2. The upper limit of normal range of SPA is higher in healthy individuals than in patients receiving anti-PLT therapy. This may be especially important when evaluating the effectiveness of anti-PLT therapy.

P-068

EXPERIENCE OF USING AVATROMBOPAG IN A TERTIARY HOSPITAL

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Background: Avatrombopag, oral 2nd generation thrombopoietin receptor agonist (TPO-RA) is approved in adults with chronic ITP (> 12 months) who are refractory or have an unsatisfactory response to other treatments and in adults with chronic liver disease (CLD) scheduled for surgery. In Galicia (Spain) it was included in the pharmacotherapeutic guide of the Galician Health Service (SERGAS) Hospitals on 12/20/2022.

Aims: Report the experience with Avatrombopag in our Center, in patients with chronic ITP or CLD with thrombocytopenia prior to invasive procedure.

Methods: Collection of data from the clinical history, retrospectively and prospectively, and record the usual clinical practice, in accordance
with the guidelines in force in our department

Results: From January/23 to 1/5/2023 9 patients with chronic ITP started treatment with AVATROMBOPAG and 1 patient with CLD and thrombocytopenia performed one multiple tooth extraction.

5 patients switch to AVA from previous treatment with another TPO-RA: reasons; 2 -loss of response to the previous agonist, 2- comfort of administration and 1- inability to maintain stable platelet count (50-150000 / ml) with previous agonist. Three patients started Avatrombopag as 2nd line, 2 after corticosteroid therapy failure and 1 relapsed after administration of IV Ig trying to avoid side effects of Corticosteroids by age (96 years) and associated comorbidities (Hypertension, Diabetes)

Initial dose was always 140 milligrams per week (mg/w) with control for adjustment according tecnichal data sheet: In table 1, you could see the controls and dose adjustment at 2 and 4 weeks and the last control. Seven patients achieved a complete response 2 weeks after the start of treatment. The mean platelet count was 115000 per milliliter (SD 68-230000/ml) and the mean dose of Avatrombopag, 142 mg/w (SD 80-220)

The patient with CLD suffers severe AH, without inhibitor, in prophylaxis with pegylated rFVIII-EHL 2 times per week; presents thrombocytopenia around 20000/ml secondary to HCV liver cirrhosis. The CLD protocol was applied by technical data sheet; he received 60 mg / day, 5 days; on the day before the procedure (5 days after the last dose), he presented PLT of 102000/ml, which he maintained 7 days later. Procedure without hemorrhagic intercurrences.

In general, well-tolerated treatment: 1 patient reported mild headache at baseline, which disappeared without analgesia, and another suffered a EP at month +2, with PLT count of 150000/ml, and positive Lupus Anticoagulant, treated without stopping the agonist, with LMWH in therapeutic dose and subsequently, VKA (target INR 2.5). This adverse event (AE) was reported to AEMPS (Spanish Agency of Medicines and Health Products)

Summary/Conclusion: Avatrombopag shows efficacy and well tolerance with adverse events similar to other TPO-RA with no new or single events not previously observed in patients treated with other TPO-RA. Oral administration, non-interference of food or divalent cations in absorption, no need to monitor liver function or its rapidity of action are highly noteworthy characteristics of Avatrombopag, in addition to the

potential use in CLD. It is true that the follow-up period is short, but based on what has been reported in the literature, we hope that the expectations will be confirmed as time passes.

P-069

THE MEAN PLATELET VOLUME VALUES IN NEW INFLAMMATORY INDICES IN COVID-19 PATIENTS

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Background: Platelets have a crucial role in haemostasis, coagulation, innate immunity and inflammatory response. Complete Blood Count (CBC) parameters are used for the calculation of several formulas as inflammation and immunity indices. Thrombocytopenia is common in SARS-CoV-2 infection and platelet count is used for the computation of inflammatory indices, such as platelet-to-lymphocyte ratio (PLR) and Systemic Immune–Inflammation index (SII). PLR has been reported to be a useful prognostic factor for COVID-19 severity and increased values of both PLR and SII are associated with increased mortality in COVID-19 patients. Platelets are activated and hyper-responsive while mean platelet volume (MPV) is associated with disease severity in COVID-19 patients.

Aims: The aim of this study is to create new formulas by replacing the platelet count by the MPV, as large platelets are more active metabolically compared to smaller platelets, and to evaluate their value in COVID-19 patients.

Methods: The demographic data and the laboratory findings upon admission (CBC on Sysmex XN-1000 automated hematology analyzer at the core Hematology Laboratory) of all patients presented to the Emergency Room of our hospital and diagnosed with COVID-19 were retrospectively recorded. Absolute neutrophil count (Neut, $x10^9$/L), absolute lymphocyte count (Lympho, $x10^9$/L), platelet count (PLT, $x10^9$/L) and mean platelet volume (MPV, fL) were incorporated into formulas. The inflammation indices were calculated as followed: PLR = PLT / lympho, SII = neut x PLT / lympho, MPV-LR = MPV / lympho & SII-MPV = neut x MPV / lympho. All study parameters were retrieved from the hospital electronic database system. Patients were divided into two groups: patients discharged and treated as outpatients (group A, N=99) and patients hospitalized in COVID–19 inpatient wards (group B, N=164). Statistical analysis: Data are referred as median and percentiles. The Mann-Whitney test, the Pearson Chi-Square test and the Receiver Operating Characteristic (ROC) curve with Area Under the Curve (AUC) were applied. P value of <0.05 was considered significant for all data analysis.

Results: The two groups do not differ in gender (P=0.287) but inpatients are older [70 (58-80) vs 59 (39-71) years, P=0.000]. The two groups differ in a statistically significant degree in all indices; Group A vs Group B: PLR = 158.33 (108.80 – 221.54) vs 188.35 (130.30 – 254.70) [P=0.039], SII = 526.37 (296.10 – 1072.23 vs 844.20 (488.54 – 1470.69) [P=0.000], MPV-LR = 9.08 (6.22 – 12.56) vs 10.52 (7.66 – 14.93) [P=0.004], SII-MPV = 27.68 (18.83 – 48.50) vs 50.40 (32.27 – 82.06) [P=0.000]. The AUC in discriminating inpatients (consider as the positive actual state) is statistically significant for all indices; PLR = 0.576 (P=0.039), SII = 0.641 (P=0.000), MPV-LR = 0.605 (P=0.004), SII-MPV = 0.685 (P=0.000).

Summary/Conclusion: Patients with COVID-19 who need hospitalization present increased levels of all study inflammation indices. The new in-house formulas (MPV-LR & SII-MPV) where the platelet count in the established indices (PLR & SII) is replaced by mean platelet volume values are well associated with the relevant treatment the patient should receive.

P-071

THE MECHANISMS OF PLATELET ACTIVATION IN INFLAMMATION

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Background: Although the platelets' role in hemostasis is well established, their contribution to the inflammatory response remains poorly understood. This is particularly true for the complex interactions between inflammatory factors and platelet agonists.

Aims: This study aims to determine the effects of inflammatory factors such as different cytokines and hormones on their own and in combination with platelet agonists.

Methods: The platelets from healthy donors are exposed to well-defined agonists, including ADP, PAR1, TRAP, Epinephrine, IL-4, IL-12, and serotonin alone or in combination to achieve a high level of platelet activation in an *in vitro* environment. The resulting changes in platelet activation will be analyzed using flow cytometry to measure various markers.

Results: Low concentrations of different individual agonists did not lead to significant platelet activation according to CD62P expression by activated platelets. PAR1 agonist with 5μM concentration caused 7.3% ±4.2% (P=0.3365) CD62P expression in comparison with Control Tube (3.06% ± 2.3%). 5μM Epinephrine agonist led to 15.8% ± 14.26% CD62P expression by activated platelet in contrast to no-agonist condition (P= 0.4594). The presence of 1μg/ml LPS agonist, displayed a very mild activation of 4.5%± 3.5% in CD62P expression (P=0.9999) in parallel with the absence of agonists test.

However, the combination of two and three agonists illustrates a significant platelet activation level increase and a remarkable synergy. The mixture of 1μg/ml LPS and 5μM PAR1 led to 17.37% ± 5.6% (P=0.3305), 1μg/ml LPS and 5μM Epinephrine caused 31.83% ± 4.4% (P=0.0045) and also, the combination of 5μM Epi and 5μM led to 73% ± 9.8% (P=<0.0001) CD62P expression by activated platelets.

The combination of all named agonists with the same concentrations could reach 66.8% ± 7.8% (P= <0.0001) CD62P expression which is a noticeable platelet activation level.

Summary/Conclusion: In conclusion, the early results from the first phase of the study using *in vitro* condition display that different agonists in combination together lead to a significant activation percentage in the platelet population, however, a similar process should be examined during *in vivo* models that would be evaluated in further study.

P-072

DISTINGUISHING PATHOLOGICAL FROM PHYSIOLOGICAL ACTIVATION OF PLATELETS POTENTIATED BY EXERCISE

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Background: Exercise is widely accepted to exert beneficial effect on human health in prevention and treatment of cardiovascular diseases (CVD). However, a paradox has emerged where exercise has been documented to result in pathological activation of platelets contributing to the development of CVDs.

Aims: This study is aimed at understanding the exact molecular mechanisms that governs the differentiation between pathological and physiological activation of platelets.

Methods: The potential effects of exercise on platelet activation are studied using an *in vitro* model that simulates exercise induced activation of platelets using whole blood samples and low concentrations of agonists (epinephrine, TRAP/thrombin receptor-activating peptide, serotonin). The levels of platelet activation are analysed using flow cytometry method.

Results: The use of the low concentrations of individual agonists did not result in significant platelet activation as shown by the expression of CD62P. The addition of 1 μM TRAP led to 3.0% ±1.1% (P=0.9549) CD62P positive platelets compared to level of expression in the absence of agonists (3.1% ±1.3%). The presence of 10 μM serotonin resulted in 5.2% ±2.4% activated platelets compared to no agonist conditions (P=0.9999). The 5 μM epinephrine led to a modest, non-significant activation (12.1% ±6.9%) compared to no agonist levels (P=0.9519). However, the combination of agonists showed a striking synergy and a significant increase in platelet activation 50.87% ±25.81 (P=0.0044) compared to no agonist levels.

Summary/Conclusion: Preliminary results using *in vitro* model showed that a combination of agonists that are likely to be present during physiological exercise act synergistically to trigger a strong platelet activation. Whether similar process indeed occurs during *in vivo* conditions will be investigated in the future study.