ECTH 2021

14 - 15 October 2021
www.ecth.org

ABSTRACT BOOK

ECTH 2021
European Congress on
Thrombosis and Haemostasis
Ghent, Belgium
14 - 15 October

TABLE OF CONTENT

BSTH 3.1

First report of a de novo iTTP episode associated with an mRNA-based anti-COVID-19 vaccination

S. De Bruijn [1,*], M.-B. Maes [2], L. De Waele [3], K. Vanhoorelbeke [3], A. Gadisseur [1 4]
[1]Department of Hematology, [2]Department of Clinical Biology, Antwerp University Hospital, Antwerp, [3]Laboratory for Thrombosis Research, Interdisciplinary Research Facility Life Sciences, Catholic University of Leuven, Campus Kulak Kortrijk, Kortrijk, [4]Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Antwerp, Belgium

Background: Thrombotic thrombocytopenic purpura (TTP) is a rare but potentially life-threatening thrombotic microangiopathy (TMA) characterized by disseminated thrombus formation in the microvasculature, microangiopathic hemolytic anemia, profound thrombocytopenia and organ failure. Predominantly, TTP is the result of immune-mediated inhibition and/of clearance of the von Willebrand Factor (vWF)-cleaving protease ADAMTS13 (immune-mediated TTP/iTTP), leading to excessive levels of ultra-large vWF multimers. These multimers are responsible for binding and removal of platelets, and ultimately to microthrombosis and thrombocytopenia. Several events, including malignancies, pregnancy, and viral infections can trigger production of anti-ADAMTS13 antibodies. iTTP has also been described after vaccination but the pathophysiology of vaccine-related TMAs is not entirely clear. The time from vaccination to onset of TTP was between 5-15 days in earlier published cases of TTP after administration of both viral and bacterial vaccines.
Aims: NA
Methods: NA
Results: A 38-year-old Caucasian female, without relevant medical history, was referred to the Hematology department because of progressive thrombocytopenia since one week, and spontaneous bruising/petechiae starting 2 weeks after receiving the first dose of the Pfizer-BioNTech mRNA anti-COVID-19 vaccine. She received both doses of the vaccine, respectively 6 and 3 weeks earlier. Further anamnesis revealed blurred vision in the left eye since 2 weeks and ophthalmological examination showed signs of central serous chorioretinopathy, caused by platelet-rich microthrombus formation in the choroid vasculature, as an ocular manifestation related to TTP. Laboratory data revealed a severe ADAMTS13-deficiency with undetectable levels of ADAMTS13 enzyme activity, and a very high autoantibody titer against ADAMTS13 (106 BU), measured by a functional Bethesda method and confirmed by a general ADAMT13 inhibitor ELISA (antibody titer of > 1000 AU/mL). Epitope mapping revealed a “classical” iTTP immunoprofile with only antibodies against the cysteine/spacer (CS) domains of ADAMTS13. Further analysis revealed an open ADAMTS13 conformation (conformation index > 0.5), both spontaneously and after addition of activating anti-CUB1 17G2 antibodies, which is a hallmark of acute iTTP. Anti-COVID-19 antibodies were also very high after vaccination. Antibodies to platelet factor 4-heparin complex were negative, using HIT testing, ruling out vaccine-induced prothrombotic immune thrombocytopenia (VIPIT/VITT). She was successfully treated with plasma exchange, corticosteroids, rituximab and Caplacizumab.
Summary/Conclusion: In this case bruising gradually occurred two weeks after first dose of the Pfizer-BioNTech mRNA anti-COVID-19 vaccine. Full diagnosis of iTTP was confirmed three weeks after second vaccine by the presence of anti-ADAMTS13 antibodies accompanied by the diagnosis of central serous chorioretinopathy. Epitope mapping only showed presence of anti-CS antibodies, and an immunoprofile not different to “classical” iTTP. The causal association was mainly suggested by the fact that no other underlying precipitating causes were present and because of a clear time-correlation. To the best of our knowledge, this is the first published case report of iTTP post mRNA-based COVID-19-vaccination in a previously TTP-naïve patient. However, this is not an unique occurrence as several databases, monitoring adverse events after COVID-19 vaccinations, contain mentions of suspected TTP.

BSTH 3.2

Neutrophils promote the progression of Staphylococcus aureus endocarditis

S. Meyers [1,*], M. Lox [1], S. Kraisin [1], C. Martens [1], L. Liesenborghs [1], L. Frederix [1], T. Vanassche [1], P. Verhamme [1], K. Martinod [1]
[1]Center for molecular and vascular biology, KU Leuven, Leuven, Belgium

Background: The interplay between coagulation and *Staphylococcus aureus* (*S. aureus*) is crucial in infective endocarditis (IE). IE is characterized by a vegetation, or an infected thrombus, containing bacteria, platelets, and fibrin. How initial bacterial adhesion to endothelium progresses towards this complex vegetation, particularly how bacteria bypass the immune system, remains unclear. Neutrophils, via release of neutrophil extracellular traps (NETs), lie at this interface between host defense and thrombosis.
Aims: We aimed to determine the role of neutrophils in *S. aureus* IE progression.
Methods: We injected mice with *S. aureus* i.v. and locally stimulated the endothelium with histamine, resulting in IE lesions on inflamed aortic valves in a subset of mice. After one day, we determined the incidence of IE with Gram staining. We investigated the presence of neutrophils and NETs by staining for Ly6G and for citrullinated histone H3 (H3Cit), extracellular DNA, and myeloperoxidase (MPO), respectively. The area of positive signal of these markers was quantified in mice infected with a methicillin susceptible (Newman), a methicillin resistant (USA300) and a clinical IE strain of *S. aureus*. The ability of these strains to induce NETs was assessed *in vitro* in peripheral blood neutrophils incubated with these various bacterial strains and the degree of NETosis measured. Subsequently, we determined the role of NETs in USA300-induced IE by using neutrophil-specific PAD4 knockout mice ($MRP8Cre^{+}xPAD4^{fl/fl}$). The impairment of NETs formation in these mice was validated by stimulating their isolated neutrophils and counting the number of released NETs. Finally, we depleted neutrophils in mice infected with USA300 or the clinical strain using antibodies against Ly6G followed by an anti-rat κ IgG2a.
Results: Immunostaining revealed the presence of neutrophils and neutrophils undergoing NETosis in inflammation-induced vegetations. More specifically, mice with USA300-induced IE had significantly more MPO (P = 0.03) and H3Cit (P = 0.0005) than mice without a vegetation (MPO: 1.2(0.5-3.4)% vs. 0.3(0.2-0.8)%, H3Cit: 0.2(0.04-1.1)% vs. 0(0-0.0007)%). Similar results were observed for Newman and clinical strains of *S. aureus*. *In vitro*, these strains differentially induced NETs. The clinical strain induced significantly more NETs than either Newman (relative OD 22.0 vs. 9.8, P = 0.04) and USA300 (22.0 vs. 7.4, P = 0.0005) strains. *In vivo* impairment of NET formation was validated in neutrophil-specific PAD4 knockout mice since their neutrophils formed less NETs *in vitro* after stimulation with USA300 than control mice (0.6(0.4-0.9)% vs. 1.8(1.6-2.3)%, P < 0.0001). Prevalence of USA300-induced IE at day one was similar (P = 0.67) between mice with impaired NETosis (2/14) and wild-type controls (4/18). On the other hand, neutrophil-depleted mice infected with USA300 developed significantly more endocarditis (12/23 vs. 4/21, P = 0.03) and had higher levels of bacteremia (log 5.0(3.7-6.1) CFU/ml vs. log 2.5(0-3.5) CFU/ml, P < 0.0001) than wild-type animals. Similar results were observed with a clinical strain.
Summary/Conclusion: Depleting neutrophils in our IE model revealed that neutrophils play a protective role against IE, whereas results with neutrophil-specific PAD4 knockout mice suggest that the microbicidal properties of NETs do not protect from IE.

SFF 1.1

Correlation of calibrated automated thrombogram parameters with clinical phenotype in a large cohort of patients with antithrombin deficiency carrying different SERPINC1 variants

C. Bravo-Perez [1,*], A. Miñano [1], R. Cifuentes [1], B. de la Morena-Barrio [1], V. Vicente [1], J. Corral [1], M. E. de la Morena-Barrio [1]

[1]Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER, Murcia, Spain

Background: Antithrombin deficiency (ATD) is the most severe thrombophilia, but exerts high clinical heterogeneity. The analysis of *SERPINC1* gene may assist prognostic stratification, but it is not available in routine laboratories. Thrombin generation assays (TGA) have been shown to reflect the hypercoagulable state of patients with thrombophilia, and calibrated automated thrombogram (CAT) parameters have been proposed as biomarkers. However, the number of patients with ATD included in these studies is very limited, and genotype-phenotype correlation has not been evaluated.
Aims: We aim to investigate the hypercoagulable phenotype of AT-deficient patients by TGA, and to correlate CAT parameters with different *SERPINC1* genotypes.
Methods: Cohort of 943 cases with ATD (1998-2021) functionally and genetically characterized. We selected patients whose defect in *SERPINC1* was present in >5 unrelated cases. Patients under anticoagulant treatment or with other thrombophilic alterations were ruled out. Thrombin generation in citrated plasma was evaluated with a fluorogenic method (Thrombinoscope®, Stago) using tissue factor (1 pM), with and without the AT cofactor, heparin, added *ex vivo*. CAT results were correlated with the risk of thrombosis and heparin resistance associated to the *SERPINC1* genotype, according to data from our cohort and/or from the literature.
Results: We selected 113 patients: 15 with type I deficiency, 26 with type II/RS deficiency (p.Ala416Ser [AT Cambridge], N=19; p.Arg425His [AT Glasgow], N=7) and 72 with type II/HBS deficiency (p.Arg45Trp, N=8; p.Pro73Leu [AT Basel], N=15; p.Arg79Cys [AT Toyama], N=21; p.Phe131Leu [AT Budapest 3], N=28).
Compared with healthy controls (N=20), patients with ATD had significantly higher endogenous thrombin potential (ETP: 1581 vs. 1336 nM_min, P=0.025). Analysis by type of deficiency and variant revealed that the differences were more elevated for the more severe cases, such as type I (ETP: 2606 nM_min, P <0.001), type II/RS Glasgow (ETP: 2753, P<0.001) and type II/HBS Budapest variants (ETP: 2065 nM_min, P=0.003); whereas no significant differences were detected for the rest of the type II variants, which were milder and more difficult to detect by anti-FXa methods.
Heparin added *ex vivo* caused a significant reduction in all CAT parameters (P< 0.001). The most sensitive CAT parameter among the different variants was the slope of the curve (VelIndex), so that its relative reduction (RR) was significantly lower in cases with mutations supposed to cause heparin resistance than in those without (P=0.03). The capacity of a regression model based on baseline ETP and heparin-induced RR of VelIndex was high to: 1) differentiate between cases and controls (AUC: 0.74, 95% CI 0.36-0.86, P<0.0001), 2) predict the odds ratio for thrombosis associated to *SERPINC1* genotype in a linear regression model (R^2=0.40, P<0.0001) and 3) predict heparin resistance (AUC: 0.71, 95%CI 0.61-0.81, P<0.0001).
Summary/Conclusion: We have performed the largest TGA-based analysis on ATD. CAT parameters reflect the hypercoagulable state of AT-deficient patients. Baseline ETP shows a good genotype-phenotype correlation. Besides, heparin added *ex vivo* reflects heparin resistance. Further efforts should focus on the standardization of the technique and on the prospective validation of CAT parameters as biomarkers of clinical utility.
Funding: ISCIII&FEDER: PI18/00598; CM20/00094. Fundación Séneca 19873/GERM/15

SFF 1.2

Inhibition of ADAMTS13 Rescues Acquired von Willebrand Syndrome in a Preclinical Left Ventricular Assist Device Animal Model

S. J. Deconinck [1,*], C. Nix [2], S. Barth [2], E. Bennek-Schöpping [2], A.-S. Schelpe [1], E. Roose [1], I. Pareyn [1], A. Vandenbulcke [1], J. Muia [3], C. Vandenbriele [4], B. Meyns [5], C. Tersteeg [1], S. Jacobs [5], S. F. De Meyer [1], K. Vanhoorelbeke [1]
[1]Laboratory for Thrombosis Research, KU Leuven Campus Kulak Kortrijk, Kortrijk, Belgium, [2]Abiomed Europe GmbH, Aachen, Germany, [3]Department of Biochemistry and Microbiology, Oklahoma State University Center for Health Sciences, Tulsa, United States, [4]Department of Cardiovascular Diseases, [5]Department of Cardiac Surgery, University Hospitals Leuven, Leuven, Belgium

Background: Bleeding is the most 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) by ADAMTS13, leading to aVWS. We previously showed that an inhibitory monoclonal antibody against ADAMTS13 prevented the loss of high molecular weight (HMW) VWF multimers in in vitro LVAD experiments using human blood. The efficacy of this drug in a preclinical animal model has not been investigated yet.
Aims: To investigate if blocking ADAMTS13 using our in-house developed inhibitory anti-ADAMTS13 monoclonal antibody (mAb) 17C7 rescues aVWS in a preclinical calf model.
Methods: Bovine blood was circulated through an in vitro Impella system (n=3) with either mAb 17C7 (20 µg/mL) or PBS. VWF multimers and function (collagen binding activity) were determined in plasma samples obtained before and 5, 30, 60, 180 and 300 minutes after the start of perfusion. Next, Impella pumps were implanted in calves (n=4) and 7 days after Impella implantation, the calves were injected with PBS. Subsequently, 7 days after PBS injection, the same calves were treated with one dose (600 µg/kg) of mAb 17C7. Blood was sampled before pump implantation and at 2, 24, 48, 72, and 168 hours after pump implantation and PBS/mAb 17C7 injection. VWF, ADAMTS13 and blood parameters were determined.
Results: Perfusion of bovine blood supplemented with PBS through the in vitro system resulted in a decrease of HMW VWF multimers (54±3% before vs 30±4%, 300 min after perfusion (p=0.005)) and VWF function (0.98±0.13 before vs 0.42±0.18, 300 min after perfusion (p=0.01)). In contrast, blocking bovine ADAMTS13 using mAb 17C7 prevented the loss of HMW VWF multimers (55±4% before vs 50±9%, 300 min after perfusion (p=0.40)) and VWF function in the in vitro system (0.89 ± 0.09 vs 0.87±0.15, 300 min after perfusion (p>0.99)). Implantation of LVADs in calves resulted in a decrease of HMW VWF multimers (51±4% before pump implantation vs 26±8%, 72 h after pump implantation (p=0.003)) and VWF function (VWF:CB/VWF:Ag ratio was 0.80±0.09 at 72 h after pump implantation (p=0.04)). Treatment with PBS had no effect (HMW VWF multimers decreased from 51±4% before pump implantation to 32±3%, 72 h after PBS injection (p=0.03) and VWF:CB/VWF:Ag ratio was 0.83 ± 0.06, 72 h after PBS injection (p=0.04)). Interestingly, ADAMTS13 inhibition using mAb 17C7 could rescue the loss of HMW VWF multimers (51±4% before pump implantation vs 42±5%, 2 h after mAb injection (p>0.99)) and VWF function (0.93±0.09, 2 h after mAb injection (p>0.99)). Importantly, blocking ADAMTS13 in calves did not lead to severe thrombocytopenia nor hemolytic anemia.
Summary/Conclusion: Blocking ADAMTS13 rescues aVWS in a preclinical calf model and could become a promising therapy to treat aVWS-induced bleeding in LVAD patients.

SFF 1.3

S100A8/A9 levels are increased in COVID-19 patients and induce procoagulant platelets in a GPIbα-dependent manner

M. Colicchia [1,*], W. Schrottmaier [2], J. H. Bourne [1], N. Beristain-Covarrubias [1], P. L. Nicholson [1], P. Murray [3], G. Taylor [3], M. Pugh [3], A. Iqbal [1], G. E. Rainger [1], S. P. Watson [1], M. R. Thomas [1], A. Assinger [2], J. Rayes [1]
[1]Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, Vincent Drive, B15 2TT, Birmingham, U.K., University of Birmingham, Birmingham, United Kingdom, [2]Center for Physiology and Pharmacology, Medical University of Vienna, University of Vienna, Vienna, Austria, [3]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, B15 2TT, U.K., University of Birmingham, Birmingham, United Kingdom

Background: Activated and procoagulant platelets are observed in COVID-19 but the mechanism is yet to be described. S100 A8/A9, a biomarker of immune cell activation, is increased in plasma of COVID-19 patients and its levels correlate with tissue damage severity and cytokine release. S100 A8/A9 is also elevated in myocardial infarction and its high concentrations predispose to late stent thrombosis, sustained myocardial inflammation and heart failure. We hypothesise that S100A8/A9 is not only a marker of inflammation but also acts as an extracellular DAMP regulating platelet function.
Aims: To investigate the concentration levels and distribution of S100A8/A9 in plasma and lung autopsies of COVID-19 patients and its effect on platelet function.
Methods: S100A8/A9 plasma levels were measured by ELISA in 87 COVID-19 patients (uncomplicated and ICU-admitted) over the first 7 days of hospitalisation. S100A8/A9 and platelets in lung sections from fatale COVID-19 patients were visualised by immunofluorescence. To assess the effect of S100A8/A9 on platelet function, human and mice platelets were stimulated *ex vivo* with recombinant human and mouse S100A8/A9. P-selectin, GPIIbIIa activation and phosphatidylserine exposure were assessed by flow cytometry. Platelet aggregation was performed using light transmission aggregometry.
Results: S100 A8/A9 is detected at the time of hospital admission in all COVID-19 patients (13µg/ml±10 µg/ml) and sustained high levels on day 5-7 associate with clinical severity. S100 A8/A9-positive cells are observed in the lung parenchyma of COVID-19 patients with and without thrombotic complications. A proportion of these cells are positive for platelets ($CD42b^+$). S100A8/A9 (20 and 40µg/ml) significantly induces P-selectin expression and GPIIbIIIa activation but not platelet aggregation on human platelets. S100A8/A9 causes a significant increase in phosphatidylserine exposure suggesting the formation of pro-coagulant platelets. Targeting CD36, one of the known receptors for S100 A8/A9, decreases platelet activation but not phosphatidylserine exposure on platelets. P-selectin exposure, but not GPIIbIIIa activation and phosphatidylserine exposure, is partially reduced by Src, Syk or Btk inhibitors. Using mouse platelets, we identified GPIbα as a novel specific receptor for S100A8/A9 on platelets mediating P-selectin and phosphatidylserine exposure as well as GPIIbIIIa activation. Recombinant GPIbα completely inhibits P-selectin, phosphatidylserine exposure and GPIIbIIIa activation on human platelets. Finally, we confirm that GPIbα is the only platelet receptor for S100A8/A9 using platelets from a rare patient with Bernard-Soulier Syndrome (GPIb-IX-V deficiency) which failed to respond to S100A8/A9.
Summary/Conclusion: Here we show for the first time that S100A8/A9 is not only a marker of inflammation but also acts as an extracellular DAMP inducing platelet activation and procoagulant platelets through one single receptor, GPIbα. We propose that GPIbα could be a target to reduce platelet activation and procoagulant platelets in COVID-19 patients. Furthermore, these data open the possibility to target GPIbα in multiple chronic inflammatory diseases where this alarmin is increased and contributes to thrombotic complications and/or the development of cardiovascular disease. This work was supported by the Wellcome Trust (204951), British Heart Foundation (FS/IBSRF/20/25039) and Medical Research Council (grant number MC_PC_19029).

SFF 1.4

Protease Nexin-1, a serpine participating in neutrophil recruitment through CD11a integrin regulation.

C. Madjene*, M.-C. Bouton [1], L. Venisse [1], K. Aymonnier [1], Y. Boulaftali [1], V. Arocas [1]
[1]Inserm U1148, Paris, France

Background: Inflammation and coagulation are two closely linked processes essential in the defense of the body. Thrombin, a key serine protease in the coagulation cascade, is also involved in the inflammatory reaction through its ability to stimulate inflammatory cells. The activity of thrombin is mainly regulated by proteins belonging to the serpin superfamily (SERine Protease inhibitor). Among these serpins, Protease Nexin-1 (PN-1) or SerpinE2 is the most effective tissue thrombin inhibitor. Although its potential role in regulating inflammation processes is not yet known, PN-1 has been detected in monocytes/macrophages and its expression has been shown to be up-regulated under pathological conditions such as pulmonary fibrosis and rheumatoid arthritis.
Aims: Our aim is to understand how PN-1 in neutrophils affects their functions.
Methods: We demonstrated the localisation of neutrophil PN-1 by immunocytochemistry. We studied, by western Blot, the expression of PN-1 in resting neutrophils and in neutrophils stimulated by LPS. We used in vivo models of peritonitis induced by LPS and Thioglycollate. We studied by intravital microscopy the recruitment of neutrophils induced by topical application of LTB4 on mesenteric veins. We analysed by flow cytometry, the expression of main integrins and neutrophil receptors.
Results: We demonstrated by immunocytochemistry studies of resting human neutrophils the presence of PN-1 within specific granules, gelatinases granules and endosomes, but not in azurophilic and secretory granules. We showed by western blot that PN-1 is secreted by neutrophils after stimulation with LPS. We have also quantified the production of reactive oxygen species (ROS) by murine neutrophils and observed a significantly reduced ROS generation in PN-1-deficient neutrophils compared to WT neutrophils. In vivo models of peritonitis induced by LPS or thioglycollate showed a decreased recruitment of PN-1-deficient neutrophils in the intraperitoneal lavages by 30% and 60% respectively, compared with WT neutrophils. We have demonstrated by intravital microscopy, that the recruitment of neutrophils induced by topical application of LTB4 on mesenteric veins, is less important in PN-1-KO mice compared to WT mice. Since PN1 is also expressed by platelets, we compared neutrophil recruitment in PF4-CRE + PN-1 Flox / Flox mice with the one observed in LY6G CRE + PN-1 Flox / Flox mice. No significant modification of neutrophil vascular recruitment was observed in mice exhibiting PN-1-deficient platelets and WT neutrophils, whereas a significant decreased neutrophil recruitment was observed in mice exhibiting PN-1-deficient neutrophils and WT platelets. Using flow cytometry, we studied the expression of the main integrins and receptors present in neutrophils. We observed a 2-fold lower Mean Fluorescence Intensity (MFI) for the integrin CD11a on neutrophils from PN-1-deficient mice compared with neutrophils from WT mice. Such a deficit may explain the reduced neutrophil recruitment observed on PN-1-KO mice.
Summary/Conclusion: Our results demonstrated that neutrophil PN-1 promotes neutrophil recruitment and activity and could play an important role in the inflammatory reaction.

OP1.1

Selective serotonin reuptake inhibitor use is associated with major bleeding during treatment with vitamin K antagonists: results of a cohort study

L. Burggraaf [1,*], S. Bakker [2], M. Kruip [3 4], F. van der Meer [5 6], W. Lijfering [1], N. van Rein [1 2]
[1]Department of Clinical Epidemiology, [2]Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, [3]Department of Hematology, Erasmus University Medical Center, [4]Star-shl Thrombosis service, Rotterdam, [5]Department of Thrombosis and Hemostasis, Leiden University Medical Center, [6]Anticoagulation Clinic Leiden, Leiden, Netherlands

Background: Selective serotonin reuptake inhibitors (SSRIs) may increase the risk of major bleeding during vitamin K antagonist (VKA) treatment by decreasing platelet activation, increasing gastric acid secretion and decreasing VKA metabolism through cytochrome P450 2C9 (CYP2C9) inhibition.
Aims: To determine whether SSRIs cause major bleeding during VKA treatment and investigate the mechanisms behind this interaction.
Methods: Information on SSRI use and bleeding complications was obtained from patient records at the Anticoagulation Clinics of Leiden and Rotterdam of VKA initiators between 2006 and 2018.To study the metabolism, we estimated the effect of SSRIs on a high INR (≥ 5) within 2 months after SSRI initiation was estimated using conditional logistic regression with up to five non-SSRI users matched to one SSRI initiator. Furthermore, we compared VKA dosage two months after SSRI initiation with before the start using a paired t-test. To study the platelet and metabolism effect, we estimated the effect of SSRIs (all, CYP2C9 inhibitors, non-inhibitors) on major bleeding using time-dependent Cox regression. In order to identify potential confounding by indication, we performed a sensitivity analyses including tricyclic antidepressants users as a negative control. Participant consent was waived because the analysis used pre-existing, coded data.
Results: 58,918 patients were included, of whom 1504 were SSRI users and 395 were tricyclic antidepressant users. SSRI initiation versus non-use was associated with a 2.41-fold (95% confidence interval [CI] 2.01-2.89) increased risk of a high INR, which was 3.14-fold (95%CI 1.33-7.43) among CYP2C9 inhibiting SSRIs and 2.30-fold (95%CI 1.70-3.12) for tricyclic antidepressant initiation. After SSRI initiation VKA dosage decreased with a mean difference of -3.4% (95%CI -4.5 to -2.3), which was -8.6% (95%CI -4.5 to -1.2) when restricting to CYP2C9 inhibiting SSRIs. For tricyclic antidepressant initiators the mean dosage difference was around zero. SSRI use versus non-use was associated with a 1.22-fold (95% CI 0.99-1.50) increased risk for major bleeding in all SSRI users, which was 1.31-fold (95%CI 0.62-2.72) in CYP2C9 inhibiting SSRIs compared to non-users. The risk estimate of major bleeding for tricyclic antidepressant users as compared to non-users was around unity (HR 1.01, 95%CI 0.66-1.53).
Summary/Conclusion: SSRIs are associated with an increased risk of high INR (≥ 5), decrease in VKA dosage and increased risk of major bleeding. These risks were slightly more elevated for CYP2C9 inhibiting SSRI users, suggesting that this was due to a pharmacokinetic interaction (by CYP2C9 inhibition) as well as the effect of SSRIs on platelet activation. Sensitivity analysis showed that tricyclic antidepressant initiation was associated with an increased risk of high INR, but not with change in VKA dosage or increased risk of major bleeding. Therefore, we would suggest to intensify monitoring of INR shortly after SSRI and tricyclic antidepressants initiation and to prefer non-CYP2C9 inhibiting SSRIs for patients already using a VKA.

OP1.2

Critical role of fibrin gamma-chain crosslinking by FXIIIa in preventing thrombus fragmentation and subsequent embolisation

C. Duval*, A. Baranauskas [1], T. Feller [1], M. Ali [1], L. Cheah [1], N. Yuldasheva [1], S. Baker [2], H. McPherson [1], Z. Raslan [1], M. Bailey [1], S. Connell [3], R. Ajjan [1], H. Philippou [1], K. Naseem [1], V. Ridger [4], R. Ariëns [1]
[1]Leeds Institute of Cardiovascular And Metabolic Medicine, University of Leeds, Leeds, United Kingdom, [2]Department of Physics, Wake Forest University, Wake Forest, United States, [3]School of Physics and Astronomy, University of Leeds, Leeds, [4]Department of Infection, Immunity & Cardiovascular Disease, University of Sheffield, Sheffield, United Kingdom

Background: Activated factor XIII (FXIIIa) crosslinks fibrin α- and γ-chains to increase resistance of the clot to mechanical strain. The role of fibrin clot mechanical properties during thrombosis and thromboembolism *in-vivo* is unknown. We have developed a new genetically modified mouse model with mutations in the γ-chains that abolish fibrin γ-γ crosslinking to test their role in thromboembolism.
Aims: To investigate the role of fibrin γ-γ crosslinking in clot stability and thromboembolism *in-vivo*.
Methods: Genetically modified FGG3X mice were designed and produced by mutating the conserved fibrinogen γ-chain crosslinking residues (γQ398N/399N/K406R). Clot formation and stability were analysed *ex-vivo* (ROTEM) and *in-vivo* (intravital microscopy, using a $FeCl_3$ femoral vein injury model). Platelet function was analysed by flow cytometry and aggregometry, whilst single fibrin fibre mechanical properties were measured using lateral atomic force microscopy single fibre pulling. A new *in-vivo* model for pulmonary embolism was developed based on $FeCl_3$ injury to the inferior vena cava of mice, with visualisation of embolisation to the lungs by live optical (fluorescence) imaging over 24hrs, and *ex-vivo* quantification of the fluorescent pulmonary emboli by light-sheet microscopy.
Results: Compared to WT (C57BL/6), FGG3X mice showed no differences in growth, blood cell counts, fibrinogen concentration, FXIII activity and tail bleeding time, but were unable to form γ-γ crosslinks whilst α-chain crosslinking was unaffected. ROTEM analysis of whole blood showed that clotting and lysis times were not significantly different. However, maximum clot firmness was significantly lower in FGG3X mice compared to WT (40.6±1.1 vs 64.6±1.0 mm; n=8, $p<0.001$), indicating a 37% reduction in clot elastic modulus. Platelet function was unchanged between both strains, and addition of Tirofiban did not alter platelet aggregation, indicating that the γ-chain mutations near the $\alpha_{IIb}\beta_3$ binding site do not alter binding of FGG3X fibrinogen to platelets. Intravital microscopy showed clots in the femoral veins of FGG3X mice exhibited a significantly larger number of breakdown events, compared to WT, during thrombosis (2.1±0.1 vs 1.0±0.3 events/mouse; n=8, $p<0.01$). Furthermore, live optical imaging at various timepoints over 24hrs showed that clots in the inferior vena cava of FGG3X mice consistently and significantly embolised more to the lungs (average 1.5-fold increase, $p<0.05$) compared to WT (n=6). This was confirmed by *ex-vivo* light-sheet microscopy analysis of lungs extracted after sacrifice, showing a 33% increase in lung emboli count for FGG3X mice compared to WT (1924±115 vs 1288±49 emboli; n=8, $p<0.001$). These data were supported *in-vitro* by single fibre pulling experiments showing that upon FXIIIa crosslinking FGG3X fibrin fibres exhibited significantly reduced strain stiffening (4.1±0.4 vs 6.2±0.4, $p<0.0001$), maximum stress before rupture (2.6±0.4 vs 5.9±0.8 MPa, $p<0.001$) and toughness (2.8±0.4 vs 6.0±0.9 MPa, $p<0.01$), compared to WT (n=20).
Summary/Conclusion: Our findings point to a central previously unrecognized role for fibrin γ-chain crosslinking in clot stability, increasing clot stability and decreasing embolism by reducing fragmentation potential. They also indirectly indicate novel mechanistic targets for prevention of thrombosis through selective modulation of fibrin α-chain, but not γ-chain, crosslinking to reduce thrombus size and burden, while maintaining clot stability and preventing embolism.

OP1.3

Platelet-specific acetyl-CoA carboxylase 1 deletion decreases phospholipid content and impairs platelet functions

M. Octave [1,*], L. Pirotton [1], A. Ginion [1], V. Robaux [1], S. Lepropre [1], S. Kautbally [1], Y. Senis [2], Z. Nagy [3], J. Ambroise [4], B. Guigas [5], M. Giera [5], L. Bertrand [1], C. Beauloye [1], S. Horman [1]
[1]Institute of experimental and clinical research, Pôle de Recherche Cardiovasculaire, UCLouvain, Brussels, Belgium, [2]Institut National de la Santé et de la Recherche Médicale (INSERM), Etablissement Français du Sang Grand Est, Unité Mixte de Recherche (UMR)-S 1255, Fédération de Médecine Translationnelle de Strasbourg, Université de Strasbourg, Strasbourg, France, [3]Institute of Experimental Biomedicine, University Hospital and Rudolf Virchow Center, University of Würzburg, Würzburg, Germany, [4]Institute of experimental and clinical research, Centre de technologies moléculaires appliquées, UCLouvain, Brussels, Belgium, [5]Leiden university medical center, Leiden, Netherlands

Background: Acetyl-CoA carboxylase (ACC) catalyzes the first step of de novo lipogenesis (DNL). Pharmacologic inhibition of ACC has been of interest for therapeutic intervention in a wide range of diseases, including cardiovascular diseases and cancers. However, the impact of its inhibition on hemostasis remains under-investigated.
Aims: We previously demonstrated that ACC1 activation promotes thrombus formation by increasing platelet phospholipid content and thromboxane A2 generation. Here, we sought to evaluate the impact of its platelet-specific deletion on platelet lipid content and functions.
Methods: We generated a new Cre transgenic mouse strain that allows megakaryocyte/platelet specific ACC1 deletion (GpIbCre+/- x ACC1 flx/flx mouse). *In vitro*, platelet functions were assessed by aggregometry and flow cytometry. Lipidomics analysis was carried out on the commercial Lipidyzer platform. Thromboxane A2 secretion was evaluated by ELISA.
Results: As expected, ACC1 deletion was restricted to the megakaryocytic lineage. Hematological parameters in platelet-specific ACC1 knockout mice (ACC1 pKO) showed a decrease in platelet count by 30% and an increase in platelet volume by 31%, compared to ACC1 floxed platelets. *In vitro*, ACC1 pKO platelets displayed an impaired thrombin and CRP-induced platelet aggregation, associated with reduced dense granules secretion. In contrast, ADP-induced platelet aggregation was higher in absence of ACC1. In agreement with our previous studies, lipidomics analyses showed that ACC1 deletion in platelets was associated with a significant decrease in arachidonic acid-containing phosphatidylethanolamine plasmalogen, and subsequently with a reduced production of thromboxane A2 upon thrombin or CRP stimulation.
Summary/Conclusion: Together, these findings indicate that ACC1 inhibition affects platelet lipidome with consequences on platelet formation and functions. This suggests clinical implications for DNL inhibitors as a new class of therapeutics.

OP1.4

TLR3 promotes venous thrombosis through endothelial cell activation

M. Najem [1,*], R. Rys [2], V. Gourdou-Latyszenok [1], F. Couturaud [1], M. Blostein [2], C. Lemarie [1]
[1]UFR Medecine, EA3878, GETBO,, Brest, France, [2]Lady Davis Institute, Montreal, Canada

Background: Venous thromboembolism (VTE), encompassing both pulmonary embolism (PE) and deep vein thrombosis (DVT), is the third leading cause of morbidity and mortality among cardiovascular diseases. Coagulation and inflammation are separate physiological processes yet an intense interconnection between these mechanisms has been recognized in VTE over the past decade. Toll-like receptors (TLRs) are key components of the innate immune system that sense endogenous and exogenous danger signals. Extracellular RNA (eRNA) released by damaged cells seem to exert procoagulant activities but mechanisms during venous thrombosis have not been elucidated.
Aims: Characterizing the effects of eRNA and TLR3 on endothelial cells during venous thrombosis.
Methods: The FeCl3 model was used to induce venous thrombosis in WT and TLR3 deficient (-/-) mice. A specific fluorescent probe for RNA (Syto RNA Select), RNase1, poly (I:C), RNA extracted from murine endothelial cells (eRNA) were injected to mice. Vascular permeability was investigated through immunofluorescence technique in HUVECs treated with poly (I:C) or eRNA in the presence of TLR3 antagonist. Gene expression and signaling pathway activation were analyzed in HEK 293 cells overexpressing TLR3 by using western blot and real-time quantitative PCR. Plasma clot formation was measured after incubating HUVECs treated with poly (I:C) or eRNA with human plasma.
Results: We found that thrombosis exacerbated RNA release in vivo and increased RNA content within the thrombus. RNase1 treatment reduced thrombus size compared to control mice. In contrast, eRNA and poly (I:C) treatments increased thrombus size in WT mice but not in TLR3-/- mice, by bolstering neutrophil recruitment and NETosis. In vitro, eRNA stimulated CXCL5 secretion and VE-cadherin downregulation in WT but not in TLR3 deficient endothelial cells. In addition, eRNA triggered plasma clot formation potentially by reducing thrombomodulin mRNA expression. eRNA mediate these effects through TLR3-dependent activation of NFkB.
Summary/Conclusion: These results suggest that eRNA/TLR3 axis contributes to venous thrombosis by enhancing neutrophil recruitment and coagulation.

OP1.5

Exploring new candidate mechanisms behind antithrombin deficiency in cases with unknown molecular basis: mosaicism and intronic profiles

P. Garrido-Rodríguez [1,*], B. de la Morena-Barrio [1], R. Cifuentes [1], J. Padilla [1], C. Bravo-Pérez [1], A. Miñano [1], V. Vicente [1], M. E. de la Morena-Barrio [1], J. Corral [1]
[1]Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER, Murcia, Spain

Background: The molecular base of a number of cases with genetic diseases cannot be solved following routine molecular methods. In antithrombin deficiency, these could account for up to 20% of patients after sequencing the gene (*SERPINC1*), evaluating gross gene defects by MLPA, and analysis of glycosylation. The search of the molecular basis responsible for these cases may benefit from the consideration of new mechanisms and the use of new technology.
Aims: Our objective was to evaluate mosaicism and intronic profiles of variants as candidate novel mechanisms for antithrombin deficiency of unknown cause.
Methods: Deep, Next Generation Sequencing with short (2500x) and long reads (130x) of *SERPINC1* in 26 cases with antithrombin deficiency, selected from a cohort of 403 non-related cases because of unknown molecular base by routine methods (Sanger sequencing of exons and flanking regions, MLPA and glycosylation analysis). Molecular study was conducted using DNA from peripheral blood cells. Detection of mosaicism and intronic profiles was performed running a custom bioinformatic pipeline. We used pathogenicity prediction tools (HSF, regSNPintron, MutationTaster, PolyPhen2). Genotyping of selected variants in 305 healthy controls was done using taqman probes.
Results: Most of the cases (86%) showed moderate deficiency (Anti-FXa activity 70-80%). For evaluation of mosaicism, variants in exons and adjacent positions (+/- 20bp) present in, at least, 5% of reads were selected for further inspection. We identified an average of 9 variants per patient. We discarded indel variants as they were considered artifacts (Song et al. Sci Rep 2017). Another two were synonymous, germline variants (c.981A>G and c.1011A>G). A missense (c.100G>C, p.Gly34Arg) as well as an intronic (c.409-12T>G) variant, both showing low frequency (MAF < 0.0001), were discarded as potential mosaics due to their low depth (<15% of reads), for being reported in 10 or more cases of our study and because of their location in homopolymeric regions.
On the other hand, our search of germline intronic profiles reported 2 haplotypes potentially associated to mild antithrombin deficiency identified in 20 patients and showed a prevalence 4 times higher than expected according to MAF (p=0.018). Each haplotype was determined by two linked SNPs (r2>0.91) located on the same allele, as proved by nanopore sequencing:
1. c.41+141G>A (intron 1, MAF 0.1024) & c.41+645G>A (intron 1, MAF 0.1036): 10 carriers; mean Anti-FXa activity: 78%. c.41+141G>A was associated with lower antithrombin levels in controls (Anti-FXa:95%; p=0.037). Intron 1 accommodates regulatory elements that could be affected by these variants. Moreover, c.41+645G>A could produce a novel cryptic donor site, according to HSF.
2. c.408+174T>A (intron 2, MAF 0.08581) & c.624+185G>T (intron 3, MAF 0.08613): 10 carriers, mean Anti-FXa activity: 77%. A patient with this profile, carrier of an additional mutation on intron 4 (c.762+329G>T) showed a more severe deficiency.
Summary/Conclusion: Our study discards mosaicism as candidate mechanism for antithrombin deficiency in cases with unknown molecular basis. Nonetheless, we identified two profiles of intronic variants that could cause a moderate reduction in antithrombin levels.
Funding: ISCIII&FEDER-PI18/00598; Fundación Séneca-19873/GERM/15; ISCIII- CM20/00094;FI19/00048.

OP2.1

A hemostatic chemical compound against bleedings induced by direct oral anticoagulants.

M. Desvages [1 2,*], D. Desmaële [3], F. Adam [1], G. Tu [1 3], S. Jaouen [1], C. Repérant [1], D. Borgel [1 2], E. P. Bianchini [1]
[1]UMR_S1176, Inserm-Université Paris Saclay, Le kremlin Bicetre, [2]Laboratoire d'Hématologie Biologique, Hôpital Necker, AP-HP, Paris, [3]Institut Galien Paris-Saclay, UMR 8612, CNRS-Université Paris-Saclay, Châtenay-Malabry, France

Background: Bleeding risk associated with direct oral anticoagulants (DOAC) remains a major concern and rapid reversion of anticoagulant activity may be required to stop bleeding or prior urgent surgery. Although specific biotherapies are available as antidote to DOAC, here we propose a hemostatic chemical compound, easy to produce, to store, to administer, and cost-effective as reversal strategy for DOAC-induced bleeding.
Aims: A chemical molecule, named NaGe5, was identified from the screening of a chemical library for its procoagulant properties. The purpose of this study is to characterize and assess the efficacy of NaGe5 as a hemostatic agent to reverse DOAC anticoagulant activity in human plasma and in-vivo.
Methods: The hemostatic properties of NaGe5 were evaluated in a thrombin generation assay (TGA, Thrombinoscope™, Stago), using PPP-Reagent low, and in a thromboelastography assay (TEG, Heamonetics), triggered with kaolin. Global coagulation assays were performed in normal human plasma spiked with DOAC (apixaban, rivaroxaban, or dabigatran). To address the efficacy of NaGe5 to reverse DOAC anticoagulant activity in-vivo, wild-type mice (C57Bl/6) were treated with apixaban (17.4 µmol/kg) and 30 minutes later with NaGe5 (117 µmol/kg), by intravenous injection. Bleeding time and volume of blood loss (during 20 minutes) were recorded after a tail clip procedure.
Results: In TGAs, NaGe5 dose-dependently promoted coagulation in plasma, resulting in shortened time-dependent parameters (lag time and time to peak) and increased quantitative parameters (thrombin peak and endogenous thrombin potential (ETP)). In plasma spiked with apixaban (435 nM), NaGe5 (6.25 µM) restored altered thrombin generation, as evidence by its ability to reduce the lag time (from 163 ± 5% to 63 ± 8% of its normal value measured in the absence of DOAC and NaGe5) and to enhance the ETP (from 53 ± 18% to 125 ± 11% of its normal value). Similarly, NaGe5 also rescued thrombin generation inhibited by addition of rivaroxaban or dabigatran as assessed in TGAs. The reversal activity of NaGe5 was also evidenced in TEG assays, since NaGe5 (50 µM) corrected both R- and K-time elongated by apixaban (435 nM) from 157 ± 24% to 105 ± 17%, and from 186 ± 74% to 86 ± 19% of their normal values, respectively. NaGe5 was also found efficient in reversing apixaban-induced bleeding in-vivo. In mice treated with apixaban (n = 12), bleeding time (974 ± 290 s) and blood loss (708 ± 587 µL) were significantly increased compared to the control group (n = 10, bleeding time = 240 ± 342 s and blood loss = 43 ± 80 µL). In mice treated with apixaban and NaGe5 (n = 10), both parameters were significantly corrected (bleeding time = 507 ± 371 s and blood loss = 65 ± 104 µL) to reach values comparable to that measured in the control group, while administration of a NaGe5 analog devoid of procoagulant activity had no significant effect on apixaban-induced bleeding phenotype.
Summary/Conclusion: NaGe5 is a small chemical compound that exhibits procoagulant properties able to counterbalance hemostatic equilibrium disrupted by thrombin inhibitor (dabigatran) or FXa inhibitors (apixaban and rivaroxaban). Its efficacy in promoting clot formation, associated with its versatility, make it a promising therapeutic alternative in the management of the hemorrhagic risk inherent to DOAC therapies.

OP2.2

Presence and evolution of NET markers and DAMPS in critically ill COVID-19 patients

J. Huckriede [1,*], S. Bülow Anderberg [2], A. Morales [3], F. de Vries [1], M. Hultström [2 4], A. Bergqvist [5], J. T. Ortiz [6], J. W. Sels [7 8], K. Wichapong [1], M. Lipcsey [2], M. van de Poll [7 9], A. Larsson [10], T. Luther [11], C. Reutelingsperger [1], P. Garcia de Frutos [3], R. Frithiof [2], G. Nicolaes [1]
[1]Department of Biochemistry, Maastricht University, Maastricht, Netherlands, [2]Department of Surgical Sciences, Uppsala University, Uppsala, Sweden, [3]Department of Cell Death and Proliferation, IIBB-CSIC, IDIBAPS and CIBERCV, Barcelona, Spain, [4]Department of Medical Cell Biology, [5]Department of Medical Sciences, Uppsala University, Uppsala, Sweden, [6]Department of Cardiology, Hospital Clinic Barcelona, Barcelona, Spain, [7]Department of Intensive Care Medicine, [8]Department of Cardiology, [9]Department of Surgery, Maastricht University Medical Centre MUMC+, Maastricht, [10]Department of Medical Sciences, Uppsala University, Uppsala, Netherlands, [11]Department of Surgical Sciences, Uppsala University, Barcelona, Sweden

Background: The coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection presents with a wide range of disease symptoms. In the more severe patients, COVID-19 is associated with respiratory failure, neutrophil extracellular trap (NET) formation, and multiple organ failure (MOF).
Aims: We investigated the presence and evolution of several damage associated molecular patterns (DAMPs) neutrophil markers and immune modulators in a group of 100 COVID-19-positive ICU patients.

Methods: Citrated plasma was collected from adult patients with confirmed COVID-19 by PCR detection of SARS-CoV-2 E and N-genes in nasopharyngeal swabs admitted to the intensive care unit (ICU) at Uppsala University hospital, Sweden. Written informed consent was obtained from the patients, or next of kin if the patient was unable to give consent. The Declaration of Helsinki and its subsequent revisions were followed. Plasma concentration of cell free DNA (cfDNA), extracellular histone H3 (H3), neutrophil elastase (NE), myeloperoxidase (MPO) and the cfDNA-MPO complex, and the immune modulators GAS6, and sAXL were measured in all COVID-19-positive and in COVID-19-negative patients and healthy controls. We determined marker levels upon admission, of their evolution, and correlation with disease severity, organ failure, thromboembolic events, mortality, and other blood parameters.
Results: The level of cfDNA, H3, NE, MPO, cfDNA-MPO complex, GAS6, and sAXL were all significantly increased in plasma of COVID-19 patients compared to controls. Importantly, a diminution of cfDNA and GAS6 levels over time was observed in patients surviving 30 days after ICU admission. Histone H3 levels were detected in 40% of the COVID-19 patient plasma at ICU admission and the presence of histone H3 during ICU stay was associated with an increased risk of thromboembolic events and secondary infection. Though NET markers were not predictive of 30-day mortality, they correlated with several parameters of tissue damage and neutrophil counts.
Summary/Conclusion: The increased presence of cfDNA, H3 and NE, MPO, and MPO-DNA illustrates the severity of cellular damage and indicates activation of NETosis in severe COVID-19 ICU patients. The evolution of cfDNA and Gas6 is able to predict disease prognosis of severely ill COVID-19 patients, where GAS6 appears to be part of an early activated mechanism in response to COVID-19. These data support treatment aimed at the reduction of NET formation in severe COVID-19 patients.

OP2.3

Global seroprevalence of pre-existing immunity against AAV serotypes in people with hemophilia A

R. Klamroth [1], G. Hayes [2], T. Andreeva [3], T. Suzuki [4], B. Hardesty [5], M. Shima [6], T. Pollock [7], P. Slev [7], J. Oldenburg [8], M. C. Ozelo [9], S.-M. Castet [10], J. Mahlangu [11], F. Peyvandi [12], R. Kazmi [13], A. D. Leavitt [14], M. Callaghan [15], B. Pan-Petesch [16], D. Quon [17], M. Li [18], W. Y. Wong [18], Sebastian Wolf* [19]
[1]Vivantes Hospital, Berlin, Germany, [2]BioMarin, Novato, United States, [3]City Outpatient Clinic, St. Petersburg, Russian Federation, [4]Ogikubo Hospital, Tokyo, Japan, [5]Indiana Hemophilia and Thrombosis Center,, Indianapolis, United States, [6]Nara Medical University, Kashihara, Japan, [7]ARUP Laboratories, Salt Lake City, United States, [8]Universitätsklinikum Bonn, Bonn, Germany, [9]Hemocentro UNICAMP, Sao Paolo, Brazil, [10]Centre de Ressources et de Compétence des Maladies Hémorragiques Constitutionnelles, CHU de Bordeaux, Bordeaux, France, [11]Haemophilia Comprehensive Care Centre, University of the Witwatersrand and National Health Laboratory Service, Johannesburg, South Africa, [12]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [13]Southampton University Hospital, Southampton, United Kingdom, [14]University of California San Francisco, San Francisco, [15]Central Michigan University, Detroit, United States, [16]Centre Hospitalier Régional Universitaire de Brest, Hôpital A. Morvan, Brest, France, [17]The Orthopedic Hemophilia Treatment Center, Los Angeles, [18]BioMarin Pharmaceutical, Novato, United States, [19]BioMarin Deutschland GmbH, Kronberg, Germany

Background: Adeno-associated virus (AAV)-mediated gene therapy may provide durable protection from bleeding events and reduced treatment burden to people with hemophilia A (PwHA). However, pre-existing immunity against AAV may limit treatment success. Global data on the prevalence of AAV serotypes are limited.
Aims: We determined the prevalence of pre-existing immunity against AAV2, AAV5, AAV6, AAV8, and AAVrh10 capsids among PwHA from 9 countries.
Methods: BMN 270-901 was a prospective study conducted in PwHA in Brazil, France, Germany, Italy, Japan, Russia, South Africa, the UK, and the USA. Plasma samples were collected from participants who provided informed consent. Antibodies against each serotype were detected using validated, electrochemiluminescent-based enzyme-linked immunosorbent assays. To evaluate changes in antibody titers over time, 20% of participants were retested at 3 and 6 months.
Results: The study enrolled 546 participants: 478 adults (aged ≥18 years) and 68 adolescents (<18 years). Global seroprevalence of antibodies against AAV2, AAV5, AAV6, AAV8, and AAVrh10 on day 1 were 58.5% (300/513), 34.8% (188/540), 48.7% (250/513), 45.6% (234/513), and 46.0% (236/513), respectively. Considerable geographic variability was observed in the prevalence of pre-existing antibodies against each serotype, but the percentage of participants positive for AAV5 was consistently the lowest among serotypes and across the countries studied. A greater percentage of adult participants were positive for AAV5 antibodies (36%) when compared with adolescents (29%). Comparative analyses of AAV serostatus in non-hemophilic individuals in select countries showed similar rates of seropositivity, as would be expected for viruses endemic to the human population. Serostatus and antibody titer were generally stable over the 6-month sampling period.
Summary/Conclusion: Among PwHA, pre-existing immunity against AAV serotypes varied across serotypes and regions, but global seropositivity was lowest for AAV5 and highest for AAV2. As clinical trials of AAV-mediated gene therapies progress, the presence of antibodies against the various AAV serotypes may become an increasingly important eligibility consideration.

OP2.4

Hypoxia augments proinflammatory activation of endothelial cells towards a prothrombotic phenotype

C. J. Ullsten-Wahlund [1,*], S. Caglayan [1], C. Esi Damoah [1], J.-B. Hansen [1 2], O. Snir [1]
[1]Department of Clinical Medicine, UiT - the Arctic University of Norway, [2]Division of Internal Medicine, University Hospital of North Norway, Tromsoe, Norway

Background: Although many risk factors of venous thromboembolism (VTE) have been described, little is known about the triggers of the disease, the sequence of events that initiate and promote the growth of venous thrombi, and the role of vascular cells. Venous thrombi originate in the venous valve pocket (VVP), a site of low and alternating oxygen levels (i.e. sustained and intermittent hypoxia). Even though intermittent hypoxia has been suggested to be a major trigger for cellular activation and thrombus formation, very few studies have investigated responses of intermittent hypoxia alone and in combination with inflammatory cytokines on prothrombotic properties of vascular endothelial cells.
Aims: To study the effects of sustained and intermittent hypoxia, alone and in combination with inflammatory cytokines, on prothrombotic properties of vascular endothelial cells.
Methods: The thrombogenic milieu in the VVP was mimicked using a state-of-the-art hypoxia chamber with sealed compartments for fully hypoxic work. Primary human endothelial cells were cultured under conditions of sustained and intermittent hypoxia, and further stimulated with pro-inflammatory VTE-associated cytokines. Phenotypic changes were studied using broad multi-color flow cytometry of pro-and anti-thrombotic molecules, as well as RT-qPCR. Furthermore, endothelial cell function and interaction with monocytes under various conditions of hypoxia and inflammatory stimuli were studied.
Results: Sustained and intermittent hypoxia augmented a cytokine-induced prothrombotic phenotype of primary endothelial cells, both at RNA and protein levels. TNF stimulation induced high expression of E- and P-selectin, as well as ICAM-1 and VCAM-1 on the surface of endothelial cells. Under hypoxic conditions, the expression levels of E-selectin and ICAM-1 were further elevated. Moreover, primary monocytes adhered to a larger degree to TNF-stimulated endothelial cells conditioned by hypoxia, compared to normoxia-conditioned endothelial cells. Interestingly, lower expression of anti-thrombotic mediators such as thrombomodulin and the endothelial protein C receptor was detected when cells were stimulated with TNF under hypoxic conditions.
Summary/Conclusion: Sustained and intermittent hypoxia augmented a cytokine-induced prothrombotic shift of both pro-, and anti-thrombotic molecules on primary endothelial cells, both on protein level and RNA levels. Hypoxia also elevated monocyte adhesion to TNF-stimulated endothelial cells, further suggesting a functional pro-thrombotic shift of the endothelial cells.

OP2.5

Derivation of a predictive score for venous thromboembolism in women using combined oral contraceptives

P. Suchon [1,*], L. Ameur [1], C. Bernard [1], D. Brunet [1], M.-C. Barthet [1], N. Saut [1], L. Goumidi [2], M.-C. Alessi [1], D.-A. Tregouët [3], P. Morange [1]
[1]APHM, [2]Aix-Marseille Université, Marseille, [3]Bordeaux Population Research, Bordeaux, France

Background: No tool is currently available for identifying women at risk for venous thromboembolism (VTE) under combined oral contraceptives (COC). In a previous work, we have shown that F11_rs2289252-A allele, non O blood groups, obesity and smoking were risk factors for VTE in COC users. However, these findings were observed in a case-control sample where controls were not representative of the general population of women taking COC but were strongly enriched in patients with family history of VTE. In order to generalize these first results, we here recruited new controls from the general population matched to the cases and extended the list of assessed common polymorphisms.
Aims: The ultimate goal being to construct and validate a predictive clinico-biological score for VTE in COC users.
Methods: The current study builds upon 766 VTE patients from the PILGRIM study that are women presenting with a personal history of objectively confirmed VTE while using COC and recruited at the Thrombophilia Center of Marseille, France. COC were classified according to the generation of progestogen. Cases were matched according to age (+/- 5 years) and generation of COC to control women from the Nutrinet-Santé French cohort including voluntaries completing online questionnaires evaluating daily habits. Controls had no personal history of VTE and were using COC at the inclusion. In all women body mass index (BMI) and smoking status were collected and the following list of variants was genotyped: F2_rs1799963, F2_3136516, F5_4524, F5_6025, F11_rs2036914, F11_rs2289252, FGG_rs2066865, KNG1_rs710446, PROC_rs867186, SLC44A2_rs2288904, CYP2C9_rs1799853, TSPAN15_rs78707713 and for ABO blood group rs579459, rs2519093, rs8176749. For the score points were then attributed using beta coefficients obtained after multivariate logistic regression. Values of sensitivity (Se) and specificity (Sp) were calculated for the different cut-offs and we generated the Receiver Operating Characteristic curve and calculated its Area under the ROC curve (AUC).
Results: In total, 766 cases and 717 controls were included. Matching could not be obtained for 49. After multivariate analysis, 10 variables remained associated with VTE at p = 0.05: obesity, smoking, ABO blood group, F2_rs1799963, F5_6025, F11_rs2036914, FGG_rs2066865, KNG1_rs710446, CYP2C9_rs1799853, TSPAN15_rs78707713. The main effect was observed for F5_6025 (Odds ratio= 6.04; 95% CI 3.71-10.27), and AB blood group (Odds ratio= 4.63; 95% CI 2.59-8.47). For all included women, each variable was attributed 0 point if absent and 1 to 5 points if present. Then the total score was calculated. The highest observed score was 19. Mean score was 7.26 in cases and 3.41 in controls ($p<0.0001$). The AUC was 0.75. Best performances were obtained when a 6 points cut-off was used allowing the identification of 64% of VTE patients with a 74% specificity.
Summary/Conclusion: In conclusion, we here proposed a new predictive score for VTE in COC users showing good performances (AUC= 0.75). The validity of this score and the performances of the 6 points cut-off will be validated in an independent case control study for which recruitment of cases is ongoing.

OP3.1

Genetic and clinical determinants of the outcome of immune tolerance induction in severe hemophilia A – preliminary results

I. Oomen [1 2,*], A. Abdi [1], S. Q. Nagelkerke [3 4], K. Fischer [5], M. Carcao [6], G.-E. Rivard [7], N. Rydz [8], J. Eikenboom [9], A. B. Mohseny [9], S. Schols [10], F. W. Leebeek [11], J. Geissler [4], M. W. Tanck [12], D. Lillicrap [13], J. Voorberg [2], T. W. Kuijpers [3 4], K. Fijnvandraat [1 2], S. C. Gouw [1 14]
[1]Department of Pediatric Hematology, Emma Children's Hospital, Amsterdam University Medical Center, [2]Department of Molecular and Cellular Hemostasis, Sanquin Research and Landsteiner Laboratory, [3]Department of Immunology and Infectious Diseases, Emma Children's Hospital, Amsterdam University Medical Center, [4]Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory, Amsterdam, [5]Department of Hematology, Van Creveldkliniek, University Medical Center Utrecht, Utrecht, Netherlands, [6]Division of Hematology/Oncology, Department of Pediatrics, , Hospital for Sick Children, University of Toronto, Toronto, [7]Division of Hematology/Oncology, Department of Pediatrics, Centre Hospitalier Universitaire Sainte-Justine, Université de Montréal, Montréal, [8]Department of Hematology and Hematologic Malignancies, Foothills Medical Center, Calgary, Canada, [9]Department of Internal Medicine, division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, [10]Department of Hematology, Radboud University Medical Center, Hemophilia Treatment Center Nijmegen-Eindhoven-Maastricht, Nijmegen, [11]Department of Hematology, Erasmus University Medical Center, Rotterdam, [12]Department of Epidemiology and Data Science, Amsterdam University Medical Center, Amsterdam, Netherlands, [13]Department of Pathology and Molecular Medicine, Richardson Laboratory, Queen's University, Kingston, Canada, [14]Department of clinical epidemiology, Leiden University Medical Center, Leiden, Netherlands

Background: Eradicating inhibitors to restore the efficacy of factor VIII (FVIII) is a desirable treatment goal for patients with severe hemophilia A (SHA) and inhibitors, as this enables treatment of bleeding episodes with FVIII concentrates. However, since immune tolerance induction (ITI) is a burdensome and costly treatment, it is important to identify determinants for ITI success to decide if worth undertaking and additionally to potentially individualize treatment. Perhaps this has never been so crucial as now as many patients/families or clinicians may feel that it may not be worth trying ITI if emicizumab is available. Several polymorphisms in various genes (Fc gamma receptor (FcγR) 2A, 2B, 2C, 3A, 3B as well as cytotoxic T lymphocyte antigen 4 (CTLA-4) and tumor necrosis factor (TNF)) postulated as contributing to inhibitor development, may also play a role in ITI outcome.
Aims: The aim of this study is to identify genetic and clinical determinants for ITI success in patients with SHA.
Methods: Dutch and Canadian patients with SHA (FVIII:C<0.01 IU/mL) who underwent ITI between 2015 and 2019 were included. The primary outcome was successful ITI, defined by (1) a negative inhibitor titer, and, (2) an adequate clinical response to standard doses of FVIII. We analyzed the following genetic determinants: *FCGR2A* (p.His166Arg, p.Gln62Trp, c.777+1G>A), *FCGR2B* (p.Ile232Thr, promotor haplotypes 2B.1/2B.4), *FCGR2C* (p.Gln57Ter, promotor haplotypes 2B.1/2B.2), *FCGR3A* (p.Val176Phe), *FCGR3B* (haplotypes NA1/NA2), CTLA-4 (rs5742909 -318C/T) and TNF (rs1800629 -308G/A) and clinical determinants included patient, treatment and inhibitor characteristics. Crude relative risks (RR) were calculated for ITI success with 95% confidence intervals [CI].
Results: The preliminary analyses, 76 patients were included. Most patients were Caucasian (52/76 (68%)), other descents included Asian (6.6%), African (7.9%) or other (15.8%). 46 of 76 (61%) had the intron-22 inversion mutation. 62 (82%) patients achieved ITI success. Most patients received recombinant FVIII (65/76 (86%)). Median age at inhibitor development was 2 years (interquartile range (IQR) 1-3), inhibitor titer at detection was 4.7 BU/ml (IQR 1.3-14.9), pre-ITI inhibitor titer was 5.8 BU/ml (IQR 2.6-20.0) and historical peak inhibitor titer was 25.3 BU/ml (IQR 4.7-194.3). Duration of ITI was median 19 months (IQR 10-38). The RR of inhibitor success in patients with historical peak titers ≥200 BU/ml was 0.48 [0.28-0.80] compared to <200 BU/ml. The RR for interval between inhibitor detection and ITI start >3 months was 0.82 [0.66-1.02] in comparison with ≤3 months, the RR for inhibitor titer at detection >5 BU/ml was 0.94 [0.75-1.18] and pre-ITI inhibitor titer >5 BU/ml was 0.80 [0.66-0.98] in comparison to ≤5 BU/ml. No clear associations were found between ethnicity, *F8* genotype and genetic variation in *FCGR*, CTLA-4, TNF with ITI success.
Summary/Conclusion: Our preliminary results suggest that historical peak titer <200 BU/ml was the most strongly associated variable with clinical ITI success, and possible associations were found for interval between inhibitor detection and ITI start ≤3 months, and inhibitor titer at detection and pre-ITI titer ≤5 BU/ml. The final GO ITI study is expected to include over 200 patients. Multivariate analyses and further analyses on genetic variants will be performed.

OP3.2

The Extracellular Protease EpiP from S. aureus Triggers Blood Coagulation by Proteolytically Activating Prothrombin and Platelet Protease-Activated Receptor 1

I. Artusi [1], G. Pontarollo [2], L. Acquasaliente [1], I. Bettin [3], A. Pagotto [1], C. M. Radu [4], G. Petrocola [5], P. Simioni [4], F. Bagnoli [6], P. Speziale [5], V. De Filippis [1,*]
[1]Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy, [2]Center for Thrombosis and Hemostasis, University Medical Center Mainz , Mainz, Germany, [3]Department of Medicine, University of Verona, Verona, [4]Department of Medicine, University of Padua, Padua, [5]Department of Molecular Medicine, University of Pavia, Pavia, [6]GSK Vaccines, Siena, Italy

Background: *Staphylococcus aureus* is a Gram-positive bacterium known for its pathogenicity in humans, responsible for both mild and systemic infections, i.e. bacteremia and sepsis. Clinical evidence, accumulated during the last decades, provide new insights for a positive relation between severe *S. aureus* infections and dramatic thrombotic complications, leading to multiple organ failure and death. So far, the secreted protein staphylocoagulase has been identified as the major non-proteolytic prothrombin (ProT) activator, initiating blood coagulation. Among the huge arsenal of virulence factors, extracellular proteases might play a role in triggering thrombotic events in infectious diseases, whereby bacterial proteases could activate the coagulation cascade by proteolytically converting ProT zymogen into active thrombin species and stimulating platelets aggregation.
Aims: In this work, we investigated if the subtilisin-like protease EpiP from *S. aureus* is involved in thrombus formation during staphylococcal infections, directly activating Prothrombin and platelets.
Methods: Activation of ProT by EpiP (1:1 mol/mol) was detected by S2238 enzymatic assay. The proteolysis reaction was followed by SDS-PAGE and RP-HPLC and the cleavage sites were identified by high resolution mass spectrometry. Fibrin generation was monitored by turbidimetry. EpiP binding to GpIbα(268-282) was observed by Surface Plasmon Resonance (SPR) while PAR1(38-60) proteolysis by RP-HPLC. Platelet aggregation was investigated by impedance aggregometry and fluorescence microscopy.
Results: This research underlines that staphylococcal EpiP serin-protease cleaves ProT to generate an active specie which can hydrolyze the thrombin-specific chromogenic substrate S2238. The highest amount of active thrombin specie was obtained after 72 h of reaction. The time-course analysis of ProT activation allowed to identify the main cleavage sites on the zymogen structure, i.e. R155-S156, R271-T272, and R320-I321 bonds. Remarkably, EpiP reveals a high substrate specificity and the cleaved peptide bonds were found to be identical to those hydrolyzed by factor Xa under physiological conditions. The activation products of ProT by EpiP can induce fibrin clot formation, either from a fibrinogen solution or platelets-free human plasma, and platelets aggregation in human whole blood. Moreover, EpiP can proteolyze the PAR1 peptide corresponding to the N-terminus of the receptor, at the same site of thrombin cleavage (R41-S42), even with a slower kinetics of proteolysis. Then, EpiP electrostatically interacts with GpIbα peptide with a dissociation constant (K_d) comparable to that of α-thrombin, as we demonstrate with SPR measurement. Ultimately, we directly observed platelets agglutination by EpiP whose effect is comparable to TRAP-6.
Summary/Conclusion: The extracellular EpiP serine protease from *S. aureus*, can directly convert the inactive ProT into an active thrombin specie which is able to trigger fibrin clot formation and platelets agglutination. Furthermore, EpiP directly induces platelet aggregation activating PAR1 receptor after binding to GpIbα on platelet surface. These results widen our understanding of biochemical mechanism whereby *S. aureus* proteases can initiate coagulation, thus establishing a direct link between infections and higher thrombotic risk and paving the way to the development of novel therapeutic strategies in the treatment of thrombotic complications in infectious diseases.

OP3.3

Activation mechanism dependent surface exposure of cellular FXIII on platelets and platelet microparticles: immunofluorescence and immune electron microscopic study

L. Muszbek [1,*], L. Somodi [1], I. Beke Debreceni [2], G. Kis [3], J. Kappelmayer [2], M. Antal [3], H. Bárdos [4], N. J. Mutch [5]
[1]Laboratory Medicine, Division of Clinical Laboratory Science, [2]Department of Laboratory Medicine, [3]Department of Anatomy, Histology and Embryology, [4]Department of Public Health and Epidemiology, University of Debrecen, Debrecen, Hungary, [5]Aberdeen Cardiovascular and Diabetes Centre, School of Medicine, Medical Science and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom

Background: Plasma factor XIII (FXIII) consists of two potentially active A subunits (FXIII-A) and two protective/inhibitory B subunits (FXIIII-B). By cross-linking fibrin chains and plasmin inhibitor to fibrin its active form, a transglutaminase, stabilizes the fibrin clot. The dimer of FXIII-A is also present in a number of cell types including platelets, monocytes, macrophages, osteoblasts, osteocytes, chondroblasts, adipocytes and corneal keratocytes. The cellular form (cFXIII) is of cytoplasmic localization, the elevation of intracellular Ca^{2+} concentration is needed to transform it into an active enzyme. Platelets are an abundant source of cFXIII, it amounts to 3% of the total platelet protein but its function has been only partially explored. It is not secreted, but becomes translocated to the surface of platelets activated though the collagen and PAR1 receptors by convulxin (CVX) and thrombin (Thr) (Mitchell et al. Blood 2014;124:3982-90, Mattheij et al. Haematologica 2016; 101:427-36). Platelet activation by these agonists also induce the transposition of phosphatidylserine (PS) from the internal to the outer membrane layer and produce microparticles (MPs).
Aims: 1/ To confirm the surface exposure of cFXIII on platelets activated by robust (CVX+Thr) receptor mediated mechanism using immune electron microscopic technique.
2/ To reveal if cFXIII also appear on the surface of platelet MPs produced by CVX+Thr activated platelets.
3/ To find out if non-receptor mediated activation by Ca^{2+}-ionophore also induces the translocation cFXIII to the surface of platelets and platelet MPs.
4/ To establish if surface translocation of cFXIII and PS during platelet activation are coinciding or independent events.
Methods: Gel-filtered platelets were stimulated by CVX+Thr or Ca^{2+}-ionophore (A23187). Platelets and platelet derived MPs were identified by anti-CD41a antibodies. Exposure of cFXIII to the platelet surface was investigated by flow cytometry, immunofluorescent and immune electronmicroscopic techniques. In immunofluorescent studies FXIII-A was labeled by rabbit anti-human FXIII-A and DyLight 488-labeled horse anti-rabbit IgG, annexin V was conjugated to Alexa Fluor 568. For double immunogold labeling rabbit anti-FXIII-A and mouse anti-CD41a were followed by goat-anti-rabbit IgG conjugated to 15 nm gold particles and goat-anti-mouse IgG conjugated to 10 nm gold particles.
Results: Following activation by CVX+Thr in over half of platelets and platelet MPs PS and cFXIII became transposed to the outer membrane surface. Most of the surface exposed cFXIII accumulated in a cap-like structure. The majority of PS-positive MPs also showed FXIII-A positivity. Electron microscopy revealed larger MPs with preserved membrane structure and smaller MPs (cytoplasmic fragments?) devoid of labeling for membrane glycoprotein CD41a. cFXIII was observed on both types of MPs but was more abundant in MPs not labelled for CD41a. Non-receptor mediated activation triggered by Ca^{2+}-ionophore resulted in PS positive platelets and MPs, however neither the cells nor the formed MPs expressed FXIII-A on their surface.
Summary/Conclusion: Receptor-mediated activation induces surface exposure of PS and cFXIII on both platelets and MPs. Transposition of PS and cFXIII to the membrane surface requires different mechanisms. Elevation of intracellular Ca^{2+} concentration is sufficient for PS transposition but insufficient for exposing cFXIII.

OP3.4

Protein S Gla domain as theranostic for early vascular calcification

A. Gentier [1,*], S. Agten [1], L. Schurgers [1], T. Hackeng [1]
[1]Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, Netherlands

Background: Vascular calcification is implicated in severe pathologies such as atherosclerosis and chronic kidney disease. Early-stage calcification (microcalcification) is considered to be life-threatening both by increasing risk of plaque rupture and evading detection by conventional imaging techniques. In addition, no therapy exists to intervene with ectopic calcification. Calcium binding properties of coagulation factors are governed by post-translationally modified γ-carboxy glutamic acid (Gla) residues, hence the Gla-domain. Previous research has shown that some of these Gla-domains are able to interact with deleterious calcium-crystal formation, as such they form an interesting, new platform for diagnostics and therapeutics of ectopic microcalcification. The protein S Gla-domain was selected because of its 11 Gla-amino acids and previous findings indicating a calcification inhibiting effect.
Aims: To investigate if protein S Gla-domain can act as both tracer and therapy for microcalcification.
Methods: Protein S Gla domain was chemically synthesized and subsequently used for detection and inhibition of calcification in *in vitro* calcification assays. For this purpose, protein S Gla domain and negative control peptide protein S Glu were assessed in an non-cellular crystallization assay and assays with human vascular smooth muscle cells (hVSMCs) cultured under calcifying conditions. The ability to detect and interfere with calcification was determined using a combination of fluorescence microscopy and quantitative calcium phosphate crystal measurements.
Results: Protein S Gla domain selectively bound to microcalcifications in an *in vitro* setting allowing specific detection. Importantly, protein S Gla-domain interfered with initiation as well as progression of calcification of hVSMCs. Finally, at low concentrations, protein S Gla domain was used to follow progression of calcification in a time-dependent manner without interfering with the calcification process.
Summary/Conclusion: Protein S Gla-domain is a promising tool for detection and follow-up of early vascular calcification and has potential as a calcification inhibitor, due to its ability to interfere with calcification nucleation sites as well as progression of calcium crystals.

OP3.5

Remote history of venous thrombosis and the risk of venous thrombosis beyond the age of 70 years

H. Wang [1,*], M. Cushman [2], F. R.Rosendaal [1], A. van Hylckama Vlieg [1]
[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands, [2]Department of Medicine, Larner College of Medicine at the University of Vermont, Burlington, United States

Background: Currently, there are no prediction model for the risk of venous thrombosis (VT) specifically in the elderly. A prior VT is associated with the risk of a future VT, but the evidence for this in the literature rarely comes from studies with longer than 10 years of follow-up.
Aims: To perform a predictor finding study to assess whether a remote history of VT, i.e., a VT >10 years ago, is a good predictor of the risk of VT in older age.
Methods: Analyses were performed in the AT-AGE study, a multi-center case-control study performed in Vermont, USA and Leiden, Netherlands, comprising of 460 VT cases and 456 control subjects, aged ≥70 years. Individuals with a VT <10 years prior to the index date (current VT date for cases and date of inclusion for controls) were excluded. We calculated the crude and independent predictive value of a self-reported history of VT, i.e., after adjustment for known predictors, by calculating odds ratios (OR) with 95% confidence intervals (CI). Two models were made to assess the independent predictive value, i.e., one adjusting for standardly available predictors of a VT at older age (age, sex, BMI, and family history of VT), and one also including genetic markers of venous thrombosis, i.e., the factor V Leiden, the prothrombin 20210A mutation and non-O blood group. Provoked VT was defined as thrombosis after hospitalization (including recent surgery), fracture, plaster cast, splint, minor injuries of lower extremities (such as a sprained ankle or contusion of the lower leg), or transient immobility at home ≥ 4 successive days in the three months before the thrombosis. This study was supported by grants from the Netherlands Heart Foundation(grant no: 2009B50) and the Leducq Foundation, Paris, France, for the development of Transatlantic Networks of Excellence in Cardiovascular Research.
Results: Compared with those without a history of VT, individuals with a history of VT >10 years prior to the index date had a 2.5-fold (95%CI: 1.6-4.1) increased risk of VT. The crude risk estimate did not differ with time since previous event, i.e., individuals with a VT 10-30 years ago had a 2.7-fold increased risk of VT (95%CI: 1.3-5.6); individuals with a VT more than 30 years ago had a 2.4-fold increased risk of VT (95%: 1.2-4.8). A VT >10 years ago was associated with a more pronounced risk of DVT (OR: 3.1; 95% CI: 1.8-5.5) than PE±DVT (OR: 2.1; 95% CI: 1.2-3.6), and of unprovoked (OR: 3.5; 95% CI: 2.1-6.0) than provoked events (OR: 1.5; 95% CI: 0.8-2.8). The independent predictive value of a remote history of VT was similar to the crude predictive value in both models.
Summary/Conclusion: A remote history of VT is a predictor of the risk of VT in the elderly. The added value of this predictor to more extensive prediction models targeted specifically to the elderly needs to be further assessed.

SESSION FRIDAY, 15 OCTOBER 2021: 10.45 – 11.45

OP4.1

COVID-19 associated coagulopathy in acute ischemic stroke patients treated with intravenous thrombolysis

Z. Bagoly [1 2,*], R. Orbán-Kálmándi [1], I. Szegedi [3], T. Árokszállási [3], L. Lóczi [1], J. Tóth [4], L. Oláh [3], L. Csiba [2 3]
[1]Department of Laboratory Medicine, Division of Clinical Laboratory Sciences, University of Debrecen, [2]ELKH-DE Neurodegenerative and Cerebrovascular Research Group, [3]Department of Neurology, [4]Department of Laboratory Medicine, University of Debrecen, Debrecen, Hungary

Background: Coronavirus disease 2019 (COVID-19) is the most overwhelming medical threat of the past few decades. COVID-19 has been associated with profound hemostasis changes and a high risk of venous thrombotic events. Little is known about hemostasis alterations in acute ischemic stroke (AIS) patients with COVID-19. Moreover, outcomes of vascular interventions (e.g. thrombolysis or thrombectomy) in COVID-19-positive AIS patients are not well studied as yet.
Aims: Here we aimed to test hemostasis alterations in COVID-19-positive AIS patients receiving intravenous thrombolysis as compared to non-infected AIS patients and to correlate results with therapy outcomes and safety.
Methods: In this prospective observational study, 69 AIS patients, all receiving intravenous thrombolysis according to standard protocols using recombinant tissue plasminogen activator were enrolled. Blood samples were taken on admission (within 4.5 hours of symptom onset) and at 24 h post-event. SARS-CoV-2 RT-PCR test was performed in all patients on admission and acute infection was confirmed in 8 cases (COVID-19+ group). An anti-SARS-CoV-2 antibody test was performed from all blood samples and convalescence or vaccination was proven in 5 patients (post-COVID/post-vaccination group). Besides routine laboratory measurements (including complete blood count, routine kidney- and liver-functions tests, high sensitivity C-reactive protein test), screening tests of coagulation, D-dimer, fibrinogen, von Willebrand factor (VWF) antigen, factor VIII (FVIII) and factor XIII (FXIII) activity, clot-lysis assay, thrombin generation test, angiotensin convertase enzyme (ACE)1, ACE2 activity and ROTEM analysis were performed from the blood samples of all patients. Stroke severity was determined by NIHSS. Therapy-associated intracerebral hemorrhage was classified according to ECASSII criteria. Short- and long-term outcomes were defined at 7 days and 3 months post-event according to the change in NIHSS and by the modified Rankin Scale, respectively. All patients or their relatives provided written informed consent.
Results: Stroke severity was significantly greater in the COVID-19+ group. VWF antigen levels were markedly elevated in the COVID-19+ group as compared to non-infected and post-COVID/post-vaccination groups (329±94% vs. 244±75% and 210±66%, respectively, p=0.027). FVIII levels were parallel to VWF levels and showed significant elevation in the COVID-19+ group (median: 286.5 [IQR: 227-295]% vs. 194 [144-254]% and 197 [127.5-233.5]%, p=0.047 in the COVID-19+ group vs. non-infected and post-COVID/post-vaccination groups, respectively). Short-term outcomes of therapy as well as the occurence of hemorrhagic transformation did not differ between groups.
Summary/Conclusion: Elevated FVIII and VWF levels in COVID-19-associated AIS seem to be linked to endothelial cell injury and are associated with more severe stroke. Efficacy of thrombolysis in COVID-19 positive AIS patients was similar to non-infected patients in this cohort.
Funding: NKFI-FK128582; K120042

OP4.2

Joint effect of multiple prothrombotic genotypes and mean platelet volume on the risk of incident venous thromboembolism.

L. Jakobsen [1,*], T. Frischmuth [1], S. K. Brækkan [1 2], J.-B. Hansen [1], V. M. Morelli [1]
[1]Thrombosis Research Center, Department of Clinical Medicine, UiT The Arctic University of Norway, [2]Department of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Background: Mean platelet volume (MPV), a marker of platelet reactivity, is associated with risk of incident venous thromboembolism (VTE). Whether established prothrombotic single nucleotide polymorphisms (SNPs) further increase the risk of VTE in the presence of a high MVP due to biological interaction remains unknown. Clarification of this question may provide novel insights into the biology of VTE risk and improve the identification of individuals at a substantially high risk of VTE in the general population.
Aims: To investigate the joint effect of a high MPV and a genetic risk score (GRS), composed of five established prothrombotic SNPs, on the risk of incident VTE in a population-based case-cohort study.
Methods: Cases with incident VTE (n= 653) and a randomly sampled subcohort weighted for age (n=1774) were identified from the Tromsø Study cohort (1994-2012). DNA was genotyped for rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2036914 (*F11*) and rs2066865 (*FGG*). Hazard ratios (HRs) of VTE with 95% confidence intervals (CIs) were estimated in Cox regression models according to predefined MPV strata (< 8.5, 8.5-9.5 and ≥ 9.5 fL) and number of risk alleles for individual SNPs and the GRS (0-1, 2-3, ≥ 4 risk alleles). Models were adjusted for age, sex, body mass index and platelet count. Ethical approval and informed consent were obtained.
Results: The combination of a high MPV and risk alleles, either as individual SNPs or as a GRS, had an additive effect on VTE risk. Subjects with a high MPV (≥9.5 fL) who were carriers of ≥4 risk alleles had a 2.80-fold (HR 2.80, CI 95% 1.77-4.43) increased risk of overall VTE compared with those with a MPV <8.5 fL and 0-1 risk allele. In subgroups, the combination of a MPV ≥9.5 fL and ≥4 risk alleles had a more noticeable effect on the risk of unprovoked VTE (HR 4.60, 95% CI 2.20-9.60). However, similar to overall VTE, a high MPV when jointly present with a high GRS did not result in an excess risk of VTE in subgroups (i.e. deep vein thrombosis, pulmonary embolism, provoked and unproved VTE).
Summary/Conclusion: The combination of a high MPV and prothrombotic genotypes, either as individual SNPs or as a GRS, resulted in an additive effect on the risk of incident VTE (i.e. no biological interaction). Our findings suggest that platelet reactivity and the studied prothrombotic genotypes act independently in the biology of VTE risk.

OP4.3

Shear-induced platelet GPIbα shedding under extracorporeal membrane oxygenation promotes platelet clearance independently from von Willebrand factor-GPIbα interaction

A. Dupont [1,*], A. Rauch [1], M. Rosa [2], C. Casari [3], R. Ruez [2], A. Ung [1], E. Jeanpierre [1], M. Didelot [1], N. Rousse [4], P. J. Lenting [3], M. Moussa [5], M. Desvages [1], F. Vincent [6], A. Vincentelli [4], C. Denis [3], E. Van Belle [6], S. Susen [1]
[1]Hemostasis Transfusion, CHU Lille, INSERM U1011-EGID, University of Lille, [2]INSERM U1011-EGID, University of Lille, Lille, [3]INSERM, UMR-S 1176, Université Paris-Sud, Université Paris-Saclay, Le Kremlin Bicêtre, [4]Cardiac Surgery, CHU Lille, INSERM U1011-EGID, University of Lille, [5]Anesthesiology and critical care, CHU Lille, INSERM U1011-EGID, University of Lille, [6]Cardiology, CHU Lille, INSERM U1011-EGID, University of Lille, Lille, France

Background: Thrombocytopenia is a frequent adverse event under Extracorporeal Membrane Oxygenation (ECMO). Different mechanisms such as platelet consumption, heparin-induced or sepsis may account for thrombocytopenia during ECMO. However, shedding of glycoprotein Ibα (GPIbα) has been recently identified as a potential mechanism of platelet clearance. There is also evidence that shear stress regulates von Willebrand factor (VWF) interaction with platelet GPIbα. Moreover, the shedding of GPIbα is sensitive to high shear stress, a rheological condition provided by ECMO.
Aims: To investigate whether a shear-induced platelet-GPIbα shedding could promote thrombocytopenia under ECMO and the potential role of increased VWF-GPIbα interaction as a trigger.
Methods: We first investigated in a cohort of 30 ECMO patients the time course of thrombocytopenia and platelet-GPIbα shedding. In an ECMO circulatory loop model, we next assessed the impact of shear stress on i) platelet-GPIbα shedding and ii) the role of VWF-GPIbα interaction on this shedding by inhibiting this interaction with monoclonal antibodies and analyzing VWF multimers distribution. Plasma levels of soluble GPIbα (assessed by ELISA), GPIbα platelet expression (determined by flow cytometry) and analysis of GPIbα in platelet pellets (by Western-Blot) were used as surrogate markers of platelet-GPIbα shedding. We further evaluated in a NOD/SCID mouse model the clearance of platelets after their exposure to ECMO rheological conditions.
Results: A decrease of mean platelet count and GPIbα platelet expression and an increase of plasma soluble GPIbα levels were observed 24 hours after ECMO implantation ($p<0.0001$, $p=0.0005$ and $p=0.005$ respectively). Two days after ECMO removal, platelet count was restored. When perfusing platelets for 180 min in an ECMO loop model, we observed a time and shear dependent increase in platelet-GPIbα shedding, which occurred independently of VWF-GPIbα interaction. We noted the appearance of a subpopulation of GPIbα-shed platelets after one hour of perfusion which was more pronounced with the high outflow condition. When transfusing those shear-exposed human platelets to NOD/SCID mice, we observed a significant increase of platelets clearance when compared to unsheared-platelets ($p<0.05$).
Summary/Conclusion: ECMO shear-induced platelet GPIbα shedding promotes platelets clearance independently from VWF-GPIbα interaction. Inhibiting platelet-GPIbα shedding is a potential approach to reduce thrombocytopenia during ECMO support.

OP4.4

Effects of dietary palmitate on blood lipid profile and calcification of aorta and aortic valve

N. Donis [1,*], Z. Jiang [1], C. D'Emal [1], R. Dulgheru [1], M. Giera [2], N. Blomberg [2], P. Delvenne [3 4], A. Nchimi [1], P. Lancellotti [1 5], C. Oury [1]
[1]Laboratory of Cardiology, University of Liège - GIGA Institute, Liège, Belgium, [2]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, Netherlands, [3]Department of Pathology, CHU University Hospital, [4]Laboratory of Experimental Pathology, University of Liège - GIGA Institute, Liège, Belgium, [5]Gruppo Villa Maria Care and Research, Anthea Hospital, Bari, Italy

Background: Vascular and valvular calcification are leading causes of life-threatening cardiovascular diseases. Dietary habits are well known risk factors of these diseases. Palmitic acid (PA) is a major dietary saturated fatty acid (SFA) found in palm oil and lard, whereas oleic acid (OA) is the principal monounsaturated fatty acid (MUFA) in olive oil. Dietary intake of PA has been associated with increased risk for cardiovascular disease, while OA would play a protective role. However, whether PA intake could promote vascular and/or valvular calcification remains unknown.
Aims: Our study aimed to assess the effect of a short-term intake of lard, as source of dietary PA and OA, on aorta and aortic valve calcification.
Methods: Twelve-week-old male New Zealand white rabbits were fed for 16 weeks with either chow diet (n=7), 0.175% lard-enriched diet (n=7) that mimics regular PA intake by children, or 5% lard-enriched diet (PA and OA-enriched diet, n=6). Blood draws were performed at baseline and at 16 weeks of diet. Blood cell counts were measured, serum and plasma samples were prepared and stored for further analyses. Heart and aorta were harvested and fixed for histological analyses of tissue structure (hematoxylin-eosin staining) and calcification (alizarin red staining). Serum levels of fetuin-A were measured by enzyme-linked immunosorbent assay and total cholesterol, triglycerides, LDL-c, and HDL-c were determined on a AU480 Chemistry Analyzer. Comprehensive quantitative lipidomics analysis was carried out in citrated plasma on the commercial LipidyzerTM platform.
Results: After 16 weeks of intake of lard-enriched diets, calcification was observed in the aortic intima and in the aortic valve. The extent of calcification did not differ between the two diets. In contrast, rabbits fed chow diet did not develop any calcification. In blood, PA enrichment resulted in decreased lymphocyte and monocyte counts, with increased neutrophil count, revealing a low-grade inflammatory response. Circulating levels of the calcification inhibitor fetuin-A were also decreased by the two diets. Of note, none of the diets changed the conventional cholesterol parameter levels (LDL-c, HDL-c, non-HDL-c). Comprehensive quantitative lipidomics analysis identified diet-related changes in plasma lipids. In total lipids, the 0.175% and 5% lard diets led to a drop of polyunsaturated fatty acid (PUFA) levels in comparison with chow diet (23.93 and 21.44 vs. 28.55%, P=0.002, P<0.001), which was reflected in cholesterylesters, free fatty acids, triglycerides and diacylglycerols. SUFA:PUFA, PA:PUFA and OA:PUFA ratios were increased in the same lipid classes. Interestingly, levels of linoleic acid, an omega-6 PUFA (18:2), were specifically reduced by lard-enriched diets as compared to chow diet (19.6 and 18.9 vs. 24.8%, P<0.001). Ratio of PA to 18-carbon PUFA in diacylglycerols was positively correlated with the extent of aortic valve calcification, and negatively with monocyte counts. PA content in blood correlated with aorta calcification.
Summary/Conclusion: Regular dietary PA intake induces vascular and valvular calcification independently of traditional risk factors. Our findings raise awareness about PA-rich food consumption and its potential deleterious effect on cardiovascular health.
Fundings: Région Wallonne (DGO6) - Fondation Léon Frédéricq

OP4.5

Lower excess mortality in an anticoagulated population during the first wave of the COVID-19 pandemic in the Netherlands

Q. Chen [1,*], M. Toorop [1], C. Visser [2], M. Nierman [3], F. van der Meer [1], L. Goede [4], A. ten Cate-Hoek [5], B. Spaetgens [6], C. den Uil [2], F. Klok [1], E. van Gorp [7], G.-J. Geersing [8], K. Meijer [9], C. Kramers [10], L. Faber [11], M. Huisman [1], N. van Es [12], R. Brüggemann [6], S. Middeldorp [10], V. Tichelaar [9], H. ten Cate [13], M. Kruip [2], W. Lijfering [1], S. Cannegieter [1]
[1]Leiden University Medical Center, Leiden, [2]Erasmus University Medical Centre, Rotterdam, [3]Atalmedial Medical Diagnostics Centre, Amsterdam, [4]Saltro, Utrecht, [5]Maastricht University Medical Centre and Cardiovascular Research Institute Maastricht, [6]Maastricht University Medical Centre, Maastricht, [7]Erasmus MC, Rotterdam, [8]University Medical Centre Utrecht, Utrecht, [9]University Medical Centre Groningen, Groningen, [10]Radboud University Medical Centre, Nijmegen, [11]Red Cross Hospital, Beverwijk, [12]Amsterdam University Medical Centre, Amsterdam, [13]Cardiovascular Research Institute Maastricht, Maastricht, Netherlands

Background: Excess mortality has been observed in the general population during the COVID-19 pandemic, but it is unknown whether long-term anticoagulation therapy benefits survival of COVID-19 patients, given that COVID-19 associated hypercoagulability adversely impacts prognosis.
Aims: The study aimed to compare the magnitude of excess mortality in an anticoagulated population during the COVID-19 pandemic with that in the general population and in a chronically ill (i.e., diabetic) population.
Methods: A nationwide cohort of atrial fibrillation patients (aged ≥50 years) receiving oral anticoagulants with a CHA_2DS_2-VASc score ≥2 on 1/1/2020 was identified using data from Statistics Netherlands. The corresponding patient population on 1/1/2019 was enrolled as a control cohort. Both cohorts were followed for 130 days and the 70th day after the start of follow-up was designated as the landmark. Excess mortality in the anticoagulated population in 2020 was evaluated by comparing the risk of all-cause mortality in 2020 with that in 2019 within the same follow-up periods before and after the landmark respectively. The contemporary Dutch population and a diabetic population (both aged ≥50 years) were identified in the same way for 2020 and 2019. Excess mortality in these two populations in 2020 was evaluated identically and compared with that in the anticoagulated population.
Results: A total of 151,155 atrial fibrillation patients were identified as the Cohort 2020 of the anticoagulated population, whose baseline characteristics were broadly the same as the control cohort (Cohort 2019, n=137,422). After the landmark, excess mortality was observed in the anticoagulated population in 2020 (a relative increase of 23.10%, 95% CI 15.98-30.66%, evaluated by multivariable Cox regression analysis, adjusted for age, sex, immigration status, and various comorbidities), which was not observed before the landmark. Similar to the anticoagulated population, excess mortality was also only observed after the landmark in the Dutch population (Cohort 2020, n=7,038,732; Cohort 2019, n=6,917,047) and the diabetic population (Cohort 2020, n=304,494; Cohort 2019, n=297,538), but here the magnitude was significantly higher (a relative increase of 32.98% (95% CI 30.81-35.19%) and 37.34% (95%CI 31.92-42.99%), respectively).
Summary/Conclusion: A patient population receiving long-term anticoagulation therapy showed a significantly lower excess mortality during the first wave of the COVID-19 pandemic in the Netherlands when compared to the general population and a chronically ill population, suggesting that early use of anticoagulants provides a potential protective effect on COVID-19 related mortality at population level.

OP5.1

A novel quali-quantitative defect of VWF

T. van de Berg [1 2,*], A. Todaro [1], J. van Beers [3], K. Wichapong [1], F. Heubel-Moenen [2], E. Castoldi [1], Y. Henskens [3], E. Beckers [2]
[1]Department of Biochemistry, Maastricht University, [2]Department of Hematology, [3]Central diagnostic laboratory, MUMC+, Maastricht, Netherlands

Background: Von Willebrand Factor (VWF) is a multimeric protein largely involved in both primary and secondary hemostasis. The diagnosis and classification of von Willebrand Disease (VWD) patients can be challenging. In this poster we explore the genetic defects and their structural consequences in a VWD patient with a disproportionately high bleeding phenotype for her VWD severity. A 31-year old female, initially diagnosed with VWD type 1, presented herself with a bleeding tendency (ISTH-BAT 13) disproportionate to the severity of her VWD with antigen levels of 36%. Additional analysis showed decreased FVIII-binding at 28%. The combination of both quantitative and functional defects of VWF was an indication for further genetic analysis in order to better define the subtype of VWD.
Aims: Genetic and structural analysis of VWF in a patient with a disproportionally high bleeding phenotype with regard to a mild decrease in VWF antigen.
Methods: Routine laboratory analysis for VWD was performed. Genetic screening was performed by exome sequencing of hemostasis related genes. VWF mRNA analysis was carried out by RT-PCR and Sanger sequencing. The X-ray structure of furin in complex with a peptide-based inhibitor (PDB ID: 6YD7) was used as a template to construct furin-VWF (759HR(R760S)SKRS764) complex. The derived structures (furin in complex with WT/R760S-VWF) were subjected to molecular dynamics (MD) simulations (200ns) and binding free energy (BFE) calculations by using standard parameters and protocols implemented in AMBER20 program.
Results: Routine analysis showed PFA-ADP and PFA EPI >300 seconds, VWF:ACT of 37% with a VWF:AG of 36%. Collagen binding and FVIII-binding were 46% and 28% respectively.
Genetic analysis of the VWF gene disclosed 2 heterozygous variants of unknown significance (VUS): c.2771 G>A (exon 21, p.Arg924Gln) has a 1-2.5% population prevalence and has been previously described in type 1 and 2N VWD. The other VUS (c.2278 C>A; exon 17) is a novel mutation predicting a major amino acid substitution (p.Arg760Ser) in the D2-domain of *VWF*. Sequencing of exons 17 and 21 in the patient's VWF mRNA revealed homozygosity for the mutated allele at both mutation sites, indicating that the two variants are in cis and that the 'normal' allele is not expressed at mRNA level.
Molecular dynamics simulations of the novel c.2278 C>A mutation (Arg760Ser) predicts a markedly decreased binding of furin to its VWF binding site, possibly decreasing or preventing VWF pro-peptide cleavage. This in turn has been shown to lead to reduced FVIII-binding of VWF.
Summary/Conclusion: Genetic analysis shows one polymorphism (c.2771 G>A) and one variation of unknown significance (c.2278 C>A) in the patient's VWF-gene. The polymorphism is known to be of low pathogenicity. The c. 2278 C>A mutation was not known in any of the mutation databases and is a novel VWF mutation. Both mutations were shown to be present on the same allele. As the wild-type allele was not expressed on mRNA level, all of the patient's VWF protein includes both amino acid substitutions. Modeling and molecular dynamics simulations show a markedly decreased affinity of furin to its cleavage site on the VWF protein due to the Arg760Ser substitution, likely resulting in a persistent pro-peptide binding to the mature VWF protein.

OP5.2

Anti-cysteine/spacer autoantibodies that open the conformation of ADAMTS13 are a common feature of the autoimmune response in immune-mediated thrombotic thrombocytopenic purpura

L. De Waele [1,*], A. Curie [1 2], K. Kangro [1 3], E. Tellier [4 5], G. Kaplanski [4 6], A. Männik [3], C. Tersteeg [1], B. Joly [7 8], P. Coppo [8 9], A. Veyradier [7 8], S. De Meyer [1], E. Roose [1], K. Vanhoorelbeke [1]
[1]Laboratory for Thrombosis Research, IRF Life Sciences, KU Leuven Campus Kulak, Kortrijk, Belgium, [2]Department of Internal Medicine , CHU Charles Nicolle, Rouen, France, [3]Icosagen Cell Factory, Össu, Kambja, Tartumaa, Estonia, [4]Aix-Marseille University, [5]INSERM, INRAE, C2VN, [6]Hôpital de la Conception, Assistance Publique-Hôpitaux de Marseille, Marseille, [7]Service d'Hématologie biologique, Hôpital Lariboisière, [8]Assistance Publique-Hôpitaux de Paris, [9]Centre de Référence des Microangiopathies Thrombotiques, Hôpital Saint Antoine, Paris, France

Background: Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a life-threatening thrombotic microangiopathy that is caused by an autoantibody-mediated deficiency of ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 repeats member 13). Patient autoantibodies have epitopes in all ADAMTS13 domains: Metalloprotease (M) and Disintegrin-like (D) domain, a first Thrombospondin type-1 repeat (T1), a Cysteine-rich (C) and Spacer (S) domain, 7 additional Thrombospondin repeats (T2-T8) and 2 CUB (CUB) domains, and almost all patients have autoantibodies against the CS domains. In healthy individuals, ADAMTS13 circulates in a folded conformation where the central S domain interacts with the C-terminal CUB domains. We recently showed that ADAMTS13 adopts an open conformation in iTTP patients and that patient immunoglobulin G's (IgGs) can open ADAMTS13. We now hypothesize that the anti-CS and anti-CUB autoantibodies can disrupt the S-CUB interaction in folded ADAMTS13 thereby inducing an open conformation.
Aims: To investigate whether patient anti-CS and anti-CUB autoantibodies can induce an open ADAMTS13 conformation.
Methods: Anti-CS and anti-CUB autoantibodies were first purified from the total IgGs of 13 acute iTTP patients by CS- or CUB-coupled affinity chromatography and their specificity was evaluated in ELISA. Next, the ability of the affinity purified anti-CS or anti-CUB autoantibodies to open ADAMTS13 was tested in our in-house developed ADAMTS13 conformation ELISA. The purified anti-CS or anti-CUB autoantibodies were incubated with closed ADAMTS13 present in healthy donor plasma after which the binding of open ADAMTS13 to an ELISA plate coated with 1C4, a monoclonal antibody that recognizes a cryptic epitope in the S domain of ADAMTS13, was studied.
Results: Affinity purified anti-CS (10/13 patients) and anti-CUB (4/13 patients) autoantibody fractions showed binding to respectively CS or CUB while little to no binding to other ADAMTS13 fragments was observed. Interestingly, all purified anti-CS autoantibody fractions (10/10 patients) were able to induce an open ADAMTS13 conformation. In contrast, only half of the purified anti-CUB autoantibody fractions (2/4 patients) opened the conformation of ADAMTS13.
Summary/Conclusion: Our data shows that the presence of anti-CS autoantibodies inducing an open ADAMTS13 conformation is a common feature of the autoantibody response in iTTP patients.

OP5.3

GPVI is a binding partner for pro-coagulant factor VIII on platelets

R. Sekar [1 2,*], V. Proulle [1 3], S. Loyau [4], S. Delignat [1], Y. Boulaftali [4], A. Mimoun [1 2], M. Jandrot-Perrus [4], S. Lacroix-Desmazes [1]
[1]Centre de recherche des Cordeliers, INSERM UMRS 1138, [2]Sorbonne Universite, [3]Service Hematologie Biologique et Hemostase Clinique, Hopital Cochin, AP-HP Centre Universite de Paris, [4]INSERM UMRS 1148, Hopital Bichat, Paris, France

Background: Factor VIII (FVIII) is an essential actor in the intrinsic coagulation pathway, where it acts as a cofactor for activated FIX to form the tenase complex that anchors at the surface of activated platelets. Binding partners for FVIII on platelets include phosphatidylserine and platelet-bound fibrin. GPVI is a platelet-specific glycoprotein, the cross-linking of which by collagen, activates platelets. Screening for FVIII interactions with platelet-expressed glycoproteins indicated binding of FVIII to GPVI.
Aims: To characterize the FVIII/GPVI interaction and decipher its potential biological relevance on platelet functions.
Methods: Binding of human full-length and B domain-deleted (BDD) FVIII (Advate®, Kogenate®, Factane®, Novoeight®, Eloctate®, FVIII^{HSQ}) was investigated on immobilized human recombinant GPVI (R&D) or home-made Fc-fused GPVI. FVIII was pre-incubated alone or with varying amounts of von Willebrand factor (VWF, Wilfactin®), or human monovalent monoclonal anti-A2 (BOIIB2), anti-C1 (KM33) or anti-C2 (BO2C11) IgG. Binding of the FVIII C1$^{R2090A/K2092A/F2093A}$, C2$^{R2215A/R2220A}$ and C1C2 variants was also investigated. Bound FVIII was revealed using SAF8C, a FVIII-specific polyclonal sheep IgG. Collagen or TRAP-induced platelet aggregation was studied on human washed platelets in the abscence or presence of FVIII (or FIX as control) by Light Transmission Aggregometry.
Results: Full-length and BDD FVIII, but not plasma-derived FVIII, bound in a dose-dependent manner to GPVI-Fc. VWF inhibited FVIII binding to GPVI-Fc. Interestingly, C1 and C2-specific, but not A2-specific, monovalent IgG also inhibited the interaction. Accordingly, FVIII variants mutated in the target epitopes of BO2C11 and/or KM33 lost binding capacity to GPVI-Fc. FVIII, but not FIX, inhibited in a dose-dependent manner collagen-mediated platelet aggregation while TRAP-mediated platelet aggregation was unperturbed. Preliminary results suggest that the FVIII C1C2 mutant did not prevent collagen-mediated platelet aggregation.
Summary/Conclusion: Phosphatidylserine and $\alpha_{IIb}ß_3$ integrin-bound fibrin are binding sites on platelets for the light chain of FVIII. Our recent data identify GPVI as a novel binding partner for FVIII on platelets. The interaction involves the C1 and C2 domains of the light chain of FVIII and is prevented in the presence of VWF. Our preliminary data suggest that supra-physiological concentrations of FVIII interfere with collagen-mediated GPVI stimulation. Future work shall investigate the potential biological significance of the interaction.

OP5.4

Highly reactive juvenile platelets express higher levels of GPVI in a size-related manner

A. Veninga [1,*], S. Handtke [2], B. M. Tullemans [1], S. L. Brouns [1], A. Greinacher [2], J. W. Heemskerk [1], P. E. Van der Meijden [1] [3], T. Thiele [2]
[1]Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, Netherlands, [2]Institute for Immunology and Transfusion Medicine, University Medicine Greifswald, Greifswald, Germany, [3]Thrombosis Expertise Center, Heart and Vascular Center, Maastricht University Medical Center, Maastricht, Netherlands

Background: Platelets within one individual display heterogeneity in reactivity, size, age, and expression of surface receptors. We and others have shown that larger platelets show increased responsiveness to activating stimuli compared to smaller platelets. Next to that, it is considered that the RNA content in juvenile platelets is associated with higher reactivity.
Aims: To investigate the combined intra-individual contribution of platelet size, platelet age, and receptor expression levels on the reactivity of platelets.
Methods: Fractions of large and small platelets from healthy donors were separated by differential centrifugation. Multicolour flow cytometry with subsequent automated high-dimensional clustering analysis (FlowSOM) was used to identify and phenotype platelet subpopulations formed in response to different doses of CRP-XL, TRAP6, and ADP. Platelet age correlations were assessed by co-staining of labelled oligo-dA/T with either anti-glycoprotein (GP)VI or anti-HLA-I antibodies. Whole blood reconstituted with size-separated platelet fractions were perfused over a collagen-coated surface to assess thrombus formation.
Results: Clustering analysis of the flow cytometric data showed that highly reactive platelet populations are characterised by highest GPVI and HLA-I (marker for juvenile platelets) expression. Platelets with high expression of GPVI also show a higher RNA content. Highly reactive juvenile platelets were found to be enriched in the large platelet fraction. Even when adjusted to the same platelet mass, the larger platelet fraction resulted in faster adhesion to collagen under flow and the formation of larger thrombi, compared to the small platelet fraction.
Summary/Conclusion: High GPVI expression is a feature of highly reactive juvenile platelets, which are predominantly found among large platelets and likely promote thrombus formation.

Funded by Landsteiner Foundation for Blood Transfusion Research

OP5.5

SARS-CoV2 associated venous thromboembolism - a one-year follow-up

A. Maino [1,*], M. Landolfo [1], S. Magnoni [2], F. Boccafoglio [3], D. Peterlana [1]
[1]Internal Medicine, [2]Intensive Care, [3]Pneumology, Azienda Provinciale per i Sevizi Sanitari di Trento, Trento, Italy

Background: Venous thromboembolism (VTE) is associated with acute SARS-CoV2 infection, especially in cases with severe interstitial pneumonia, and influences its short-term prognosis. Data on long-term consequences and follow-up of SARS-CoV2 associated VTE are still lacking.
Aims: To investigate recurrences, resolution rates and outcome of SARS-CoV2 associated VTE.
Methods: We conducted a cohort study of all SARS-CoV2 patients consecutively admitted for severe pneumonia to the intensive and sub-intensive care units at S. Chiara Hospital, Trento, from March 21st to May 4th, who developed an episode of VTE (either pulmonary embolism, PE, or deep vein thrombosis, DVT). During hospitalization, all patients were screened for DVT and, when appropriate, for PE. After discharge, we followed patients with confirmed VTE for thrombotic and haemorrhagic events at three, six and 12 months. Thrombus resolution and cardiac involvement were evaluated by venous and cardiac ultrasound. The post-COVID19 functional status scale (PCFS) was used to investigate residual symptoms and dysfunction. Thrombophilia screening (including factor V Leiden, prothrombin G20210A mutation, antiphospholipid antibodies, protein S, protein C and antithrombin levels) was performed at least one month after termination of anticoagulation. Descriptive results are presented by mean and standard deviation where appropriate.
Results: Of 101 patients admitted for severe SARS-CoV2 pneumonia, 16 (16%) had VTE (mean age 63 ± 9 years, 14 males). Three patients died during hospitalization (all males, mean age 61 ± 7 years). Of the 13 remaining, three had PE and ten DVT. After six months of follow-up, all but two patients had complete resolution of DVT, and none had signs of pulmonary hypertension at the cardiac ultrasound. Thrombophilia screening was performed in ten patients, with negative results. After discharge, most patients were treated with direct anticoagulants. Anticoagulant therapy was stopped after three months in most patients (8, 62%), and six months in four patients (31%). Only one patient continued anticoagulation for 12 months. After a mean follow-up of 13 ± 0.6 months, three minor bleeding and no VTE recurrence occurred. Seven patients (54%) reported no limitations or symptoms at the PCFS, and the remaining reported mild to moderate limitations with a mean PCFS grade of 13.
Summary/Conclusion: We did not observe recurrences of SARS-CoV2 associated VTE. Our preliminary data suggest that stopping anticoagulation after a standard treatment period of three (for DVT) or six (for PE) months might be a reasonable approach in SARS-CoV2 related VTE. The risk of bleeding might be not negligible.

OP6.1

Prosthetic valve bioactive surface coating to reduce the prevalence of thrombosis

P. Lancellotti [1], A. Aqil [2], N. Jacques [2], M. Debuisson [2], A. Gougnard [3], C. Sandersen [3], J.-P. Cheramy-Bien [4], N. Sakalihasan [4], C. Jérôme [5], C. Oury [2,*]
[1]Cardiology, University Hospital Liège, [2]GIGA Institute, University of Liège, [3]Faculty of veterinary medicine, University of Liège, [4]Surgical research centre, University Hospital Liège, [5]CESAM Research Unit, University of Liège, Liège, Belgium

Background: Valvular heart disease affects more than 100 million people worldwide, and the problem is growing because of the increasing burden of degenerative valve disease in the ageing population. About 800 000 prosthetic heart valves are implanted every year, and this remains the only treatment for the majority of patients with severe valvular heart disease. Heart valve prostheses are currently among the most widely used cardiovascular devices. Continuing advances in heart valve prosthesis design and in techniques for implantation have improved survival of patients who receive these devices. However, the ideal valve prosthesis does not exist. Mechanical valve prostheses, made of metallic and polymeric components, are prone to thrombosis and therefore require permanent anticoagulation and monitoring, which often leads to higher risks of thromboembolism or hemorrhage, and impacts patient's quality of life.
Aims: The present project aims to reduce mechanical valve thrombosis to improve their long-term in vivo performance by using a novel bioactive coating.
Methods: We used a catechol-based approach to produce a layer-by-layer coating made of cross-linked nanoscale particles, referred to as nanogels, that adheres on the entire valve prosthesis, its metallic (pyrolytic carbon, titanium) and polymeric (polyethylene terephthalate) constituents. Nanogels were loaded with the antiplatelet drug ticagrelor. The hemodynamic performance of coated mechanical valves in aortic position was verified by using an ISO-compliant heart model tester (pulse duplicator). Coating durability on the long-term was assessed in a dedicated prosthetic valve durability tester producing accelerated cardiac cycles. The surface antithrombotic activity was evaluated both in vitro with human plasma or whole blood under static and flow conditions and in vivo, after surgical valve implantation in pig thoracic aorta.
Results: Several layers of nanogels loaded with ticagrelor could be attached on the surface of mechanical valve prosthesis. This technology enabled us to achieve the expected antithrombotic effect. The coating did not increase the contact phase activation of coagulation and it prevented plasma protein adsorption, platelet adhesion and thrombus formation under flow. Moreover, our coating did not alter prosthetic valve hemodynamic performance. We found that the chemistry of our coating remained unaltered for a period equivalent to two years of human life. Finally, and more importantly, the in vivo demonstration of our coating hemocompatibility and antithrombotic activity was obtained upon surgical implantation of coated and non-coated mechanical valves in the pig. Even though none of the implanted animals received anticoagulation during 1-month follow-up, our coating led to impressive reduction of valve thrombosis. Almost no thrombi were observed on the surface of explanted coated valve, whereas massive thrombosis occurred on non-coated valves.
Summary/Conclusion: We have developed a bioactive coating for mechanical prosthetic heart valves. We demonstrated the hemodynamic performance, hemocompatibility and thromboresistance of the bioactive surface coated prosthetic heart valve in vitro and in vivo. Thus, we provide a solution to reduce the need for anticoagulant in patients with mechanical valve, as well as the number of revision surgeries due to valve thrombosis despite anticoagulation.

OP6.2

Venous Thromboembolism after Incident Colorectal Cancer in the Netherlands: Incidence, Predictors, and Prognosis

R. Anijs*, Q. Chen [1], W. Lijfering [1], S. Cannegieter [1]
[1]Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands

Background: Cancer patients have an increased risk of developing venous thromboembolism (VTE), resulting in increased morbidity and mortality. In The Netherlands, colorectal cancer (CRC) is the third most prevalent type of cancer, with an overall 5-year survival rate of 60%. The occurrence and predictors of VTE after CRC diagnosis and how VTE influences subsequent mortality has not been well examined, especially at a nationwide level with recent data.
Aims: The aim of this study is to determine the incidence, predictors and prognosis of VTE occurring 1 year after incident CRC in the Netherlands.
Methods: Data from the Statistics Netherlands (CBS) and the Netherlands Comprehensive Cancer Organization (IKNL) were used to identify patients with an incident diagnosis of CRC in the Netherlands between 1 January 2013 and 31 December 2018. The patients were followed for 1 year from incident diagnosis of CRC. Incidence rates (IR) and cumulative incidences of developing VTE during follow-up were estimated and predictors of developing VTE were explored by univariable Cox regression analyses. The association between developing VTE and all-cause mortality was evaluated by multivariable Cox regression analyses where developing VTE was treated as a time-dependent exposure. The patients were followed for 1 year from diagnosis of CRC.
Results: A total of 67588 patients with incident CRC were included with a mean age of 69.8 years and a male sex proportion of 55.6%. The cumulative incidences of VTE after 1, 3, 6, 9, and 12 months of cancer diagnosis were 0.31% (95% CI 0.27-0.36%), 0.86% (95% CI 0.79-0.93%), 1.43% (95% CI 1.34-1.52%), 1.72% (95% CI 1.62-1.82%), and 1.88% (95% CI 1.78-1.99%), respectively. The risk of VTE increased accordingly with higher cancer stage (cumulative incidence at 12 months ranging from 0.76-3.76% from stage 1 to 4) and with the presence of systemic chemotherapy and radiotherapy treatment.
Significant predictors for developing VTE were increasing clinical cancer stage, overlapping lesion of the colon, presence of systemic chemotherapy, previous history of asthma, heart failure, diabetes mellitus and VTE. Developing VTE in CRC patients was found to be associated with increased risk of all-cause mortality (hazard ratio 3.99, 95% CI 3.59-4.43), compared to cancer patients without VTE. After adjusting for age, sex, cancer morphology and cancer stage, the HR for mortality was 2.58 (95% CI 2.33-2.87).
Summary/Conclusion: In this Dutch nationwide study, a VTE risk of 1.9% one year after incident CRC diagnosis was demonstrated. Occurrence of VTE was associated with worse survival, independent of tumor morphology and cancer stage, indicating a VTE related effect. Significant predictors for VTE were identified and may help to identify CRC patients at higher risk of developing VTE and result in a lower burden for colorectal cancer patients.

OP6.3

Identification of two new ligands of the platelet CLEC-2 receptor

L. A. Moran [1,*], J. C. Clark [1], E. Martin [1], Y. Di [1], M. Sowa [2], L. Hermidia-Nogueira [2], M. Barrachina [2], D. Moreira [2], E. Dominguez [2], M. Loza [2], A. Garcia [2], S. Watson [3]
[1]Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, United Kingdom, [2]University of Santiago de Compostela, Santiago de Compostela, Spain, [3]University of Birmingham, Birmingham, United Kingdom

Background: CLEC-2 is a novel target in thrombo-inflammation diseases such as deep vein thrombosis and infection. Structural and molecular studies have suggested clustering of CLEC-2 as an activation mechanism of the single transmembrane receptor and consequent platelet aggregation.
Aims: To develop new ligands for CLEC-2 based on small molecules and nanobodies against CLEC-2.
Methods: We have screened a small molecule chemical library on an ALPHA screen platform assay based on the podoplanin and CLEC-2 interaction to identify small molecules ligands of the receptor. Potential hits have been characterised using light transmission aggregometry and western blotting. For our second approach, we have screened high-affinity nanobodies raised against human CLEC-2. The most potent nanobody has been multimerised using a short crosslinking sequence of repeated GGGGS, developing dimeric and tetrameric forms.
Results: We have identified a “small molecule” with polymeric nature, which binds to CLEC-2 and induces platelet aggregation in an all-or-none mechanism. Activation is mediated through Src and Syk tyrosine kinases and associated with phosphorylation of CLEC-2.
We have raised over 20 nanobodies and shown that the most efficient of these, Nb4, blocks activation of platelets by podoplanin-expressing cells. Nb4 binds to CLEC-2 with an affinity of ~140nM as shown by SPR. Dimerisation increases the affinity to 0.5nM and generates a potent-blocking agent. However, tetramerisation of Nb4 induces platelet aggregation which is blocked by Src and Syk kinase inhibitors, and by the divalent CLEC-2 antibody fragment AYP1 f(ab)2, showing that activation is mediated through CLEC-2.
Summary/Conclusion: Polymeric and tetrameric ligands are required to induce platelet aggregation and potentially induce clustering of CLEC-2, while dimerisation does not induce platelet aggregation. The novel tools will enable us to further probe CLEC-2 function and mode of activation.

OP6.4

Elevated plasma levels of the complement activating enzyme MASP-2 are associated with risk of future incident venous thromboembolism

C. E. Damoah [1,*], O. Snir [1], K. Hindberg [1], P. Garred [2], J. K. Ludviksen [3], S. K. Brækkan [1 4], V. M. Morelli [1], T. E. Mollnes [1 3 5 6], J.-B. Hansen [1 4]
[1]Thrombosis Research Center, Department of Clinical Medicine, UiT – The Arctic University of Norway, Tromsø, Norway, [2]Laboratory of Molecular Medicine, Department of Clinical Immunology, Section 7631, Rigshospitalet , Copenhagen, Denmark, [3]Reaserch Laboratory, Nordland Hospital, Bodø, [4]Division of Internal Medicine, University Hospital of North Norway, Tromsø, [5]Department of Immunology, Oslo University Hospital and University of Oslo, Oslo, [6]Centre of Molecular Inflammation Research, Norwegian University of Science and Technology, Trondheim, Norway

Background: Experimental studies have shown that the complement activating enzyme MBL-associated serine protease 2 (MASP-2) exhibits a thrombin-like activity, and that inhibition of MASP-2 protects against thrombosis.
Aims: We investigated whether plasma MASP-2 levels were associated with risk of future venous thromboembolism (VTE), and whether genetic variants linked to MASP-2 levels were associated with VTE risk.
Methods: We conducted a population-based nested case-control study involving 410 VTE patients and 842 age- and sex-matched controls derived from the Norwegian Tromsø Study. Logistic regression was used to estimate odds ratios (ORs) for VTE across MASP-2 quartiles. Whole exome sequencing and protein quantitative trait loci (pQTL) analyses were performed to assess genetic variants associated with MASP-2 levels. A two-sample Mendelian randomization study, also including data from the INVENT consortium, was performed to assess causality.
Results: Subjects with plasma MASP-2 in the highest quartile had a 48% higher OR for VTE (OR: 1.48; 95% CI:1.06-2.06) and 83% higher OR for deep vein thrombosis (OR:1.83, 95% CI:1.23-2.73) compared with those with MASP-2 levels in the lowest quartile. The pQTL analysis revealed that three previous described gene variants, rs12711521 (minor allele frequency (MAF) =0.153) and rs72550870 (MAF=0.045) (missense variants in MASP2 gene) and rs2275527 (MAF=0.220) (exon-variant in the adjacent MTOR gene) explained 39% of the variation of MASP-2 plasma concentration. The OR for VTE per 1 SD increase in genetically predicted MASP-2 was 1.03 (95% CI:1.01-1.05, p=0.0011).
Summary/Conclusion: Our findings suggest that high plasma MASP-2 levels are causally associated with risk of future VTE.

OP6.5

Role of Matrix Gla Protein in vascular calcification: hard chemistry for soft vessels

S. Agten [1,*], D. Suylen [1], A. Gentier [1], L. Schurgers [1], T. Hackeng [1]
[1]Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht, Netherlands

Background: Vascular calcification is implicated in severe pathologies such as atherosclerosis and chronic kidney disease. First perceived as a passive process, calcification is now considered a tightly regulated active process. However, much is unclear regarding initiation and inhibition of calcification while detection can only be achieved at a late stage, hampering intervention. A key player in this process is Matrix Gla Protein (MGP), a heavily post-translationally modified inhibitor of calcification. While MGP is recognized as one of the strongest inhibitors of ectopic calcification, its mechanism of action is unknown. Previous research has shown the importance of carboxylation for its function and, in addition, presence of circulating uncarboxylated MGP has been associated with vascular calcification. Further research into MGP's function is hampered by the inability in obtaining homogenously modified MGP.
Aims: We believe research into MGP function will lead to elucidation of mediators and mechanisms of calcification inhibition for early detection and repair.
Methods: We have employed a 3-fragment, one-pot, chemical protein synthesis approach to synthesize all possible post-translationally modified variants of MGP (unmodified, phosphorylated, carboxylated, phosphorylated-carboxylated). These different MGP variants were next tested for their ability to inhibit formation of mineral deposits in a calcium phosphate precipitation assay. Cellular effects involved with calcification were evaluated in an *in vitro* vascular calcification assay where MGP variants were added to human vascular smooth muscle cells (hVSMC) cultured under calcifying conditions.
Results: Chemical protein synthesis approach has provided access to homogenously post-translationally modified samples of MGP. This will open up research into MGP's structure and function relationships. First results of biological evaluation could show that unmodified MGP is unable to interfere with calcium phosphate precipitation whereas all post-translationally modified variants showed a dose-dependent inhibitory effect. Moreover, we could show a dose-dependent inhibitory effect of post-translationally modified MGP on calcifying hVSMCs.
Summary/Conclusion: Results of both the non-cellular and cellular assay confirm importance of carboxylation but also show a clear effect of phosphorylation. Currently, we investigate effects of addition of different MGP variants on hVSMC differentiation and phenotype switching.

BLEEDING

P-001

Selection of immuno-dominant T cell epitopes from the repertoire of FVIII derived peptides presented on MHC class II

M. Miranda [1,*], J. Voorberg [1]
[1]Molecular Hemostasis, Sanquin, Amsterdam, Netherlands

Background: The main complication of hemophilia A treatment is the development of neutralizing antibodies (inhibitors) against factor VIII (FVIII). The eradication of FVIII inhibitory antibodies relies on the immune tolerance induction (ITI). Since ITI is efficient in only 60-80% of the cases, novel strategies are needed to more efficiently induce tolerance in hemophilia A patients with inhibitors.
Aims: Within the EDUC8-consortium we are exploring novel approaches for ITI which includes tolerogenic targeting of FVIII derived $CD4^+$ T cell epitopes coupled to different classes of nanoparticles. Here we employed bioinformatics and advanced peptide presentation assays to identify promiscuously presented FVIII derived peptides with the ultimate goal of selecting suitable candidate-peptides for tolerogenic nanoparticle-mediated targeting.
Methods: A database of FVIII derived peptides presented on different MHC class II alleles was assembled and compared to predicted MHC class II presented epitopes using the 'Immune Epitope Database (IEDB)' website. An additional data-set of naturally processed FVIII peptides was generated by incubating human FVIII with immature monocytes-derived DCs from HLA-typed healthy donors. Specific attention was directed towards the identification of FVIII peptides presented on HLA-DP4 since this MHC class II allele is highly prevalent in the Caucasian population.
Results: The 'Immune Epitope Database' website based analysis of FVIII presented peptides revealed a large number of FVIII core peptides. Detailed inspection of the data-set revealed that several FVIII derived peptides were presented by multiple HLA-DR and HLA-DQ alleles. To supplement the current data-set we successfully developed a protocol to study peptide presentation on HLA-DP utilizing a monoclonal antibody that specifically bound to HLA-DP4. This provides a basis for studying the HLA-DP4 presented peptide repertoire of FVIII.
Summary/Conclusion: Taken together, our data provide an inventory of promiscuously presented FVIII-derived peptides which will guide novel approaches of nanoparticle mediated induction of tolerance in hemophilia A.

P-002

Genetic and non-genetic determinants of the outcome of immune tolerance induction in patients with hemophilia A and inhibitors – preliminary data of a systematic review

I. Oomen [1 2,*], R. M. Camelo [3], S. M. Rezende [3], J. Voorberg [2], M. E. Mancuso [4], J. Oldenburg [5], M. Carcao [6], D. Matino [7], D. Lillicrap [8], K. Fischer [9], K. Fijnvandraat [1 2], S. C. Gouw [1 10]
[1]Department of Pediatric Hematology, Emma Children's Hospital, Amsterdam University Medical Center, [2]Department of Molecular and Cellular Hemostasis, Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, [3]Department of Internal Medicine, Faculty of Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil, [4]Center for Thrombosis and Hemorrhagic Diseases, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy, [5]Department of Internal Medicine, University Clinic Bonn, University of Bonn, Bonn, Germany, [6]Division of Hematology/Oncology, Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, [7]Department of Medicine, McMaster University, University of Hamilton, Hamilton, [8]Department of Pathology and Molecular Medicine, Richardson Laboratory, Queen's University, Kingston, Canada, [9]Department of Hematology, Van Creveldkliniek, University Medical Center Utrecht, Utrecht, [10]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands

Background: Immune tolerance induction (ITI) is the only therapy to eradicate anti-factor VIII antibodies (inhibitors) in patients with hemophilia A. However, this burdensome and costly treatment fails in 10-40%. In order to determine whether to attempt ITI, it is important to identify predictors of ITI success.
Aims: This systematic review aims to assess association between genetic and non-genetic factors and ITI success in patients with hemophilia A.
Methods: A comprehensive literature search was conducted on February 17 2021, in the MEDLINE and Embase databases using the following search terms; hemophilia and immune tolerance. Cross-sectional, longitudinal and cohort studies and registries reporting on predictors for ITI outcome in patients with congenital hemophilia A who underwent ITI therapy were eligible for inclusion. Data were extracted on study, patient and treatment characteristics, ITI outcome definitions and genetic and non-genetic factors associated with ITI success or failure. Two independent reviewers performed the study selection, data extraction and methodological quality assignment using an adapted checklist of the Joanna Briggs Institute for cross-sectional or cohort studies. Studies were classified as high-quality when ≥11/13 criteria were met. Incidence of ITI success were reported for each study. If heterogeneity is limited, pooled effect estimates of individual determinants will be assessed.
Results: The literature search yielded 1021 unique papers of which 932 were excluded after title and abstract screening. Another 51 articles were excluded after full text screening. After exclusion of five papers that represented duplicate cohorts and 3 low quality studies, 30 articles were included (24 intermediate and 6 high quality). The following factors were reported to be associated with ITI success in a majority of studies investigating these determinants; lower titer at start of ITI, lower peak titer pre-ITI and lower peak titer on ITI. Factors reported to be correlated to ITI failure in a majority of studies were interruption of treatment, younger age at inhibitor development, longer interval between inhibitor detection and ITI initiation and inhibitor against the factor VIII A2 domain. No association with ITI outcome was reported for concomitant use of immunomodulatory drugs and inhibitor titer at detection. Factors on which conflicting results on ITI outcome were reported, were ethnicity, *F8* genotype, ITI product, ITI dose, and cumulative number of exposure days at inhibitor development.
Summary/Conclusion: The preliminary results of the systematic review summarizes the current evidence on factors associated with ITI outcome. Further research is required on predictors of ITI outcome. Our future aim is to create a prediction model in order to tailor treatment on the basis of predicted ITI outcome.

P-003

Analysis of natural and recombinant missense variants involving Cysteine residues in factor XI: implications on folding, dimerization and function

C. Bravo-Perez [1,*], A. Rubio [1], A. Palomo [2], J. Esteban [3], F. Bauduer [4], A. Miñano [1], R. Cifuentes [1], B. de la Morena-Barrio [1], P. Garrido [1], V. Vicente [1], J. Corral [1], M. E. de la Morena-Barrio [1]
[1]Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER, Murcia, [2]Servicio de Hematología y Hemoterapia del centro Materno-Infantil del Hospital Regional Universitario Carlos de Haya, Malaga, [3]Servicio de Hematología, Hospital Virgen del Castillo, Yecla, Spain, [4]Service d'Hématologie, Centre Hospitalier de la Côte Basque, Bayonne, Francia; Laboratoire PACEA UMR 5199, Université de Bordeaux, Pessac, France

Background: Factor XI (FXI) has 17 intrachain disulfide bonds and circulates in plasma as a disulfide-linked homodimer. Although missense variants causing FXI deficiency that eliminate or introduce cysteines (Cys) have been identified, there is no systematic study on the role of disulfides in the folding and function of this molecule, nor on the pathogenic mechanisms in these cases.
Aims: We aimed to analyze the consequences of mutations causing FXI deficiency that eliminate or introduce Cys.
Methods: Review of variants involving Cys described in the literature (FXI.org and HGMD®), and functional and biochemical characterization of selected cases from our cohort of 105 unrelated patients with FXI deficiency. We used a recombinant model based on HEK293 cells for the expression of wild-type and mutated *F11* cDNA.
Results: Overall, 40 missense variants eliminating or introducing Cys have been described. Nine out of 13 variants from our cohort involved Cys. Twenty-five variants removed Cys (five from our cohort). Only one, pCys599Tyr (from our cohort), located in the catalytic domain, caused CRM+ deficiency, with the presence of non-functional protein in plasma. As regards cases with CRM- deficiency, mean FXI:C levels were below 50% in heterozygotes (mean FXI:C=32%), suggesting a dominant-negative effect. Besides, we highlight a CRM- variant that eliminated two Cys in the same allele according to family studies: p.Cys255Tyr and p.Cys339Phe, the last one involved in the interchain disulfide bond. Despite the relevance of Cys339 on FXI dimerization, previous works in BHK cells suggested that its mutation might not impair protein secretion (PMID: 11895778). Our recombinant model demonstrated that the mutation of this residue (p.Cys339Ala) precluded dimer formation, but not monomer secretion. Moreover, mutation of the only free Cys of FXI (Cys29) reduced the efficiency of dimer formation in the recombinant model. Finally, p.Cys416Tyr, which affected an intrachain disulphide bond, caused CRM- deficiency and impaired both dimer and monomer secretion in the recombinant model.
Fifteen missense variants introduced new Cys (four in our cohort). Three of them caused CRM+ deficiency. We highlight the p.Phe295Cys variant, identified in hemizygosity in a case from our cohort due to concurrent *de novo* deletion of the wild-type allele. This variant allowed the secretion of two dimeric forms of FXI, one with normal size and an aberrant one with less electrophoretic mobility. The recombinant model confirmed these two conformations in both the dimer and the monomer. This new Cys did not form an interchain disulfide bond, nor did it interact with the free Cys29.
Summary/Conclusion: Our study confirms the importance of intrachain disulfide bonds in FXI folding and secretion, generating a CRM- deficiency. Only one variant identified in our cohort that affects a disulfide bond in the catalytic domain allows the secretion of an inactive form of FXI. The mutation of Cys339 only prevents dimerization, but it causes CRM- deficiency *in vivo*. Finally, the introduction of new Cys can lead to the formation and secretion of aberrant dimers without coagulant activity, as we have shown for the p.Phe295Cys mutation. Intramolecular disulfide bond exchanges may explain these findings.
Funding:ISCIII&FEDER:PI18/00598;CM20/00094. FundaciónSéneca:9873/GERM/15

P-004

Optimization of thrombin generation for hemophilia A

T. Van De Berg [1 2,*], E. Beckers [2], T. Hackeng [1]
[1]Department of Biochemistry, Maastricht University, [2]Department of Hematology, MUMC+, Maastricht, Netherlands

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 one-stage 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 baseline in the lower FVIII ranges.
Methods: In order to minimize patient specific sensitivity a hemophilia A pool plasma (HAPP) was created using the plasma of 10 severe hemophilia patients. Influence of contact activation inhibitors, assay temperature, phospholipid concentration and sample handling were taken into account in the optimization of the assay. Ideal tissue factor (TF) concentrations were determined by titration in our HAPP. Titrations were aimed at providing a reliable baseline curve, even in severe HA plasma.
Results: Commonly used tissue factor (TF) initiated TG alone at varying concentrations was unable to significantly differentiate in FVIII levels below 20%. In contrast, TG activation with low concentrations of TF in presence of FXIa appeared to be highly sensitive for FVIII changes both in high and low ranges. Additionally, a representative baseline TG-curve in severe HA plasma could only be produced using this dual TF/FXIa-activation. The sensitivity of dual activated thrombin generation to FVIII changes was superior to TF only activated thrombin generation. The influence of contact activation inhibitors, assay temprature and phospholipids were taken into account as part of the optimilization process.
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.

P-005

Induction of tolerance to therapeutic factor VIII in HA by modification with α2,3 sialic acid

E. Nardini [1,*], E. R. Li [1], Y. van Kooyk [1]
[1]Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Background: Our awareness of the importance of glycosylation in the regulation of the immune system has greatly broadened the way see autoimmune diseases. There is now growing evidence that sialic acid–terminating glycans act as "self-associated molecular patterns", thus contributing to the maintenance of peripheral tolerance. Indeed, dendritic cells (DCs) are equipped with inhibitory sialic acid-binding Ig-type lectin (Siglecs) receptor. Recent data has shown that targeting the sialic-acid-Siglec axis on DCs promoted CD4+ T cell skewing to Tregs at the expenses of effector T cells. Thinking out of the box, this strategy could prove useful also in the treatment of hemophilia A. The major complication in replacement therapies is the development of anti-FVIII neutralizing antibodies (inhibitors) in ~25% of severe patients. Finding novel approaches to tackle FVIII immunogenicity is therefore an urgent concern.
Aims: Here, we aim to modify therapeutic FVIII with an α2,3-sialic acid to induce preventive or therapeutic antigen-specific tolerance.
Methods: A database of FVIII derived peptides presented on HLA – DRB1*1501 molecules with a demonstrated degree of promiscuity was built and compared to predicted mouse H2 – IA^b FVIII - T cell epitopes using the 'immune epitope database' (IEDB). The common sequences were selected, synthetized by solid phase, and conjugated to α2-3 sialic acid (NeuAcα2-3) using the GlycoDC technology patented by DC4U. Specific binding to Siglecs was investigated in an ELISA with Siglec – Fc chimeras.
Results: The interrogation of the IEDB generated a substantial number of FVIII core peptides with a significant predicted score of binding to mouse H2 - IA^b. This haplotype is expressed by C57BL/6 strain, and it was therefore selected to prepare for follow up experiments *in vivo*. Alignment of the retrieved mouse sequences with the database of human FVIII – core peptides presented by multiple HLA-DR alleles identified several common sequences, which were chosen to be conjugated to NeuAcα2-3.
Preliminary data obtained from different peptide sequences, but similarly sialylated, showed that the binding to Siglec9 is dependent on the presence of sialic acid and stronger for the α2-3 linkage.
Summary/Conclusion: Altogether, our data identify a plethora of FVIII-derived peptides promiscuously presented on HLA-DR. Upon sialyation of the retrieved epitopes, this assembled database comprise promising candidates to tolerizing HA patients to FVIII concentrates.

P-006

Large-scale mapping of CD4 T-cell epitopes of recombinant full length FVIII in healthy individuals.

V. Porcheddu [1,*], B. Maillere [1]
[1]CEA Paris-Saclay, Saclay , France

Background: 5-30% of the patients with Hemophilia A (of all severities) develop inhibitor alloantibodies against pro-coagulant factor VIII, limiting the use of current treatment options. The only ITI strategy available offer a transient efficacy and fails in 30% of the cases.
Recent studies in this field have demonstrated the presence of a FVIII specific T-cell population under physiological conditions. CD4 T cells specific for FVIII are present in healthy donors and comprise of naïve and memory cells. Furthermore, up to 20% of healthy individuals possess immunoglobulin G reactive to endogenous FVIII. Whereby, loss of tolerance to endogenous FVIII may occur in individuals without previous abnormality in hemostasis. These ulterior evidences complicate our understanding of pro-coagulant FVIII immunogenicity that therefore requires further characterization.
Aims: Identification of CD4-T cells specific to HLA-DR restricted pro-coagulant FVIII epitopes in healthy donors.
Methods: We performed *in silico* prediction analyses on the whole sequence of full-length FVIII, in order to determinate a group of 9-mer cores with the highest binding score affinity to restricted HLA-DR alleles, selected as representative of the most expressed in the European and North American population. We used healthy donors as a source of T cells to identify CD4 T-cell epitopes, generating specific T cell lines by *in vitro* T cell amplification assay, using recombinant full length FVIII and keyhole limpet hemocyanin as control. The antigen specificity of the CD4 T-cell lines were analyzed by two independent interferon-γ (IFN-γ) enzyme-linked immunospot (ELISPOT). Firstly, testing the response to the 7 pools of 9 FVIII peptides and rFVIII. Secondly, based on the results from the first ELISPOT, the positive T cell lines were stimulated with all the single peptides present in the reactive pool.
Results: 63 CD4 potential epitopes were tested on 4 healthy donors. All donors reacted against recombinant full length FVIII with a mean of 24 T cell lines per donor. The range of CD4 T cell precursors per million cells in our experimentation follows what already shown by previous study, approximately 6 cells/M, one log higher than other preexisting T cells specific for immunogenic therapeutic proteins. A total of 146 T cell lines were specific for the CD4 epitopes tested. Thirty-five of the 63 predicted peptides induced a T cell response in at least one donor.
Summary/Conclusion: Here we present preliminary data on the first 4 healthy donors screened for the identification of specific CD4 T cell FVIII epitopes *in vitro.* An extended epitope mapping study will pave the way for a better understanding of the peptide specificity of FVIII-specific T cells in healthy donors.

Acknowledgment: This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 859974

P-007

Detection of endogenous FVIII variants in IPS derived vEC from Hemophilia A patients with null mutations.

P. Chawla*, M. Rath [1], K. Czogalla-Nitsche [1], F. Forin [1], R. Al-Rifai [1], O. El-Maarri [1], H. Singer [1], J. Oldenburg [1]
[1]IHT, Ukb, Bonn, Germany

Background: FVIII specific nonsense mutations are a type of null mutation that have a higher prevalence of alloantibodies during the treatment of Hemophilia A (HA). The location of the pre-terminal stop codon (PTC) in the light chain (A3-C1-C2) of the FVIII molecule has a higher risk of inhibitor development contrary to the heavy chain (A1-A2). We generated IPS cells from ten severe HA patients with varying null mutations and further differentiated them into venous-like endothelial cells (vEC). Using ELISA with monoclonal antibodies against A2 & C1 domain we were able detect FVIII variants for wild type, I22I patient and for two nonsense mutations located in the FVIII light chain.
Aims: 1)To verify endogenous FVIII detection using immunostaining in primary endothelial cells. 2) To localize endogenous FVIII in IPS derived vECs
Methods: Primary HDLEC (positive control) and HUVEC (negative control) were used to test specificity of different monoclonal antibodies (GMA8021, GMA012 & GMA8011) against low concentrations of endogenous FVIII (mU). Subsequently our IPS derived vEC from wild type vEC (Cm), *F8* knock out (F8KO), Intron 22 inversion (P-I22I), large deletion mutation (P-LDA2 [Deletion Ex 7-9]), nonsense mutation in HC (P-Y431X[ex9/A2] and P-L705X[ex14/A2]) and nonsense mutation in LC (P-Q1874X[ex17/A3], P-R1941X[ex18/A3] and P-R2209X[ex24/C2]) were stained with the highly specific FVIII antibody GMA8021. Selected samples were also co-stained with anti-Sec31A (COPII-marker).
Results: Using GMA8021 we are able to detect a positive signal for vEC wild type and I22I, while this signal was lacking in both negative controls (F8KO and LDA2). Wild type cells are partly co-localizing with COPII coated vesicles, while I22I is clearly accumulated and may be surrounded by these vesicles. vEC samples derived from nonsense mutation in the A3 (R1941X) and C2 domain (R2209X) of the light chain show occasional FVIII signal not co-localizing with COPII vesicles.
Summary/Conclusion: We are able to detect endogenous FVIII in wt and FVIII variants from three different null mutations in our in vitro model. Further co-localization studies of the FVIII protein and of eluted peptide molecules will show where endogenous FVIII protein is localized and if FVIII variants are presented by MHC-I in a different peptide pattern than wild type.

P-008

Characterization of endothelial cell function in a patient with recurrent gastrointestinal bleeding using patient-derived endothelial colony forming cells

S. Laan [1 2,*], S. de Boer [2], R. Dirven [2], I. van Moort [1], J. Eikenboom [2], R. Bierings [1]
[1]Hematology, Erasmus University Medical Centre, Rotterdam, [2]Thrombosis and Hemostasis, LUMC, Leiden, Netherlands

Background: In ~50% of bleeding patients referred to tertiary centres, the underlying mechanisms that cause clinically relevant bleeding problems remain unidentified, so-called bleeding of unknown cause (BUC). The contribution of the vessel wall in BUC is unclear. Here, we investigate a patient with recurrent gastrointestinal bleeding who was historically diagnosed with von Willebrand disease (VWD) type 1 based on low von Willebrand factor (VWF) parameters (VWF:Ag 0.61 U/mL, VWF:CB 0.31 U/mL, VWF:act 0.30 U/mL, FVIII:C 0.71 U/mL). However, upon recent re-evaluation, VWF parameters were normal (VWF:Ag 0.81 U/mL, VWF:CB 1.11 U/mL, VWF:act 1.13 U/mL, FVIII:C 1.06 U/mL) and no mutation in *VWF* was detected. Therefore, VWD is not likely the correct diagnosis and the cause of bleeding is currently unclear.
Aims: To identify the cause of bleeding using *ex vivo* patient endothelial colony forming cells (ECFCs).
Methods: Patient and healthy control ECFCs were derived from peripheral blood. Intra- and extracellular markers were analysed using flow cytometry and confocal microscopy. Cell migration was analysed by scratch assay. RNA expression was examined by qPCR. VWF production/secretion were measured via ELISA, collagen binding and VWF multimer assay.
Results: No obvious changes in cell surface expression of endothelial markers were observed. Morphological analysis indicated no differences in Golgi or endoplasmic reticulum. Patient ECFCs had reduced VWF production and secretion, and delayed closing in the scratch assay. VWF:Ag/VWF:CB ratio was normal, suggesting a regular multimer pattern, which was confirmed in VWF multimer assay. qPCR analysis showed reduced expression of the angiogenic mediators angiopoietin-2 and interleukin-8.
Summary/Conclusion: Reduced levels of angiopoietin-2 and interleukin-8 could affect angiogenesis, which might explain the delayed closing of ECFCs.
Reduced production and secretion suggest a mild quantitative VWF defect, however the cause is not yet clear. Further analysis, including proteomic expression profiling, is needed to elucidate the cause of bleeding.
on behalf of the SYMPHONY consortium. NWO-NWA.1160.18.038 "SYMPHONY: Orchestrating personalised treatment for patients with bleeding disorders”

P-009

Successful immunosuppressive therapy to eradicate an anti-factor V inhibitor responsible for two life-threatening hemorrhagic episodes

N. Jad [1], B. Yasmine [2*],C. Ladislas [1], E. Syrine [1], S. H. Mama [1], D. Jerôme [1], F. Michaela [1], A. Jean [3], P. Valérie [1]
[1]Service Hematologie Biologique et UF Hemostase Clinique, hôpital Cochin, APHP, paris, [2]Sysmex France, Villepinte, [3]HYPHEN BioMed, Neuville sur Oise, France

Background: Acquired auto-anti-factor V inhibitor (aFVi) is a rare and challenging hemostatic disorder ranging from asymptomatic biological abnormality to severe bleeding tendency. AFVi may be idiopathic or related to surgical procedures, antibiotic treatment, auto-immune disorders or cancers
Aims: We report the case of a patient who developed an aFVi responsible for two life-threatening hemorrhagic episodes, who was successfully treated after immunosuppressive therapy.
Methods: PT, aPTT, fibrinogen, FV were measured on a STAR-Max (Stago) according to the manufacturer's instructions. The aFVi level was measured using the modified Bethesda method (BU). The level of specific anti-FV IgG was measured using an in-house ELISA.
Results: The patient is a 74 years-old man with comorbidity (obesity, diabetes, hypertension, dyslipidemia) with no personal or familial bleeding history. He had a chronic wound below his left knee prosthesis following a fall the year before. At that time, PT, aPTT and fibrinogen were normal. He was shortly hospitalized for a septic arthritis caused by *Citrobacter fruendii* and received ceftriaxone, cefepim and metronidazole. One month later, he was admitted for an acute hemorrhagic syndrome from the knee scar. He had prolonged PT (60/17 sec) and aPTT (> 120 sec) and elevated fibrinogen (8.1 g/l) with an isolated FV<3%. PT, aPTT and FV were not normalized on mixing studies. The aFVi was estimated at 128 UB. Using ELISA, elevated levels of IgG directed against FV and FVa were evidenced in the patient's plasma. Bleeding episode required the transfusion of 2 packed red blood cells (RBC), the use of activated prothrombin concentrates (APCC: 5000 IU/12hx2d), tranexamic acid (TXA: 1gx3/d) and intravenous immunoglobulin (IVIG: 60g/dx2d) sequentially. The development of the aFVi, characterized as a strongly positive anti-FV IgG, was attributed to the association of antibiotics treatment, surgery, and sepsis, no autoimmune nor lymphoproliferative disorders or neoplasms were found. An immunosuppressive therapy was started, using mycophenolic acid (1000 mg/12h), to eradicate the aFVi. Unfortunately, 3 months later, he was admitted for a septic shock due to a purulent collection caused by *Proteus mirabilis*, requiring surgical and antibiotic treatment (tazocilline). Surgery was required and the combination of APCC and TXA was prescribed because of the persistence of the aFVi at 114 UB. Unfortunately, he developed a severe bleeding episode requiring transfusion (5 RBC) and resuscitation after a 30 sec cardiac arrest with signs of septic and hemorrhagic shock. Bleeding was eventually controlled with aggressive hemostatic replacement (APCC, TXA) and local pressure bandage. The patient was discharged 3 weeks later, with antibiotics (levofloxacin) and mycophenolic acid. The following 12 months, there was an increase of FV from <3% to 12-32-47%. Concurrently, aFVi level drop from 128 UB, to 144-28-1 and <0.4 UB. Mycophenolic acid was withdrawn after 12 months, FV was at 60%. Two years later, the patient had a tooth extraction that went well without any replacement therapy.
Summary/Conclusion: Acquired aFVi are rare and can lead to severe bleeding tendency, including life-threatening hemorrhage. Hemostatic therapeutic strategy is not clear to date but is based on the use of by-passing agent. Immunosuppressive drugs are often proposed to eradicate the inhibitors. The question remains of the benefit-risk ratio of this therapy, which exposes patient to a risk of severe infections.

P-010

Impact of Heparin neutralization on Thrombin Generation Assay

M. Didembourg [1 2,*], J. Douxfils [1 2 3], A. Carlo [4], L. Morimont [1 2 3]
[1]Pharmacy Department, University of Namur, [2]Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostatis Centre (NTHC), [3]QUALIblood sa, Namur, Belgium, [4]Diagnostica Stago, Asnière-sur-Seine, France

Background: Heparins are sulphated glycosaminoglycans mainly used as an anticoagulant therapy to prevent venous thromboembolism. However, anticoagulants as heparinoids are known to impact coagulation tests by increasing clotting times or reducing thrombin generation in the test sample. Therefore, to ensure an unbiased evaluation of the patient coagulability status, neutralization of the anticoagulant effect of heparinoids is necessary. This can be achieved by using neutralizing agents such as hexadimethrine bromide, also known as polybrene, or Hepa-remove® (5-Diagnostics, Switzerland), a newly developed synthetic tripeptide.
Aims: This study aimed to assess the capacity of polybrene and Hepa-remove® to neutralize the anticoagulant effect of enoxaparin on thrombin generation assay (TGA) and to evaluate their impact on this test.
Methods: Low molecular weight heparin was spiked in a normal pooled plasma composed of 50 healthy individuals at different concentrations, 0 [=sterile saline solution], 0.2, 0.4 and 0.6 UI/mL. TGA was assessed on the ST Genesia system using STG-ThromboScreen -TM (Stago, France) as triggering reagent. Polybrene and Hepa-remove® were added into the triggering reagent upon reconstitution in order to obtain final plasma concentrations of 0.025mg/mL and 0.166mg/mL, respectively. Analyses were performed in duplicate and assessed in three independent runs. The impact of these neutralizing agents on TG was assessed by comparing plasma (without heparin) treated with and without Polybrene or Hepa-remove®. The neutralizing capacity was measured as the percentages of change from baseline in which the plasma without heparin treated with Polybrene or Hepa-remove® was considered as the baseline condition.
Results: No TGA parameter was impacted by the presence of polybrene at the concentration tested in this study. On the opposite, Hepa-remove® significantly reduced the Lag Time (LT) and the Time-to-peak (Ttp) (p-value< 0.05). Polybrene permits to fully neutralize the effect of enoxaparin. The residual Endogenous Thrombin Potential (ETP), mean Velocity Rate Index (mVRI) and Peak Height (PH) after treatment by polybrene were increased by 5.1%, 8.6% and 6.6% compared to baseline TG on NPP spiked with saline, even at the highest enoxaparin concentration tested (i.e., 0.6 IU/mL). The LT decreased by 6.4%. On the other hand, the Ttp decreased by 3.9% which was significant compared to the baseline (p-value = 0.048). The Hepa-remove® permitted the recovery of the baseline ETP, mVRI and PH values regardless of the heparin concentration. However, as it impacted the LT and Ttp even in absence of heparin, it does not permit to recover the baseline value of these parameters in absence of enoxaparin.
Summary/Conclusion: This study showed that polybrene had no impact on TG under our assay conditions; while the Hepa-remove® impacted the LT and Ttp. Regarding their neutralizing capacity, polybrene was able to fully neutralize enoxaparin up to a concentration of 0.6 UI/mL with no impact on TG. The Hepa-remove® showed acceptable neutralizing capacity, but impacted some TG parameters independently to its neutralizing capacity on enoxaparin.

P-011

Tolerance induction towards FVIII in Haemophilia A patients through CAR-transduced Tregs

S. Scatigna*, A. Schmidt [1], C. Königs [1]
[1]University Hospital Frankfurt am Main, Frankfurt am Main, Germany

Background: Haemophilia A (HA) is a severe X-linked bleeding disorder characterized by a deficiency of blood coagulation factor VIII (FVIII). To restore coagulation, patients can be treated by protein replacement therapy but one third of severe HA patients develop antibodies to FVIII referred to as inhibitors. Thus, it is important to develop an effective tolerogenic therapy to prevent and reverse inhibitor formation. The immune response to FVIII therapy can be reduced by regulatory T cells (Tregs) that have a naturally suppressive function. Indeed, recently it has been shown that FVIII-specific second generation human chimeric antigen receptor (CAR) transduced Tregs are able to inhibit the activation of FVIII-specific helper T cells *in vitro*, resulting in the inhibition of differentiation of murine FVIII-specific memory B cells into antibody-secreting cells. In this study we are aiming to design and characterise different third generation CAR constructs designed for Tregs that may inhibit and/or reverse the anti-FVIII immune response.
Aims: The main aim of this study is to induce tolerance towards FVIII in HA patients through third generation FVIII-specific CAR Tregs. For this, we aim to decipher essential criteria to design/generate FVIII-specific CAR constructs able to suppress humoral and cellular responses in vitro and in vivo, combining FVIII-specific scFvs isolated from phage display libraries with specific intracellular domains.
Methods: The designed CAR constructs are ligated into lentiviral vectors and used to transduce naïve T cells or Tregs isolated from human peripheral blood cells of healthy donors. Cells are characterized following transduction and FVIII stimulation to identify CAR parameters associated with a stable T cell phenotype and a robust FVIII-specific response.
Results: *In vitro* and *in vivo* experiments are conducted to decipher the therapeutic potential of CAR-transduced Tregs with different *properties.* Essential parameters, including the need for the presence of co-stimulatory CAR domains or the required affinity of the scFv, will be deciphered. The mechanisms underlying the suppression of effector T cells by CAR-transduced Tregs will also be validated, with a focus on cytokine profiles, expression of costimulatory receptors and markers of T cell activation and exhaustion.
Summary/Conclusion: Third Generation FVIII CAR Tregs may effectively suppress anti-FVIII immune response controlling FVIII inhibitor formation and restoring tolerance towards FVIII. This will hopefully lead the way to the translation of CAR-transduced Tregs to HA patients and other types of disease.

P-013

Pooled real-world data of rVIII-SingleChain compared with standard- and long-acting FVIII products for prophylaxis of haemophilia A in Germany, Italy, and the United States

M. Olivieri [1], M. Simpson [2], S. Yan [3,], J. Fedorovsky [4], X. Zhang [3], R. Tomic [5*], K. Pinachyan [6], M. E. Mancuso [7]
[1]Pediatric Thrombosis and Hemostasis Unit, Pediatric Hemophilia Centre, Dr. von Hauner Children´s Hospital, LMU Munich, Munich, Germany, [2]Rush Hemophilia and Thrombophilia Center, Rush University Medical Center, Chicago, IL, [3]CSL Behring, King of Prussia, PA, [4]Adivo Associates, San Francisco, CA, United States, [5]CSL Behring, [6] CSL Behring, Mechelen, Belgium, [7]Center for Thrombosis and Hemorrhagic Diseases, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy

Background: Different technologies have been used to modify factor VIII (FVIII) to extend dosing intervals. Several standard- (SA) and long-acting (LA) products, including rVIII-SingleChain, are available to treat patients with haemophilia A (HA).
Aims: To analyse real-world data on rVIII-SingleChain prophylaxis in HA compared with two SA FVIII products and one LA FVIII product in Germany, Italy, and the United States (US).
Methods: De-identified patient chart data were collected from treatment centres in Germany, Italy, and the US and combined for rVIII-SingleChain, two SA FVIII products (octocog alfa and BAY 81-8973) and one LA rFVIII (rFVIIIFc). Patients on each product for ≥12 weeks were included in the analysis. Descriptive statistics were summarized for key variables. Regression analyses were conducted for FVIII consumption, annualised bleed rate (ABR), annualised spontaneous bleed rate (AsBR) and corresponding percentages of patients with zero bleeds, adjusting for available potential covariates.
Results: Analysis included 616 patients (rVIII-SingleChain, n=129; octocog alfa, n=181; BAY 81-8973, n=147; rFVIIIFc, n=159). Mean age was 30.5, 28.8, 31.0, and 30.0 years and percentage of patients with severe HA was 65.9%, 69.6%, 68.0%, and 73.6% for rVIII-SingleChain, octocog alfa, BAY 81-8973, and rFVIIIFc, respectively. The proportion of patients ≥12 years was 89.1%, 83.4%, 82.3%, and 85.5%, respectively. Differences in ABR and AsBR were not significant ($p>0.05$ for all comparisons) among products. In all patients the percentage with zero bleeds was significantly higher for rVIII-SingleChain (59.7%) vs octocog alfa (45.3%, $p<0.001$) and BAY 81-8973 (44.9%, $p=0.003$), and was similar to rFVIIIFc (62.3%, $p=0.916$). In patients ≥12 years the percentage with zero bleeds was significantly higher for rVIII-SingleChain (58.3%) vs octocog alfa (45.0%, $p=0.002$) and BAY 81-8973 (42.1%, $p=0.006$), and was comparable to rFVIIIFc (61.0%, $p=0.967$). Results were similar for patients with severe HA (all or ≥12 years). In all patients the percentage with AsBR=0 was significantly higher for rVIII-SingleChain (76.0%) vs octocog alfa (68.5%, $p=0.025$), and was comparable for rVIII-SingleChain vs BAY 81-8973 (73.5%, $p=0.529$) and rFVIIIFc (77.4%, $p=0.976$); results were similar in patients with severe HA. Similar numerical trends hold for patients ≥12 years (all or severe), although differences were not significant. Percentage of all patients who infused ≤2 times a week was 65.9%, 25.4%, 40.1%, and 75.5% for rVIII-SingleChain, octocog alfa, BAY 81-8973, and rFVIIIFc, respectively. Corresponding values in patients ≥12 years were 65.2%, 29.1%, 36.4%, and 75.0%. In all patients, rVIII-SingleChain showed lowest consumption (overall $p<0.001$) at 83.0 (mean IU/kg/week) vs 108.6 (octocog alfa, $p<0.001$), 104.3 (BAY 81-8973, $p=0.001$), and 96.9 (rFVIIIFc, $p=0.055$). Results were similar (overall $p<0.001$) in patients ≥12 years, with rVIII-SingleChain at 81.6 vs 97.5 (octocog alfa, $p<0.001$), 96.2 (BAY 81-8973, $p=0.001$), and 88.6 (rFVIIIFc, $p=0.119$). Similar trends were observed in all patients with severe HA (92.8, 120.0, 113.4, 104.9, respectively) and in those ≥12 years with severe HA (mean IU/kg/week 92.0, 105.5 102.2, 95.3, respectively).
Summary/Conclusion: These data demonstrate that prophylaxis with rVIII-SingleChain may provide improved bleed protection, less frequent dosing, and lower consumption compared with the two SA products, and it is comparable to the LA product.

P-014

Effectiveness of FVIII-poor plasma-derived von Willebrand factor concentrate (WILFACTIN®) in patients with von Willebrand disease who underwent surgery

L. Rugeri [1,*], R. Benoit [2]
[1]Service de Gynécologie Obstétrique Croix Rousse, Hospices Civils de Lyon, Lyon, [2]Laboratoire Hématologie Biologique, CHRU , Nancy, France

Background: Patients with symptomatic forms of von Willebrand disease (VWD) require specific treatments during high bleeding-risk situations such as surgical procedures. Treatment strategies have to be adapted for each patient, depending of the type of VWD, the plasma clotting factor activity of von Willebrand factor (VWF) and of factor VIII (FVIII), the bleeding risk associated with the surgical procedure, and the comorbidities.
Aims: To evaluate the efficacy of modalities of treatment with a FVIII-poor plasma-derived VWF concentrate (WILFACTIN®) for patients with VWD who underwent surgery.
Methods: This observational retrospective single-center study was conducted in the Hemophiliac care center of Lyon (Hospices Civils de Lyon, Lyon, France). VWD patients who underwent elective surgical procedures between 2003 and 2020 for which treatment with WILFACTIN® (LFB-Biomedicaments, France) was required were analyzed. The preoperative loading dose, the total dose and the duration of treatment were analyzed for both major and minor surgeries. And when available, residual VWF:RCo and FVIII:C levels were collected.
Results: A total of 63 patients who underwent 87 surgical procedures (n=48 major and n=39 minor procedures) were included. There were 41 (65%) women, the median (range) age at surgery was 47 (1 – 80) years. There were 18 (28.5%) patients with type 1 VWD, 40 (57.1%) with type 2 VWD (17 type 2A, 9 type 2B, 4 type 2M, 4 type 2N, 3 type 2B/2N, and 3 with undetermined VWD subtype), and 5 (7.9%) patients for whom the subtype had not been determined yet. In 72% (63/87) of surgical procedures, the preoperative infusion was WILFACTIN® alone, without FVIII. For major procedures, the median (range) preoperative loading dose was 50 (35 – 67) IU VWF:RCo.kg^{-1}, the median (range) total dose was 190 (37 – 897) IU VWF:RCo.kg^{-1}, and the median (range) duration of treatment was 2 (1 – 15) days. For minor procedures, the median (range) preoperative loading dose was 50 (40 – 60) IU VWF:RCo.kg^{-1}, the median (range) total dose was 163 (38 – 412) IU VWF:RCo.kg^{-1}, and the median (range) duration of treatment was 2 (1 – 10) days. Plasma VWF:RCo and FVIII:C levels were monitored after 35 and 36 surgeries, respectively. During the first 2 days after surgical procedures, the median (range) residual VWF:RCo levels were 70 (32 – 220) IU.dL^{-1} for major procedures and 74 (18 - 150) IU.dL^{-1} for minor procedures; the FVIII:C levels were 99 (39 - 150) IU.dL^{-1} for major procedures, and 97 (80 – 150) IU.dL^{-1} for minor procedures. Only 2 hemorrhagic complications were reported after surgical procedures. No thrombotic event associated with WILFACTIN® was observed in this study.
Summary/Conclusion: These results suggest that WILFACTIN® is effective and safe for the perioperative management of patients with VWD, despite a shorter duration of treatment than recommended.

P-016

Plasma thrombin generation in the presence of TIX-5 may contribute significantly to a prediction model for major bleeding in patients on VKA anticoagulant therapy

A. Maag, H. Peter-Sengers [1], M. Bos [2], T. van der Poll [1], N. van Rein [3], C. van 't Veer [1,*]
[1]Amsterdam University Medical Centers - AMC, Amsterdam, [2]Division of Thrombosis and Hemostasis, [3] Division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, Netherlands

Background: Oral anticoagulant therapy comes at the cost of a significant bleeding risk. However, why certain patients bleed during oral anticoagulant therapy is largely unknown and hard to predict. As of today, there are only a few laboratory measurements used to predict major bleeding. Recently, we observed that the lagtime of tissue factor (TF)-initiated plasma thrombin generation analyzed with Calibrated Automated Thrombography (CAT) was prolonged in patients with major bleeding on vitamin K antagonist (VKA) therapy compared to controls that did not bleed on VKAs. This difference was more pronounced when an inhibitor of the factor Xa (FXa)-mediated activation of factor V (FV), TIX-5, was added to the thrombin generation reaction. The latter indicated a greater dependence of thrombin generation on FV activation by FXa in patients with major bleeding in the BLEED study, a cohort especially powered to find new biomarkers of major bleeding during VKA therapy.
Aims: Here we aimed to investigate the predictive capability for major bleeding of CAT parameters obtained in the absence and presence of TIX-5 in comparison to, or in combination with, other biomarkers and clinical parameters.
Methods: We estimated hazard rations (HRs) and 95% confidence intervals (CIs) by means of Cox regression models. We employed a modelling strategy to first conduct univariable analyses of a total of 15 biomarkers which include the CAT parameters measured in the absence and presence of TIX-5 and the TIX-5/vehicle ratio of the particular CAT parameter.
Results: Univariable Cox regression analyses showed highest predictive value among the laboratory measures for thrombin generation lagtime in the presence of TIX-5 (TIX-5 lagtime per 25% increase, (HR:1.10,95%CI:1.05-1.16,p=0.0002) and TFPIα (HR 1.12, 95%CI 1.05-1.19, p=0.0005), which remained independently associated with major bleeding after backwards elimination: TIX-5 lagtime (HR:1.11,95%CI:1.05-1.17,p=0.0001); TFPIα (HR:1.13, 95%CI:1.06-1.20,p=0.0002). The addition of TIX-5 lagtime and TFPIα to the clinical parameters improved the model significantly ($X^2(1)=5.1$, p=0.0248).
Summary/Conclusion: In conclusion, we established predictive value of the lagtime of thrombin generation measured in the presence of TIX-5 for the risk of a major bleeding of patients on VKA therapy, further indicating that the activation of FV by FXa may be involved in the bleeding risk provoked by this anticoagulant therapy.

P-017

Structural variants in FXI deficiency: diagnosis, characterization by nanopore sequencing and mechanisms of formation.

B. De La Morena-Barrio*, A. Palomo [1], A. Rubio [2], J. Padilla [2], L. Martin [3], R. Cifuentes [2], C. Bravo-Pérez [2], A. Miñano [2], V. Vicente [2], F. Vidal [4], J. Corral [2], M. de la Morena-Barrio [2]
[1]Servicio de Hematología y Hemoterapia del centro Materno-Infantil del Hospital Regional Universitario Carlos de Haya, Málaga, [2]Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER, Murcia. Spain., Murcia, [3]Unitat de Genòmica de Malalties Complexes. IIB-Sant Pau. , [4]Laboratori de Coagulopaties Congènites, Banc de Sang i Teixits (BST), Barcelona, Spain. Medicina Transfusional, Vall d'Hebron Institut de Recerca, Universitat Autònoma de Barcelona (VHIR-UAB), Barcelona, Spain., Barcelona, Spain

Background: FXI deficiency has been considered a rare disorder in Caucasians, but probably it is underestimated due to the mild clinical manifestations and the difficult diagnosis, especially by aPTT screening and in patients with moderate deficiency. The molecular diagnosis, normally done by sequencing the exons of *F11* gene (4q35.2), has identified up to 280 variants, mostly SNVs or Indels. Only 3 cases with structural variants (SV) have been reported worldwide: two whole gene deletions and one partial deletion.
Aims: The objective of this study is the identification and characterization of new SV causing FXI deficiency.
Methods: We studied a cohort of 272 patients with FXI deficiency from 105 families identified by prolonged aPTT. Identification of SVs was done by MLPA in cases with negative results by whole gene sequencing. The characterization of the extension and breakpoint resolution of SVs was obtained by long-read sequencing of genomic DNA by nanopore sequencing using real-time enrichment methods in a MinION device. The duplication was validated by Long-range PCR and NGS sequencing. Paternity was evaluated by analysis of 8 STRs. Mosaicism was studied by high depth NGS sequencing of the *F11* gene by PGM and MinION devices. The FXI deficiency was characterized by coagulometric (FXI:C) and immunologic (Western Blot) methods.
Results: Two cases with FXI deficiency, both CRM-, carried SVs affecting one allele that were identified by MLPA. P1 had a partial duplication of exons 8 and 9 in intron 4. The duplication segregated with FXI deficiency in 5 members of the family with moderate FXI deficiency (FXI:C=44.3%). P2, a child with severe FXI deficiency (FXI:C=5%), had a complete deletion of *F11* gene in one allele, and a deleterious variant of maternal origin in the other allele (p.Phe295Cys), detected by sanger sequencing. His father and sister had normal FXI levels and no *F11* defects. STR analysis confirmed paternity. Mosaicism was discarded by Deep NGS analysis of the p.Phe295Cys mutation in 2 tissues of the patient and in the father discarded mosaicism and supported a germline transmission. Nanopore sequencing allowed the identification of the breakpoints in both cases, identifying repetitive elements (Alu) implied on them. This study also determined the extension of the deletion: 7 MB affecting 142 genes. Despite the high number of genes affected, only FXI deficiency and moderate hemorrhagic signs were the only clinical phenotype observed in the patient.
Summary/Conclusion: This study shows the heterogeneity in type and extension of SVs affecting *F11* that cause FXI deficiency, always CRM-. These SVs are probably generated by recombination of repetitive elements and may be relatively frequent according to the incidence in our cohort, (2%), and the detection of a de novo deletion, which probably occurred during the spermatogenesis. All these data support the screening of SVs affecting *F11* in FXI deficiency. MLPA is able to detect these SVs, but nanopore sequencing emerges as the best method to identify and characterize at nucleotide level resolution these SVs, determining the extension, type and the breakpoint.
Funding: Fundación Séneca 20261/FPI/17, FundaciónSéneca-19873/GERM/15;ISCIII-CM20/00094; SETH Emergentes 2018; FI19/00048

P-018

Genotype-phenotype relations in hereditary hemorrhagic telangiectasia

Z. Bereczky [1,*], R. Gindele [1], G. Pfliegler [2], G. Balogh [1], Z. Szabó [1], T. Major [3]
[1]Department of Laboratory Medicine, UNIVERSITY OF DEBRECEN, FACULTY OF MEDICINE, DIVISION OF CLINICAL LABORATORY SCIENCE, [2]Center of Expertise for Rare Diseases3, University of Debrecen, Clinical Center, [3]Division of Otorhinolaryngology and Head and Neck Surgery, University of Debrecen, Kenézy Campus, Debrecen, Hungary

Background: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant multisystemic vascular disease with a prevalence of 1:5,000-1:10,000. Diagnosis is based on clinical Curacao criteria. Approximately 85% of HHT cases have heterozygous family-specific mutations in the *ENG* or *ACVRL1* genes.
Aims: We present our results of systematic genetic and clinical screening of Hungarian HHT families in a 10-year period.
Methods: Probands were diagnosed by otorhinolaryngological examination, visceral arteriovenous malformation screening and genetic testing for *ENG/ACVRL1/SMAD4/RASA1/GDF2* genes. At-risk individuals (family members) underwent clinical examination and family-specific mutation testing.
Results: Eighteen *ENG*, 16 *ACVRL1* and 1 SMAD4 mutations were identified in 21, 26 and 1 families, respectively, with 117 individuals carrying the family-specific mutation (92 with definite, 20 with suspected and 5 with unlikely HHT by Curacao criteria). We found a novel mutation in *RASA1* gene in a patient with only 1 Curacao criterion present.
Summary/Conclusion: As no laboratory assay is available in HHT, genetic testing has a major role. It is important in the confirmation of HHT in young asymptomatic HHT family members. Moreover there are mutations with specific clinical consequences (eg. *ACVRL1* and *MADH4* can associate with pulmonary hypertension, *RASA1* may associate with basal cell carcinoma). Identifying founder effects simplify the genetic diagnosis in the corresponding geographic region.

P-020

The (im)possibility of diagnosing PAI-1-deficiency based on lab values in childhood

M. van der Meeren [1 2,*], E. Talsma [3 4], S. Lako [5], D. Meijer [6], M. te Loo [1]
[1]Pediatric Haematology, Amalia Children's Hospital, Radboudumc, Nijmegen, [2]Internal Medicine, Rijnstate Hospital, Arnhem, [3]Pediatrics, Alrijne Hospital, Leiderdorp, [4]Pediatric Heamatology, Amalia Children's hospital, Radboudumc, [5]Anesthesiology, [6]Laboratory Medicine, Laboratory of Haematology, Radboudumc, Nijmegen, Netherlands

Background: Plasminogen activator inhibitor type 1 (PAI-1) is an important regulator of fibrinolysis. Patients with a PAI-1-deficiency may present with mild to severe bleeding symptoms. PAI-1-deficiency cannot be cured and management includes avoidance of contact sports and anti-fibrinolytic medication before surgery or after injury. The diagnosis PAI-1-deficiency is based on clinical symptoms, absent levels of PAI-1 activity and antigen, and/or genetic testing. The absence of normal values of PAI-1 antigen/activity in pediatric age makes it almost impossible to diagnose PAI-1 deficiency in childhood.
Aims: The primary objective of this study was to determine normal values of PAI-1 activity and antigen in children. Based on these values we re-assessed patients previously diagnosed with PAI-1-deficiency to evaluate whether this diagnosis was accurate.
Methods: Children visiting the hospital for a clinical procedure with need for an intravenous catheter were screened for this study. Inclusion criteria were age 0 to 12 years and a negative pediatric bleeding questionnaire, in which bleeding symptoms were assessed prior to their hospital visit. Informed consent was obtained from parents or legal representatives of all included children. Blood samples were collected early in the morning, for it is known that PAI-1 values peak in the morning due to the human circadian rhythm. Samples were subsequently analyzed for PT, APTT, PAI-1 activity and antigen.
Results: In total 299 children were approached. Of them, 218 patients were excluded. Most common reasons for exclusion were inability to make contact before the hospital visit (n=89), failed sample collection (n=35) or no informed consent (n=22). Finally, 81 children were included to analyze normal values. Median age was 5.5 years (range 0.3-11.7) and 55.6% were male. Values of PT and APTT corresponded with the age of the patients (median PT 14.8 seconds and APTT 32.4 seconds). PAI-1 activity and antigen was undetectable in respectively 83% and 15% of participants. Median level of PAI-1 antigen was 6.3 ng/L. A positive correlation between PAI-1 activity and PAI-1 antigen was found (p=0.002). PAI-1 levels tended to increase with age, but this did not reach statistical significance. Of the 17 patients previously diagnosed with PAI-1-deficiency (mean age of 4.6 years), six patients met the diagnostic criteria for PAI-1 deficiency. Interestingly, none of them had severe bleeding symptoms. In eight out of 17 patients follow-up values of PAI-1 were available. In all but one patient PAI-1 levels increased with age and were in the normal range, indicating that these patients had no PAI-1-deficiency (Spearmans' rho=0.711, p=0.0006).
Summary/Conclusion: Our study shows, that at young age low values of PAI-1 activity/antigen are common. Furthermore, increasing age is accompanied with increasing values of PAI-1 values. Based on the data of this study we postulate that the diagnosis PAI-I deficiency cannot be made at young age based on laboratory testing. Therefore, we advise to postpone laboratory testing until older age (preferentially above 12 years), although genetic testing, if the pathogenetic variant is known, can be performed at younger age. When there is a higher suspicion of PAI-1-deficiency, a more defensive treatment strategy seems appropriate.

P-021

Role of next generation sequencing in identifying hemorrhagic disorders and its use in differential diagnosis

Z. Bereczky [1,*], R. Gindele [1], A. Kerényi [2], J. Kállai [1], Z. Bagoly [1], Z. Szabó [1], J. Kappelmayer [2]
[1]Department of Laboratory Medicine, UNIVERSITY OF DEBRECEN, FACULTY OF MEDICINE, DIVISION OF CLINICAL LABORATORY SCIENCE, [2]Department of Laboratory Medicine, UNIVERSITY OF DEBRECEN, FACULTY OF MEDICINE, Debrecen, Hungary

Background: Diagnosis of rare bleeding disorders is challenging and there are several differential diagnostics issues. Molecular genetic studies including next-generation sequencing (NGS) are useful tools to overcome these problems.
Aims: Aim of our work was to provide correct diagnosis by NGS for cases with uncertainty in laboratory diagnosis.
Methods: Two large gene panels were constructed, one covered 14 genes (NGS panel 1) selected on the basis of unresolved differential diagnostic issues in von Willebrand disease (vWD), fibrinogen disorders. and in hereditary hemorrhagic telangiectasia (HHT). The second one (NGS panel 2) covered 14 candidate genes in platelet secretion disorders. Libraries were created by QIAseq targeted DNA custom panel, the MiSeq System and Sanger sequencing were used for NGS and for validation, respectively. N=96 patients were recruited into each NGS study. Clinical data were collected and detailed laboratory studies were performed before patient-selection.
Results: The mutation detection rate in NGS panel 1 was almost 100%, and the causative mutations (n=28) were found in vWD (4 patients were re-classified as hemophilia A based on our results). N=10 mutations were identified in fibrinogen disorders helping to predict clinical phenotype and two rare variants of HHT were explored. The mutation detection rate in NGS panel 2 -as expected- was lower, n=37 variants were found, some of them with uncertain significance.
Summary/Conclusion: Genetic testing provides a higher-level evidence for diagnosing bleeding disorders, however in case of novel variants pathogenicity should be carefully determined before providing interpretative result.

P-022

Social participation is reduced in type 3 Von Willebrand disease patients

E. K. Kempers [1,*], C. B. van Kwawegen [1], K. P. van Galen [2], J. G. van der Bom [3 4], M. H. Cnossen [5], K. Meijer [6], K. Fijnvandraat [7 8], J. Eikenboom [9 10], F. Atiq [1], F. W. Leebeek [1]
[1]Department of Hematology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, [2]Van Creveldkliniek, University Medical Center Utrecht, Utrecht University, Utrecht, [3]Department of Clinical Epidemiology, Leiden University Medical Center, [4]Jon J van Rood Center for Clinical Transfusion Medicine, Sanquin Research, Leiden, [5]Department of Pediatric Hematology, Erasmus MC-Sophia Children's Hospital, University Medical Center Rotterdam, Rotterdam, [6]Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, [7]Pediatric Hematology, Amsterdam UMC, University of Amsterdam, Emma Children's Hospital, [8]Department of Molecular Cellular Hemostasis, Sanquin Research, Amsterdam, [9]Department of Internal Medicine, Division of Thrombosis and Hemostasis, [10]Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Netherlands

Background: Patients with bleeding disorders may experience limitations in their participation in school or work. The impact of hemophilia on social participation has previously been demonstrated, however, it is unknown yet whether Von Willebrand disease (VWD) also affects social participation of patients.

Aims: The aim of this study was to examine the social participation of a large cohort of VWD patients in the Netherlands and to compare this with the general Dutch population. In addition, we aimed to identify factors associated with reduced social participation in VWD patients.
Methods: Patients participated in the nationwide cross-sectional “Willebrand in the Netherlands” (WiN) study. Participants completed a questionnaire, containing questions on educational level, absenteeism from school or work, occupational disability, and a self-administered version of the condensed Tosetto bleeding score. Data on the general population were provided by the Central Bureau of Statistics Netherlands (CBS).
Results: We included 788 VWD patients, of whom 136 children aged <16 years. In total, 470 patients had type 1, 292 had type 2, and 26 had type 3 VWD. The proportion of patients aged ≥16 years with a low educational level was higher in type 3 VWD (52.9%) compared to type 1 (40.2%) and type 2 VWD (36.8%) and the general population (36.4%) (p=0.005). Moreover, in patients aged ≥16 years the number of absent days in the year prior to inclusion in the study differed significantly between the three VWD types, namely median 0.0 days (interquartile ranges [0.0, 2.0] and [0.0, 4.0]) among type 1 and type 2 VWD patients, respectively, and median 4.0 days [0.0, 10.0] among type 3 VWD patients (p=0.011). In negative binomial regression analyses, the occurrence of bleeding episodes requiring treatment in the year preceding inclusion in the study was significantly associated with the number of absent days among patients aged ≥16 years (IRR=6.7, 95%CI 3.5-13.2). Among children aged <16 years, the presence of at least one comorbidity was significantly associated with the number of absent days in the year prior to inclusion in the study (IRR=3.7, 95%CI 1.7, 9.3). Furthermore, in multivariable logistic regression analysis, we found that a higher total bleeding score (OR=1.07, 95%CI 1.04, 1.11), older age (OR=1.03, 95%CI 1.01-1.05) and the presence of at least one comorbidity (OR=3.33, 95%CI 1.88, 6.12) were significantly associated with occupational disability in patients aged ≥16 years.
Summary/Conclusion: Our study shows that social participation and educational level is lower in type 3 VWD and patients with a more severe bleeding phenotype. Total bleeding score and bleeding requiring treatment in the year prior to inclusion were important associated factors.

P-023

Discrepancies on VWF activity results between techniques due to genetic variants in the A1 domain: risk of misdiagnosis of VWD

F. Lassalle [1,*], E. Jeanpierre [1], C. Zawadzki [1], C. Pouymayou [2], S. Castet [3], Y. Huguenin [3], R. Marlu [4], A. Voyer [5], P. Chamouni [6], C. Lavenu-Bombled [7], S. Moyrand [8], J. Goudemand [1], S. Susen [1]
[1]Institut d'Hématologie - Transfusion, CHU Lille, Lille, [2]Service d'hématologie biologique, Hôpital de la Timone, Marseille, [3]Service d'hématologie biologique, CHU Bordeaux Pellegrin, Bordeaux, [4]Laboratoire d'hémostase, CHU Grenoble, Grenoble, [5]Laboratoire d'hémostase, CHU Amiens, Amiens, [6]Service d'hématologie biologique, CHU Rouen, Rouen, [7]Service d'hématologie, Hôpital Bicêtre, Paris, [8]Laboratoire d'hématologie, CHU Poitiers, Poitiers, France

Background: The diagnosis of von Willebrand disease (VWD) relies on measurements of von Willebrand factor antigen (VWF:Ag) and functional activity (VWF:Act). Measuring VWF:Act is based on the quantification of VWF binding to platelets through GPIbα, with or without using ristocetin. Some polymorphisms are known to cause discrepancies between the different techniques, as the variant D1472H that falsely decreases the ristocetin cofactor activity (VWF:RCo) by interacting with ristocetin.
Aims: To identify if other variants located in the A1 domain of VWF (containing the binding sites for GPIbα) can interfere with the different techniques for measuring VWF:Act.
Methods: After being notified that 2 VWD type 2M patients (one carrying E1359K and the other one V1360A in exon 28 of *VWF*) completely normalized their ratio VWF:Act/VWF:Ag measured with the Acustar® VWF:GPIbR assay (using ristocetin) comparing to the VWF:RCo technique, we decided to retest all the patients from the database of the French Reference Center for VWD with the same variants. We tested in total 15 patients and compared the results obtained with the Acustar® assay and the Innovance® VWF:GPIbM assay (no ristocetin).
Results: All the 15 patients had a low ratio with the Innovance® assay (mean = 0.20, interval range = <0.1–0.37). Among them, 11 had a completely normal ratio with the Acustar® assay (mean = 0.86 interval range = 0.70–1.21). Two patients had a very low ratio (<0.1) with the 2 techniques including one who has a second variant responsible for VWD type 3. The variants E1359K and V1360A, located successively in the A1 domain of VWF, might interfere with the Acustar® assay, either with the recombinant GPIb or with the chemiluminescent detection, rendering it insensitive to the binding defect.
Summary/Conclusion: This study is another proof about the complexity to measure VWF:Act and to choose the right technique. Due to their VWF genetics, the diagnosis of VWD for these patients could have been missed if only the Acustar® assay has been performed.

P-025

Safety of oral anticoagulation in Caucasian patients with FXI deficiency

C. Bravo-Perez [1,*], M. J. Serna [2], J. Esteban [2], E. Fernandez-Mellid [3], E. Fontanes-Trabazo [3], A. Lorenzo [3], M. Calviño [3], A. Miñano [1], J. Padilla [1], V. Roldan [1], V. Vicente [1], J. Corral [1], M. E. de la Morena-Barrio [1]
[1]Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER, Murcia, [2]Servicio de Hematología, Hospital Virgen del Castillo, Yecla, [3]Servicio de Hematología, Hospital Universitario Lucus Augusti, Lugo, Spain

Background: The bleeding phenotype of FXI deficiency is unpredictable. Bleeding is usually mild, and mostly occurs after injury and/or in highly fibrinolytic tissues. Although FXI deficiency renders antithrombotic protection, some patients might eventually develop thrombotic complications during lifetime, then requiring anticoagulant therapy. There is almost no evidence on the bleeding risk in this scenario.
Aims: We aim to investigate the safety of anticoagulation and its management in patients with FXI deficiency.
Methods: Retrospective, descriptive analysis of our cohort of 269 Caucasian subjects with congenital FXI deficiency collected from two Spanish centers (Murcia: 238, Lugo: 31, 1995-2021). FXI plasma activity and antigen levels were characterized by functional and immunological methods. Sequencing of the exons and flanking regions of the *F11* gene by Sanger or next-generation sequencing and analysis of gross gene variations by MLPA were carried out in cases displaying FXI deficiency. We selected patients ≥18 y.o. with chart-based evidence of anticoagulation at any time of the study period. Subjects treated exclusively with prophylactic-dose heparin or antiplatelets were excluded.
Results: We identified 15 FXI-deficient patients treated with anticoagulant therapy. Seven patients were women (46.7%). Median age of this group was 70 y.o. (IQR: 64.5–79 y.o.). They harbored eight different *F11* gene variants: seven point mutations and one insertion (1653 bp). All patients had mild/moderate deficiency (FXI:C 20–70%). Two subjects (13.3%, 95%CI: 3.7-37.9%) had a positive history of hemorrhage before starting treatment, in both cases mild events secondary to injury. Atrial fibrillation was the main indication for anticoagulants (12/15, 80%). Almost all patients (14/15, 93.3%) started therapy with vitamin K antagonist (VKA), but four subjects were on direct oral anticoagulants (DOACs) at the end of follow-up.
Over the accumulative course of more than 1000 months of oral anticoagulant treatment, only two mild bleeding episodes in two patients (13.3%, 95%CI: 3.7-37.9%) were recorded, in both cases mild events. No major bleeding events were reported. One of the two subjects who bled after anticoagulant start had prior hemorrhagic history, and the “pre-post” characteristics of bleeding were similar in terms of localization and severity.
As regards VKA, median dose of acenocoumarol was 10.75 mg/week (IQR: 8–16 mg/week) and median TTR was 70% (IQR: 60–81%). Only two patients with moderate FXI deficiency had a TTR<65%, in both cases it was due to INR<2, and this was the reason for switching to a DOAC. VKA dosing and TTR did not differ significantly from the standards, and seemed to be unaltered by FXI deficiency.
Summary/Conclusion: We provide the largest descriptive analysis of oral anticoagulation in FXI deficiency, and the first cases receiving DOACs. Although further studies are needed, our valuable clinical observations suggest that moderate FXI deficiency does not relevantly interfere with bleeding risk or anticoagulant management.
Funding: ISCIII&FEDER: PI18/00598; CM20/00094. Fundación Séneca 19873/GERM/15

P-026

A Canadian, multi-center, retrospective, non-interventional study of clinical outcomes from early use of N9-GP compared with previous treatment in patients with hemophilia B in a real-world setting

D. Matino [1], S. Matta [2,*], A. Iorio [1], A. Keepanasseril [1], F. Germini [1], A. Caillaud [3], M. Carcao [4], J. Hews-Girard [5], E. Iserman [1], P. James [6], A. Lee [7], C. Phua [8], H. Sun [9], J. Teitel [10], M.-C. Poon [11]
[1]McMaster University, Hamilton, Canada, [2]Novo Nordisk, Anderlecht, Belgium, [3]Novo Nordisk, Mississauga, [4]The hospital for sick children, Toronto, [5]Foothilss Medical Centre, Calgary, [6]Queen's University, Kingston, [7]University of Calgary, Calgary, [8]Western University, London, [9]University of Alberta, Edmonton, [10]St Michael's Hospital, Toronto, [11]Cumming School of Medicine, Calgary, Canada

Background:
The Canadian Blood Disorders Registry (CBDR) captures data collected from hemophilia treatment centers and directly from patients. With the recent availability of nonacog beta pegol (N9-GP) in Canada, the CBDR provides a unique opportunity.
Aims: To assess real-world outcomes following N9-GP use in a clinical setting.
Methods:
This non-interventional, retrospective study used CBDR data to describe and analyze real-world outcomes in Canadian hemophilia B patients receiving N9-GP for ≥3 months in any setting (prophylaxis, on-demand treatment, treatment of breakthrough bleeding [BTB]). For comparison with previously-used products, only patients for whom data existed in CBDR for the 6-month period pre-switch to N9-GP were included.
Results:
At the data cut-off (September 30, 2019), 40 patients were included in the analysis, with a median age of 44 years. Distribution of disease severity was 2.5% mild, 40% moderate, 55% severe, and 2.5% unknown. At study start, 10 target joints were present in 5 patients. Most patients had previously received rFIXFc (55% versus 40% rFIX), with most previously receiving prophylactic treatment (85% versus 15% on-demand). No patients had present or previous inhibitor development.
During a median treatment period of 11.11 months on N9-GP, 106 BTBs were reported in 22 patients; 42% of patients reported zero bleeds. Median time from last recorded prophylactic injection to start of bleeding was 7.1 days and the mean number of injections required to treat a bleed was 1.23.
Summary/Conclusion: This is the first study to investiage treatment patterns and clinical outcomes with N9-GP in hemophilia B patients in a real-world setting. Initial data suggest improved bleeding outcomes with lower factor consumption after switching to N9-GP, regardless of whether patients previously received standard or extended half-life products.

P-027

MOREtogether: a dynamic video-based hemophilia community in Belgium

P. Maes [1,*], V. Janssens [2], A. Devos [1], P. De Smet [3], N. Colasuonno [3]
[1]Pediatrics, [2]Hematology, University Hospital Antwerp, Edegem, [3]AHVH - Hemophilia Patient Association Belgium, Anderlecht, Belgium

Background: In Belgium there are around 1267 people with hemophilia (PwH) A & B of all severities (*WFH annual global survey 2019*). To screen for potential unaddressed needs within the hemophilia community in Belgium a serie of interviews with PwH, parents, patient organization and physicians were done in 2019. Out of these interviews 3 clear needs were defined.
1. A need for additional information and education, not only towards the PwH and parents, but also towards the larger family, friends, teachers and sport trainers.
2. A connection to the hemophilia community was perceived as valuable and helped PwH and parents to communicate about and cope with their hemophilia.
3. Current communication channels that existed in Belgium (websites, email, Facebook, brochures) were not adapted and attractive to a younger target audience.
In each interview with PwH below 25 years old YouTube was mentioned as the platform to search for information. This is also reflected in the global online statistics were YouTube is the 2nd most visited website after Google and that video traffic accounts for 84% of all consumer internet traffic (*Cisco VNI forecast highlights; Hubspot global internet user survey).*
Aims: To address the 3 defined needs a video-based patient-focused project was initiated together with 3 Belgian hemophilia reference centers (UZ Antwerp, UCL St-Luc and HemoWaB), the patient organization AHVH and Bayer. The aim of this project was a partnership to co-create a dynamic, virtual hemophilia community channel where everyone can find information and share their stories related to hemophilia. This community channel is named MOREtogether, which stands for 'Move Outside, Reach Everyone Together'.
Methods: The MOREtogether channel consist of 2 parts:
1. An educational part with hemophilia experts explaining specific topics related to hemophilia. These short educational videos include metaphors and animations to increase the comprehensibility and attractiveness of the message. Here 10 topics were defined together with the physicians and patient organization ranging from bleeds and treatment to sport and travel with hemophilia.
2. A community part that includes stories from PwH and their environment via vlogs. The goal of this part is to inspire others and help them taking ownership of and living with their hemophilia.
Videos are created in both Dutch and French and are therefore useable for Belgium, The Netherlands and France. The YouTube platform provides the opportunity to act as a social medium by sharing content via different other channels and offers a tool to the hemophilia community to spread relevant information.
Results: In March 2021 the MOREtogether community channel was launched. In a period from March to June a total of 527 views have been logged across the channel with the best watched video being a patient story on kayaking with 139 views. The goal by end 2021 is to have 20 educational videos and 10 patient vlogs online with at least 1000 views across the channel. Awareness materials have been developed and will now be spread by all the partners with the ambition to stimulate the involvement of the PwH in this online community even further. The channel can be accessed via the link go.bayer.com/MOREtogether
Summary/Conclusion: MOREtogether is the first open, online hemophilia community in Belgium and shows a unique partnership and co-creation between the hemophilia reference centers, the patient organization and the industry to address clear defined community needs.

P-028

Healthcare resource utilisation associated with the management of intracranial haemorrhage in patients receiving direct oral anticoagulants: a rapid literature review

D. Bem [1], S. Schiavo [1], T. Johnson [1], J. Kirby [1], K. Cadwell [1], S. Walsh [2,*]
[1]Health Economics and Outcomes Research Ltd, Cardiff, [2]Norgine Ltd, Harefield, United Kingdom

Background: Direct oral anticoagulants (DOACs) are a class of anticoagulants prescribed for the prevention and treatment of thrombosis in several cardiovascular diseases. As with any anticoagulation therapy, adverse side effects of DOACs may result in haemorrhagic complications, including life-threatening intracranial haemorrhage (ICH). Despite a favourable efficacy and safety profile compared to older anticoagulants, patients treated with DOACs have an annual rate of ICH of 0.2-0.5% and the healthcare resource utilisation (HRU) of managing these patients is substantial.
Aims: The aim of this rapid review was to explore the HRU burden of managing major bleeding (MB) in patients prescribed DOACs. The HRU findings of the review, stratified by the site of MB with a specific focus on ICH, are presented here.
Methods: The rapid review adopted a systematic approach for searching, screening and data extraction, which was performed by one screener while excluding a risk of bias assessment. The search was conducted in Ovid Embase (2010-2021). All studies which met the pre-determined eligibility criteria were included in the review. Data extraction was completed by a single reviewer with outcomes checked for accuracy by a second reviewer. All HRU outcomes related to MB were reported. Review outputs were qualitatively summarised.
Results: Eleven studies were included in the review with five of those focusing on ICH-associated HRU in patients receiving DOACs. Of those, a retrospective observational analysis of an Italian cohort showed that among all patients hospitalised for uncontrolled severe bleeding 21.1% (40/190 patients) sustained an ICH and 5.8% (11/190 patients) experienced gastrointestinal (GI) bleeding. Mean hospital length of stay (LOS) was 17 days in the total population; however, ICH patients had a longer LOS compared with GI patients (31.8 versus 13.5 days, respectively). In addition, a retrospective cohort study in USA hospitals reported that mean [SD] LOS was 11.4 [18.7] days in patients with ICH and 8.8 [8.6] days in patients with GI bleeding. Moreover, two retrospective reviews of USA hospital trauma databases showed that the median LOS for hospitalised patients with a traumatic or spontaneous ICH ranged from 4.0 to 6.3 days and the median intensive care unit (ICU) LOS ranged from 0.9 to 2.7 days. Finally, a post-hoc analysis of interim data from a global phase III trial (RE-VERSE AD) assessed HRU in patients receiving the reversal agent idarucizumab for reversal of the DOAC dabigatran due to MB. The analysis showed a prolonged median [min, max] hospital LOS in patients with ICH (subdural: 7 [6, 71] days; deep intracerebral/subarachnoid: 9 [4, 47] days) compared to those with GI bleeding (6 [1, 24] days) or other bleeds (6.5 [1, 18] days); patients with subdural haemorrhage also stayed longer in ICU (24 [4, 44] days) compared to patients with other types of bleeds (range: 3-4 [1, 11] days).
Summary/Conclusion: These results indicate a high HRU for the management of ICH in patients prescribed DOACs. These patients incurred higher HRU for treatment than patients with GI bleeding.

P-029

Activated clotting time (ACT) can be used safely in patients treated with emicizumab

L. Capdevilla [1,*], C. Frère [2], M. Desvages [1], A. Harroche [3], C. Bally [3], R. d'Oiron [4], L. Frenzel [3], D. Borgel [1], D. Lasne [1]
[1]Laboratoire d'hematologie, Hôpital Necker-Enfants malades, [2]Service d'hematologie biologique, Hôpital Pitié-Salpétrière, [3]Service d'hematologie clinique, Hôpital Necker-Enfants malades, AP-HP, PARIS, [4]Service d'hematologie, Hôpital de Bicêtre, AP-HP, LE KREMLIN BICETRE, France

Background: Activated clotting time (ACT) is recommended to monitor unfractionated heparin (UFH) during cardiopulmonary bypass (CPB) or cardiac catheterization. Emicizumab, a new treatment for hemophilia A patients shortens coagulation times in assays based on an activated partial thromboplastin times (aPTT) and is expected to shorten ACT.
Aims: To know whether patients treated with emicizumab can benefit from ACT for heparin monitoring during cardiac surgery with CPB or during cardiac catheterization.
Methods: Citrated whole blood samples from healthy donors, patients undergoing CPB, and haemophilia A patients treated with emicizumab, were spiked with increasing concentrations of UFH (0 (baseline ACT), 1, 3, and 5 IU/mL for ACT-HR (High Range) or 0, 0.5, 1 and 2.5 UI/mL for ACT-LR (Low Range)) and/or increasing concentrations of emicizumab (0, 10, 25 and 50 µg/mL). Calcium chloride (10 mM) was added in each sample just before performing ACT in order to obtain whole blood close to the usual conditions of use. Statistical analysis were performed with GraphPad Prism Software. Results are presented as median [interquartile range].
Results: In healthy donors under baseline conditions, emicizumab didn't shorten ACT. Even at the maximum concentration of emicizumab tested (50 µg/mL in whole blood equivalent to 90 µg/mL plasma concentration when hematocrit is 45%), ACT-HR was not different with or without emicizumab (129 s [123-138] vs 136 s [115-141] respectively, n=8). Moreover, the addition of increasing concentrations of UFH resulted in a prolongation of ACT-HR regardless of the concentration of emicizumab. Similarly, the addition of increasing concentrations of UFH to whole blood from patients treated with emicizumab for more than 4 weeks induced a concentration-dependent prolongation of ACT-HR. When ACT-HR was performed with blood from patients undergoing cardiac surgery before, during and after CPB, ACT values were not different with or without 50 µg/mL emicizumab (before CPB: 157 s [115-159] vs 118 s [108-131] s; during CPB: 411 s [407-417] vs 405 s [399-452] s, after heparin neutralization by protamine: 145 s [139-153] vs 139 s [138-151] s; n=3). Experiments performed under the same conditions in healthy donors and emicizumab-treated patients with ACT-LR cartridges and lower heparin concentrations led to the same conclusion, namely that emicizumab does not alter UFH-induced ACT prolongation.
Summary/Conclusion: Our study shows that UFH increases ACT values similarly in the presence or absence of emicizumab, regardless of the cartridge used (ACT-HR or ACT-LR). Even in the absence of factor VIII, ACT values were prolonged as expected when UFH was added to the blood of patients with severe haemophilia A treated with emicizumab. Thus, the use of ACT for anticoagulation monitoring during CPB or cardiac catheterization is possible in haemophilia patients treated with emicizumab with or without factor VIII treatment.

P-030

Towards personalized care and patient empowerment: user perspectives on a personal health record in hemophilia care

M. Brands [1,*], J. Muis [2], M. Driessens [3], F. van der Meer [4 5], S. Meijer [3], F. Bellinck [3], M. de Jong [6], G. Goedhart [4 5], J. van der Bom [7], M. Beestrum [8], R. Cronin [9], M. Cnossen [10], K. Fijnvandraat [1 11], L. Haverman [2], S. Badawy [8 12], S. Gouw [1 5]
[1]Pediatric Hematology, [2]Psychosocial Department, Amsterdam University Medical Center, University of Amsterdam, Emma Children's Hospital, Amsterdam, [3]Netherlands Haemophilia Patient Society (NVHP), Nijkerk, [4]HemoNED Foundation, [5]Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, [6]Patient (not affiliated), Utrecht, [7]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands, [8]Feinberg School of Medicine at Northwestern University, Chicago, [9]The Ohio State University, Columbus, Ohio, United States, [10]Pediatric Hematology, Erasmus University Medical Center, Erasmus University Rotterdam, Sophia Children's Hospital, Rotterdam, [11]Department of Molecular Cellular Hemostasis, Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, [12]Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, United States

Background: Congenital bleeding disorders have a large impact on patients. Digital health portals, including patient portals and personal health records, can significantly help engage patients, resulting in better self-management and improved health outcomes.
We set out to develop a nationwide digital personal health record for patients with congenital bleeding disorders in the Netherlands, to manage and share relevant medical information. Therefore, we aimed to assess the effectiveness of digital health portals and perspectives of patients and healthcare providers on the use of portals in clinical care.
Aims: (1) To evaluate the effect of current digital health portals, designed for patients with chronic health conditions, on health outcomes.
(2) To assess patient and healthcare provider's attitudes towards a personal health record for patients with a congenital bleeding disorder in the Netherlands.
Methods: This mixed methods study consists of a systematic literature review and qualitative interviews. First, we searched multiple databases on studies investigating the effects of digital health portals on health outcomes in chronic, non-hospitalized patients. Health outcomes included clinical and self-reported outcomes, healthcare utilization, treatment adherence and patient satisfaction.
Second, we conducted semi-structured interviews with 19 adult and pediatric patients with a congenital bleeding disorder and their caretakers, as well as 18 healthcare providers (including (pediatric) hematologists, nurses, pharmacists, psychologists and physiotherapists).
Results: From the literature search, 82 studies were included. We found a positive association between the use of digital health portals and clinical outcomes in over half of the included studies. This effect was more noticeable for hematological parameters, such as HbA1C and cholesterol. For self-reported outcomes, this positive association is found in slightly less than half of cases. Patients satisfaction, feasibility and acceptability were high.
In the interviews, most patients on prophylaxis or frequent on-demand therapy and most healthcare providers indicated a positive attitude towards personal health records. Most participants expected it will enhance patient empowerment, coordination of care and patient safety. Yet, participants expressed concerns about privacy, data ownership and healthcare accessibility.
Summary/Conclusion: Our study shows promising effects of digital health portals on health outcomes, and positive attitudes of potential users. Therefore, the development of a personal health record for patients with congenital bleeding disorders is expected to facilitate integrated care and enhance self-management.
Research done for the SYMPHONY consortium

P-031

Assessing the impact of COVID-19 on hemophilia patients aged ≥ 40 years and the HTCs that care for them: an observational cohort study from the ADVANCE group

C. Hermans [1,*]
[1]Hemostasis and Thrombosis Unit, St-Luc University Hospital, Brussels, Belgium

Background: Older people are at greater risk of severe infection with COVID-19, as has been widely reported. ADVANCE provides a unique opportunity to investigate the impact of the pandemic on this population of older PWH and provide guidance on the management of COVID-19 for the broader hemophilia community.
Aims: To compare the rates of infection and outcomes between hemophilia and non-hemophilia populations within ADVANCE-represented countries. To identify any specific issues with the treatment and management of older people with hemophilia (PWH) with COVID-19 infection. To provide guidance on the management of older PWH during the COVID-19 pandemic to the broader hemophilia community.
Methods: A retrospective and prospective observational cohort study of all PWH (A or B) aged ≥ 40 years registered at ADVANCE HTCs. The study will comprise two surveys, each with a Part A and Part B, undertaken at two different time points. Survey 1 will collect data on patients seen between March and November 2020, while Survey 2 will collect data from December 2020 to end of May 2021.
Surveys 1A and 2A will capture a snapshot of the situation relating to COVID-19 infection in the ADVANCE population aged ≥ 40 years and the situation seen within local healthcare environments during the specified time points. Surveys 1B and 2B will provide more detailed information on patients identified with COVID-19 infection, methods of diagnosis, treatment and implications for the treatment of hemophilia and/or age-related comorbidities.
Results: Preliminary data from Survey 1 (parts A and B), representing 17 ADVANCE centers and 3,707 people with hemophilia A/B aged ≥ 40 years, reveal 24 patients had a confirmed diagnosis of COVID-19 of which five were admitted to hospital and 19 were treated without hospital admission.
Hypothesized reasons for the low patient numbers in the preliminary data are a higher awareness of risk / increased caution in PWH; highest risk of severe COVID-19 impact is at the top end of the age group studied, where patient numbers in this cohort are likely to be lower; possible lower rates of obesity in the hemophilia population could reduce the risk of severe COVID-19.
Summary/Conclusion: While it is reassuring to see relatively low absolute numbers of diagnosed cases and hospitalizations in the preliminary data, further data collection is expected to expand on the situations seen in second and third waves of COVID-19 infections across Europe. This will then be compared to infections rates, disease severity and hospitalizations/outcomes in non-hemophilia populations by country.

P-032

Healthcare resource utilisation associated with the management of major bleeding in patients receiving direct oral anticoagulants: a rapid literature review

D. Bem [1], S. Schiavo [1], T. Johnson [1], J. Kirby [1], K. Cadwell [1], S. Walsh [2,*]
[1]Health Economics & Outcomes Research Ltd, Cardiff, [2]Norgine Ltd, Harefield, United Kingdom

Background: Direct oral anticoagulants (DOACs) are a class of anticoagulants prescribed for the prevention and treatment of thrombosis in several cardiovascular diseases. Since 2010, five DOACs are available (dabigatran, rivaroxaban, apixaban, edoxaban and betrixaban) and approved across various indications including stroke, venous thromboembolism, and systemic embolism in non-valvular atrial fibrillation. All anticoagulation therapies increase the risk of major bleeding (MB), and this is accompanied by substantial healthcare resource utilisation (HRU) and costs.
Aims: The aim of this rapid review was to explore the economic burden, in terms of costs and HRU, of managing MB in patients prescribed DOACs. The HRU findings of the review are presented here.
Methods: The rapid review adopted a systematic approach for searching, screening and data extraction, which was performed by one screener while excluding a risk of bias assessment. The search was conducted in Ovid Embase (2010-2021). All studies which met the pre-determined eligibility criteria were included in the review. Data extraction was completed by a single reviewer with outcomes checked for accuracy by a second reviewer. All HRU and cost outcomes related to MB were reported. Review outputs were qualitatively summarised.
Results: The search yielded 1,158 citations with 20 studies in total (costs=9, HRU=1, costs and HRU=10) included in the review. Among these, 11 studies reported on HRU associated with MB (USA=8, Canada=1, Italy=1, multinational=1); intracranial haemorrhage and gastrointestinal bleeds were the most reported sites of MB. All, apart from one study (a global phase III trial, RE-VERSE AD), were of a retrospective observational design. HRU data associated with managing MB specifically in patients who received a DOAC reversal agent as a MB management strategy was reported in 4/11 studies. Hospitalisations and associated length of stay (LOS) were presented in 9/11 studies. Across all treatments, mean LOS per patient admission ranged from 7.8 to 11.4 days and median LOS ranged from 4.0 to 7.2 days. A higher rate of hospitalisations and outpatient visits was also observed in patients with a MB event compared to those without a MB event (mean difference per patient per year [95% CI]: 1.6[1.5–1.7] and 3.9[2.5–5.2], $p<0.001$, respectively), as reported in one study. The second most reported HRU (6/11 studies) was intensive care unit (ICU) admission and associated LOS. ICU utilisation among patients hospitalised with MB ranged from 27.7 to 33.0% of patients with a median ICU LOS between 0.9 and 4.0 days across all treatments. Hospital readmissions for any cause within 30 days of discharge varied between studies (5/11 studies) and different treatments used (DOACs alone or in combination with a reversal agent) ranging from 0.3 to 25.6%. Other HRU reported in five studies were transfusions, use of blood products or administration of reversal agents.
Summary/Conclusion: These results indicate a high HRU for the management of DOAC-associated MB, primarily driven by hospitalisations and LOS including time in ICU.

P-033

The ethics of gene transfer for haemophilia: a systematic review

L. Baas [1,*], R. van der Graaf [1], S. van Hoorn [2], A. Bredenoord [1], K. Meijer [3]
[1]Department of Medical Humanities, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, [2]Department of Public Health, Erasmus MC, University Medical Center Rotterdam, Rotterdam, [3]Department of Haematology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Background: Research done for the SYMPHONY consortium. Gene transfer is expected to become a very promising treatment for haemophilia, relieving many of the burdens associated with conventional factor replacement therapy. Several gene transfer trials are currently ongoing or have been conducted in the last few years, and it is expected that a gene transfer product will receive market approval in the coming years. However, besides its potential benefits, several ethical issues have been described that may occur in gene transfer trials or treatment, which require clarification before gene therapy enters the market.
Aims: We aimed to obtain an overview of the ethical issues of gene transfer trials and treatment that have been described in the literature to date.
Methods: We conducted a systematic review of the literature published before March 2021. We searched databases PubMed, Embase and Web of Science, with a search string combining synonyms for haemophilia, gene transfer/therapy, and ethics. This yielded 161 unique articles, which were screened by two authors (LB and SvH). We selected 53 articles for full-text screening. To be included, articles had to be written in English and describe one or more ethical issues related to gene transfer for haemophilia. Discrepancies in the decision on inclusion were resolved by discussion within the research team.
Results: We included 24 articles, which were published between 2002 and 2019. The majority of articles discussed ethical aspects only in a sub-section of the text, whereas a handful focussed on ethical aspects of gene transfer specifically. The ethical aspects of gene transfer for haemophilia can be divided into three main categories: aspects regarding gene transfer in general, aspects related to gene transfer trials, and aspects related to gene transfer as a treatment. General aspects concern whether gene transfer will ever be desirable for haemophilia, as a good standard of care already exists, and concerns surrounding the proportion of research budget and attention spent on gene transfer, potentially at the expense of the development of other innovative treatments. Ethical aspects in gene transfer trials concern the selection of trial participants, informed consent, and the occurrence and acceptability of risks. Ethical aspects surrounding gene transfer as a treatment concern its benefits and potential to live up to the expectations of a cure, latent risks that may occur and the requirement for long-term follow-up of patients, the high costs of gene therapy, and its potential social impacts.
Summary/Conclusion: Our review demonstrates there are various ethical aspects to gene transfer for haemophilia, which occur in different stages of its life cycle. These have to be taken into account in designing trials and developing the treatment further. However, the majority of the articles was published in the first decade of the 2000's and may therefore not reflect the ethical aspects arising from most recent progress in the field. Therefore, further empirical and normative research is required to obtain a more thorough insight into the ethical aspects of gene transfer for haemophilia.

P-034

Safety profile of enoxaparin in noncritical hospitalized patients with COVID-19. Results of a retrospective multicenter study

M. Pérez Pinar [1], A. M. Lizcano Lizcano [2], J. Portillo Sánchez [2], A. I. Farfán Sedano [3], J. Leal Martínez-Bujanda [4,*], E. García Aguilar [4], C. Nieto Magro [4], J. Carriel Mancilla [5]
[1]Internal Medicine, Hospital Virgen de la Luz, Cuenca, [2]Internal Medicine, Hospital General Universitario de Ciudad Real, Ciudad Real, [3]Internal Medicine, Hospital Universitario de Fuenlabrada, Fuenlabrada, [4]Medical Affairs, ITF Research Pharma S.L.U., Alcobendas, [5]Internal Medicine, Hospital de Emergencias Enfermera Isabel Zendal, Madrid, Spain

Background: Currently, most clinical guidelines recommend the use of low molecular weight heparins (LMWH) or other anticoagulants in hospitalized patients with medical diseases. Moreover, COVID-19 is a pro-inflammatory and procoagulant condition that may further increase the risk of coagulopathies. Although several clinical and observational trials evaluating the risk/benefit ratio of LMWH in COVID-19 patients were developed in critical units or with very seriously ill patients, few were conducted with a less severe population. Given that the benefit of using these medications has been widely endorsed for the majority of hospitalized patients, we aimed to study if the use of prophylactic or even higher doses of enoxaparin in less severe COVID-19 patients could lead to a relevant increase in the risk of suffering bleeding complications.
Aims: To assess the safety of enoxaparin administration in noncritical hospitalized patients with COVID-19 infection.
Methods: We collected data of admitted patients with COVID-19 from three Spanish hospitals during the initial months of the pandemic: March and April of 2020. We excluded patients who were initially admitted in critical conditions. Data from 359 patients receiving enoxaparin at different doses were analyzed and compared to data from the 56 patients who did not receive enoxaparin or any other antithrombotic therapies during the same time-period. We studied the evolution of these patients from admittance until discharge or decease, collecting the incidence of both clinically relevant and major bleedings.
Results: Globally, the incidence of bleeding in our study was very low: only 3.9% of the patients receiving enoxaparin presented a bleeding, while only 0.8% of them suffered from major bleedings. Importantly, 25.9% of enoxaparin treated patients (93 patients) received during their admission intermediate and/or therapeutic enoxaparin doses. There were no statistical differences between the bleeding pattern observed with enoxaparin and that of patients who did not receive enoxaparin (3.6% of clinically relevant and 1.8% of major bleeding). The incidence of thrombotic complications described in enoxaparin treated patients was small, present only on 2.2% of them.
Moreover, 25.6% of the patients that received enoxaparin were treated while suffering from baseline thrombocytopenia (thrombocyte count < 150.000/µl), being at a higher risk of presenting bleeding complications. However, within this subpopulation, the incidence of bleeding in enoxaparin patients remained similar to that of the whole population (4.3% of clinically relevant bleedings, no major bleedings).
Summary/Conclusion: The incidence of bleeding with different doses of enoxaparin in noncritical COVID-19 patients was very low, with a particularly small prevalence of major bleedings. Furthermore, enoxaparin was equally safe in higher-risk patients suffering from thrombocytopenia.
In summary, the use of enoxaparin in noncritical hospitalized COVID-19 patients showed remarkably good safety results.

P-035

Inhibitor to factor V in a patient with severe factor V deficiency after exposure to fresh frozen plasma

S. De Bruijn [1,*], M.-B. Maes [2], A. Gadisseur [1 3]
[1]Department of Hematology, [2]Department of Clinical Biology, [3]Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Antwerp, Belgium

Background: Congenital isolated factor V (FV) deficiency is a rare inherited bleeding disorder, with an estimated prevalence in heterozygous and homozygous form of 1: 1000 and 1: 1 000 000 persons, respectively. Deficiencies of FV can also rarely arise due to acquired inhibitor antibodies directed against FV. Factor V specific inhibitors have been most often identified in patients after exposure to bovine thrombin products, used as topical hemostatic agents during surgery. In patients with hereditary FV deficiency, the development of alloantibodies to FV is a potentially challenging complication after fresh frozen plasma (FFP) replacement therapy.
Aims: NA
Methods: NA
Results: A 35-year-old Turkish female, known with a severe congenital homozygous FV deficiency (FV level <3%), was admitted to the Hematology department because of left shoulder pain after recent bariatric surgery, which turned out to be a symptom of pneumonia in the left lower lung lobe. Laboratory analysis revealed an inflammatory blood count with a severely prolonged APTT of 243.9 seconds and a PT of 141.3 seconds, where these had been 72.0 and 33.6 seconds, respectively, 3 months earlier. Imaging did not shown any signs of bleeding. FFP (15- 20 ml/kg) had earlier been used perioperative as a substitute for the missing factor V and n-butyl cyanoacrylate, which does not contain fibrin, as wound adhesive
FFP was started and additional blood analysis showed an isolated reduced FV activity level of <3%. Mixing studies were positive, suggesting the presence of an acquired inhibitor, which was confirmed using Bethesda assay (5.5 BU). We started high dose corticosteroids and intravenous immunoglobulins, in association with cyclophosphamide, with improvement of the APTT. On day 9, Rituximab IV (375 mg/m²) was started on a weekly basis as the APTT and PT prolonged again under the existing therapy. Subsequently, the patients FV inhibitor decreased to 1.3 BU, and she could be discharged. A total of 4 doses of Rituximab have been administrated. On day 25 we did not detected any level of FV inhibitor and coagulation values APTT/PT were back to the patients baseline levels.
Summary/Conclusion: As FV-specific concentrates are still unavailable, FFP remains the mainstay as replacement therapy perioperatively in FV-deficient patients. Unfortunately, the administration of FFP is occasionally complicated by alloantibodies to FV in these patients, comparable to immunization to transfused FVIII concentrate in hemophilia A patients. The management of bleeding in the presence of a FV inhibitor is challenging as there are no evidence-based guidelines available. In general the treatment is, as with every other inhibitor, based on a 2-step approach, consisting of (1) controlling bleeding by using FV replacement therapy, in which platelet concentrates are preferred over FFP, and (2) eradicating the inhibitor with several immunosuppressive agents, IVIG, and plasmapheresis.
The remaining problem in this patient concerns future need for coagulation correction in case of bleeding or surgery. She has also expressed a wish for another pregnancy. We can never be certain that new inhibitor issues will not arise again with the use of FFP and except for platelet concentrates no other treatment option is available.

CLOTTING

P-037

An association of low-density lipoproteins and fibrinolysis in patients with severe aortic stenosis- valvular expression of fibrinolytic proteins

M. Kopytek [1 2,*], A. Undas [1 2], J. Natorska [1 2]
[1]Institute of Cardiology, Jagiellonian University Medical College, Kraków, [2]Centre for Medical Research and Technologies, John Paul II Hospital, Krakow, Poland

Background: Aortic stenosis (AS) is associated with hyperlipidemia and hypofibrinolysis. However, the contribution of serum lipids to the valvular expression of fibrinolytic proteins is not known.
Aims: To evaluate whether fibrinolytic factors are present in loco within stenotic aortic valves and to determine their potential associations with LDL cholesterol concentrations, their oxidized forms (oxPL-LDL), and AS severity.
Methods: We enrolled 75 patients with isolated severe AS aged 66±8 years (mean gradient 52 mmHg, max gradient 84 mmHg) undergoing valve replacement surgery. Aortic valve leaflets obtained from AS patients and from age-matched apparently healthy autopsy donors (n=3) were used to evaluate in loco the presence of plasminogen (PLG), tissue type plasminogen activator (tPA), and fibrinolysis inhibitors (α2-antiplasmin and plasminogen activator inhibitor, PAI-1). PLG activity and plasma concentrations of tPA and PAI-1, along with serum cholesterol LDL and oxPL-LDL were assayed. Clot lysis time (CLT) was performed to assess the overall activity of the fibrinolytic system. The Ethical Committee approved the study and all participants provided written informed consent in accordance with the Declaration of Helsinki.
Results: AS patients with serum LDL cholesterol ≥3.0 mM (n=25, 56% patients received statin therapy) compared to patients with LDL cholesterol <3.0 mM (n=50, 86% patients received statin therapy) were characterized by higher oxPL-LDL levels (+80%, p=0.0014). Moreover, AS subjects with LDL cholesterol ≥3.0 mM, compared to those with LDL cholesterol <3.0 mM had longer CLT (+22.4%, p=0.0022) along with higher plasma concentrations of tPA (+31.5%, p=0.004) and PAI-1 (+47.4%, p=0.01), while PLG activity tended to be higher (+10.8%, p=0.056). For the first time the valvular expression of fibrinolytic proteins was detected in AS patients, but not in healthy donors. The expression of studied proteins was observed mainly on the aortic side of the leaflets and only trace amounts were detectable in deeper layers of valves. The immunopositive valve area for PLG was 4.4±1.8%, for tPA 3.6±0.8%, while the expression of fibrinolysis inhibitors was 4.2±1.7% of the valvular area for α2-AP and 6.9±2.3% for PAI-1. In the whole AS group, oxPL-LDL, but not LDL cholesterol concentrations showed associations with transvalvular pressure gradients (PGmean: r=0.34, p=0.041; PGmax: r=0.3, p=0.016). Serum concentrations of LDL cholesterol correlated with CLT (r=0.42, p=0.0002), blood PLG activity (r=0.56, p=0.0012) and with tPA (r=0.43, p=0.0001) and PAI-1 (r=0.32, p=0.006) levels. Similarly, oxPL-LDL were associated with CLT, PLG activity, and tPA concentrations (r=0.34; r=0.59; r=0.47, all p<0.01, respectively). Valvular expression of fibrinolytic proteins did not correlate with LDL cholesterol, ox-PL-LDL or transvalvular gradients (all p>0.05).
Summary/Conclusion: This study is the first to demonstrate that in AS patients hypercholesterolemia and enhanced lipid oxidation are associated with impaired systemic fibrinolysis, which supports the concept of beneficial effects of cholesterol-lowering therapy in mild-to-moderate AS. Moreover, valvular expression of fibrinolytic factors suggests that these factors are delivered with circulating blood but not synthesized de novo.
Funding: This work was supported by the grant from the Polish National Science Centre (UMO-2018/29/B/NZ5/02629 to J.N).

P-038

Socioeconomic status and risk of incident venous thromboembolism

H. Jørgensen [1 2], E. Horváth-Puhó [3 4], K. Laugesen [3 4], S. Brækkan [1 2], J.-B. Hansen [1 2*], H. Toft Sørensen [3 4]
[1]Thrombosis Research Center (TREC), Department of Clinical Medicine, UiT - The Arctic University of Norway, [2]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway, [3]Department of Clinical Epidemiology, Aarhus University Hospital , [4]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Background: Although venous thromboembolism (VTE) is a leading cause of morbidity and mortality, and socioeconomic status (SES) affects human health and health behavior, few studies have explored the association between SES and VTE.
Aims: The aim of this nationwide case-control study was to investigate the association between SES, assessed individually and in a composite score by levels of education, income, and employment status, and incident VTE.
Methods: We used Danish national registries to identify 51,350 persons aged 25-65 years with incident VTE during 1995-2016. For each case we used incidence density sampling to select five age-, sex-, and index-year-matched population controls from the general Danish population (n=256,750). SES indicators, including education, income, and employment status, were assessed one and five years prior to the VTE. We used conditional logistic regression to compute odds ratios (ORs) with 95% confidence intervals (CIs) for VTE according to individual SES indicators and a composite SES score in analyses adjusted for age, sex, and comorbidities.
Results: Compared to low levels of SES indicators, high educational level (OR 0.74; 95% CI 0.71-0.77), high income (OR 0.70; 95% CI 0.68-0.72), and high employment status (OR 0.66; 95% CI 0.64-0.68) were associated with decreased risk of VTE, even after adjusting for comorbidities. A composite SES score was superior to the individual indicators in assessing VTE risk (OR for high vs. low score: 0.61; 95% CI 0.59-0.63). In sensitivity analyses with SES indicators measured 5 years prior to the VTE event, the risk estimates remained essentially the same.
Summary/Conclusion: High levels of both individual SES indicators and a composite SES score were associated with decreased VTE risk, even after adjustment for comorbidities.

P-039

Identification of potential thrombotic biomarkers in advanced gastric cancer

D. Zaragoza Huesca [1,*], P. Garrido Rodríguez [1 2], P. Jiménez Fonseca [3], E. Martínez de Castro [4], M. Sánchez Cánovas [5], L. Visa [6], A. Custodio [7], A. Fernández Montes [8], J. Peñas Martínez [1], V. Vicente [2 5], I. Martínez Martínez [1 2], A. Carmona Bayonas [5]
[1]Medical Oncology and Haematology service, Centro Regional de Hemodonación from Murcia, Murcia, [2]CIBERER, Madrid, [3]Medical Oncology Service, Central University Hospital in Asturias, Oviedo, [4]Medical Oncology Service, University Hospital Marqués de Valdecilla, Santander, [5]Medical Oncology and Haematology service, University Hospital Morales Meseguer, Murcia, [6]Medical Oncology Service, Hospital del Mar, Barcelona, [7]Medical Oncology Service, University Hospital La Paz, Madrid, [8]Medical Oncology Service, University Hospital Complex in Ourense, Ourense, Spain

Background: Advanced gastric cancer (AGC) is one of the most thrombogenic neoplasms. However, genetic mechanisms responsible for this complication are still not known, and molecular heterogeneity in this neoplasm points to contribution of multiple and specific biological processes in its development.
Aims: To identify candidate genes whose expression was associated to thrombosis 1) independently on Lauren histopathological subtype (intestinal or diffuse), and 2) conditioned to molecular classification from TCGA (The Cancer Genome Atlas).
Methods: A case-control nested study was designed on the cohort from AGC national registry AGAMENON. Ninety-seven patients were selected, 48 with venous thromboembolism (VTE) and 49 VTE-free, and a differential gene expression array (Clariom D Human) was carried out on primary tumor samples. Propensity score matching was used in order to adjust by confounding factors. Analyses were made with Student´s t-tests stratified by histopathological subtype (intestinal or diffuse), based on |fold change|>1 and 5% signification level, adjusted to a 10% false positive rate. Gene selection was based on the criteria that expression sense between thrombosis and thrombosis-free patients was conserved in both subtypes. By histopathology conditioned logistic regression, correlation between thrombotic risk and expression from selected genes was calculated in the whole cohort. Differential gene expression was also analyzed in each TCGA category, by applying |fold change|>1.5 and 5% signification level.
Results: From histopathology stratified analysis, 15 thrombosis-associated genes were obtained in both intestinal and diffuse subtypes. In thrombotic patients, CRELD1, MAGEB16, SAA1 and CCDC169 were downexpressed in both subtypes (adjusted p-value (p)<0.05). Regarding overexpressed genes in thrombotic patients, MIR5683 and PRKD3 were highlighted (adjusted p<0.05). Interestingly, PRKD3 has already been related to gastric cancer through anaerobic glycolysis activation (Warburg effect), a process which is associated to venous thrombi formation. Indeed, increased expression of PRKD3 was associated to a major thrombotic risk in the whole cohort (odd ratio=9.11; p=0.004). According to the analysis of the differentially expressed transcripts based on the TCGA classification, in chromosome instability category, PIGR was downexpressed (p=0.024) in patients with VTE. Among patients with Epstein-Barr Virus-associated tumors, ACP1, implicated in antiplatelet and antiangiogenic functions, and EPS8, which regulates vascular permeability, were downexpressed (p=0.004) and overexpressed (p=0.012), respectively, in patients with VTE. In genomic stability group, AGR2 was overexpressed (p=0.011) in thrombotic patients. Finally, among subjects with microsatellite instability tumors, SGK1, which promotes coagulation in type II diabetes, was overexpressed (p=0.047) in patients with VTE.
Summary/Conclusion: Our study suggests participation of multiple genetic mechanisms, general and specific of each subtype, in the thrombogenesis of AGC patients. If validated, these genetic risk factors, individually or as molecular signatures, could contribute to improve capacity to predict individual thrombotic risk in these patients.

P-040

Genetic background of prothrombotic plasma fibrin clot properties in patients with acute pulmonary embolism and its association with post-PE syndrome

A. Klajmon [1,*], C. Jakub [2], M. Ząbczyk [1 2], J. Natorska [1 2], A. Undas [1 2]
[1]John Paul II Hospital, [2]Institute of Cardiology, Jagiellonian University Medical College, Kraków, Poland

Background: Acute pulmonary embolism (PE), isolated or combined with deep-vein thrombosis (DVT), is the major cause of mortality and hospitalization due to venous thromboembolism (VTE). Patients following PE suffer from its long-lasting complications such as post-PE syndrome. To our knowledge, the impact of fibrinogen alpha chain gene (*FGA*) c.991A>G (rs6050), fibrinogen beta chain gene (*FGB*) -455G>A (rs1800790) or factor XIII gene (*F13*) c.103G>T (rs5985) polymorphisms on plasma fibrin clot phenotype in acute PE as well as on post-PE syndrome occurrence was not reported to date.
Aims: We investigated associations of *FGA* rs6050 C___2892877_10, *FGB* rs1800790 or *F13*rs5985 polymorphisms with plasma fibrin clot properties or thrombin generation capacity in patients with acute PE and occurrence of post-PE syndrome.
Methods: *FGB* rs1800790 C___7429790_20 and *F13* rs5958 C___1639938_20 were genotyped by TaqMan assay in normotensive non-cancer patients with acute PE (n=126; aged 58±14 years). Fibrin clot permeability (K_s), clot lysis time (CLT), and endogenous thrombin potential (ETP) were assessed on admission. Post-PE syndrome was diagnosed at 6 months since the index PE event.
Results: *FGA* rs6050 polymorphism was found in 62 patients (49.2%), *FGB* rs1800790 in 40 subjects (31.7%), while the *FXIII* c.103T allele in 49 individuals (38.9%). As many as 25 (19.7%) subjects were both A in *FGB* rs1800790 gene and T in *F13* rs5985 gene alleles carriers. Patients with the *FGB* rs1800790 A allele (4 homozygous) had 15.7% reduced K_s, 11.1% prolonged CLT, and 10.9% higher ETP compared with major homozygotes (all $p<0.05$). The *F13* rs5985 T allele (9 homozygous) was associated with 18.3% reduced K_s, 9.8% prolonged CLT, and 9.2% higher ETP compared with major homozygotes (all $p<0.05$). There were no associations between the *FGA* rs6050 polymorphism and fibrin clot phenotype or ETP. There were no differences in the frequency of studied polymorphism between post-PE (n=31, 24.6%) and non-post-PE patients. However, the *FGB* rs1800790 A allele carriers with post-PE had 12.7% lower K_s compared to carriers without post-PE ($p=0.022$). Similarly, patients with post-PE and the *FXIII* c.103T allele had 16% lower K_s compared to non-post PE individuals with the same polymorphism ($p=0.0005$). No differences were found regarding CLT and ETP with regard to post-PE and both polymorphisms.
Summary/Conclusion: The influence of the *FGB* rs1800790 and *F13* rs5985 polymorphisms on fibrin clot structure and thrombin potential is strong enough to be observed in the acute phase of PE. Moreover, presence of *FGB* rs1800790 or *F13* rs5985 polymorphisms associated with denser fibrin network might be associated with post-PE syndrome development.
This work was supported by the Polish National Science Centre (UMO-2015/B/NZ5/02215).

P-041

Molecular Interactions of Coagulation Factor XIII B Subunit

K. Penzes-Daku [1,*], B. Bécsi [2], F. Erdődi [2], L. Muszbek [3]
[1]Department of Laboratory Medicine, Division of Clinical Laboratory Science, [2]Faculty of Medicine, Department of Medical Chemistry, University Of Debrecen, [3]Department of Laboratory Medicine, Division of Clinical Laboratory Science, Department of Laboratory Medicine, Division of Clinical Laboratory Science, Debrecen, Hungary

Background: Coagulation factor XIII (FXIII) consists of two potentially active A subunits and two inhibitory/protective B subunits (FXIII-A_2B_2). FXIII-B is in excess, half of it circulates in non-complexed form. It contains 8.5 % carbohydrate; the glycan part has a role in keeping FXIII-A_2B_2 in the circulation. A major polymorphism of FXIII-B is due to a novel splice acceptor site in intron K (IK-FXIII-B) and the replacement of 10 C-terminal amino acids by 25 novel amino acids. FXIII-B is associated to fibrinogen through its γ-chain. Peak 1 fibrinogen (Peak1; 85% of plasma fibrinogen) contains two γA-chains, peak 2 fibrinogen (Peak2) also possesses alternatively spliced γ'-chain. Byrnes et al have reported that a heptapeptide present in the C-terminal part of both types of γ-chains (γHP) is involved in the binding to FXIII-B.
Aims: To evaluate whether the glycan residue and IK polymorphism influence the binding of FXIII-B to FXIII-A and how FXIII-B and its polymorphic variant binds to Peak1 and Peak2.
To investigate the binding of γHP and the C-terminal peptide of the γ' chain to FXIII-B.
Methods: The following peptides were synthesized by Merrifield solid phase peptide synthesis: fibrinogen γ'-chain 20-mer peptide: H-VRPEHPAETEYDSL**Y**PEDDL-OH with sulfated and unsulfated Y side chains, a nonapeptide corresponding to fibrinogen γ-chain sequence 389-397 (γA-chain peptide) H-FNRLTIGEG-OH. The latter peptide contains the γHP sequence in the middle. The purity of the prepared peptides was validated by HPLC and mass spectrometry.
Binding studies between proteins and large peptides were carried out by surface plasmon resonance technique (Biacore 3000 and Biacore X instruments). Association rate constant (ka), dissociation rate constants (kd), and equilibrium dissociation constants (Kd) were calculated.
Association of the short γHP peptide to FXIII-B was investigated by isothermal titration calorimetry.
Results: Using recombinant proteins Byrnes et al demonstrated that the sequence γHP is involved in the binding of fibrinogen to FXIII-B. We confirmed their results and showed by ITC, that the synthetic γHP binds to FXIII-B (Kd = $2.40x10^{-8}$ M) Deglycosylated FXIII-B (DG-FXIII-B) showed decreased affinity to FXIII-A. The Kd for the binding of wild type and IK FXIII-B variant to FXIII-A were comparable.
Interestingly, FXIII-B as analyte also bound to the ligand FXIII-B (Kd = $2.75x10^{-8}$). FXIII-B bound to Peak2 with somewhat higher affinity than to Peak1 (Kd = $2.89x10^{-8}$ versus Kd = $5.03x10^{-7}$).
The sulfated γ'-chain 20-mer peptide also showed relatively high affinity toward FXIII-B (Kd = $7.42x10^{-8}$).
Summary/Conclusion: Intron K polymorphism does not influence the binding to FXIII-A
The glycan structure on FXIII-B contributes to its binding to FXIII-A.
Our ITC result supports previous finding that a heptapeptide present in both fibrinogen γ-chain variants is involved in the binding of FXIII-B to fibrinogen. The results obtained with γ'-chain 20-mer peptide indicate that this sequence present only in the γ'-chain also contributes to the binding of FXIII-B to Peak2 fibrinogen. Posttranslational sulfation of the tyrosine side chains strengthens this interaction.
Multiple interactions are involved in the binding of fibrinogen to FXIII-B, and very likely also to FXIII-A_2B_2.

P-043

New procoagulant components from gastric cancer secretome

D. Zaragoza Huesca [1,*], J. Peñas Martínez [1], M. C. Rodenas Bleda [1], S. Espín [1], V. Vicente [2 3], C. Martínez [1], A. Carmona Bayonas [2], I. Martínez Martínez [1 3]
[1]Medical Oncology and Haematology service, Centro Regional de Hemodonación from Murcia, [2]Medical Oncology and Haematology service, University Hospital Morales Meseguer, Murcia, [3]CIBERER, Madrid, Spain

Background: Gastric cancer is associated with a high venous thromboembolism risk. Cells from gastric and other digestive tumors can express procoagulant molecules, among which are tissue factor or plasminogen activator inhibitor-1. Cancer cells also secrete other molecules that might prone a hypercoagulant state in patients. Consequently, finding of new procoagulant molecules secreted by gastric tumors could help in thrombotic risk prediction or antithrombotic molecular targeted therapy.
Aims: To identify new procoagulant molecules in secretomes from gastric cancer cells.
Methods: Kato-III (gastric cancer) and 293T (human embryonic kidney) cells were cultivated in CD-CHO protein free medium. After 48 hours, secretomes were harvested and centrifuged at 200*g*, 5 min, and later at 2000*g*, 20 min, to discard death cells and apoptotic bodies, respectively. Clot formation by Kato-III and 293T secretomes was measured by absorbance spectrophotometry at 405nm after incubation with plasma for 60 min and later addition of 12.5mM CaCl2. Proteomic analysis from secretomes was conducted by SWATH-MS, and differential protein expression was analysed. Procoagulant effect of selected proteins was tested in clot formation assay, both in plasma with cell secretomes and in secretome-free plasma. Tissue factor pathway inhibitor (TFPI) levels in plasma with different concentrations of selected proteins were assessed by western-blot.
Results: We firstly checked that clot formation lag time for Kato-III secretome was shorter than 293T secretome (15 vs 20 min), confirming the higher prothrombotic potential of gastric cancer cells. Among the proteins differently expressed in Kato-III vs 293T secretomes identified by SWATH-MS, we selected NGAL (Fold change:21.3; p-value:8.52E-5). Interestingly, high levels of NGAL have been described in different cardiovascular diseases and as a thrombotic biomarker in myelodysplastic leukemias. In clot formation assay for plasma plus 293T secretome, purified NGAL addition increased clot absorbance maximum levels in a dose-dependent manner. Additionally, clot formation lag time was shorter when adding NGAL in comparison with the absence of NGAL (31 vs 51 min). In clot formation assay for plasma plus Kato-III secretome, addition of NGAL increased clot absorbance maximum levels in comparison with the same assay without NGAL (0.897 ± 0.05 vs 0.805 ± 0.004). Additionally, NGAL addition to plasma without any cell secretome shortened clot formation lag time (5 vs 50 min for plasma without NGAL). It has been described that NGAL protects MMP9 from proteolytic degradation, and this protease is implicated in TFPI degradation, thus promoting tissue-factor activation. Therefore, NGAL could increase TFPI degradation by stabilizing MMP9. To probe this hypothesis, we evaluated TFPI levels in plasma after purified NGAL addition. According to western blot and densitometry techniques, TFPI in plasma was reduced in presence of NGAL in a dose-dependent manner (9.6% and 28.3% reduction when 5 µg and 10 µg were added, respectively).
Summary/Conclusion: This study describes, for the first time, a possible functional mechanism by which NGAL could increase hypercoagulant state in gastric cancer patients. For this reason, anti-NGAL molecular targeted therapy could become a potential treatment in order to reduce thrombotic risk in these patients. However, further studies are needed to validate these results.

P-044

Extracellular vesicles derived from activated platelets display an increased procoagulant activity and drive the production of TF by monocytes

E. Guerreiro [1,*], S. Swamy [1], N. Latysheva [1], L. Wilgård [1], J.-B. Hansen [1], O. Snir [1]
[1]Thrombosis research center - University of Tromsø, Tromsø, Norway

Background: Extracellular vesicles (EVs) are small nanostructures (<1000 nm) confined by a lipid bilayer membrane and released by all cells. Such EVs serve as intercellular mediators under physiological and pathophysiological conditions. Of particular note, EVs also have procoagulant properties and thus contribute to hemostasis and blood clotting. More specifically, platelet-derived EVs were reported to be highly procoagulant due to a high content of phosphatidylserine (PS) on their surface membrane and their activity was associated with several pathological conditions. Elevated levels of platelet-derived EVs have been detected in plasma of venous thromboembolism (VTE) patients. VTE is a multicausal disease in which venous thrombi are generated in the deep veins with severe outcomes and complications. The pathology of VTE is also associated with increased activation of platelets and the involvement of monocytes and activation of coagulation.
Aims: To comprehensively study the procoagulant potential of EVs derived from resting and activated platelets, and to examine the effects of such EVs on monocyte activation and tissue factor (TF) activity.
Methods: Platelets were isolated, washed and resuspended in tyrodes buffer prior to a short, 15 min, stimulation with TRAP6 or calcium ionophore. Expression of CD41a and CD62P was monitored to determine platelet purity and activation. EVs derived from *(i)* resting platelets, *(ii)* TRAP6- and *(iii)* calcium ionophore-stimulated platelets were isolated by centrifugation at 20,000 xg, which was followed by a 100,000 xg ultracentrifugation. Size distribution and number of platelet-EVs were determined by Nanoparticle Tracking Analysis (NTA), and their procoagulant potential was studied by Calibrated Automated Thrombogram (CAT) and Procoagulant Phospholipid (PPL) activity assay. Furthermore, TF activity and expression on the surface of monocytes were monitored following stimulation of primed monocytes with EVs derived from either resting or activated platelets.
Results: Platelet-derived EVs isolated following centrifugation at 20,000 xg were larger and showed more potent PPL activity in comparison to EVs isolated by a subsequent ultracentrifugation at 100,000 xg. This 20,000 xg EV-fraction was therefore further investigated in this study. EVs derived from activated platelets displayed an increased procoagulant potential in comparison with those isolated from resting platelets. EVs from activated platelets showed increased PPL activity and induced significantly higher thrombin generation measured by CAT. Interestingly, EVs-derived from activated platelets induced the expression and activity of TF in monocytes that were primed with suboptimal concentration of LPS. In comparison, EVs from resting platelets did not induce TF expression or activity in monocytes.
Summary/Conclusion: Platelet activation leads to the release of EVs with an increased procoagulant potential. Moreover, EVs from activated platelets trigger TF activity and expression by monocytes.

P-045

β2-Glycoprotein I (β2GpI) is a Fibrinogen Binding Protein

L. Acquasaliente [1], A. Pagotto [1], D. Peterle [2], V. Pengo [3], V. De Filippis [1,*]
[1]Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy, [2]Department of Chemistry and Chemical Biology, Northeastern University, Boston, United States, [3]Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, Padua, Italy

Background: β_2GpI is an abundant plasma protein of yet unknown function and the main autoantigen in the antiphospholipid syndrome (APS). We have recently discovered that β_2GpI may function as a mild anticoagulant, as it binds to α-Thrombin (αT) exosite-2, impairing binding to GpIbα platelet receptor [Pozzi et al., JTH (2013); Acquasaliente et al., Biochem J (2016)]. Earlier studies have shown that β_2GpI Domain-I is the major epitope for APS autoantibodies.
Aims: The aim of this work is to check whether β_2GpI interacts with fibrinogen (Fb); identify the binding regions on β_2GpI and Fb involved in β_2GpI-Fb complex formation; study the effect of β_2GpI binding to Fb on fibrin structure.
Methods: Binding measurement were performed by fluorescence and Surface Plasmon Resonance (SPR). Fibrin structure was studied by turbidimetry, Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Fb fragment-X and β_2GpI nicked at Domain-V were both prepared by limited proteolysis with plasmin [Furlan and Beck, 1972; Ohkura et al., Blood (1998)]. Domain-I was produced by solid-phase synthesis [Pozzi et al., Protein Sci (2010)].
Results: Fluorescence and SPR analyses indicate that β_2GpI interacts with Fb at two sites, with K_{d1} = 15±4nM and K_{d2} > 1μM. Identical results were obtained with Domain-I and Domain-V clipped β_2GpI. Binding of β_2GpI to Fb fragment-X, lacking the Cα-domains, resulted in affinity drop. Turbidimetric, DLS, and SEM measurements indicate that 4μM β_2GpI induces formation of thinner and shorter fibrin fibers.
Summary/Conclusion: Given the plasma concentrations of β_2GpI (4μM) and Fb (7μM), and the affinity of β_2GpI for Fb (K_{d1} = 15nM), we conclude that under physiological conditions β_2GpI circulates in the fibrinogen-bound form. β_2GpI-Fb complex formation is driven by β_2GpI Domain-I and Fb Cα-domains. Considered that Domain-I is also the major epitope for APS autoantibodies, our results challenge the current view on the role of β_2GpI in the pathogenesis of APS.

P-046

Transcriptomic analysis of anti-tumour effect of prelatent antithrombin on glioblastoma multiforme cells

J. Peñas-Martínez [1,*], D. Zaragoza-Huesca [1], E. Navarro Manzano [1], E. J. Cuenca-Zamora [1], S. Espín [1], V. Vicente [1], A. Carmona-Bayonas [1], I. Martínez-Martínez [1]
[1]Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER, Murcia, Spain

Background: Antithrombin (AT) is the main inhibitor of the coagulation cascade, but it has other functions beyond hemostasis that depend on its conformation: native, activated by heparin, cleaved, latent or prelatent. We have recently shown that prelatent AT reduces migration and invasion processes of glioblastoma multiforme cells U-87 MG and inhibits the expression of vascular endothelial growth factor A (VEGFA), showing a potential anti-angiogenic effect. Prelatent AT also reduces pSTAT3 (Tyr705) and pERK1/2 (Thr202/Tyr204) phosphorilation, and STAT3 levels, which have been associated with less resistance to treatment.
Aims: Due to the wide spectrum of prelatent AT effects in glioblastoma multiforme cells, the aim of this study was to investigate those genes that could be over- or underexpressed using transcriptomic analysis.
Methods: Native and prelatent AT were purified from healthy donor plasma. U-87 MG cells were treated with prelatent AT (2.16 µM) or phosphate-buffered saline (PBS) for 12 hours. Then, a microarray was carried out using Human Clariom D chip and the results were analyzed with Partek Genomics Suite software. The criterion for selection of differentially expressed genes was a fold-change (FC) >1.5, taking into account a significance level of 0.1%. Exploratory data analysis was performed with principal component analysis. . Before each validation, U-87 MG cells were treated with native or prelatent AT (2.16 µM) or PBS for 12h. Differences in proteins expression were validated by western blot (n=4/group). Cell cycle and proliferation were assessed by measuring 7-AAD and BrdU incorporation by flow cytometry (n=4/group).
Results: PCA showed the separation of control samples and samples treated with prelatent AT based on the expressed transcripts. Prelatent AT induced overexpression of 2467 transcripts and under-expression of 6738. Among the altered pathways after prelatent AT treatment, we highlight cell cycle process, where different cyclin genes and regulatory proteins involved in cell cycle progression were inhibited (p-value and FC, respectively): *CCND3* ($8.46 \cdot 10^{-5}$, -1.65), *CDK4* ($2.60 \cdot 10^{-5}$, -1.64), *CCNE1* ($3.99 \cdot 10^{-4}$, -1.62), *CCNE2* ($2.65 \cdot 10^{-6}$, -2.06), *E2F1* ($6.47 \cdot 10^{-6}$, -1.87), *E2F3* ($7.11 \cdot 10^{-6}$, -1.94) and *RB1* ($1.37 \cdot 10^{-7}$, -1.58). We further evaluated CDK4 and RB1 expression by western blot as they participate in the control of transition from G1 to S phase, confirming that CDK4 was inhibited after prelatent AT treatment (p=0.020). Using flow cytometry, a higher number of events (~10%) in G0-1 phase and a halfway inhibition of S phase were observed after U-87 MG treatment with native or prelatent AT. Furthermore, cell proliferation also decreased by 40.63% compared to control cells.
Summary/Conclusion: Our results show that prelatent AT has surprisingly versatile anti-tumor properties on U-87 MG glioblastoma multiforme cells. In addition to the potential treatment resistance-reducing effect and its anti-angiogenic function already described, our results support that prelatent AT in glioblastoma multiforme cells induces cell cycle arrest. These results support the potential therapeutic role of prelatent AT in glioblastoma multiforme.
Founding: PI17/00050 & FEDER, Crowdfunding Precipita (FeCyT).

P-047

Identification of small interfering RNAs for allele-selective silencing of murine von Willebrand factor

Y. Jongejan [1 2,*], R. Dirven [1 2], K. Paunovska [3], K. van der Gouw [1 2], E. Schrader [3], A. de Jong [1 2], J. Dahlman [3], B. van Vlijmen [1 2], J. Eikenboom [1 2]
[1]Department of Internal Medicine, Division of Thrombosis and Hemostasis, [2]Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Netherlands, [3]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, United States

Background: High von Willebrand factor (VWF) plasma levels are associated with (arterial) thrombosis. Antiplatelet therapy increases bleeding risk and often fails to prevent arterial thrombosis. Lowering of VWF through allele-selective silencing of *VWF* would be an approach which averts complete *VWF* knockdown and thus minimizes bleeding risk.
Aims: We aimed to identify small interfering RNAs (siRNAs) that can distinguish between strain-specific differences in murine *Vwf* to be used in allele-selective knockdown studies in heterozygous mouse models.
Methods: Two commonly used mouse inbred strains, C57BL/6J and 129S1/SvImJ, were selected based on genetic differences between their *Vwf* genes and comparable plasma VWF levels. 14 siRNAs were designed *in silico* to target one or two of 11 genetic differences between these strains. Activity and allele/strain-selectivity of the siRNAs were determined, dose-dependently, in HEK293 cells transiently expressing both C57BL/6J and 129S1/SvImJ *Vwf*. A non-selective siRNA (si*Vwf*) was also designed to test feasibility of *in vivo Vwf* silencing by means of polymeric nanoparticle-mediated endothelium-specific siRNA delivery.
Results: 6 out of 14 strain-selective siRNAs effectively inhibited the targeted allele (≥80% at 5 nM siRNA), with minimal inhibition of the untargeted allele. Out of these 6 siRNAs 2 lead candidates were chosen based on potency (good inhibition at concentrations 62.5 pM–1 nM), strain-selectivity and ability to target one nucleotide difference. si*Vwf* showed inhibition of *Vwf in vitro* for both strains (~90% at 0.5 nM). It also strongly reduced *Vwf* at the level of lung mRNA (~90%) and plasma protein (~80%) in C57BL/6J mice at dose 1.5 mg siRNA/kg body weight.
Summary/Conclusion: We have identified strain-selective siRNAs that can distinguish between C57BL/6J and 129S1/SvImJ *Vwf* based on one or two nucleotide(s) difference between their *Vwf* genes. We will test these siRNAs in F1 of C57BL/6J x 129S1/SvImJ mice, permitted by the successful *in vivo* silencing approach.

This study was funded by the Dutch Thrombosis Foundation grant # 2018-01

P-048

Simultaneous Assessment of DOACs Effects on Clot Formation and Fibrinolysis with the FibWave

J. Evrard [1,*], V. Maloteau [1], J.-M. Dogné [1], J. Douxfils [1 2]
[1]Pharmacy, University of Namur, [2]Qualiblood, Namur, Belgium

Background: Direct oral anticoagulants (DOACs) are more and more used for the prevention and treatment of thromboembolic events. The DOACs are also known to impact coagulation assays and enhance FX-dependent fibrinolysis and plasmin generation.
Simultaneous assessment of the coagulation and fibrinolysis processes could facilitate the global understanding of the pharmacodynamics of DOACs. To date, three methods including the thromboelastometry (TEG/ROTEM), the thrombin generation assay (TGA) and the clot waveform analysis (CWA) are able to monitor the formation of thrombin or fibrin and recognized to reflect complete processes of coagulation and/or fibrinolysis. We have previously reported the performance of the FibWave (microplate reader) to assess the impact of DOACs on the clot formation process. In this study, the FibWave has been adapted in order to measure both the clot formation and fibrinolysis processes simultaneously.
Aims: To evaluate the suitability of the FibWave for simultaneously assessing the impact of DOACs on coagulation and fibrinolysis.
Methods: Apixaban, edoxaban, rivaroxaban and dabigatran were spiked at several concentration in normal pooled plasma (NPP) : 0 (buffer, 10, 30, 50, 100 and 250 ng/mL. The fibrin clot formation and the fibrinolysis were measured on the FibWave using a mixture of tissue factor, phospholipids and tissue plasminogen activator (tPA) at near physiological concentrations. Briefly, the plasma samples were incubated for 5 minutes at 37°C and the clot formation and fibrinolysis (CFF) process was triggered by the addition of 20 µL of $CaCl_2$ at 100 mM and monitored during 25 minutes.
Results: In absence of tPA, the velocity (FW-Max_1), the acceleration (FW-Max_2) and the deceleration (FW-Min_2) of coagulation were reduced and the FW-Ttpeak was prolonged with factor Xa (FXa) inhibitors while dabigatran prolonged the FW-Ttpeak and had little impact on other parameters.
The addition of tPA to different DOACs spiked in the NPP showed a tendency to enhance DOACs effects on the clot formation. As observed in coagulation phase, DOACs impacted differently the parameters of clot fibrinolysis. FXa inhibitors showed a reduction of maximum fibrinolysis velocity (|FW-Min_1|) while dabigatran showed a prolongation of fibrinolysis time (FW-TFib). The time length between fibrinolysis and coagulation velocities (FW-TFib – Ttpeak) was reduced for all anticoagulants.
Summary/Conclusion: Thanks to its capacity to assess coagulation and fibrinolysis, the FibWave provided insight the global pharmacodynamics of DOACs and could be useful for understanding the global haemostatic potential. Once standardized and automated, the FibWave may be a suitable assay as diagnostic and/or prognostic laboratory tool for evaluating the effects of haemostatic treatment in diagnosed patients and the bleeding risk from the fibrinolysis.

P-049

Identification of Tumor-expressed MicroRNAs Associated with Venous Thrombosis in Colorectal Cancer

R. Anijs [1,*], E. H. Laghmani [1], B. Ünlü [1], S. Kielbasa [2], H. Mei [2], F. Klok [1], P. Kuppen [3], H. Versteeg [1], J. Buijs [1]
[1]Thrombosis & Hemostasis, [2]Medical Statistics and Bioinformatics, [3]Surgery, Leiden University Medical Center, Leiden, Netherlands

Background: Colorectal cancer (CRC) patients have an increased risk of developing venous thromboembolism (VTE), resulting in increased morbidity and mortality. Available risk prediction tools for identifying patients at high risk of VTE show poor clinical performance. MicroRNAs (miRNAs) are small RNAs, which regulate a variety of cellular processes, are relatively stable and are detectable in body fluids.
Aims: The aim of this study is to identify novel tumor-expressed miRNAs associated with VTE.
Methods: In a cohort of 418 CRC patients diagnosed between 2001-2015 at the Leiden University Medical Center (LUMC), 23 patients developed VTE 1 year before or after cancer diagnosis. Based on availability of frozen tumor material, age, gender and tumor stage, tumor cells of 17 patients with VTE and 18 patients without VTE were isolated using laser capture microdissection and subsequently analyzed on the Illumina sequencing platform NovaSeq600 using a 150 bp paired-end sequencing. Differential miRNA expression was analysed using edgeR. Sub-analyses were performed depending on date of VTE diagnosis.
Results: A total of 547 miRNAs were detected. Applying a minimum 1.5 fold change (FC) difference and a false discovery rate value of < 0.1, 14 miRNAs were differentially regulated in CRC patients with VTE, compared to without VTE. In a sub-analysis, we assessed miRNAs associated with VTE in the early disease course and not affected by cancer treatment (1 year before cancer diagnosis). Seven significant miRNAs were identified.
Summary/Conclusion: We identified 19 tumor-expressed miRNAs significantly expressed in cancer-associated VTE, which may have the potential to serve as novel, non-invasive predictive biomarkers for VTE in CRC.

P-051

IDENTIFICATION OF STRUCTURAL VARIANTS IN ANTITHROMBIN DEFICIENCY USING MULTIPLE LIGATION-DEPENDENT PROBE AMPLIFICATION

R. Cifuentes [1], J. Padilla [1], B. de la Morena-Barrio [1], M. E. de la Morena-Barrio [1], C. Bravo-Perez [1], P. Garrido [1], A. Miñano [1], V. Vicente [1], J. Corral [1,*]
[1]Servicio de Hematologia y Oncologia Medica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER,,, Murcia, Spain

Background: Structural variants (SVs) are genetic alterations > 50bp with great pathological impact. They are studied in many disorders using multiple ligation-dependent probe amplification (MLPA), a multiplex PCR technique that uses two oligonucleotides that recognize adjacent sites.
In antithrombin deficiency (AT), 3-5% of cases are due to SVs that affect *SERPINC1*. However, the high concentration of intragenic Alu sequences (repetitive elements involved in the formation of SVs), and the identification by our group of *de novo* SVs in this gene, suggest that these SVs may be underestimated.
Aims: To study SVs in *SERPINC1* in patients with AT deficiency using MLPA.
Methods: We studied a cohort of 403 unrelated cases with AT deficiency characterized by functional, biochemical and molecular methods. SVs were evaluated by MLPA (Probemix P227, MRC Holland), and their nucleotide resolution was achieved by nanopore sequencing and/or long PCRs (Long Amp Taq NEB) and next generation sequencing (NGS).
Results: Sequencing methods identified 243 single nucleotide variants (SNVs) or small indels. MLPA identified 15 SVs: 9 complete deletions, 5 partial deletions, and 2 partial duplications. The nucleotide characterization of these SVs showed 2 cases in which the diagnosis of MLPA was not precise: in one case the deletion of an exon detected by MLPA was not complete, while in another, a partial deletion of an exon was not detected by MLPA. Furthermore, 4 SVs affecting introns were not detected by MLPA.
As the molecular diagnosis algorithm ends when a potentially pathogenic variant is detected by the first sequencing method, we speculated that small SVs linked to SNVs or indels affecting exons or introns might be lost. To resolve this issue, we analyzed by MLPA 40 cases with type I deficiency theoretically caused by a SNV or indel detected by sequencing. This analysis revealed one case with a potential deletion of exon 7. However, the patient had a 29bp deletion that partially affected one MLPA probe. In order to identify other cases in which the MLPA could generate incorrect results, we analyzed 28 patients with genetic alterations of different exons affecting the probes used in MLPA: 23 SNVs, 3 small deletions, 1 small insertion and 1 small complex variant. In two cases, the MLPA mistakenly identified SVs, both deletions of the whole exon that indeed was: 1) a 28bp duplication and 4bp deletion, and 2) a SNV that affects the last nucleotide of a probe, in the zone of interaction of the probes.
Summary/Conclusion: MLPA was able to identify 15 SVs in *SERPINC1* responsible for AT deficiency in our cohort. However, this method has some limitations. Small insertions or deletions, or even point mutations that affect the hybridization of the MLPA probes can lead to an incorrect complete exon deletion diagnosis. Moreover, MLPA does neither detect intronic SVs.
Funding: ISCIII&FEDER: PI18/00598, FI19/00048, CM20/00094; Seneca Fundation-19873/GERM/15

P-052

EVALUATION OF STA-ECA II REAGENT FOR ARGATROBAN MONITORING

A. GENIN [12], K. Carrière, T. Flor[2*]
[1]laboratory of hematology, Hospital, AIX EN PROVENCE , France, [2]Stago, Leiden, Netherlands

Background: The use of argatroban is indicated in the management of patients with suspected HIT or proven HIT but also in case of thrombotic thrombocytopenia mediated by PF4 due to COVID-19 vaccination (Vaccine-Induced Immune Thrombotic Thrombocytopenia).
Short half-life and extra-renal metabolism of argatroban make its use necessary in sever renal impairment or in need of rapid cessation of the anticoagulant action (urgent surgery).
Biological monitoring of argatroban with APTT is defined by an increase of 1.5 to 3 times patient's initial APTT. Nevertheless, APTT is sensitive to multiple interference. Its lengthening is not proportional to argatroban concentration and depends on the reagent used, making it an unreliable witness of anticoagulant activity of argatroban.
Ecarin chromogenic methods (ECA) are described in the literature since more than 15 years as able of monitoring the anti-IIa activity of direct thrombin inhibitors as dabigatran or argatroban. The principle of dosage free itself from the interference impacting the APTT and is therefore suitable for argatroban monitoring.
Aims: The aim of this study was to determine performance of STA-ECA II reagent (Stago), in order to check its suitability for routine use on STA-R Max system to monitore the anticoagulant action of the argatroban.
Methods: The performance of STA-ECA II have been evaluated in the laboratory of hematology "Centre Hospitalier Intercommunal d'Aix-Pertuis" (France) in may 2021. Dosing protocol has been configured on the STA-R Max following the recommendations of Stago.
A method comparison has been carried out between STA-ECA II and the TTD HTI reagent, chronometric dosing method based on diluted thrombin time (Hyphen) on 28 samples. Dosing protocol has been configured on the STA-R Max following the recommendations of Hyphen.
Results: Calibration curve shows a good correlation between anti-IIa activity and the level of coloration of the reaction medium over the concentration range studied (0 to 2 µg/ml).
Automatic relaunch at 1/20th of sample with a concentration greater than 2µg/ml allows to obtain results up to 4 µg/ml.
The analytical performances obtained at the native dilution of 1/10th are excellent, both on terms of repeatability and reproducibility with a coefficient of variation below 10%. Automatic relaunch doesn't impact this performance level.

During this study, we had a limit of quantification below 0.2 µg/ml allowing to keep a coefficient of variation lower than 20% on a 10-pass repeatability test. This limit is adapted to biological monitoring of argatroban which has a therapeutic range between 0.5 and 2 µg/ml.

Method comparison gave consistent results and a good correlation between the 2 methods for the determination of argatroban on the whole concentration range (0.3 to 4 µg/ml).
Summary/Conclusion: The use of STA-ECA II reagent is a valuable alternative to APTT for biological monitoring of anticoagulation action of argatroban. It can be used routinely on STA-R Max system. Using a unique reagent,STA-ECA II, for the biological monitoring of dabigatran and agatroban is a perfect way to reduce the costs of carrying out specific dosing and to contribute to their distribution within laboratories to ensure an optimal follow-up of these anticoagulants.

P-053

Inter-laboratory variability of the standardized ETP-based APC resistance assay

L. Morimont [1 2 3,*], M. Didembourg [2 3], H. Haguet [2 3], E. Modaffari [1], M. Tillier [4], A. Lebreton [4], C. Bouvy [1], J.-M. Dogné [2 3], J. Douxfils [1 2 3]
[1]Qualiblood sa, [2]Pharmacy Department, University of Namur, [3]Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Namur , Belgium, [4]Service d'hématologie biologique , CHU Clermont-Ferrand, Clermont-Ferrand, France

Background: In 2004, the endogenous thrombin potential (ETP)-based activated protein C (APC) resistance assay was listed by the European Medicines Agency for the evaluation of APC resistance during the development of steroid contraceptives in women. However, one of the main limitations of this technique was its lack of standardization and harmonization, which hampered study-to-study comparison. A standardized methodology that met all the regulatory requirements in terms of analytical performances has been developed recently. The final step of this validation was the transfer of this methodology to different laboratories in order to ensure a wide implementation of this promising test.
Aims: The aim of this study was to assess the inter-laboratory transferability of the ETP-based APC resistance assay.
Methods: The ETP-based APC resistance assay was implemented in two testing laboratories and results were compared to those obtained in the reference laboratory. First, dose-responses curves were performed at each unit to define APC concentration in order to obtain 90% of inhibition of the ETP on healthy donors. Intra- and inter-run repeatability were assessed on a commercial reference plasma and three levels of quality controls. To investigate the variability in results of donor samples among the originating unit and both receiving units, 60 plasma samples were analyzed at each site. Results were expressed either as inhibition % of the ETP or as normalized APC sensitivity ratio (nAPCsr).
Results: The APC concentration was defined at 1.21 µg/mL and 1.14 µg/mL in the two receiving units. Intra- and inter-run repeatability showed standard deviations below 3%. Analyses of plasma samples from the 60 individuals showed no statistically significant difference (Friedman P >0.05). The Spearman correlations between nAPCsr values from each receiving unit and nAPCsr values from the originating unit showed significant effective pairings (rs >0.98). Linear regressions showed the following equations $Y = 1.154 x – 0.2409$ ($r^2 = 0.98$) for receiving unit 1 and $Y = 0.9213 x + 0.08939$ ($R^2 = 0.99$) for receiving unit 2.
The sensitivity of the test in the different laboratories is maintained and subgroup analyses still report significant differences between women not using hormonal contraception and women using 2nd generation combined oral contraceptives (COCs) (P = 0.0081) or 3rdgeneration COCs (P = 0.002). The same tendency was observed for men and women using COCs (P <0.001 regardless of COC generation). On the other hand, there was no significant difference between women without COC and men (P = 0.3635), and between women using 2nd and 3rd generation COC (P >0.9999).
Summary/Conclusion: This study is the first reporting the inter-laboratory variability of the validated ETP-based APC resistance assay. Data revealed excellent intra-laboratory precision and inter-laboratory reproducibility. These results support the concept that the normalized APC sensitivity ratio obtained with our validated and standardized methodology, provides an appropriate sensitivity irrespective of the laboratory in which the analysis is performed.

P-054

Clot growth from a surface results in layers of fibrin with variation in lysability.

C. Kluft*, C. Krijnen [1], A. Ramlal [1], P. de Kruijff [1]
[1]biomarkers, Good Biomarker Sciences, Leiden, Netherlands

Background: Clot growth and clot size are read outs that can be studied with the thrombodynamics analyser where plasma is clotting from a surface of immobilised tissue factor. By incorporating a thrombin substrate in the cuvette the thrombin formation can be followed at various distances from the start. By incorporating t-PA in the clot we can follow the lysis at several distances from the start.
Aims: To evaluate in layers the thrombin formation and lysis by t-PA
Methods: Clot growth and lysis with the thrombodynamics equipment
Results: In the growing clot, we can identify layers with different thrombin peak levels such as in normal plasma there is a high peak close to the surface with TF and a practically constant peak further on (> 1mm) .
We tested in normal plasma lysis at 0.35; 1,0 and 2,0 mm from the starting surface. We observed slow lysis at 0,35 mm and faster lysis at 1,0 and 2,0 mm. This pattern is congruent with the thrombin tested at the same places.
In haemophilia plasma we observed very low thrombin at 0,35 mm and also low at 1,0 and 2,0 mm compared to normal plasma. This coincided with a rapid lysis at 0,35 mm and slower but still rapid lysis at 1,0 and 2,0 mm.
Spiking HA plasma with purified factor VIII resulted in decrease of lysis in all layers.

Summary/Conclusion: Thrombin profiles coincide with lysability suggesting a dominant role of thrombin in stabilisation of fibrin. The thrombin detected in the clot during growing is proposed as a proxy of lysability
There is a gradient of thrombin and lysability in growing clots, showing that the outer layers of the clots lyse better.

P-055

HIGH THROUGHPUT B-CELL EPITOPES PROFILING

P. Tomar [1,*]
[1]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy

Background: Deficiency in clotting factor VIII causes hemophilia A, an X-linked bleeding disorder. Patients with this genetic disorder are treated with factor VIII products (plasma-derived or recombinant), in which the greatest barrier is the development of inhibitors (anti-factor VIII alloantibodies). Recent research studies have shown that type of factor VIII product is one of the most important risk factors for inhibitor development.
Aims: In this research project, we are focusing mainly on two tasks, (a) creating an immunoprofile (IP) of patients treated with factor VIII products developing inhibitors or not, (b) analysing the difference in IPs of patients treated with plasma-derived factor VIII products or with recombinant factor VIII products.
Methods: Next generation phage display technology (Mimotope Variation Analysis - MVA) was used to examine the antigenic repertoire of plasma of patients with hemophilia A (tot = 124 patients, of whom 39 developed inhibitors and 83 did not) before and after replacement therapy (plasma-derived or recombinant factor VIII product). A modified random peptide display library (PhD12), based on the M13KE phage vector carrying billions of different 12-mer peptide sequences was used. Captured phages were lyzed and resulting amplicons were pooled for next generation DNA sequence analysis. The data cleaning was performed on the generated data, in which target unrelated peptides are removed by comparing the generated data with biopanning database (BDB) using a command line tool SAROTOP - a tool for scanning, reporting and excluding possible target-unrelated peptides from mimotopes.
Results: The MVA was performed on pre-treatment samples, post-treatment samples, and samples pre-incubated with factor VIII (competition MVA). On the basis of MVA, we have generated an IP of (a) hemophilia A patients before the start of the treatment, (b) hemophilia A patients after the treatment, (c) hemophilia A patient samples pre-incubated with factor VIII. Further, IP of patients were analysed on the basis of replacement therapy and inhibitors development. Data cleaning process searches for peptides binding to unintended materials and removing unrelated peptides, resulting in a reduction (up to 10%) in whole peptide sequences.
Summary/Conclusion: A comprehensive 372 IPs were analysed for 124 unique patients, comprising 130 million unique and 1.2 billion whole 12-mer peptide sequences. After preliminary cleaning and analysis, we summed the copy number of each unique peptide over all dataset. After the cleaning process, we conditioned the data to make it ready for subsequent data analysis.

P-056

Transferability of the ETP-based APC resistance assay on the ST Genesia system

L. Morimont [1 2 3,*], M. Didembourg [2 3], A. Carlo [4], J.-M. Dogné [2 3], J. Douxfils [1 2 3]
[1]Qualiblood sa, [2]Pharmacy Department, University of Namur, [3]Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Namur , Belgium, [4]Diagnostica Stago , Asnières-sur-Seine, France

Background: Regulatory bodies recommend assessing the endogenous thrombin potential (ETP)-based activated protein C (APC) resistance assay during the development of combined oral contraceptives (COC) in women. In 2019, this assay was validated and standardized on the Calibrated Automated Thrombogram (CAT) device using commercially available reagents to ensure batch-to-batch traceability, and reproducibility of the method over time. However, in view of its screening potential, its implementation in clinical routine is essential.
Aims: This study aimed at implementing the ETP-based APC resistance assay on the automated ST Genesia system (Stago, France), using reagent STG-ThromboScreen -TM (TS -TM) (Stago, France) with exogenous APC added (Stago, France).
Methods: Healthy pooled plasma (HPP) constituted of 20 healthy individuals (10 men and 10 women not using COC, not carrier of FV Leiden nor G20210A mutation) was used to define APC concentration leading to 90% of inhibition of the ETP. Dose-response curves were performed on two different batches of TS -TM. The intra- (N=5) and inter-run (N=10) variability were assessed on three samples: STG-RefPlasma TS, STG-QualiTest Norm TS and STG-QualiTest High TS. STG-QualiTest Low TS was not assessed as thrombin generation was completely inhibited in presence of APC. Fifty-six samples from healthy individuals (32 men and 24 women not using hormonal contraception, not carrier of FV Leiden or G20210A mutation) were analyzed to define reference ranges. To assess the sensitivity of the test, 36 women taking hormonal therapy were recruited and stratified based on their contraceptive product: women using COC containing ethinylestradiol (EE) with levonorgestrel (LNG) (N=18); women using COC containing EE associated with desogestrel (DSG) or gestodene (GSD) (N=12) and women using COC containing EE associated with drospirenone (DRSP), cyproterone acetate (CPA) or dienogest (DNG) (N=6).
Results: The APC concentration [IC95%] leading to 90% of inhibition of the ETP, on both batches of TS -TM was defined at 652 mU/mL [534-805 mU/mL]. Intra- and inter-run variability showed standard deviations (SD) values below 2.0% and 3.5 % respectively, regardless of the tested samples. The mean inhibition % [±SD] over the 56 healthy subjects was 89.5% [87.3%-91.6%]. Mean inhibition % ± SD of each subgroup were: 63%±13% for women using EE+LNG; 59±11% for women using EE+GSD/DSG; and 44%±6% for women using EE + DRSP/CPA/DNG. Women using COC, regardless of the estro-progestin association, showed significantly lesser response to APC than healthy individuals (Holm-Sidak's P <0.05). Among women using COC, significant differences were observed for EE associated with DRSP, CPA or DNG compared to other combinations (Holm-Sidak's P <0.05).
Summary/Conclusion: This study is the first reporting the transferability of the ETP-based APC resistance assay on the ST Genesia system, an automated clinical device for evaluating thrombin generation. Data revealed excellent precision (within- and between-run repeatability) and provided an appropriate sensitivity depending on the hormonal status of women. Confirmation of promising results on thrombogenicity identification for all COC users is still needed before this test can be commercially available.

P-057

Influence of Tissue-Factor Pathway Inhibitor on Thrombin Generation Assay

M. Didembourg [1 2,*], J. Douxfils [1 2 3], A. Carlo [4], F. Mullier [2 5], M. Hardy [2 6], L. Morimont [1 2 3]
[1]Department of pharmacy, University of Namur, [2]Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), [3]QUALIblood sa, Namur, Belgium, [4]Diagnostica Stago, Asnières-sur-Seine, France, [5]CHU UCL Namur, Laboratory Hematology, [6]CHU UCL Namur, Department of Anesthesiology, Université catholique de Louvain, Yvoir, Belgium

Background: Tissue-factor pathway inhibitor (TFPI) is an anticoagulant protein that exerts its activity by forming an inactive FXa-TFPI-TF-FVIIa quaternary complex. In plasma, TFPI is normally found at a concentration of about 70 ng/mL but many pathologies impact its levels, modifying the hemostatic function of individuals. In COVID-19 patients, in spite of a hypercoagulability state, increased levels of TFPI up to 400 ng/mL were observed.
Aims: This study aimed to assess the impact of TFPI on thrombin generation assay (TGA), when performed on the Calibrated Automated Thrombogram (CAT) and on the ST-Genesia system.
Methods: Normal pooled plasma (NPP) constituted of 50 healthy individuals was used as matrix. Recombinant TFPI was spiked in NPP at five relevant plasma concentrations (0 [=Phosphate buffer saline], 50, 100, 200 and 400 ng/mL). TGA was assessed on both platforms by 3 independent runs and the triggering reagent was the STG-ThromboScreen-TM (Stago, France). The percentages of change from baseline (i.e., 0 ng/mL) were calculated for each TGA parameter.
Results: On both platforms, based on mean values, statistically significant differences were observed between the five tested concentrations (p-value<0.05). Though not statistically significant, percentages of change from baseline were more pronounced on the ST-Genesia compared to the CAT system, especially for the endogenous thrombin potential (ETP) (decrease of 25% and 17%, respectively), for the lag time (LT) (prolongation of 17% and 10%, respectively) and for the peak height (PH) (decrease of 47% and 35%, respectively).
Summary/Conclusion: Regarding the physiological role of TFPI, impact on the LT parameter was expected. On the other hand, the important inhibitory effect of TFPI on the ETP and PH is a novel observation. This study showed that TFPI levels impacted in a dose-dependent manner TGA performed either on the CAT or on the ST-Genesia system. Any acute or mechanistic situations in which TFPI levels may be impacted will thus interfere with TGA.

P-058

Molecular diagnosis and optimization of third generation sequencing methods in hemostasia and thrombosis: globalization of molecular analysis

J. Padilla Ruiz [1,*], M. E. de la Morena-Barrio [1], P. Garrido [1], R. Cifuentes [1], C. Bravo-Perez [1], A. Miñano [1], V. Vicente [1], J. Corral de la Calle [1], B. de la Morena-Barrio [1]
[1]Servicio de Hematología y Oncología Médica,, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER,, Murcia, Spain

Background: The development of next generation sequencing (NGS) was a revolution in molecular diagnosis. However, these sequencing technologies are restricted to specialized laboratories due to the expensive equipment and high technical expertise requirements. NGS, based on short readings and high depth, allows easy point mutation detection, but can limit the characterization of structural variants (SVs) and haplotype analysis. The development of third-generation sequencing may solve these limitations with the obtention of ultra-long reads. Moreover, the portable device MinION, with low cost and requirements, takes a step forward in globalizing the access to genomics.
Aims: To evaluate nanopore sequencing in a clinical genomic laboratory and to implement new methods to increase the yield of sequencing using a MinION device
Methods: Sequencing *SERPINC1* (7 exons/13Kb) using NGS (PGM), Sanger, and nanopore (MinION) in 96 patients with antithrombin deficiency.
For the MinION device, 3 overlapping long PCRs with barcodes were used.
In order to increase the yield of MinION in direct DNA sequencing, enrichment of specific target genomic regions were done in six patients with SVs affecting *SERPINC1* detected by other methods, following two strategies:
1) Experimental enrichment, using LR-PCR amplicons that only had a forward primer targeting the region of interest.
2) Informatics real time *in silico* enrichment of 3MB-genomic region using *readuntil* tool.
Bioinformatic analysis used a local pipeline including alignment, structural and punctual variants calling and haplotyping
Results: *SERPINC1* sequencing of LR-PCR on the MinION device was the fastest (0.5h) and cheapest (0.0001€/bp) system. This nanopore sequencing reached high depth allowing somatic variant detection. Furthermore, this system detected SNVs, large indels, certain SVs, and long reads facilitated the identification of haplotypes for each allele. However, detection of small indels, especially those located in homopolymeric regions was not guaranteed and large SVs spanning the PCR primers were not detected.
The experimental enrichment approach obtained a mean depth of 186x in 38 minutes sequencing with a cost of 257€/sample. The mean length of reads was 1508bp. This method characterized all partial SVs studied but not SVs affecting the whole gene.
Informatics enrichment provided a depth of 3.3x after 20 hours of sequencing with a cost of 491€/sample. The mean read length was 4732bp. This allowed detection of all SVs, regardless its size or type, as well as epigenetic analysis.
Summary/Conclusion: Our study shows a highly efficient sequencing system that allows universal sequencing processes due to its simplicity and cost. It requires an initial investment 100 times lower, has a costs 70 times lower, and render 8 times faster procedures than other sequencing systems. The possibilities of this equipment range from the identification of genetic variants, including SVs, to the determination of haplotypes and epigenetic studies, both for DNA and RNA.
Finally, we have implemented two approaches that allow the enrichment of target regions using the minION device, allowing the sequencing of not manipulated DNA to detect any kind of SVs and epigenetic modifications.

Funding: ISCIII&FEDER: PI18/00598, FI19/00048, CM20/00094; Fundación Séneca-19873/GERM/15

P-059

Fragmentation of Histone H3 in severe COVID-19 patients

G. A. Nicolaes [1,*], J. Huckriede [1], F. de Vries [1], M. Hultstrom [2], K. Wichapong [1], C. Reutelingsperger [1], M. Lipcsey [2], P. Garcia de Frutos [3], R. Frithiof [2]
[1]Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht, Netherlands, [2]Surgical Sciences, section Anaesthesia & Intensive Care, Uppsala University, Uppsala, Sweden, [3]Department of Cell Death and Proliferation, , IIBB-CSIC, IDIBAPS and CIBERCV, Barcelona, Spain

Background: The severity of coronavirus disease 19 (COVID-19) is associated with neutrophil extracellular trap (NET) formation. During NET formation, cytotoxic extracellular histones are released from activated neutrophils. Extracellular histones are known as damage-associated molecular patterns (DAMPs) but their presence is not merely a reflection of ongoing damage: they actively contribute to damage promotion, largely due to their cytotoxic and cell-activation properties. In fact, the presence of histones in circulation is linked to the initiation and progression of several prothrombotic processes as it is to acute inflammatory diseases.
Aims: With this study we aimed to quantitate the presence and evolution of extracellular histone H3 and several other neutrophil-related molecules and DAMPs as cell-free DNA (cfDNA), neutrophil elastase (NE), myeloperoxidase (MPO), and the DNA-MPO complex in the plasma of 117 COVID-19-positive ICU patients.
Methods: The methods employed used a combination of biochemical approaches (ELISA, semi-quantitative Westernblotting), routine clinical chemical analyses, along with recording of routine clinical observations at the ICU and statistical analysis.
Results: We show that at the time of ICU admission, the levels of histone H3, cfDNA, NE, MPO and DNA-MPO complex were all significantly increased in COVID-19-positive patients compared to control samples. Furthermore, in a subset of 54 patients from whom we could obtain multiple samples during their stay at the ICU, the levels of each marker remained increased after 4+ days compared to admission. Histone H3 was found in 28% of the patients on admission to the ICU and in 50% of the patients during their stay at the ICU. Notably, in 47% of histone positive patients we observed proteolysis of histone in their plasma as was evidenced from the appearance of lower molecular weight forms of this protein upon Western blot analysis.Overall presence of histone H3 during ICU stay was associated with thromboembolic events and secondary infection, and non-cleaved histone H3 was associated with the need for vasoactive treatment, invasive ventilation and the development of acute kidney injury.
Summary/Conclusion: Our data show for a clear association between histone presence, their cleavage and clinical events like thromboembolic events and secondary infection, need for vasoactive treatment, invasive ventilation and the development of acute kidney injury. Collectively, these data support the validity of treatments that aim to reduce NET formation and additionally underscore that more targeted therapies focused on the neutralization of histones should be considered as treatment options for severe COVID-19 patients.

P-060

LUPUS ANTICOAGULANT-HYPOPROTHROMBINEMIA SYNDROME (LAHS) ASSOCIATED TO MARGINAL ZONE LYMPHOMA

A. Peleteiro Raíndo [1,*], E. Fernández Mellid [1], P. Cadahía Fernández [1], P. Melero Valentín [1], A. Abuin Blanco [1], M. Cid López [1], A. Mosquera Orgueira [1], J. Á. Díaz Arias [1], N. Alonso Vence [1], L. Bao Pérez [1], A. De Andrés y Jacob [1], M. J. Rabuñal Martínez [1], M. M. Pérez Encinas [1]
[1]Hematology, Hospital Clínico Universitario de Santiago de Compostela (CHUS), Santiago de Compostela, Spain

Background: Autoantibodies toward clotting factors are rare disorders that can be developed in people suffering from autoimmune or neoplastic diseases, after drug intake or even in healthy subjects. They are more commonly directed against factor VIII or von Willebrand factor, but can also develop against other clotting factors and course with different clinical manifestations ranging from minimal or no bleeding to life-threatening events.
Aims: Our objective is to clarify the diagnosis of a 76 year-old patient with a Marginal Zone Non Hodgkin Lymphoma (MZL) detected in 2020 and a personal history of several bleeding manifestations since then (massive hemoptysis, bleeding after splenectomy, knee hemarthrosis). When MZL was detected, she has a prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT), alterations already present in studies carried out the previous year. The prolonged APTT was prolonged with a 1:1 mixture of the patient plus normal plasma, Factor II (FII:C) was 19% in the absence of other clotting factor deficits and a positive lupus anticoagulant (LA) was detected by Rusell and Sylica clotting time hemosil, with no evidence of FII inhibitor at that time.
Interestingly, when MZL remission was achieved after treatment, the coagulation study returned to normal. However, the alterations described reappeared when MZL relapse was detected in May 2021.

Methods: Once relapse and recurrence in coagulation abnormalities was detected, we initially performed a Kasper test by incubating a 1:4 mixture of the patient sample with normal plasma for two hours at 37 degrees, both together and separately, that was compatible with LA. Subsequently, we ruled out the presence of a possible associated factor II inhibitor facing a denatured sample from the patient incubated 2 hours at 56 degrees against a pool of plasma with a known amount of factor II, to make a parallelism factor assay with mixtures of 50%, 25% and 12.5% of the test sample and we did not observe a significant effect of the inhibitor. Finally, we performed an anti prothrombin antibody test by ELISA.

Results: Once the relapse of MZL was detected, a lymph node biopsy was necessary, so we evaluate the hemorrhagic risk of the patient. After ruling out the presence of another type of inhibitor with the Kasper test and the parallelism above described, the antiprothrombin IgM antibody test was positive at high title, which together with LA confirms the presence of LAHS.
Summary/Conclusion: The isolated factor II deficiency can be observed in patients with LA. This uncommon association appears to beat mostly associated with autoimmune disorders, but it has been reported in a few other conditions, including primary antiphospholipid syndrome, infections, drugs and lymphoma. A correct diagnosis is essential in order to rule out the presence of another type of factor II inhibitor and to avoid spontaneous or after procedures bleeding complications.
This result is somewhat discordant with the patient's bleeding history, but this may be due to the fact that many other bleeding risk factors are involved in vivo (capillary fragility, drugs). The literature already reflects that the bleeding pattern of these entities shows great variability.

P-062

Association between cardiovascular risk factors and venous thrombosis in the elderly

H. Wang [1,*], M. Cushman [2], F. R.Rosendaal [1], A. van Hylckama Vlieg [1]
[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands, [2]Department of Medicine, Larner College of Medicine at the University of Vermont, Burlington, United States

Background: Several cardiovascular risk factors have been associated with the risk of venous thrombosis (VT) in young and middle-aged populations. Limited information about these associations is available in the elderly.
Aims: To investigate whether the cardiovascular risk factors BMI, smoking, alcohol intake, hypertension, and diabetes are associated with the risk of VT in elderly and to assess the combined effect between these cardiovascular risk factors and genetic risk factors for VT (factor V Leiden (FVL), prothrombin 20210A (PT20210), positive family history of VT, and non-O blood group).
Methods: Analyses were performed in the AT-AGE study, a multi-center case-control study performed in Vermont, USA and Leiden, NL, comprising of 401 cases with a first VT and 431 control subjects, all aged ≥70 years. All participants were visited at home by a trained research nurse. Body weight, body height, and blood pressure were measured by the research nurse, other cardiovascular risk factors were self-reported by an interview. A blood sample was drawn for genotyping. To assess the risk of VT, odds ratios (OR) with 95% confidence intervals (CI) were calculated, after adjustment for potential confounders. Confounding factors considered were age, sex, study center, cardiovascular risk factors (BMI, smoking, alcohol intake, hypertension, diabetes; depending on the exposure) and comorbidities (heart failure, angina, myocardial infarction, cerebral bleeding, transient ischemic attack and cerebral infarction). All participants provided written informed consent. This study was supported by grants from the Netherlands Heart Foundation (grant no: 2009B50) and the Leducq Foundation, Paris, France, for the development of Transatlantic Networks of Excellence in Cardiovascular Research.
Results: Both body height and weight were positively associated with VT risk: ORs were 2.2 (95%CI: 1.2-3.9) for height and 1.5 (95%CI: 1.0-2.4) for weight, when comparing individuals in the top quartile with those in the lowest quartile. These risks were more pronounced for unprovoked VT (defined as thrombosis without hospitalization (including recent surgery), fracture, plaster cast, splint, minor injuries of lower extremities (such as a sprained ankle or contusion of the lower leg), or transient immobility at home ≥ 4 successive days in the three months before the thrombosis. No association with VT was observed for smoking, alcohol intake, and diabetes. Higher systolic (OR for individuals in the highest quartile compared with the lowest quartile: 0.4; 95% CI 0.3-0.7) and diastolic blood pressure (OR for individuals in the highest quartile compared with the lowest quartile: 0.4; 95% CI 0.2-0.6), as well as hypertension (OR: 0.7; 95% CI 0.4-1.0), were associated with a decreased risk of VT. In the presence of a genetic predisposition, height and weight further increased the risk of VT, while hypertension lowered the risk of VT.
Summary/Conclusion: In the elderly, height and weight were positively associated with the risk of VT. With genetic predisposition, being higher levels of height and weight further affect the risk of VT. High blood pressure decreased the risk of VT in the elderly.

P-063

Tailored anticoagulant treatment after a first venous thromboembolism: protocol of the Leiden Thrombosis Recurrence Risk Prevention (L-TRRiP) study, a cohort based randomized controlled trial

L. Burggraaf [1,*], N. van Rein [1], R. Bemelmans [2], J. W. van den Berg [3], M. Coppens [4], A. Dees [5], L. Faber [6], W. de Jong [7], T. Koster [8], R. Mairuhu [9], K. Meijer [10], M. Nijkeuter [11], M. van de Ree [12], E. van den Akker - van Marle [13], S. le Cessie [1 13], M. Huisman [14], E. Klok [14], S. Cannegieter [1 14]
[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, [2]Department of Internal Medicine, Ziekenhuis Gelderse Vallei, Ede, [3]Department of Pulmonology, Isala Hospital, Zwolle, [4]Department of Vascular Medicine, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, [5]Department of Internal Medicine, Ikazia Ziekenhuis, Rotterdam, [6]Department of Internal Medicine, Red Cross Hospital, Beverwijk, [7]Department of Pulmonology, Ziekenhuis Gelderse Vallei, Ede, [8]Department of Internal Medicine, Groene Hart Hospital, Gouda, [9]Department of Internal Medicine, Haga Teaching Hospital, The Hague, [10]Department of hematology, University Medical Center Groningen, Groningen, [11]Department of Internal Medicine, University Medical Center Utrecht, [12]Department of Internal Medicine, Diakonessenhuis, Utrecht, [13]Department of Biomedical Data Sciences, [14]Department of Medicine - Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, Netherlands

Background: Patients with a first venous thromboembolism (VTE) are at risk of recurrence. A recurrent VTE can be prevented by prolonged anticoagulant therapy, but this may come at the cost of major bleeding. Currently the decision to prolong anticoagulant therapy is based on whether the VTE was provoked or not. However, this strategy is not optimal since the risk of major bleeding is not taken into account, a uniform definition of provoked VTE is lacking and the strong difference in risk of recurrent VTE between individual patients within the provoked and unprovoked VTE groups is disregarded. Previously, the L-TRRiP score has been developed to predict the risk of recurrent VTE in all patients with a first event and to classify it as low, intermediate or high. Furthermore the VTE-BLEED score is developed to classify the risk of major bleeding as low or high. However, their combined use in finding the optimal balance to minimize both long-term risks is unclear.
Aims: To evaluate tailored duration of long-term anticoagulant treatment based on individualized risk assessments of recurrent VTE and major bleeding.
Methods: The L-TRRiP study is a multicenter, open-label, cohort based randomized controlled trial with blinded endpoint assessment including 1600 participants with a first VTE. Patients with cancer, antiphospholipid syndrome or other long-term indications for anticoagulants, as well as patients with indications for platelet inhibitors despite the use of anticoagulants are excluded.
After provision of written informed consent, each patient's individual recurrent VTE and major bleeding risk will be determined using the L-TRRiP and VTE-BLEED prediction scores, respectively. The L-TRRiP score includes sex, clinical characteristics of the first VTE (type and location and presence of known risk factors), history of cardiovascular disease and genetic information (blood group and factor V Leiden) to predict the 2-years risk of VTE recurrence. Patients with a predicted 2-years recurrence risk <6% are considered at low risk, patients with a predicted 2-years risk >14% are classified as high risk. The VTE-BLEED score includes age, comorbidity (anemia, renal insufficiency, uncontrolled hypertension, malignancy) and previous bleeding to classify patients at low vs. high risk of major bleeding.
After the initial three months of anticoagulant treatment, patients with a low recurrent VTE risk will discontinue anticoagulants, whereas patients with a high recurrent VTE risk and low major bleeding risk will continue. The other groups (i.e. patients with an intermediate recurrent VTE risk or a high recurrent VTE combined with a high major bleeding risk), with unclear benefit of prolonged treatment, will be randomized to continue or discontinue anticoagulants. The primary outcome is the incidence of recurrent VTE and major bleeding in the randomized group after 2 years follow-up. Secondary outcomes are quality of life, cost-effectiveness and functional outcomes in all groups and incidence of recurrent VTE and major bleeding in the non-randomized groups. The study has been approved by the Institutional Review Board Leiden-Den Haag-Delft.
Results: Results are expected in 2025.
Summary/Conclusion: The L-TRRiP study will assess whether a tailored strategy, based on classification of both recurrent VTE risk and major bleeding risk, leads to minimized risks of both complications.

P-064

Plasma levels of ADAMTS13, von Willebrand Factor/ADAMTS13 ratio and future risk of incident venous thromboembolism

M. S. Edvardsen [1,*], E.-S. Hansen [1], K. Hindberg [1], V. M. Morelli [1], T. Ueland [1 2], P. Aukrust [2 3], S. K. Brækkan [1 4], J.-B. Hansen [1 4]
[1]Thrombosis Research Center (TREC), Department of Clinical Medicine, UiT - The Arctic University of Norway, Tromsø, [2]Research Institute of Internal Medicine, [3]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, [4]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Background: Plasma von Willebrand Factor (VWF) is a risk factor for venous thromboembolism (VTE).
Aims: As ADAMTS13 is a fundamental enzyme in the regulation of VWF, we aimed to investigate whether plasma ADAMTS13 levels or the ratio between VWF and ADAMTS13 levels were associated with risk of future VTE.
Methods: A nested case-control study consisting of 383 VTE cases and 780 controls was derived from a population-based cohort study. Plasma ADAMTS13 and VWF levels were measured in blood samples drawn at baseline, and the participants were followed up for 12 years. Odds ratios (OR) with 95% confidence intervals (CI) for VTE were estimated across quartiles of ADAMTS13 and VWF/ADAMTS13 ratio. Informed written consent was attained from all participants, and the regional committee for medical and health research ethics approved the study.
Results: Participants with ADAMTS13 in the lowest quartile had an OR of 1.40 (95% CI 0.99-1.99) for VTE compared with those with high ADAMTS13. The ORs were higher when the time between blood sampling and VTE events were shortened. A VWF/ADAMTS13 ratio in the highest quartile yielded an OR of 3.07 (95% CI 2.06-4.57) for VTE compared to those in the lowest quartile. The VTE risk increased linearly across quartiles of VWF/ADAMTS13 ratio (*p* for trend<0.001). The association was most prominent for unprovoked events (OR 3.76, 95% CI 2.11-6.69) and deep-vein thrombosis (OR 3.49, 95% CI 2.15-5.65). The risk estimates were only negligibly affected by adjustments for age, sex, body mass index and C-reactive protein.
Summary/Conclusion: Our results indicate that low plasma ADAMTS13 levels are associated with increased future risk of VTE, and that the VWF/ADAMTS13 ratio is linearly associated with VTE risk. Our findings suggest that a disequilibrium in plasma levels of VWF and ADAMTS13 represents a risk factor for VTE.

P-065

COVID-19 diagnosis and its impact on thrombotic risk in a cohort of consecutive patients hospitalized due to Acute Respiratory Distress Syndrome

A. Coy Canguçu [1,*], G. A. Locachevic [2], J. C. Silva Mariolano [2], K. H. De Oliveira Soares [2], J. D. Oliveira [2], C. De Oliveira Vaz [2], G. Vieira Damiani [2], B. Mazetto [2], J. Annichino-Bizzacchi [2], E. V. de Paula [2], F. Andrade Orsi [2]
[1]Pontifícia Universidade Católica de Campinas (PUCC), [2]Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil

Background: Previous evidence suggest that the thromboembolic risk is greater among patients with COVID-19 than those affected by other types of acute respiratory distress syndrome (ARDS). However, such comparison has been mainly evaluated in historical cohorts.
Aims: To evaluate thromboembolic events in patients with COVID-19 and other ARDS hospitalized in the same time period.
Methods: We have selected consecutive patients admitted from March to June, 2020 at the UNICAMP Clinical Hospital who met the ARDS clinical criteria established by the Brazilian Ministry of Health and the Berlin Definition. COVID-19 diagnosis was confirmed by real time polymerase chain reaction or enzyme-linked immunosorbent assay. Descriptive analysis, chi-square and t-tests were used to compare COVID-19 and non-COVID-19 patients.
Results: Of the 377 patients admitted during this period, 100 COVID-19 patients and 100 non-COVID-19 patients were included in this study. 67% and 55% were men (P=0.08), respectively. The median age was 57.36 years (IQR 45.84 to 65.83) in the COVID-19 group, and 53.87 years (IQR 43.94 to 68.91, P=0.3) in the non-COVID-19 group. Both groups had a similar baseline risk of thrombosis, assessed by: previous thromboembolic events; recognized "thrombophilia"; infarction, stroke, trauma and/or surgery within the past 4 weeks. Oxygen saturation at admission was lower in COVID-19 patients (92% IQR 90% to 97%) than in non-COVID-19 patients (95% IQR 89% to 96%, P=0.03); accordingly, the need for invasive oxygen support was greater and more lasting (44%; 16.00 days IQR 8.50 to 22.50) in the COVID-19 group than in the non-COVID-19 group (33%, P = 0.05; 12.50 days IQR 4.75 to 21.25, P=0.002). Coagulation markers such as activated thromboplastin time, prothrombin time, platelet count, fibrinogen, and D-dimer levels (1,700.00ng/mL IQR 752.00 to 3,417.00 non-COVID-19; 1,426.50ng/mL IQR 744.25 to 3,461.00 COVID-19, P=1.0) were similar between groups. Although thromboprophylaxis was more frequently administered to COVID-19 (76%) than non-COVID-19 patients (42%, P<0.0001), thrombotic events were more recurrent in the former group: there were 28 reported events in 22 COVID-19 patients against 14 events in 12 non-COVID-19 patients (P=0.06). The most common events in the COVID-19 group were pulmonary embolism (46.43%), thrombosis in unusual sites (25%), and deep vein thrombosis (21.42%), which represented 50% (P=0.07), 7.14% (P=0.23), and 28.57% (P=0.51) of non-COVID-19 thrombotic events, respectively. Unusual sites included upper limb and neck veins and abdominal arteries.
Summary/Conclusion: In this study, we provide clinical evidence that the risk of thrombosis is more pronounced in COVID-19 than in other ARDS, even though COVID-19 patients received anticoagulants more frequently. Laboratory parameters classically used as hypercoagulability markers, such as D-dimer levels, were not able to differentiate the thromboembolic risk between the two study populations. These findings indicate the need to improve clinical screening and treatment for thrombosis in COVID-19 in order to prevent its occurrence and reduce disease mortality.

P-066

Risk factors and predictors for the occurrence of venous thromboembolism in patients with ischemic stroke: A systematic review

B. G. Tøndel [1,*], V. M. Morelli [1], J.-B. Hansen [1 2], S. K. Brækkan [1 2]
[1]Thrombosis Research Center (TREC), Department of Clinical Medicine, UiT – the Arctic University of Norway, [2]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Background: Patients with ischemic stroke are at increased risk of developing venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), especially in the acute phase following the stroke. Identification of stroke patients at high risk of VTE is crucial for targeted, individualized thromboprophylaxis. Increased knowledge on risk factors and biomarkers for VTE in stroke patients may guide development of future risk prediction models and aid clinical decision making. We therefore set out to systematically summarize the available literature on risk factors and predictors for VTE in ischemic stroke.
Aims: To identify risk factors and predictors for VTE in patients with ischemic stroke by performing a comprehensive systematic review of the existing literature.
Methods: We conducted a systematic literature search of Medline and Embase from January 1990 through February 2020. Two reviewers screened all retrieved records, independently and in duplicate. The electronical searches were supplemented by a manual search of reference lists of included papers.
We included studies that investigated demographic, clinical and/or laboratory factors for the occurrence of objectively confirmed DVT and/or PE, in adult patients with ischemic stroke. Studies on ischemic and hemorrhagic stroke were included as long as the majority of the patients had ischemic stroke. We excluded studies of selected subgroups of ischemic stroke patients.
A standardized, pre-piloted form was used for data extraction. Characteristics, results and overall risk of bias in the included studies were presented in tabular and narrative formats. An overview of the range of the reported relative risks (RR) was provided for risk factors and predictors associated with VTE in multivariate models in two or more studies.
The study protocol of this systematic review was published in the PROSPERO database (ID: CRD42020176361, available at https://www.crd.york.ac.uk/prospero).
Results: Of 4674 identified records, 26 studies were included in our systematic review. The following demographic, clinical and laboratory factors were associated with VTE in multivariate models in two or more studies (with RR ranges in brackets): advancing age (per 1-year increase: 1.03-1.11, ≥ 60 years: 1.6-4.0), female sex (1.7-5.0), increasing National Institutes of Health Stroke Scale (NIHSS) score (per 1-unit increase: 1.06-1.27, lower limb NIHSS score ≥ 2: 1.9-4.6), low Barthel Index (3.0-8.3), increasing lesion volume of the brain infarct (per 1 mL-increase: 1.02-1.14), previous history of VTE (1.1-3.7), atrial fibrillation (1.3-1.9), cancer (3.3-5.2), elevated levels of D-dimer (1.05-3.40), C-reactive protein (per 1 mg/dL-increase: 1.35-1.44, > 10 mg/dL: 3.2-10.1) and homocysteine (per 1-unit increase: 1.14-1.15), and indices of dehydration (3.4-8.8). The majority of the included studies were of poor quality and had an overall high risk of bias.
Summary/Conclusion: This systematic review informs on several risk factors and predictors for the occurrence of VTE in patients with ischemic stroke. However, there were few high-quality studies on each factor, and future research should focus on further identification and confirmation of risk factors which can be used to guide development of risk prediction models and improve patient stratification.

P-067

Changes in prescription pattern of oral anticoagulants and prognosis in incident non-valvular atrial fibrillation in the Netherlands

Q. Chen [1,*], M. Toorop [1], W. Lijfering [1], S. Cannegieter [1]
[1]Leiden University Medical Center, Leiden, Netherlands

Background: Whether advances made in anticoagulation management in non-valvular atrial fibrillation (NVAF) have actually benefitted the patients is not well known.
Aims: To investigate changes in prescription pattern of oral anticoagulants (OACs) and prognosis in incident NVAF patients in the Netherlands.
Methods: Incident NVAF patients between 2014 and 2018 were identified from Dutch national statistics. Prescription records of OACs and clinical events including ischemic stroke and major bleeding within one year after the first NVAF diagnosis were examined per calendar year of the first NVAF diagnosis.
Results: A total of 332,743 incident NVAF patients were included. Similar patient profiles were observed between different calendar years. The proportion of patients receiving OACs within one year after the first NAVF diagnosis was 68.22% in patients diagnosed in 2014 and increased to about 73% in 2015 and in the following years. Direct oral anticoagulants (DOACs) were found to gradually replace vitamin K antagonists (VKAs) as the first option (from 13.61% in patients diagnosed in 2014 to 71.97% in 2018). The incidence rate of ischemic stroke within one year after the first NAVF diagnosis in the complete patient population remained similar between patients diagnosed in 2014 and 2016 but appeared to increase from 2016 onwards, while for major bleeding it was continuously decreasing.
Summary/Conclusion: With DOACs being increasingly favoured over VKAs, the risk of ischemic stroke remained stable but the risk of major bleeding steadily decreased in incident NAVF patients in the Netherlands.

P-068

COVID-19-associated coagulopathy in pregnancy: a prospective, case-control study

Z. Bagoly [1,*], E. L. Tóth [2], R. Orbán-Kálmándi [1], T. Deli [2], O. Török [2], L. Lóczi [1], J. Tóth [3], S. Molnár [2], Z. T. Krasznai [2]
[1]Department of Laboratory Medicine, Division of Clinical Laboratory Sciences, [2]Department of Gynecology and Obstetrics, [3]Department of Laboratory Medicine, University of Debrecen, Debrecen, Hungary

Background: The new coronavirus infection (COVID-19) is associated with significant changes in hemostasis parameters and an increased risk of thromboembolic events. Despite its significance, little is known about COVID-19-associated coagulopathy in pregnancy and its effect on the clinical course.
Aims: We aimed to test hemostasis alterations in COVID-19-positive pregnant women as compared to non-infected healthy pregnant women of the same gestational age and correlate results with the clinical course and maternal and perinatal outcomes of pregnancy.
Methods: In this single-center observational case-control study, 39 women with acute COVID-19 infection at 36-40 gestational weeks of their singleton pregnancy (COVID-19+ group) and 39 healthy age- and gestational week-matched pregnant women were enrolled (COVID-19- group). All women were outpatients with mild or no symptoms at admission, and acute infection was confirmed or ruled out using SARS-CoV-2 RT-PCR or antigen test. In addition to routine laboratory tests (including complete blood count, liver-and kidney function tests, high sensitivity C reactive protein assay) screening tests of coagulation, D-dimer, fibrinogen, von Willebrand factor antigen, chromogenic factor VIII (FVIII) activity, factor XIII (FXIII) activity, in vitro clot-lysis assay, thrombin generation, angiotensin convertase enzyme (ACE) 1 and ACE2 activity, and anti-SARS-CoV-2 antibody levels were measured from blood samples. All pregnancies were followed and detailed clinical parameters of the course of pregnancy, labour and post-partum period (including pregnancy-related complications e.g. pre-eclampsia, HELLP syndrome, delivery options: unassisted/assisted or C section, Apgar status of newborn, post-partum hemorrhagic or thrombotic complications, medications, etc.) were registered up to the period of 6 weeks after childbirth. All pregnant women gave written informed consent to participate in the study.
Results: In the COVID-19+ group, APTT was significantly prolonged, while PT, TT, fibrinogen and D-dimer were not significantly different as compared to the COVID-19-group. FVIII activity was significantly lower in the COVID-19+ group (183.7±47.7%) as compared to COVID-19- group (226.7±62.5%, p=0.01). Similarly, FXIII activity was significantly reduced in the COVID-19+ group (82.3±23.5% vs. COVID-19- group: 96.2±26.6%, p=0.04). Pregnancy-associated complications including HELLP syndrome were observed in 2 cases of COVID-19+ group, with marked alterations of coagulation screening tests, clot-lysis assay results and highly increased D-dimer levels. Of all newborns, only one tested positive for SARS-CoV-2 and all were born in good clinical condition without signs of illness. In this cohort, post-partum thrombotic or severe hemorrhagic complications were not observed.
Summary/Conclusion: Pregnancy associated with SARS-CoV-2 infection in the third trimester leads to reduced levels of FVIII and FXIII activity, most likely as a result of increased coagulation activation and consumption. SARS-CoV-2 infection in pregnancy may result in complications of the clinical course accompanied with marked alterations of hemostasis and fibrinolysis.

P-069

Body height and risk of venous thromboembolism in men versus women

C. A. L. Arnesen [1], K. Hveem [2 3], M. E. Gabrielsen [2], J.-B. Hansen [1 4*], S. K. Brækkan [1 4]
[1]Thrombosis Research Center (TREC), Department of Clinical Medicine, UiT-The Arctic University of Norway, Tromsø, [2]K. G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, [3]Hunt Research Center, Department of Public Health and Nursing, Norwegian University of Science and Technology, Levanger, [4]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Background: Sex-related differences in risk of venous thromboembolism (VTE) in men and women have been described, with men having a higher risk than women, particularly among middle-aged individuals. Previous studies have shown that body height is associated with VTE, and the risk appears to be more pronounced in men, likely due to their taller stature. A previous study suggested that differences in body height may explain a higher VTE risk in men versus women. However, the impact of body height on the VTE risk in men versus women in different age groups has not been extensively studied.

Aims: To investigate the sex-specific effect of body height on risk of VTE, and to assess the impact of body height on the VTE risk in men versus women in the young, middle-aged and elderly, using a population-based cohort study.

Methods: Participants of the Tromsø Study (1994-2016) and the Nord-Trøndelag Health Study (1995-2008) served as the study cohort (N=94,377). Cox proportional hazards regression models with age as time-scale were used to estimate hazard ratios (HR) with 95% confidence intervals (CI) of VTE per 10 cm increase in body height in men and women separately. To assess the impact of body height on the VTE risk in men versus women, crude and height-adjusted HRs with 95% CIs were estimated for the total population and in age groups 19-49, 50-69 and ≥70 years.

Results: The median follow up-time was 12.4 years, and 1,945 incident VTEs were identified during follow-up. Taller stature was associated with increased VTE risk in both women (HR: 1.18, CI: 1.06-1.31) and men (HR: 1.28, CI: 1.16-1.42). Overall, the risk of VTE was 17% higher in men versus women (HR: 1.17, CI: 1.07-1.27), but this risk disappeared after adjustment for body height (HR: 0.91, CI: 0.80-1.04). Similarly, subgroup analyses showed that the risk of VTE was higher in men versus women in the age groups 19-49 years (HR: 1.13, CI 0.94-1.36) and 50-69 years (HR: 1.34, CI: 1.17-1.53), and the estimates were substantially attenuated after adjustment for body height (HR 0.89, CI 0.68-1.16 and HR 0.89, CI 0.73-1.09, respectively). In those ≥70-years, the risk was similar in men and women (HR: 0.97, CI: 0.82-1.14) and did not change after adjustment for body height (HR: 0.95, CI: 0.74-1.20).

Summary/Conclusion: Our findings confirm that taller stature is a risk factor for VTE in both men and women, and that differences in body height may explain the observed higher VTE risk in men versus women, particularly in the young and middle-aged.

P-070

Stability of oral anticoagulant treatment with vitamin K antagonists in COVID-19 patients

E. Camilleri [1,*], N. van Rein [1 2], F. J. M. van der Meer [3], M. C. Nierman [4], W. M. Lijfering [1 5], S. C. Cannegieter [1 5]
[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, [2]Department of Pharmacy, Amsterdam University Medical Centers – Location AMC, University of Amsterdam, Amsterdam, [3]Anticoagulation Clinic Leiden, Leiden, [4]Department of Thrombosis and Anticoagulation, Atalmedial Medical Diagnostics Centers, Amsterdam, [5]Department of Internal Medicine, Division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, Netherlands

Background: Coagulopathy has been reported in severely ill patients with COVID-19, but data are lacking in outpatient settings. In patients treated with vitamin K antagonists (VKAs), whose anticoagulant effect is monitored through international normalized ratios (INRs), such coagulation abnormalities might lead to unstable control of anticoagulation. This could influence their thrombosis and bleeding risk. No data are currently available on the quality of anticoagulant therapy during COVID-19 infection.
Aims: To assess stability of VKA therapy in COVID-19 patients through a case-crossover study.
Methods: Between February-July 2020, we included VKA-treated patients with a positive COVID-19 test from two anticoagulant clinics in the Netherlands. We collected INRs between 26 weeks prior to diagnosis up to 12 weeks after. We calculated Time in Therapeutic Range (TTR) and stability between INRs expressed as the Variance Growth Rate (VGR). Both were compared within patients, in order to minimize confounding, with paired sample t-test. We compared the first 6 weeks after diagnosis and the period between 6 and 12 weeks after diagnosis with the 26 weeks before infection. Moreover, we calculated the proportion of INR≥5.0 and ≥8.0 in the three time frames and the risk ratios (RRs) and 95% confidence interval (95%CI) of having an INR≥5 and ≥8.0 after COVID-19 infection, compared with the period before diagnosis.
Results: 51 COVID-19 patients (mean age 84, standard deviation 11) were included, of whom 15 (29%) were men. The most common indication for anticoagulation was atrial fibrillation (40%) and patients had been taking VKA for a median of 8 years (interquartile range 4-10) prior to their COVID-19 diagnosis. Mean TTR in the 26 weeks prior to COVID-19 diagnosis was 80% (95%CI 75-85) compared to 59% (95%CI 51-68) in the 6 weeks after. Mean TTR difference was -23% (95%CI -32 to -14), with a time above therapeutic range that increased from 17% (95%CI 13-22) in the 26 week before diagnosis to 38% (95%CI 30-47) in the 6 weeks after. The TTR rose again to 79% (95%CI 69-89) between 6 and 12 weeks after diagnosis. Also, VGR increased from 1.4 (95%CI 0.8-2.0) in the 26 weeks before COVID-19 diagnosis to 5.7 (95%CI 3.0-8.5) in the first 6 weeks after infection, with a mean increase of 4.8 (95%CI 2.1-7.5). Between 6 and 12 weeks after diagnosis, the increase in VGR was less pronounced (2.5, 95%CI -1.4 to 6.4). In the period prior to infection, we registered 19 out of 641 (3%) INR≥5.0 compared with 35 out of 247 (14%) in the 6 weeks after (RR 4.8, 95%CI 2.8-8.2). Similarly, 3 out of 641 (0.5%) INR samples were ≥8.0 in the 26 weeks prior to the infection compared with 10 out of 247 (4%) in the 6 weeks after (RR 8.6, 95%CI 2.4 to 31.2).
Summary/Conclusion: COVID-19 infection was associated with a strong decrease in TTR and increase in VGR in VKA users compared to their values before infection, with five times more INRs ≥5.0 shortly after COVID-19 diagnosis. Additional monitoring during COVID-19 infection is advised to maintain therapeutic stability, particularly to prevent supratherapeutic INRs.

P-071

Heart failure and subsequent thrombotic complications in a population with low baseline risk of thromboembolism: A nationwide cohort study

Q. Chen [1,*], M. Toorop [1], W. Lijfering [1], S. Cannegieter [1]
[1]Leiden University Medical Center, Leiden, Netherlands

Background: Heart failure (HF) is associated with increased risk of thromboembolic events, including stroke, myocardial infarction (MI), and venous thromboembolism (VTE), which is supported by experimental and clinical studies. This association is also observed in HF patients without atrial fibrillation (AF), and limited evidence suggests oral anticoagulants should be considered in these patients in some circumstances.
Aims: To investigate whether the association of HF with thrombotic complications holds true in a population with low baseline risk of thromboembolism.
Methods: Using data from Statistics Netherlands, all Dutch inhabitants who were alive and aged ≥18 years on 1/1/2019 were included as the study population, but individuals who had any diagnosis record(s) of HF, AF, rheumatic mitral stenosis, mechanical heart valves, ischemic stroke, transient ischemic attack (TIA), arterial thromboembolism, MI, or VTE within 5 years before 1/1/2019, or those who had any prescription record(s) of antithrombotic agents within 6 months before 1/1/2019 were excluded to study a population with low baseline risk of thromboembolism. The included individuals were then followed from 1/1/2019 for 1 year (i.e., until 31/12/2019), or the date of death, whichever came first. Occurrence of HF was determined by the presence of at least one diagnosis record of HF during the follow-up. Cumulative incidence of HF during the follow-up was estimated by cumulative incidence competing risk (CICR) method, and baseline characteristics associated with the occurrence of HF were explored by univariable Cox regression. A composite of ischemic stroke/TIA/arterial thromboembolism, MI, and VTE was studied as the main study outcome, and each type of the thromboembolic events and all-cause mortality were also studied separately. By treating occurrence of HF during the follow-up as a time-dependent exposure, the associations between HF and the studied outcomes were evaluated by time-dependent Cox regression (i.e., the Mantel-Byar method), in which baseline information (including age, sex, immigration background, and standard household income) and time-varying covariates (i.e., various comorbidities) were considered as confounding factors and adjusted for.
Results: A total of 12,179,366 individuals were included with a mean age of 46.91±17.46 years and a male sex proportion of 48.3%. Malignant tumor (2.1%), hypertension (1.6%), and diabetes mellitus (1.0%) were the three most prevalent comorbidities in the study population at baseline. The cumulative incidence of developing HF was 0.12% (95% confidence interval (CI) 0.12-0.12%) within the 1-year follow-up. Baseline characteristics associated with increased risk of developing HF were older age, male sex, low household income, and various comorbidities. As compared with no HF, occurrence of HF was associated with increased risk of the studied thrombotic complications (for the composite outcome: adjusted hazard ratio (HR) 1.79, 95% CI 1.57-2.03; for ischemic stroke/TIA/arterial thromboembolism: HR 1.37, 95% CI 1.15-1.62; for MI: HR 2.47, 95% CI 2.04-2.98; for VTE: HR 1.89, 95% CI 1.44-2.49), and all-cause mortality (HR 3.08, 95% CI 2.79-3.39). The associations remained consistent when restricting the study population to 10-years age categories.
Summary/Conclusion: Incident HF increased the risk of thrombotic complications in a population with low baseline risk of thromboembolism.

P-072

Evaluation of the carboxypeptidase U (CPU, TAFIa, CPB2) system in patients with SARS-CoV-2 infection

K. Claesen [1,*], Y. Sim [1], A. Bracke [1], M. De bruyn [1], E. De Hert [1], G. Vliegen [1], A. Hotterbeekx [2], S. Kumar-Singh [3], K. Vercauteren [4], I. De Meester [1], D. Hendriks [1]
[1]Laboratory of Medical Biochemistry, University of Antwerp, [2]Global Health Institute, University of Antwerp, [3]Laboratory of Cell Biology & Histology, University of Antwerp, Wilrijk, [4]Institute of Tropical Medicine, Antwerp, Belgium

Background: Coronavirus disease 2019 (COVID-19) is a viral lower respiratory tract infection caused by the highly transmissible and pathogenic SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Besides respiratory failure, systemic thromboembolic complications are frequent in COVID-19 patients and suggested to be the result of a dysregulation of the coagulation and fibrinolytic system in these patients. Although several markers of coagulation and fibrinolysis have been studied extensively, little is known about the effect of SARS-CoV-2 infection on the potent antifibrinolytic enzyme carboxypeptidase U (CPU, TAFIa, CPB2).
Aims: In this observational study, we aimed to assess to effect of SARS-CoV-2 infection on the CPU system.
Methods: Patients with laboratory confirmed SARS-CoV-2 infection and non-COVID-19 controls were enrolled in the study between April 2020 and February 2021. A baseline blood sample was collected from all participants at in inclusion. In patients with laboratory confirmed SARS-CoV-2 additional blood samples were collected every 7 days during hospitalization. Plasma procarboxypeptidase U (proCPU, TAFI, proCPB2) levels were determined with an activity based proCPU assay using the selective and specific substrate Bz-*o*-cyano-Phe-Arg based on the method described by Heylen *et al*. Total active and inactivated CPU (CPU+CPUi) antigen levels were measured with a commercially available ELISA (Asserachrom TAFI a/ai, Stago). Additionally demographic and clinical data of all included subjects were collected. Approval for the study was obtained by the local ethics committee and all patients gave their informed consent.
Results: 56 patients with laboratory confirmed COVID-19 (35% male, mean age 58 ± 14 years) were included. 31 subjects that had no SARS-CoV-2 infection (65% male, mean age 45 ± 13 years) served as non-COVID-19 controls. Upon hospital admission, mean proCPU levels were significantly lower (466 ± 178 U/L vs. 492 ± 105 U/L; $p<0.05$) and CPU+CPUi antigen levels significantly higher (51 ± 17 ng/mL vs. 44 ± 28 ng/mL; $p<0.001$) in patients with laboratory confirmed COVID-19 compared to non-COVID-19 controls. Moreover, CPU+CPUi antigen levels on admission were related to disease severity, with higher CPU+CPUi antigen levels observed in patients presenting with more severe symptoms of COVID-19. ProCPU levels did increase over time and were even higher than in non-COVID-19 controls by the time the patients were discharged from the hospital (527 ± 179 U/L vs. 492 ± 104 U/L; $p<0.05$). CPU+CPUi antigen levels on the other hand did not significantly decrease over time and were still elevated in patients with laboratory confirmed COVID-19 upon discharge (53 ± 16 ng/mL vs. 44 ± 28 ng/mL; $p<0.01$).
Summary/Conclusion: Assessment of proCPU levels and CPU+CPUi antigen levels in patients with laboratory confirmed COVID-19 shows that there is a generation of CPU with concomitant proCPU consumption during SARS-CoV-2 infection. This may contribute to impaired fibrinolysis and enlarge the risk of thrombosis in COVID-19 patients.

P-073

Elevated plasma levels of plasminogen activator inhibitor-1 are associated with risk of future incident venous thromboembolism

T. Frischmuth [1,], K. Hindberg [1], P. Aukrust [1 2 3 4], T. Ueland [1 3 4], S. K. Brækkan [1 5], J.-B. Hansen [1 5*], V. M. Morelli [1]
[1]Thrombosis Research Center, Department of Clinical Medicine , UiT - The Arctic University of Norway, Tromsø, [2]Section of Clinical Immunology and Infectious Diseases, [3]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, [4]Faculty of Medicine , University of Oslo, Oslo, [5]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Background: Plasminogen activator inhibitor-1 (PAI-1), the main inhibitor of fibrinolysis, is expressed by different cell types, including adipocytes. Elevated plasma levels of PAI-1 and hypofibrinolysis are common features in obesity, which is a major risk factor for venous thromboembolism (VTE). PAI-1 could therefore potentially serve as a mediator for the VTE risk in obese subjects. However, whether PAI-1 is associated with VTE remains uncertain.
Aims: To investigate the association between plasma PAI-1 levels and risk of future incident VTE and explore whether PAI-1 could mediate the risk of VTE in obesity.
Methods: A population-based nested case-control study, comprising 383 VTE cases and 782 age- and sex-matched controls, was derived from the Tromsø Study cohort (1994-2007). PAI-1 antigen was measured in plasma samples collected at cohort baseline (1994-95). Logistic regression was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE according to tertile cut-offs of PAI-1 levels determined in controls. Further, we applied a mediation analysis to quantify how much PAI-1 could account for the effect of obesity on VTE risk. Ethical approval and informed consent were obtained.
Results: The risk of VTE increased linearly across PAI-1 tertiles (*P* for trend <0.001) in the age-and sex-adjusted model. The OR of VTE for the highest versus lowest tertile of PAI-1 levels was 1.73 (95% CI 1.27-2.35). The association was slightly attenuated after further stepwise adjustment for body mass index (BMI) (OR 1.59, 95% CI 1.16-2.17) and C-reactive protein (CRP) (OR 1.54, 95% CI 1.13-2.11). Similar results were obtained for provoked and unprovoked events, deep vein thrombosis and pulmonary embolism, and after excluding subjects with arterial cardiovascular disease at baseline. In obese subjects (BMI of ≥30kg/m^2 vs. <25kg/m^2), adjustment for PAI-1 levels resulted in a modest attenuation of the risk estimate for VTE, with PAI-1 mediating 14.9% (95% CI 4.1%-49.4%) of the association between obesity and VTE risk in analyses adjusted for age, sex and CRP.
Summary/Conclusion: Our results indicate that elevated plasma levels of PAI-1 are associated with increased risk of future VTE. In addition, our findings suggest that PAI-1 partially mediates the association between obesity and VTE. Further research is warranted to explore the role of PAI-1 in the pathogenesis of VTE and as a potential target for VTE prevention in obese individuals.

P-075

Plasma levels of factor VIII and risk of future incident venous thromboembolism

E.-S. Hansen [1,*], M. S. Edvardsen [1], K. Hindberg [1], N. Latysheva [1], P. Aukrust [1 2 3 4], T. Ueland [1 2 3], J.-B. Hansen [1 5], S. K. Brækkan [1 5], V. M. Morelli [1]
[1]Thrombosis Research Center (TREC), Department of Clinical Medicine, UiT – The Arctic University of Norway, Tromsø, [2]Faculty of Medicine, University of Oslo, [3]Research Institute of Internal Medicine, Oslo University Hospital, [4]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, [5]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Background: Coagulation factor VIII (FVIII) has been associated with risk of a first venous thromboembolism (VTE) in several studies, but the majority of data arises from case-control studies, which may be susceptible to bias because of reverse causation and selection of controls. Only a few studies have prospectively investigated the relationship between FVIII and a first lifetime VTE in the general population. Moreover, it remains uncertain whether FVIII has a differential impact on the risk of VTE subgroups, particularly deep vein thrombosis (DVT) and pulmonary embolism (PE).
Aims: To investigate the association between plasma levels of FVIII and future risk of incident VTE, including the subgroups DVT and PE, in a population-based nested case-control study.
Methods: The study population consisted of 373 VTE cases, and 732 age- and sex-matched controls who were randomly selected from the Tromsø Study cohort (1994-2007). FVIII antigen was measured in plasma samples collected at cohort baseline (1994-95). Logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE according to quartiles cut-offs of FVIII levels determined in controls, with models adjusted for age, sex, body mass index and C-reactive protein. Ethical approval and informed consent were obtained.
Results: The risk of VTE increased in a dose-response manner across FVIII quartiles (*P* for trend <0.001). Participants with FVIII levels in the highest quartile (≥121%) had an OR for VTE of 1.92 (95% CI 1.31-2.82) compared with those with FVIII in the lowest quartile (<83%). A dose-response relationship between FVIII levels and thrombosis risk was also observed for the VTE subgroups, except for PE. FVIII levels were more strongly associated with risk of DVT (OR for highest vs. lowest quartile: 2.35, 95% CI 1.50-3.70) than with risk of PE (OR for highest vs. lowest quartile: 1.32, 95% CI 0.74-2.35). The risk estimates across FVIII quartiles were somewhat more pronounced for unprovoked than for provoked VTE, with ORs of 2.31 (95% CI 1.30-4.11) and 1.72 (95% CI 1.10-2.71) for the highest vs. lowest quartile, respectively.
Summary/Conclusion: Our results provide additional evidence on the association between high plasma FVIII levels and risk of future incident VTE in the general population. In addition, our findings suggest a stronger association of FVIII with DVT than with PE. Further research is needed to clarify the potential mechanism behind the differential impact of FVIII on the risk of DVT and PE.

P-076

Predictive value of d-dimers in the clinical outcome of severe COVID19 patients: are we giving them too much credit?

J. P. Cidade*, V. Costa [1], R. Morais [1], P. Fidalgo [1], A. Tralhão [1], C. Paulino [1], D. Nora [1], B. Valerio [1], V. Mendes [1], L. Coelho [1], C. Tapadinhas [1], P. Póvoa [1]
[1]Intensive Care Unit Hospital Sao Francisco Xavier, Lisboa, Portugal

Background: COVID-19 is a new and complex form of hypoxemic acute respiratory failure, with several pathological pathways, leading to frequent progression to acute respiratory distress syndrome, multiorgan failure and ICU admission. Furthermore, COVID19 can be associated with prolonged PT, fibrinogen and high D-dimer levels, representing clear signs of active coagulation pathways and thrombosis, preponderant in early stages of the disease. In fact, gathered evidence suggest that a 3 to 4-fold rise in D-dimer concentrations is linked to poor prognosis in these patients, predicting disease severity and potentially indicating its usefulness in signaling subsets of patients with higher mortality rates.
Aims: This study aimed to describe D-dimer admission profile in severe COVID19 patients admitted to ICU and analyze its predictive role regarding clinical outcomes and mortality rate.
Methods: We performed a single-center retrospective cohort study. All adult patients admitted to ICU with COVID19 were eligible and were further divided in 3 groups according to D dimer serum levels at admission and maximum d-dimer serum levels registered during ICU stay: (1) Lower-values group (D-dimer levels < 3-fold normal range value (500ng/mL)), Intermediate-values group (D-dimer >3-fold and <10-fold normal range value) and Higher-value group (>10-fold normal range value). COVID19 was diagnosed using clinical and radiologic criteria with a SARS-CoV-2 positive RT-PCR test. Qui-square test was used for categorical variables and Kruskal-Wallis and logistic regression were used on continuous variables for statistical assessment of outcomes between groups. Kaplan-Meier survival curve and Cox-regression were also obtained.
Results: 118 patients (mean age 63 years, 73% males) were included in the analysis (N=73 Lower-values group, N=31 Intermediate-values group; N=11 Higher-values group). SOFA score at ICU admission (p=0.75) and ICU length of stay(p=0.9) were similar between groups. The number of mechanical ventilated patients, ventilator-free days, number of patients requiring vasopressor therapy and vasopressor-free days were not statistically different between groups. Similarly, mortality was not different between groups when considering D-dimer level at admission (p=0.69) or maximum registered D-dimer levels during ICU stay (p=0.48). In spite of a tendency to higher mortality in the Higher-value group, log rank test of Kaplan-Meier survival curves revealed no differences (p=0.63) between groups, nor it was verified even when age, SOFA score at admission, ICU length of stay, D-dimer level at admission and during ICU stay, vasopressor-free days and ventilator-free days were considered as covariables, during cox regression (p= 0.58).
Summary/Conclusion: In severe COVID19 patients, the D-dimer profile does not appear to retain a predictive value to patient survivability and should not be used as a surrogate of disease severity.

P-077

Galectin-3-binding protein and risk of future venous thromboembolism

S. Brækkan [1 2,*], T. Ueland [1 3], N. Latysheva [1], P. Aukrust [1 4], V. Morelli [1], J.-B. Hansen [1 2]
[1]Thrombosis Research Center, UiT- The Arctic University of Norway, [2]Division of Internal Medicine, University Hospital of North Norway, Tromsø, [3]University of Oslo, [4]Oslo University Hospital, Oslo, Norway

Background: Galectin-3 binding protein (gal3bp) and its receptor/ligand Galectin-3 (gal-3) are secreted proteins involved in cell adhesion and initiation of proinflammatory signaling cascades. In a small case-control study (n=24), elevated levels of gal3bp were found in microvesicles derived from patients with acute deep vein thrombosis (DVT) and gal3bp was also linked to inflammatory changes in VTE in a mouse model. However, whether plasma gal3bp levels are associated with risk of future VTE remains unknown.
Aims: To investigate the association between plasma gal3bp levels and risk of future VTE.
Methods: A nested case-control study was derived from the Tromsø study, a cohort of the general population (n=27 158) of the municipality of Tromsø, Norway, with follow-up in the period 1994/95-2007. The nested case-control study comprised 415 VTE cases and 847 randomly sampled age- and sex-matched controls. Gal3bp was measured with a standard immunoassay in plasma samples collected at baseline inclusion (1994/95). Gal3bp levels were categorized based on quartile cut-offs in the control population. Logistic regression models were performed to estimate odds ratios (ORs) with 95% confidence interval (CIs) for VTE according to quartiles of gal3bp levels. All participants provided written informed consent, and the study was approved by the regional committee for research ethics.
Results: We found no association between gal3bp levels and risk of future incident VTE. The OR of VTE for the highest (>5.88 µg/mL) versus lowest (<3.14 µg/mL) quartile of plasma gal3bp was 1.04 (95% CI: 0.75-1.46) in analyses adjusted for age, sex and body mass index (p for linear trend across quartiles: 0.7). Similar results were found in subgroup analyses of DVT and PE, as well as unprovoked and provoked VTE.
Summary/Conclusion: In this population-based nested case-control study, gal3bp levels were not associated with risk of future incident VTE. Our findings suggest that gal3bp is not a biomarker for incident VTE, and that gal3bp most likely is not involved in the pathogenesis of VTE.

P-078

Impact of aortic valve replacement on the contact pathway

M. E. De La Morena-Barrio [1,*], J. Corral [1], C. López-García [2], V. Jiménez-Diaz [3], A. Miñano [1], P. Salvadores [3], M. A. Esteve-Pastor [2], J. A. Baz Alonso [2], A. M. Rubio [1], F. Sarabia Tirado [4], M. García-Navarro [2], J. García de Lara [2], F. Marín [2], V. Vicente [1], E. Pinar Bermudez [2], S. Cánovas [5], G. de la Morena [2]
[1]Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER, [2]Servicio de Cardiología, Hospital Clínico Universitario Virgen de la Arrixaca, Universidad de Murcia, IMIB-Arrixaca, CIBERCV., Murcia, [3]Unidad de Investigación Cardiovascular. Servicio de Cardiología, Hospital Álvaro Cunqueiro, Vigo, [4]Servicio de Radiología, Hospital Clínico Universitario Virgen de la Arrixaca, [5]Servicio de Cirugía Cardiovascular. Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain

Background: Despite antithrombotic treatment, aortic valve replacement is not exempt from thrombotic complications of the prosthetic valve (DVT). The role of the contact pathway in coagulation and inflammation, together with the fact that this pathway is activated by artificial surfaces, encourages exploring the impact of aortic valve replacement on this pathway. Of special interest is factor XI (FXI); high levels have a high thrombotic risk, while their reduction determines an important antithrombotic protection that has led to the development of different anti-FXI treatments with excellent efficacy and low adverse effects (bleeding).
Aims: To analyze the factors involved in the contact pathway during aortic valve replacement and to assess its impact on the development of DVT.
Methods: We studied 232 consecutive patients undergoing aortic valve replacement; 155 transcatheter (TAVR) and 77 surgical (SAVR). Laboratory and clinical data, including thrombotic events, were recorded and evaluated by computed tomography and transthoracic echocardiography at 6 months. Plasma samples were collected 24 hours before and 48 hours after replacement. Contact pathway factors FXII, FXI and (pre) kallikrein were evaluated by Western Blot.
Results: 19 patients presented thrombotic events: 13 systemic embolic events and 6 subclinical DVT patients. Regardless of the procedure, aortic valve replacement did not activate FXII, nor did it induce kallikrein generation. Moreover, these procedures did not affect FXII or prekallikrein levels. However, the FXI levels observed in the post-procedure sample were significantly lower than baseline, a reduction that was statistically more pronounced in SAVR than in TAVR. In addition, the cases with higher FXI reductions (<80%) had a lower incidence of embolic events during the procedure than those with increased FXI levels (> 150%): (2.7% vs 16.7%; p: 0.04). This reduction was not associated with the type of prosthesis, age, or anticoagulation.
Summary/Conclusion: This is the first study to evaluate the contact pathway in a large cohort of consecutive patients undergoing aortic valve replacement. Neither procedure (TAVR or SAVR) elicited a relevant activation of this pathway, but a significant reduction in FXI was observed associated with a lower incidence of thrombotic events, particularly in SAVR. These results encourage evaluating the usefulness and safety of antithrombotic treatments directed to FXI in these patients.
Funding: CIBERCV, Fundación Séneca (19873/GERM/15), CIBERER (ACCI18-04; ER19P5AC765/2019), Sociedad Española de Trombosis y Hemostasia (SETH: grupos emergentes).

P-079

Pleiotropic influence of rosuvastatin on apolipoproteins and their association with coagulation factors: results from the START trial

E. Camilleri[1,*], N. van Rein[1 2], B. J. van Vlijmen[3 4], J. S. Biedermann[5], M. J. Kruip[5 6], F. W. Leebeek[5], F. J. M. van der Meer[7], C. M. Cobbaert[8], S. C. Cannegieter[1 3 4], W. M. Lijfering[1 3 4]
[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, [2]Department of Pharmacy, Amsterdam University Medical Center, Amsterdam, [3]Department of Internal Medicine, Division of Thrombosis and Hemostasis, [4]Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, [5]Department of Haematology, Erasmus MC, University Medical Center, [6]Thrombosis Service Star-shl, Rotterdam, [7]Anticoagulation Clinic Leiden, [8]Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, Netherlands

Background: The STAtin Reduces Thrombophilia (START) trial showed that the cholesterol-lowering drug rosuvastatin decreased various coagulation factor levels, in particular factor FVIII, in patients with prior venous thrombosis, through an unknown mechanism. Apolipoproteins, which mediate lipoprotein metabolism, have been associated with levels of coagulation factors and shown to be predictive for venous thromboembolic events. Here, we investigated whether statins decrease of coagulation parameters is associated with changes in apolipoproteins.

Aims: To study the changes of apolipoprotein levels during rosuvastatin treatment and their association with coagulation parameters.

Methods: We measured the levels of apolipoprotein (apo)A-I, A-II, A-IV, (a), B-100, B total, C-I, C-II, C-III and E in the 126 patients of the START trial who were randomized to rosuvastatin. We used linear regression analysis to assess the association with 95% confidence intervals (95%CIs) between apolipoproteins and coagulation parameters (FVII, FVIII, FIX, FXI, von Willebrand factor antigen [vWF] and endogenous thrombin potential [ETP]) at baseline. The mean difference (95%CIs) of apolipoproteins between baseline and after 28 days of rosuvastatin was determined through linear regression. Furthermore, to determine if lowering of apolipoproteins by rosuvastatin was associated with coagulation factors, we added differences of coagulation parameters (between baseline and after 28 days of rosuvastatin) to this model, adjusting for age, sex and body mass index.

Results: The mean age of the 126 participants randomized to rosuvastatin was 57 years (range 19-82) and the majority (86, 54%) were men. At least one cardiovascular risk factor was present in 89 (71%) patients, with overweight and obesity being the most prevalent, in 54 (43%) and 29 (23%) patients respectively. At baseline, levels of all apolipoproteins, except apo(a), were positively associated with FVII, FIX and FXI. Increased levels of apoA-I were associated with increased levels of FVII, whereas increased levels of apoBs and apoCs were associated with increased levels of FIX and FXI. Increased levels of apo(a), but not of the other apolipoproteins, were associated with increased ETP. Apolipoproteins levels, except for apoA-I and apo(a), had decreased after 28 days of rosuvastatin. ApoB-100 showed the largest mean decrease of -0.43 g/L (95%CI -0.46 to -0.40). ApoC-I, C-II and C-III also decreased (absolute mean difference -4.98, -14.45 and -16.17 mg/dl respectively), with apoC-II showing the largest relative decrease (30%). The decrease in levels of apoA-IV, C-I and C-III was associated with a decrease in FVII, whereas the decrease of apoA-II, B-100 and B total was associated with a decrease in FXI. The decrease in apolipoproteins levels was, in contrast, not associated with FVIII and vWF decrease or with changes in ETP.

Summary/Conclusion: Rosuvastatin decreased the level of several apolipoproteins but this decrease was only associated with the decrease of liver derived coagulation FVII and FXI and not with FVIII/vWF, while the latter two were the factors that showed the biggest decrease during rosuvastatin treatment.

P-080

Plasma levels of adiponectin and risk of future incident venous thromboembolism

V. M. Morelli [1,*], T. Frischmuth [1], N. Latysheva [1], P. Aukrust [1 2 3 4], T. Ueland [1 2 3], S. K. Brækkan [1 5], J.-B. Hansen [1 5]
[1]Thrombosis Research Center, Department of Clinical Medicine, UiT - The Arctic University of Norway, Tromsø, [2]Faculty of Medicine, University of Oslo, [3]Research Institute of Internal Medicine, [4]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, [5]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Background: Adiponectin is an adipocyte-derived protein with anti-inflammatory properties that has been shown to downregulate tissue factor expression *in vitro*. Adiponectin levels are generally decreased in obese individuals, particularly among those with excess visceral adiposity. Adiponectin could therefore potentially influence the risk of venous thromboembolism (VTE) in obesity. However, whether adiponectin is associated with risk of future VTE remains unknown. We hypothesized that increasing adiponectin levels would be related to a reduced VTE risk.
Aims: To investigate the association between plasma adiponectin levels and risk of future incident VTE.
Methods: A population-based nested case-control study, consisting of 381 VTE cases and 772 age- and sex-matched controls, was derived from the Tromsø Study cohort (1994-2007). Adiponectin was measured using a standard immunoassay in plasma samples collected at cohort baseline (1994-95). Logistic regression was used to calculate odds ratios (ORs) of VTE with 95% confidence intervals (CIs) across quartiles of adiponectin. Given the sex-difference in adiponectin levels, analyses were performed separately in men and women based on sex-specific quartile cut-offs of plasma adiponectin determined in controls. Ethical approval and informed consent were obtained.
Results: Among controls, the median adiponectin level was higher in women (7.1 µg/mL, interquartile range [IQR] 5.6-8.8) than in men (5.7 µg/mL, IQR 4.1-7.4). In addition, we found a significant, albeit weak, inverse correlation between body mass index (BMI) and adiponectin levels in men (r_s= -0.22, P<0.001) and women (r_s = -0.15, P=0.003). In models adjusted for age, the ORs of VTE decreased with increasing adiponectin levels, but only in men. The ORs of VTE for the highest versus lowest quartile of adiponectin were 0.60 (95% CI 0.35-1.02) in men and 0.78 (95% CI 0.48-1.28) in women. No consistent association was observed between adiponectin levels and VTE risk across quartiles after further adjustment for BMI in both sexes, with ORs of 0.69 (95% CI 0.40-1.19) in men and 0.83 (95% CI 0.50-1.37) in women for the highest versus lowest quartile. Similar results were obtained in subgroups (i.e. deep vein thrombosis, pulmonary embolism, provoked and unprovoked VTE) in both sexes.
Summary/Conclusion: Our results suggest that plasma adiponectin levels are not associated with risk of future VTE. Further, our findings imply that the inhibitory effect of adiponectin on the inflammatory response and expression of tissue factor reported *in vitro* may not be clinically relevant for the biology of VTE risk in obesity.

P-081

Hypercoagulation detected by routine and global laboratory hemostasis assays in patients with infective endocarditis

E. Koltsova [1 2,*], M. Sorokina [1], A. Pisaryuk [3 4], N. Povalyaev [3 4], A. Ignatova [1 2], D. Polokhov [1], E. Kotova [4], A. Balatskiy [5], F. Ataullakhanov [1 2], M. Panteleev [1 2], Z. Kobalava [4], A. Balandina [1 2]
[1]Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, [2]Center for theoretical problems of physico-chemical pharmacology RAS, [3]City Clinical Hospital named after V.V. Vinogradov, [4]Peoples' Friendship University of Russia (RUDN), [5]Lomonosov Moscow State University, Moscow, Russian Federation

Background: Infectious endocarditis (IE) is an infection of the heart inner surface mainly affecting heart valves. Embolic risk is very high in IE, with embolic events occurring in 20–50% of patients, while mortality reaches 30%. Vegetations are structures formed on the endocardium of heart valves or heart chambers and consisting of fibrin, platelets and bacteria. Coagulation system is heavily involved into the process of IE vegetation formation and can facilitate further embolization.
Aims: In this study we aimed to assess the coagulation and platelet state in IE implementing a wide range of standard and global laboratory assays. We also aim to determine whether prothrombotic genetic polymorphisms play any role in embolization and mortality in IE patients.
Methods: 37 patients with IE were enrolled into the study. Coagulation was assessed using standard coagulation assays (activated partial thromboplastin time (APTT), prothrombin, fibrinogen, D-dimer concentrations) and integral assays (thromboelastography (TEG) and thrombodynamics (TD)). Platelet functional activity was estimated by flow cytometry. Single nuclear polymorphisms of coagulation system genes were studied.
Results: Fibrinogen concentration and fibrinogen-dependent parameters of TEG (MA) and TD (D) were increased in patients indicating systemic inflammation (Median [IQR] healthy volunteers vs patients: Fg 2.46 [2.02-2.88] g/l vs 5.12 [4.06-6.45] g/l; MA 59.3 [55.3-60.5] mm vs 71.8 [65.9-75.3] mm; D 25559 [22406-28376] a.u. vs 28823 [25527-30514] a.u.). In majority of patients clot growth rate (V) in thrombodynamics was significantly shifted towards hypercoagulation (Median [IQR]: V 35.2 [30.6-38.7] μm/min vs 29.0 [27.4-31.2] μm/min] in consistency with D-dimers elevation (Median [IQR]: D-dimers 158 [131-223] μg/l vs 1009 [528-2952] μg/l). However, in some patients prothrombin, thromboelastography and thrombodynamics were shifted towards hypocoagulation. Resting platelets were characterized by glycoprotein IIb-IIIa activation (Median [IQR]: PAC1 3.1 [2.8-3.7]% vs 3.8 [3.1-5.9]%), increased procoagulant activity (Median [IQR]: Annexin V 0.88 [0.62-1.25]% vs 1.22 [0.72-1.92]%) and degranulation (Median [IQR]: Mepacrine uptake 100 [90-109]% vs 65 [54-74]%) with subsequent refractoriness upon activation (Median [IQR]: PAC1 99 [87-115]% vs 49 [42-71]%; Annexin V 18.4 [13.8-29.5]% vs 8.6 [3.6-12.1]%); dense granule release 77 [67-84] vs 32 [26-41]). In patients with fatal IE, we observed a significant decrease in fibrinogen (Median [IQR] fatal IE vs non-fatal IE: Fg 3.9 [3.3-4.6] g/l vs 5.3 [4.1-6.8] g/l) and thrombodynamics (Median [IQR]: 22717 [19011-27637] a.u. vs 29072 [26764-30546] a.u.). In patients with embolism, we observed a significant elongation of the TEG R parameter (Median [IQR] embolic IE vs non-embolic IE: R 9.2 [6.9-11.7] min vs 6.2 [3.1-8.8] min). No association of embolism or mortality with genetic polymorphisms was found in our cohort.
Summary/Conclusion: Our findings suggest that coagulation in patients with infective endocarditis is characterized by general hypercoagulability and platelet pre-activation. Some patients, however, have hypocoagulant coagulation profile, which presumably can indicate progressing of hypercoagulation into consumption coagulopathy.
Funding: The study was supported by a grant from the Russian Science Foundation (project No. 20-75-00057) to EMK

P-082

Short-term incidence of thrombotic complications in male-to-female transgender Individuals

C. Steuwer [1 2,*], S. Bhoelan [2 3], K. van der Tuuk [2 4], K. Meijer [2 3]
[1]University Medical Centre Groningen, [2]University of Groningen, [3]Department of Hematology, [4]Department of Obstetrics and Gynecology, University Medical Centre Groningen, Groningen, Netherlands

Background: Cis-women using estrogen have an increased risk of thrombosis. Estrogens are part of many trans-women's cross-sex hormonal therapy (CSHT). Studies with proper adjudication evaluating this risk in the trans-women population are lacking. Furthermore, many trans-women's CSHT is administered transdermally via estrogen plasters which achieve a therapeutic concentration of estrogen with a lower dose than with oral administration.
Aims: To determine the incidence of venous and arterial thrombosis in trans-women during the first year of CSHT.
Methods: Trans-women treated with estrogen-containing CSHT at the gender clinics of the UMCG between 1985 and 2020 were retrospectively followed for one year after the start of CSHT. Contra-indications for CSHT, according to UMCG guidelines, include history of thrombosis, thrombophilia and abnormal liver function, thus narrowing down the study population to a lower-risk sample.
Data on demographic factors, medical history, and characteristics of CSHT were collected. The outcome was a composite of venous and arterial thrombotic events. If diagnostic data was not available, clinical and treatment characteristics were evaluated (i.e. at least three months of anticoagulation therapy or start of antiplatelet therapy).
The incidence rate of thrombotic events was calculated.
A waiver of informed consent was granted by the Medical Ethics Committee of the University Medical Centre Groningen.
Results: In total, 242 trans-women contributed 233 person-years follow-up (median 12.0 months of follow-up per person). Median age was 32 years (interquartile range (IQR) 23-44), median BMI was 23.9 (IQR 21.0-25.8) and 75(31%) of the patients were smokers.
The estrogen therapy took the form of plasters for 188(78,3%) trans-women, oral supplementation for 48(20,0%), and gel for 3(1,3%). The most common regimen of the UMCG was estrogen plasters 50 mcg/24h, which was administered to 146(40,9%) trans-women. Anti-androgens were also given in 226(93.4%) transwomen and progestagens in 5(2,1%).
Three thrombotic events occurred: one deep vein thrombosis of the leg in a 30 year-old non-smoking overweight (BMI 27,7) caucasian patient, one pulmonary embolism in a 21 year-old smoking caucasian patient, and one ischemic stroke in a 55 year-old non-smoking caucasian patient with a history of arthrosis. All three patients were administered transdermal estradiol via plasters with a dosage of 50mcg/24h. This corresponds to an incidence rate of 1.28/100 person-years during the first year of CSHT. Objective diagnostics were available for these three events. Considering only trans-women treated with the most common UMCG CSHT regimen, the incidence rate was 2.12/100 person-years.
Summary/Conclusion: In this population of trans-women predominantly treated with estrogen-containing plasters, the risk of thrombotic events during the first year of CSHT was lower than reported in women on oral contraceptives. Possible explanations are the predominant use of plasters instead of oral estrogen and the consideration of thrombosis risk factors before estrogens are started.

P-083

Association of single nucleotide polymorphisms with major bleeding risk during Vitamin-K Antagonists treatment: a case-cohort study

E. Camilleri[1,*], M. H. A. Bos[2 3], P. H. Reitsma[2 3], F. J. M. van der Meer[2], J. Swenn[4], S. C. Cannegieter[1 2 3], N. van Rein[1 5]

[1]Department of Clinical Epidemiology, [2]Department of Internal Medicine, Division of Thrombosis and Hemostasis, [3]Einthoven Laboratory for Vascular and Regenerative Medicine, [4]Department of Clinical Pharmacy & Toxicology, Leiden University Medical Center, Leiden, [5]Department of Pharmacy, Amsterdam University Medical Centers – Location AMC, University of Amsterdam, Amsterdam, Netherlands

Background: Major bleeding occurs annually in 1-3% of patients on vitamin-K antagonists (VKAs), despite close monitoring. Single nucleotide polymorphisms (SNPs) in proteins involved in VKAs' pharmacokinetics and pharmacodynamics may affect this risk. Previous studies reported conflicting results, but have been underpowered, included not clinically relevant bleeding events and were affected by prevalent user bias. Moreover, data are lacking for acenocoumarol and phenprocoumon users.

Aims: To determine the association of SNPs in five VKA-related enzymes (vitamin K epoxide reductase complex subunit-1 [VKORC1, rs9923231], cytochrome P450 enzymes CYP2C9 [rs1799853, rs1057910] and CYP4F2 [rs2108622], gamma-glutamyl carboxylase [GGCX, rs12714145, rs11676382]) with major bleeding in a cohort of initiating VKAs users.

Methods: We used the BLEEDS cohort, which consisted of 16,570 patients who initiated VKA treatment between 2012-2014. In the cohort, we established a case-cohort study, which included all 326 major bleeding cases that occurred during 17,613 years of follow-up and a random subcohort of 978 control patients at baseline. We determined SNPs in VKORC1, CYP2C9, CYP4F2, GGCX and estimated hazard ratios (HRs) of major bleeding with 95% confidence intervals (95%CI) by means of weighted Cox regression, using the wild-type genotype (or the extensive metabolizer in CYP2C9) as reference category. We repeated the analysis categorizing patients as being carrier of the variant alleles (or intermediate/poor metabolizer in CYP2C9) for 0, 1 or ≥2 SNPs. Furthermore, we stratified the analyses for a treatment period of one, two, three or six months, to investigate whether the major bleeding risk might increase or decrease over time.

Results: DNA was available for 261 cases and 791 subcohort members. Patients with major bleeding were slightly older (mean age 76) than patients in the subcohort (mean age 71). Phenprocoumon was the most used VKA in both groups. The AA genotype in VKORC1 was associated with a 1.5-fold (95%CI 1.0-2.3) increased risk of major bleeding compared with GG carriers. CYP4F2 TT carriership was also associated with a 1.6-fold (95%CI 0.9-2.8) increased risk of major bleeding compared with wild-type. For the CYP2C9 and GGCx SNPs, the risk of major bleeding was around unity. Carrying ≥2 SNPs was associated with a 1.6-fold increased risk of major bleeding (95%CI 0.9-2.0) compared with 0 SNPs. The major bleeding risk of AA carriers in VKORC1 compared to GG carriers was increased in the first month of treatment (HR 2.2, 95%CI 0.9-5.0) and remained stable over time (HR at 6 months 2.0, 95%CI 1.2-3.4). For the TT genotype in CYP4F2 the major bleeding risk compared to CC carriers was increased with longer follow-up and the majority of bleeding occurred after 6 months of follow-up.

Summary/Conclusion: The AA genotype in VKORC1 and TT genotype in CYP4F2 were associated with an increased risk of major bleeding, compared with wild-type. The major bleeding risk for the AA variant allele in VKORC1 and TT in CYP4F2, compared to the wild-type, was increased in the first month and with longer follow-up, respectively. Major bleeding risk was also increased in carriers of ≥2 SNPs.

P-084

Thrombolysis in patients with pulmonary embolism and in-hospital cardiac arrest

C. Epstein [1,*], J. Frithiofsson [1 2], S. Bruchfeld [1 2], E. Bendz [1 2], M. Bruzelius [1 3], T. Djärv [1 2]
[1]Department of Medicin SOLNA, Karolinska Insitutet, [2]Medical Unit of Emergency Medicin, [3]Department of Hematology, Karolinska University Hospital, Stockholm, Sweden

Background: Five percent of in-hospital cardiac arrest (IHCA) is caused by Pulmonary embolism (PE). The prognosis is poor, only one forth is alive at discharge. In European Resuscitation Council guidelines, systemic thrombolysis is recommended despite limited evidence. Previous studies have suggested a better survival in patients who received thrombolysis, but these were mainly prehospital studies on CA.
Aims: To investigate the proportion of patients with IHCA caused by PE. To further describe the characteristics of patients with PE-related IHCA and compare the proportion of survival and bleeding between patients who received thrombolytic therapy and those who did not.
Methods: This retrospective cohort study included all patients over 18 years of age in a Swedish tertiary hospital with IHCA from 2007 to 2020. Patients were identified from the Swedish Registry for Cardiopulmonary Resuscitation, and data was collected from the registry and medical records. For comparison of variables either Fisher's exact test or the Mann-Whitney U-test were used. A p-value of <0.05 was considered significant.
Results: Out of 2128 IHCA cases occurring 2007-2020, 64 (3 %) were identified as caused by PE and confirmed with either computer tomography or autopsy in 77 %. 16 (25 %) patients received thrombolysis whereas 10 patients got it during CA, three before CA and three after CA. Seven out of these 16 patients (44 %) survived to hospital discharge, compared to 4 out of 48 patients (8 %) that did not receive thrombolysis. This difference was statistically significant (p-value <0.01). A significant association was also seen between thrombolysis and minor bleeding (31 % vs 4.2 %, p-value <0.01). Major bleeding was not seen in any patient, but the autopsy rate was low. The median age was significantly lower in patients that received thrombolysis (62 vs 71 years, p-value 0.03), and the median BMI significantly higher (31 vs 26, p-value <0.01).
Summary/Conclusion: IHCA caused by PE was uncommon but associated with high mortality. Our results suggest that thrombolysis improved survival without an increased risk for major bleeding but needs larger studies and registers to be further confirmed.

P-085

Clinical evolution of non-critical COVID-19 hospitalized patients treated with or without enoxaparin. Results of a retrospective multicenter study: HEPAVID study

C. de Ancos Aracil [1], J. Portillo Sánchez [2], N. Casillas Ramos [3], J. A. Nieto Rodríguez [3], E. García Aguilar [4], J. Leal Martínez-Bujanda [4,*], C. Nieto Magro [4], A. Martínez Francés [5]
[1]Internal Medicine, Hospital Universitario de Fuenlabrada, Fuenlabrada, [2]Internal Medicine, Hospital General Universitario de Ciudad Real, Ciudad Real, [3]Internal Medicine, Hospital Virgen de la Luz, Cuenca, [4]Medical Affairs, ITF Research Pharma S.L.U., Alcobendas, [5]Hematology, Hospital General Universitario Santa Lucía, Cartagena, Spain

Background: Although during the initial months of the pandemic the use of anticoagulant therapy such as low molecular weight heparin (LMWH) was not standardized, currently it is recommended for all admitted COVID-19 patients as it has been correlated with a reduced incidence of thrombotic complications and mortality in these patients. Preliminary data suggest that anticoagulant therapy could also play a role in improving certain parameters related with the clinical course of the disease, in addition to those strictly related to coagulation.
Aims: To evaluate the early management of anticoagulation and the effect of the administration of enoxaparin in admitted (non-ICU) patients in clinical outcomes.
Methods: COVID-19 patients who were admitted during March and April of 2020 in three public hospitals from Spain were included if they were not initially in ICU. Data from 415 patients were analyzed; 87.1% of the included patients used enoxaparin (Hepaxane®/Ghemaxan®) during hospitalization while the rest of them did not use any anticoagulant therapy. We compared baseline clinical data and we evaluated ventilation and oxygen-related parameters, inflammatory parameters, and several clinical scales (WHO ordinal scale, SOFA and SIC scores) as clinical indicators of evolution in both groups of patients, alongside with overall survival and ICU admission data.
Results: The patients that received enoxaparin were a more severe population at baseline than those that did not receive anticoagulant therapy: there was a higher rate of patients with a severe disease according to WHO clinical management criteria (p=0.0439) and to WHO ordinal scale (p=0.0006). Moreover, patients who received enoxaparin showed increased levels of LDH (mean 407.5 vs 322.3; p=0.0143) and a tendency towards lower oxygen saturation (mean 91.2 vs 93.1; p=0.0916).
Despite the differences in severity described above, there were no statistical differences regarding ICU admittance or deceases during the study (total of 5.1% and 12,8%, respectively), but the population treated with enoxaparin needed more frequently oxygen support (p=0.0118) due to their baseline lower oxygen saturation values.
Only the group treated with enoxaparin achieved reductions in both SIC (-33.3%) and SOFA (-21.7%) scores. According to the mixed model fit, the reduction in the SIC score was significative (p=0.0112) and that on the SOFA score achieved a tendency towards significance (p=0.0768). Both populations had a favorable evolution regarding WHO ordinal scale (p<0.0001) and oxygen saturation (p<0.0001), although only the patients that received enoxaparin showed a tendency towards a lower air flow requirement during the study (p=0.0776).
Summary/Conclusion: Enoxaparin was widely used during the first months of the SARS-CoV-2 pandemic in non-ICU patients, even though no specific guidelines had directly recommended its use in this population yet. Admitted patients who received enoxaparin were usually more severe than those that did not receive enoxaparin and, although both populations showed a favorable evolution, certain clinical outcomes indicate a more favorable evolution of the patients that received enoxaparin.
In summary, this study supports the convenience of the use of enoxaparin in non-critical hospitalized COVID-19 patients, not only for its antithrombotic effect but also considering that it may help to improve other clinical outcomes.

P-086

Predictors of Recurrence and Mortality Following First Venous Thromboembolism among Saudi Population: Single-Centre Cohort Study

A. AlMoneef*, R. AlManea [1], N. Shalash [1], N. AlRajhi [1], S. AlMousa [1], K. Abuelgasim [2], F. AlEidan [2]
[1]Ministry of National Guard - Health Affairs, Riyadh, Saudi Arabia, [2]Hematology, Ministry of National Guard - Health Affairs, Riyadh, Saudi Arabia

Background: Little is written about recurrence and mortality rates after a first episode of venous thromboembolism (VTE) among Saudi population.
Aims: To determine the incidence rates and assess the predictors associated with recurrence and mortality following first VTE events.
Methods: We retrospectively followed up 1124 patients aged ≥18 years with a symptomatic VTE confirmed by imaging test. In this single-centre Saudi cohort study, the incidence of VTE recurrence and mortality were assessed. The association between characteristics of patients and VTE recurrence and mortality were explored by estimating hazard ratio (HR) and 95% confidence interval (CI) using univariate and multivariate cox regression. Furthermore, we also explored the difference between cancer-related VTE, provoked and unprovoked VTE in terms of recurrence and mortality using Kaplan-Meier curves and compared groups using the log-rank test.
Results: Of the 1124 patients with first VTE, 214 patients developed recurrent VTE and 192 patients died with an overall incidence rates of 15.8 per 100 patient-year (95%CI, 13.8-18.0) and 10.0 per 100 patient-year (95% CI, 8.7-11.5), respectively. Admission to intensive care unit (ICU) for more than two days (HR, 2.15; 95% CI, 1.67-3.10; p<.001), Presence of active cancer (HR, 2.97; 95% CI, 1.87-3.95; p<.001), pulmonary embolism (HR, 2.22; 95% CI, 1.56-3.16; p<.001), and proximal deep vein thrombosis (HR, 1.91; 95% CI, 1.39-2.62; p<.001) were found independent predictors associated with recurrent VTE. Recurrence carries a high mortality rate (HR, 5.21; 95%CI, 3.61-7.51; p<.001). Using Kaplan-Meier, the estimate recurrence median time (months) was significantly lower in cancer-related VTE (18.7 months) than provoked (29.0 months) and unprovoked VTE (28.4 months, p<.001 by the log-rank test). The estimate survival median time was significantly lower in cancer-related VTE (21.8 months) than in provoked (30.5 months) and unprovoked VTE (29.8 months, p<.001 by the log-rank test).
Summary/Conclusion: ICU admission, presence of active cancer, PE and proximal DVT were significant predictors for recurrent VTE. Patients who developed recurrent VTE had 5.2-fold higher mortality rate than patients with no recurrence.

P-087

Quality of life and productivity of Hemophilia patients in Montenegro

E. Zaric [1,*], M. Kavaric [2], A. Popovic [1], V. Vujicic [1], M. Dapcevic [1], N. Bakic [1], D. Ljubic [2], D. Loncar [3]
[1]Center for hematology, [2]Department of hematology and oncology with day hospital, Pediatric clinic, Institute of Children's Diseases, Clinical Center of Montenegro, Podgorica, Montenegro, [3]Faculty of Economics, University of Belgrade, Belgrade, Serbia

Background: Hemophilia A is a rare disease affecting 30 adult and 13 pediatric patients in Montenegro. One adult patient has developed inhibitors to factor VIII and is currently being treated with emicizumab, while the remaining population is treated with factor VIII.
Aims: The objective of the study is twofold: to measure the quality of life and productivity of hemophilia patients in Montenegro and to analyze the difference in treatment with factor VIII and emicizumab.
Methods: The analysis was based on questionnaires filled by 31 persons, of which 16 were adult hemophilia patients, 6 were caregivers of adult patients, 7 were children aged 7-18 and 2 were younger children. Information gathered was confirmed through interviews with individual patients. The study uses monetized (PRODISQ and WPAI-GH V2.0), and non-monetized productivity indicators (SPS and HPQ). The PRODISQ indicator arrives at the total cost of lost productivity through a quantitative and qualitative approach, while the WPAI-GH indicator calculates the total cost of decreased productivity based on absenteeism and presenteeism. SPS provides insight into the subjective feelings of patients, while the HPQ indicator allows us to estimate non-monetized absenteeism and presenteeism. Quality of life of patients is measured through the Haemo-QoL, GPAQ, EQ-5D-5L and the SF-36 surveys as well as questions adapted from the PROBE study. Haemo-QoL is a hemophilia-specific quality of life indicator, while EQ-5D-5L and SF-36 are non-hemophilia specific, self-reported measures of health, which aim to assess the domains of health most affected by the disease. The GPAQ indicator measures patients' activity levels, while questions from the PROBE study are used to describe the population.
Results: Results of the PRODISQ analysis show that the cost of lost productivity of the working population with hemophilia in Montenegro amounts to 27,778 EUR or 354 workdays each year. The total cost of work disability could be higher, as results suggest that caretakers suffer a bigger loss in work hours, by as much as 25% because of this illness. The non-monetized indicators, show that despite their condition, patients show a relatively high level of productivity at work. The burden of disease shows through absenteeism, as the working adult population surveyed missed work some 30% of the time in the last 4 weeks due to illness. Quality of life indicators show that patients are primarily concerned by their physical health and emotional problems, but also that patients tend to manage these issues better over time. The patient who started treatment with emicizumab after developing inhibitors to factor VIII rates almost all aspects of their quality of life higher than the rest of the population, rating their overall health 26% higher than the rest of the population. The difference is especially significant in physical health parameters. Interviews with patients confirmed results, with patients additionally identifying workplace discrimination and the inconvenience of using factor VIII as negatively impacting their lives.
Summary/Conclusion: Findings suggest high potential benefits of using emicizumab to treat hemophilia A patients, as the patient with inhibitors shows significantly higher self-reported health parameters albeit on a limited sample.

P-088

Coagulation factors levels during and after anabolic androgenic steroid use: data from the HAARLEM study

E. Camilleri [1,*], D. L. Smit [2], N. van Rein [1 3], S. Le Cessie [1], O. de Hon [4], M. den Heijer [5], S. C. Cannegieter [1 6], W. de Ronde [2]
[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, [2]Department of Internal Medicine, Spaarne Gasthuis, Haarlem, [3]Department of Pharmacy, Amsterdam University Medical Center, Amsterdam, [4]Doping Authority Netherlands, Capelle aan den IJssel, [5]Department of Internal Medicine, Section of Endocrinology, Amsterdam UMC, Amsterdam, [6]Department of Internal Medicine, Division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, Netherlands

Background: Anabolic androgenic steroids (AAS) are frequently used by amateur strength athletes to enhance muscle mass. An increased risk of cardiovascular events and venous thrombosis has been reported after AAS use, while the mechanism is unknown. We hypothesize that AAS use causes changes in coagulation parameters associated with thromboembolic events. At present, only case-reports and small cross-sectional studies have been performed, which by design prohibit drawing etiological conclusions between the effect of AAS and the coagulation system. Moreover, no studies describe the extent of recovery of coagulation factor levels after AAS are withdrawn.

Aims: To determine whether AAS use influences coagulation, permanently or temporarily, by assessing changes in coagulation factor levels during and after AAS use.

Methods: The HAARLEM study enrolled 100 men intending to start a self-initiated AAS cycle between 2015-2018. Coagulation parameters (factor[F]II, FVIII, FIX, FXI, von Willebrand factor [vWF], protein S [PS], D-Dimer [DD]) and clot lysis time were measured at baseline (before AAS use) and during follow-up. We compared changes in coagulation parameters in the last week of the cycle (T_1) and 3 months after discontinuation (T_2) with baseline (T_0) using linear mixed models. We also studied the association between AAS dose and cycle duration with the outcomes ($\Delta T_1 = T_1-T_0$ and $\Delta T_2 = T_2-T_0$) by means of multivariable linear regression, adjusted for potential confounders (i.e., number of AAS used, use of oral AAS, use of other performance enhancing drugs and drugs of abuse).

Results: The participants performed an AAS cycle with a median duration of 13 weeks (range 2-52) and a median dose of 901 mg per week (range 88-3721). Mean levels of procoagulant factors increased during use (T_1-T_0) for factors FII (mean difference 14%, 95%CI 10-18), FIX (20%, 95%CI 15-25) and FXI (4%, 95%CI 0.3-8). DD levels were 1.3 times higher at T_1 compared to baseline (95% CI 1.2 to 1.5). In contrast, FVIII levels were unchanged and vWF levels decreased (-7%, 95%CI -14 to -0.4). The largest increase was observed in the natural anticoagulant PS levels (22%, 95%CI 15-29). Clot lysis time was 8 min longer (95%CI 5-10) at T_1 compared with T_0, indicating longer time to fibrinolysis. A high weekly AAS dose and short cycle duration were associated with an adjusted increase in PS during use (mean ΔT_1 increase was 2.4 [95%CI 1.0-3.7] by 100 mg increase of AAS dose and -0.3 [95%CI -1.0 to 0.4] by 1 week increase of cycle length). All coagulation parameters returned to baseline levels at T_2; neither weekly dose nor cycle length were associated with the recovery of coagulation parameters (ΔT_2).

Summary/Conclusion: AAS use was associated with a reversible increase in levels of both procoagulant and anticoagulant factors. All coagulation parameters, except for FVIII and vWF, increased during AAS use and were restored to baseline after three months of AAS withdrawn. Fibrinolysis, measured as clot lysis time, was impaired during AAS use. It remains unclear whether these changes reflect in a true shift of the balance towards a procoagulant status.

P-090

Cerebral Venous Sinus Thrombosis as first Manifestation of Primary Antiphospholipid Syndrome in a Patient with end-stage Renal Disease.

R. Koliogeorgi [1,*], C. Siafarikas [1], A. Georgopoulou [1], K. Reppas [1], M. Mylona [1], A. Toskas [1], A. Euthimiou [2], P. Sfikakis [1]
[1]1st Department of Propaedeutic & Internal Medicine, [2]2Neurology Department, Laiko General Hospital, ATHENS, Greece

Background: Cerebral venous sinus thrombosis (CVST) represents 0.5% of cerebrovascular events. It is a multifactorial condition, while its clinical symptoms depend on the site and size of the thrombosis, the patient's age as well as the underlying disease. Its clinical presentation varies from focal neurological symptoms to symptoms related with increased intracranial pressure. Antiphospholipid syndrome (APS) is an acquired autoimmune disorder that manifests clinically as recurrent venous or arterial thrombosis and/or fetal loss. Characteristic laboratory abnormalities in APS include persistently elevated levels of antibodies directed against anticardiolipin [aCL] or their associated plasma proteins, predominantly beta-2 glycoprotein I or evidence of a circulating lupus anticoagulant. APS is primary or occurs in association with SLE and other rheumatic/ autoimmune disorders.
Aims: Aim of this presentation is to highlight an interesting case of Antiphospholipid Syndrome (APS)-associated CVST presenting with headache and blurred vision.
Methods: A 51-year old Caucasian woman, with rheumatoid arthritis under treatment and end stage renal disease under peritoneal dialysis, was admitted to our clinic due to sudden onset of blurred vision and occipital headache for the last week. The patient had never experienced similar symptoms neither was there any family history of similar conditions to be noted. She was afebrile, experienced no previous head trauma and her physical examination was unremarkable.
Results: Fundus examination revealed bilateral papilledema as well as decreased visual acuity, while cranial nerves examination was normal. She underwent brain MRI with presence of cerebral edema and no space occupying lesion, brain MRA detected no aneurysms, while MR Venography revealed superior and inferior sagittal sinus, transverse sinus and right sigmoid sinus thrombosis and maxillary sinus mucosal thickening in the right petrous part of the temporal bone. Cerebrospinal fluid analysis revealed increased intracranial pressure and no other specific abnormalities. Her blood testing for thrombophilia and connective tissue disorders were normal apart from anticardiolipin antibodies IgM and lupus anticoagulant, which were highly elevated. The patient was treated with ciprofloxacin IV and low molecular weight heparin SC followed by warfarin administration. The symptoms resolved gradually while the patient' s repeated brain MRA 3 months later showed revascularization around the affected sinuses.
Summary/Conclusion: CSVT is a medical emergency and a potentially lethal entity. APS-related CSVT is also a rare phenomenon, which requires high suspicious index and early detection. Despite the decreasing mortality rates after the introduction of neuroimaging, early recognition and treatment are still crucial for a good prognosis.

P-092

Critical upper limb ischemia

B. Karar [1,*], A. SMILI [1]
[1]vascular surgery , Hmruo , oran, Algeria

Background: Primary polycythemia or polycythemia vera can be complicated by arterial or venous thrombosis .
An arterial ischemic episode occurs in 24 to 43% of patients, particularly those with associated cardiovascular risk factors (especially tobacco).
We report here a case revealed by a critical ischemia of the left upper limb.
Aims: Polycythemia vera is a risk factor for venous or arterial thrombosis by various mechanisms (blood hyperviscosity, platelet activation, microcirculatory disorder, etc.).
The thrombotic phenotype is very varied, but peripheral arterial thrombosis is reported in about a fifth of patients. In our case, the diagnosis was easily made by preoperative FNS showing high hemoglobinemia.
The management consisted of the treatment of the complication (critical ischemia) associated with the treatment of the cause (vaquez disease)
Methods: 31-year-old patient, chronic tobacco user. Admitted to the emergency room for a critical ischemia of the upper left limb, the symptoms of which went back to 01 month previously marked by permanent pain in the left hand with paresis and paresthesia. Clinical examination found a painful, slightly cold, cyanotic left upper limb with an axillary pulse present and an absent humeral and radial pulse. Doppler ultrasound and CT angiography performed at the entrance found an occlusion of the humeral artery at its origin up to the lower 1/3 middle 1/3 junction. The preoperative laboratory assessment revealed polycythemia with Hb at 18.7 g / dl Ht at 64.5%. The patient was operated on, benefitting from an axillo-humeral bypass with an inverted ipsilateral basilic venous graft. The post-operative consequences were good with recovery of a radial pulse.
in the absence of anomalies in the blood count. hematology or the diagnosis of primary polycythemia vera. The patient was referred when he left the ward and the pain and sensitivo-mo- disorders (Vaquez's disease) disappeared. A basic hydrea + aspirin type treatment was started at his home.
Results: one year later, the patient returns with a coldness of the hand, and the appearance of pulp necrosis of the 2nd finger with paralysis of the 3rd finger of the left hand and absence of axillary,

humeral and radial pulses on an occluded bypass. the patient underwent a subclavo-humeral bypass with an inverted saphenous venous graft

the postoperative results are marked by nutrition a hematoma that has regressed spontaneously and the recovery of the radial pulse
and the recovery of motor skills and sensitivity of the 3rd finger
Summary/Conclusion: This clinical case illustrates the interest of the research of logical polycythemia terials in the absence of other etiology evident even in the thromboembolic assessment of arterial pathologies.

PLATELETS

P-095

Plasma levels of platelet-derived microvesicles are associated with risk of future venous thromboembolism

O. Snir [1,*], L. Wilsgård [1], N. Latysheva [1], C. J. E. Wahlund [1], S. Brækkan [1 2], K. Hindberg [1], J.-B. Hansen [1 2]
[1]TREC, Department of Clinical Medicine, UiT - the Arctic University of Norway, [2]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Background: Microvesicles (MVs) are small double-membrane encapsulated particles shed from cells. Case-control studies have reported elevated plasma levels of platelet-derived MVs (PDMVs) in patients with venous thromboembolism (VTE). However, it is not known whether high PDMV-levels is a risk factor or a consequence of the acute VTE event.
Aims: To investigate the association between the PDMVs in plasma and risk of future incident VTE.
Methods: We performed a population-based nested case-control study with 314 VTE cases and 705 age- and sex-matched controls (from the Tromsø study) to investigate the association between the proportion of PDMVs (PDMVs%) in plasma and risk of future incident VTE. MVs isolated from plasma sampled at baseline (i.e. before VTE) were stained for platelet markers and analyzed by flow cytometry. PDMVs% were defined as the number of PDMVs divided by the total number of MVs. Odds ratios (ORs) with 95% confidence intervals (CI) for VTE risk were estimated across quartiles of PDMVs%.
Results: Subjects with PDMVs% in the highest quartile had an OR for VTE of 1.76 (95% CI: 1.20-2.61) compared to those in the lowest quartile. The OR for VTE by high PDMVs% was moderately attenuated by multivariable adjustment for age, sex, BMI, CRP and platelet count. The OR for VTE was higher when the time between blood sampling and event was shorter.
Summary/Conclusion: Our results show that high proportions of PDMVs are associated with future risk of incident VTE and imply a role of platelet activation in the pathogenesis of VTE.

P-096

Plasma and whole blood measurements of platelet (dys)function in HFpEF patients

G. D'Italia [1,*], D. M. Coenen [2], T. Lemmens [1], S. Wielders [1], B. M. Tullemans [1], J. Weerts [3], B. Schroen [3], V. P. van Empel [3], J. M. Cosemans [1]
[1]Department of Biochemistry , Maastricht University, Maastricht, Netherlands, [2]Department of Molecular and Cellular Biochemistry, University of Kentucky College of Medicine, Kentucky, United States, [3]Department of Cardiology, Maastricht University Medical Centre (MUMC+), Maastricht, Netherlands

Background: Heart failure with preserved ejection fraction (HFpEF) is a complex systemic syndrome characterized by abnormal diastolic function. In recent years, the importance of platelets in vascular inflammation and endothelial dysfunction emerged, suggesting an unexplored role for platelets in microvascular dysfunction (MVD), a central mechanism underlying HFpEF and its morbidities. However, the role of platelets in HFpEF is still poorly examined.
Aims: To investigate whether HFpEF patients present alterations in platelet function and content.
Methods: Endothelial and platelet activation markers were measured in plasma from HFpEF patients with and without type 2 diabetes mellitus (T2DM) and hypertensive controls. Moreover, platelet integrin $\alpha_{IIb}\beta_3$ activation and platelet α-granule secretion were measured by flow cytometry using freshly isolated platelets from HFpEF patients (N=40) and age- and sex-matched hypertensive controls (recruitment in progress), stimulated with different agonists. Microfluidics assays with whole blood from HFpEF patients and controls were performed to measure adhesion and activation of platelets and thrombus growth under arterial flow conditions.
Results: Markers for endothelial cell activation (ICAM-1, VCAM-1) are increased in HFpEF patients compared to hypertensive controls, reflecting the inflammatory state in HFpEF, while platelet activation markers (β-TG, PF4, TSP-1) are decreased, especially in HFpEF patients with type 2 diabetes mellitus. Moreover, platelets show decreased platelet integrin $\alpha_{IIb}\beta_3$ activation and platelet α-granule secretion upon stimulation with collagen-related peptide and TRAP-6 (flow cytometry), and a tendency for overall reduction in platelet surface coverage, thrombus formation and platelet activation markers (CD62P, fibrin(ogen), annexin A5) under flow.
Summary/Conclusion: In sum, our preliminary results show endothelial cell activation and platelet dysfunction in HFpEF patients compared to hypertensive controls, suggesting a possible unrecognized role of platelets in HFpEF.

P-097

Role of platelet GARP in TGF beta activation

J. Bodart [1,*], C. Dufeys [1], Y. A. Senis [2], Z. Nagy [3], L. Bertrand [1], C. Beauloye [1], S. Lucas [1], S. Horman [1]
[1]Université catholique de Louvain, Brussels, Belgium, [2]Université de Strasbourg, Strasbourg, France, [3]Université de Würzburg, Würzburg, Germany

Background: Transforming growth factor (TGF)β is known to be a central player in the control of cardiac fibroblast properties and fibrosis. However, cellular and molecular mechanisms that trigger its activation remain poorly understood. Platelets are considered as a major source of TGFβ and recent evidence suggest that they are involved in TGFβ activation via Glycoprotein A Repetitions Predominant (GARP) present on their surface.
Aims: The present study sought to evaluate the role of platelet GARP in TGFβ activation using platelet specific GARP knockout mice.
Methods: We generated a new Cre transgenic mouse strain that allowed megakaryocyte/platelet specific invalidation of GARP (GpIba-Cre x GARPfl/fl). The impact of GARP deficiency on platelet function was measured in vitro by flow cytometry using thrombin and CRP. Serum production of total and active TGFβ was assessed by ELISA.
Results: Platelet count and other hematological parameters were normal in platelet specific GARP knockout mice, except platelet volume, which was increased by 10.3%, as compared to wild-type platelets. Stimulation by thrombin and CRP increased GARP exposure at platelet surface. However, platelets without GARP displayed normal agonist induced activation, as reflected by CD62P and αIIbβ3 exposure. Interestingly, the generation of active TGFβ was drastically impaired in the serum of platelet specific GARP knockout mice, while the amount of total TGFβ was not affected.
Summary/Conclusion: We provided evidence that platelet GARP is a crucial contributor to the systemic activation of TGFβ. Future work will aim to determine its role in cardiac fibroblast myodifferentiation and fibrosis.

P-098

Acetyl-CoA carboxylase inhibition alters tubulin acetylation and aggregation in thrombin-stimulated platelets

L. Pirotton [1,*], M. Octave [2], A. Ginion [1], V. Robaux [1], S. Lepropre [1], S. Kautbally [1], V. Darley-Usmar [3], J. Ambroise [2], B. Guigas [4], M. Giera [4], M. Foretz [5], L. Bertrand [1], C. Beauloye [1], S. Horman [1]
[1]Université Catholique de Louvain, IREC (Institute of Experimental and Clinical Research), Pôle de Recherche Cardiovasculaire, [2]Université Catholique de Louvain, Institut de Recherche Expérimentale et Clinique, Centre de technologies moléculaires appliquées , Brussels, Belgium, [3]University of Alabama at Birmingham, Center for Free Radical Biology, Department of Pathology, UAB Mitochondrial Medicine Laboratory, Birmingham, United States, [4]Leiden university medical center, Leiden, Netherlands, [5]Université Paris Descartes, Institut Cochin, INSERM, U1016-CNRS UMR8104, Paris, France

Background: Acetyl-CoA carboxylase (ACC) is the first enzyme regulating de novo lipid synthesis via the carboxylation of acetyl-coA into malonyl-coA. Inhibition of its activity decreases lipogenesis and, in parallel, increases the content in acetyl-CoA which can serve as a substrate for protein acetylation. Several findings support a role for acetylation signaling in the coordination of signaling pathway that drives platelet cytoskeletal changes and aggregation.
Aims: To demonstrate that ACC inhibition may affect tubulin acetylation and platelet functions.
Methods: Human platelets were treated for 2 hours with CP640.186, a pharmacological ACC inhibitor, prior to thrombin stimulation. Platelet functions were assessed by aggregometry and flow cytometry. Lipogenesis was measured via ^{14}C-acetate incorporation into lipids. Lipidomics analysis was carried out on the commercial Lipidyzer platform. Protein phosphorylation and acetylation were evaluated by western blot.
Results: As expected, CP640.186 drastically decreased platelet lipogenesis. However, the quantitative lipidomics analyses showed that 2 hours treatment did not affect global platelet lipid content. Interestingly, this short-term ACC inhibition was sufficient to increase the level of tubulin acetylation, at the basal state. The deacetylation of tubulin following stimulation with thrombin was also reduced. This was associated with an impaired platelet aggregation which was likely not due to alterations in platelet secretion processes, as CP640.186 did not decrease P-selectin surface exposure or ATP secretion upon stimulation with thrombin. Similar results were obtained when human platelets were pretreated with tubacin, an inhibitor of the tubulin deacetylase HDAC6. Inhibition of ACC and HDAC6 also blocked key platelet signaling events such as Rac1 GTPase activation and phosphorylation of its downstream effector, the p21-activated kinase 2 (PAK2). Surprisingly, neither CP640.186 nor tubacin affected actin cytoskeleton remodeling but both treatments significantly decreased ROS production in response to thrombin.
Summary/Conclusion: In washed human platelets, ACC inhibition limits tubulin deacetylation upon thrombin stimulation, which impairs platelet aggregation. Our data indicate that the mechanism involves a downregulation of the Rac1/PAK2 pathway with subsequent decreased ROS generation.

P-099

Differences in platelet proteomic profiles between healthy children and adults.

A. K. Garzon Dasgupta [1 2,*], V. Zgoda [3], A. Martyanov [2 4 5], A. Sveshnikova [1 2 5]
[1]Faculty of Physics, Lomonosov Moscow State University, [2]Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology Ministry of Healthcare of Russian Federation, [3] Orekhovich Institute of Biomedical Chemistry, [4]N.M. Emanuel Institute of Biochemical Physics RAS (IBCP RAS), [5]Center for Theoretical Problems of Physico-Chemical Pharmacology, Russian Academy of Sciences, Moscow, Russian Federation

Background: Platelets' function play a key role in hemostasis. It is known that plasma and platelet protein profiles are age-specific, which may reflect some differences in platelet functioning. Accurate protein quantification is key to understanding these differences. The selection of the mass-spectrometry-based proteome quantification technique significantly affects the accuracy and precision of obtained data. Label-free quantification methods are now widely used, as they do not require complex sample preparation and are based primarily on analyzing already obtained raw LC-MS/MS data.
Aims: Here we present our proteomic workflow for accurate protein identification and data on determining differences in platelet protein profiles of healthy children and adults.
Methods: Washed platelets were obtained from two cohorts of healthy donors: 4 adults (mean age: 23.5±2.6 years) and 3 children (mean age: 13.0±3.6 years). Platelet protein lysates were analyzed using LC-Orbitrap MS. The analysis of obtained raw data was performed by MaxQuant software. The resulting relative abundances analysis for each protein was performed in Python 3.7. In addition, a UniProt-based and Gene Ontology (GO) – based study was conducted to differentiate proteins by their function and preferential intracellular localization.
Results: We applied label-free quantitative proteomics to detect protein abundances in samples from healthy donors and children. Recalculating protein concentrations into "copy number per cell" gave reliable values correlated with previously published data with r=0.96±0.01 (Burkhart et al., 2012). We then made a comparative analysis of qualitative and quantitative data from all samples. It appears that within different runs of one donor sample and different donors inside one cohort relative standard deviation of protein abundances does not exceed 30%. On the other hand, there is a number of proteins that differ significantly between adult and children healthy donors. We have selected 22 platelet proteins with statistically significant >2-fold difference in copy number between adult and children groups, with copy number more than 5000 per platelet at least for one group. Surprisingly, 9 out of them were related to vesicle trafficking (several Rab proteins, VAMP7, VPS29, and USO1) and 4 of them were related to tyrosine phosphorylation/dephosphorylation.
Summary/Conclusion: Therefore, this work gives a comprehensive platelet protein profile, demonstrating age-specific differences for more than 1% of determined proteins. Preliminary results suggest, that the trafficking/cargo filling of vesicles differs the most between the groups. All parts of this study were supported by the Russian Science Foundation grant 21-74-20087.

P-100

An automated network biology approach to identify novel key players involved in platelet activation and inhibition

T. Lemmens [1,*], D. Coenen [2], M. Kutmon [3], F. Swieringa [1], S. Coort [4], J. Cosemans [1]
[1]Biochemistry, Maastricht university, Maastricht, Netherlands, [2]Molecular and Cellular Biochemistry, Kentucky College of Medicine, Kentucky, United States, [3]Maastricht Centre for Systems Biology , [4]Department of Bioinformatics -BiGCaT, Maastricht university, Maastricht, Netherlands

Background: The healthy endothelium controls platelet activity through the release of antiplatelet agents. In the case of endothelial dysfunction, there is a loss of this natural brake on platelet activity, which can cause platelets to become hyperreactive and contribute to the progression of cardiovascular diseases. One of the main ways the endothelium attenuates platelet activation is by release of prostaglandin I2 (PGI2). PGI2 binding to the IP receptor on platelets leads to stimulation of cyclic adenosine monophosphate (cAMP) production and subsequent phosphorylation events by protein kinase A (PKA). The inhibitory effects of these phosphorylation events on platelets are not yet fully understood.
Aims: In our current study, we aim to identify proteins/processes downstream of the cAMP-PKA pathway that regulate platelet activation and inhibition and can serve as a putative “switch” in platelet activity.
Methods: We designed a network biology approach to explore the entangled platelet signaling pathways downstream of PGI2 and adenosine diphosphate (ADP). Starting from existing datasets of the phosphoproteomics response to ADP and prostacyclin, the STRING database was used to build a protein-protein interaction network. Furthermore, we visually integrated a quantitative platelet proteome dataset, pathway information, relative RNA expression of hematopoietic cells, the likelihood of the proteins being phosphorylated by PKA, and drug-target information from DrugBank. The approach was then automated using R.
Results: Ultimately, we were able to distill 30 proteins from existing phosphoproteomics datasets that putatively can be “turned on” after ADP-mediated platelet activation and subsequently switched “off” after platelet inhibition with iloprost. Enrichment analysis revealed biological processes related to vesicle secretion and cytoskeletal reorganization to be overrepresented coinciding with topological clusters in the network. Our method highlights novel proteins related to vesicle transport, platelet shape change, and small GTPases as potential switch proteins in platelet activation and inhibition.
Summary/Conclusion: Our novel approach demonstrates the benefit of data integration by combining tools and datasets and visualization to obtain a more complete picture of complex molecular mechanisms such as the regulation of platelet activation and inhibition.

P-101

Novel multimeric anti-GPVI nanobody and modelling reveals mechanism of GPVI clustering and activation

H. Y. F. Cheung [1 2 3,*], Z. Maqsood [1 4 5], A. Slater [1], N. S. Poulter [1], C. Wierling [4], S. P. Watson [1]
[1]Institute of Biomedical Research, University of Birmingham, Birmingham, United Kingdom, [2]Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, Netherlands, [3]Leibniz-Institut fur Analytische Wissenschaften-ISASe. V., Dortmund, [4]Alacris Theranostics GmbH, Berlin, [5]Rudolf Virchow Center for Experimental Biomedicine and Institute of Experimental Biomedicine, University of Würzburg and University Hospital of Würzburg, Würzburg, Germany

Background: The collagen receptor GPVI initiates platelet activation in the injured vessel driving shape change, secretion of secondary agonists and integrin activation. The clustering of GPVI drives Src and Syk tyrosine kinase-mediated phosphorylation of YxxL signalling motifs and downstream signalling. NB2 is a novel anti-GPVI nanobody that inhibits collagen-induced platelet aggregation (Slater et al. Blood, 2021).
Aims: To study GPVI clustering and activation using multimeric NB2 and agent-based modelling (ABM).
Methods: Monomeric, dimeric and tetrameric NB2 were expressed in transfected E. coli WK6 cells. Each NB2 domain in multimeric NB2 is connected by a flexible $(GGGGS)_3$ linker. Platelets were stimulated in a Born aggregometer and protein phosphorylation analysed using phosphospecific antibodies. ABM was used to simulate spatial dynamics of GPVI dimers clustering based on a series of autonomous, decision-taking agents in the presence of a multivalent ligand.
Results: Dimeric NB2 blocked collagen (3 µg/mL) induced aggregation with an IC50 of 4.68 ± 0.48 nM, 6 times more potent than monomeric NB2 (IC50 = 30.7 ± 1.9 nM). Tetrameric NB2 (16 nM) induced GPVI-mediated platelet aggregation, which was inhibited by Src and Syk kinase inhibitor PP2 (20 µM) and PRT-060318 (10 µM), and GPVI inhibitor JAQ1-Fab (200 nM) and monomeric NB2 (100 nM). Tetrameric NB2 induced phosphorylation of Syk Y525/526 and LAT Y200, which was inhibited by PP2 and PRT-060318 or severly delayed by monomeric NB2. ABM is being used to model the interaction of dimeric and tetrameric Nb2 with a dimeric receptor.
Summary/Conclusion: These findings indicate that monomeric NB2 and dimeric NB2 are GPVI inhibitors while tetrameric NB2 is a GPVI agonist. The results show that dimerisation of GPVI does not induce activation of human platelets, in contrast to results on mouse platelets, and higher order multimers are required for activation.

The study is funded by the European Union's Horizon 2020 research and innovation program (Marie Sklodowska-Curie grant agreement No. 766118)

P-102

Combined antiplatelet therapy reduces the pro-inflammatory properties of activated platelets

A. C. C. Heinzmann [1,*], D. M. Coenen [1], T. Vajen [2], J. M. Cosemans [1], R. R. Koenen [1]
[1]Biochemistry, Maastricht University, Maastricht, Netherlands, [2]Cardiology, Pulmonology and Vascular Medicine, Heinrich-Heine-University, Düsseldorf, Germany

Background: The cause of atherothrombosis is rupture or erosion of atherosclerotic lesions, leading to myocardial infarction or stroke. Here, platelet activation plays a major role, leading to the release of bioactive molecules, *e.g*. chemokines and coagulation factors, and to platelet clot formation. Several antiplatelet therapies have been developed for secondary prevention of cardiovascular events, in which anticoagulant drugs are often combined. Besides playing a role in haemostasis, platelets are also involved in inflammation. However, it is unclear whether current antiplatelet therapy also affects platelet immune functions.
Aims: The aim of this study is to investigate the influence of common antiplatelet drugs on inflammatory functions of platelets and whether this influence is distinct from their established anti-haemostatic effects.
Methods: In this study, the possible anti-inflammatory effects of antiplatelet medications were investigated on chemokine release using ELISA and on the chemotaxis of THP-1 cells towards platelet releasates. Platelets were isolated from healthy subjects in agreement by the local Maastricht ethics committee (METC) and studies were performed in accordance with the declaration of Helsinki.
Results: We found that antiplatelet medication acetylsalicylic acid (ASA) led to reduced Chemokine (C-C motif) ligand 5 (CCL5) and chemokine (C-X-C motif) ligand 4 (CXCL4) release from platelets, while leukocyte chemotaxis was not affected. Depending on the agonist, $\alpha_{IIb}\beta_3$- and $P2Y_{12}$-inhibitors also affected CCL5 or CXCL4 release. The combination of ASA with a P2Y12 inhibitor or a phosphodiesterase inhibitor did not provide an additive reduction on CCL5 or CXCL4 release. Interestingly, these combinations did reduce leukocyte chemotaxis.
Summary/Conclusion: This study provides evidence that combined therapy of ASA and a P2Y12 or PDE3 inhibitor can decrease the inflammatory leukocyte recruiting potential of the releasate of activated platelets.

P-103

Pathologic desialylation of platelets in ITP can be caused by platelet activation in circulation

O. I. An [1 2 3,*], A. A. Martyanov [3 4 5], A. A. Ignatova [3 6], M. A. Panteleev [3 6], A. N. Sveshnikova [3 4]
[1]Laboratory of Intracellular Signaling and Systems Biology, Center for Theoretical Problems of Physicochemical Pharmacology of the Russian Academy of Sciences, [2]International School "Medicine of the Future", Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation, [3]Laboratory of Cellular Hemostasis and Thrombosis, Dmitriy Rogachev National Medical Research Center of Pediatric Hematology, Oncology Immunology Ministry of Healthcare of Russian Federation, [4]Laboratory of Intracellular Signaling and Systems Biology, Centre for Theoretical Problems of Physico-Chemical Pharmacology Russian Academy of Sciences, [5]N.M. Emanuel Institute of Biochemical Physics RAS (IBCP RAS), [6]Laboratory of Molecular Mechanisms of Hemostasis, Centre for Theoretical Problems of Physico-Chemical Pharmacology Russian Academy of Sciences, Moscow, Russian Federation

Background: Immune thrombocytopenia (ITP) is a common acquired bleeding disorder characterized by enhanced platelet consumption. No definitive diagnostic approaches to ITP exist. In the debute of ITP, significant amounts of antibodies against platelet glycoproteins are produced. Alongside cytotoxic T-lymphocytes, antiplatelet antibodies lead to platelet opsonization and clearance. Among the key markers for the platelet clearance is desialylation – loss of the sialic acids from the platelet glycocalyx. Desialylation inhibitors (oseltamivir) help to increase the platelet count in ITP. Antibodies to platelet GPIb induce P-selectin exposure and increase the desialylation, which leads to thrombocytopenia. Indeed, platelet pre-activation in ITP-patients has been explicitly shown. Determination of exact mechanisms of platelet desialylation can remove ITP diagnostics from the “grey zone” and provide the basis for more effective therapeutic approaches.
Aims: To determine the mechanism of platelet desialylation upon pre-activation in ITP-alike conditions.
Methods: Blood of healthy donors was collected into sodium citrate-containing tubes according to the declaration of Helsinki. Platelets were isolated by centrifugation in the presence of sodium citrate and resuspended in Tyrode’s buffer without BSA (pH 7.3). Platelet concentration was adjusted to 10.000 cells/ul, and platelets were incubated with lectins from *R. communis* and *E. cristagalli* to assess the desialylation ratio. Mepacrine, anti-GPIb, and anti-P-selectin antibodies were added to quantify platelet activation. Platelets were activated with 4 uM of SFLLRN. The maximum level of desialylation was evaluated with Neuraminidase from *C. perfringens*. A computational model of the platelet desialylation was developed for the determination of its mechanisms. In the model, the PAR-1 activation leads to the secretion of platelet dense granules and their content, including Neuraminidase-1 and beta-galactosidase, which mediates desialylation. Model parameters were obtained from the literature or fitted to previously published experimental data.
Results: Beta-galactose and N-acetyl-glucosamine exposure were significantly increased upon activation with TRAP-6. Furthermore, platelet activation markers correlated with the desialylation ratio, which is in line with previously published data of Ignatova et. al 2021. Computational modeling showed that, on average single dense granule exposure leads to the desialylation of 30% of the glycocalyx. The degree of desialylation was governed mainly by the rate of neuraminidase inactivation due to the pH change upon dense granule secretion. Interestingly, variation of the neuraminidase activity modestly altered predicted platelet desialylation. Finally, the model predicted that a single granule release could be enough to achieve the maximal degree of platelet desialylation.
Summary/Conclusion: The developed protocol allows to determine the level of platelets desialylation upon activation. Computational modeling allows proposing a mechanism of Neuraminidase secretion and action upon PAR-1 activation and dense granules consecutive release. The model predicts that strong activation is not required for platelet desialylation as only one dense granule secretion can result in the platelet sialic acid removal.
Funding: The work was supported by the Russian Presidential Fellowship SP-2675.2019.4 and a grant from the endowment foundation "Doctors, Innovations, Science for Children".

P-104

Identification of a novel de novo SLFN14 variation associated with inherited thrombocytopenia: a case report

M. Ibrahim-Kosta [1,*], C. Falaise [1], M. Poggi [1], S. Véronique [1], P. Saultier [1], H. Chambost [1], N. Saut [1], E. Tomei [1], M.-C. Alessi [1]
[1]Aix Marseille Univ, INSERM 1263, INRAE 1260, C2VN, APHM, National Reference Center on Constitutional Platelet Disorders (CRPP), APHM, C2VN, Marseille, France

Background: Schlafen 14 (SLFN14), an endoribonuclease, which belongs to the subgroup III of the SLFN family, is implicated in megakaryocyte maturation and platelet production. Few variations in the *SLFN14* gene have been associated with inherited thrombocytopenia, excessive bleeding and dense granule secretion defect.
Aims: To report the case of an eight-year-old girl with a new variation in the *SLFN14* gene.
Methods: Platelet counts, morphological platelet studies, platelet functional tests (light transmission aggregation, flow cytometry (FC), dense granule content) and a targeted gene sequencing (300 genes related to platelets) (KAPA library preparation (Roche®), Illumina sequencing (MiSeq)) were performed for the index case and her parents. Expression of SLFN14 protein was evaluated by Western-Blot.
Results: The proband had a moderate bleeding history with epistaxis, bruising and provoked large hematomas (forehead, knee, lower limbs). Platelet count was mostly close to 120 × 10^9/L with 10% of macroplatelets. We observed a reduced and reversible aggregation to ADP (2.5µM, 5µM and 10µM), a reduced response to collagen (3.3µg/mL, 6.6µg/mL), platelet aggregation did not occur in presence of epinephrine (7.5µM, 15µM) and thrombin receptor activating peptide 10µM (TRAP6). Response to arachidonic acid was slightly affected. A reduced expression of PAC1 after stimulation by ADP 10µM and TRAP 50µM was observed by FC. Reduced intra-platelet serotonin and dense granule count by Whole Mount showed partial dense granules deficiency.
A novel de novo heterozygote missense variation in the *SLFN14* gene (c.785T>A; p.V262E) was identified in the proband. This variation is not reported in the general population databases. It is located in a well established functional domain (ATPase-AAA domain). Proband's parents described no bleeding history. They presented normal platelet phenotype and no sequence variation was identified within *SLFN14* gene. Quantification of platelet SLFN14 protein in the three family members by Western Blot highlighted a reduction (56%) of SLFN14 within proband's platelets compared to platelets from both parents.
Summary/Conclusion: SLFN14 variations are a rare cause of inherited thrombocytopenia. Here, we reported a fifth pathogenic variation according to the ACMG criteria, in an eight-year-old girl with moderate bleeding, thrombocytopenia and partial dense granule defect. More functional investigations are needed to understand the mechanisms of the platelet dysfunction.

P-105

Software to make mathematical models of platelet regulation useful for biologists; allowing prediction, interpretation of experimental data, and hypothesis generation

F. Balabin [1 2,*], A. Martyanov [1 2 3], J. Dunster [4], J. Gibbins [4], A. Sveshnikova [1 2 5 6]
[1]Center for Theoretical Problems of Physico-Chemical Pharmacology, Russian Academy of Sciences, [2]Dmitry Rogachev National Medical Research Center Of Pediatric Hematology, Oncology and Immunology, [3]N.M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russian Federation, [4]Institute for Cardiovascular & Metabolic Research, University of Reading, Reading, United Kingdom, [5]Faculty of Physics, Moscow State University, [6]Department of Normal Physiology, Sechenov First Moscow State Medical University, Moscow, Russian Federation

Background: Many different mathematical models exist that enable the simulation of different aspects of platelet behaviour, both the molecular machinery and functional changes. These models are used to generate hypotheses, make predictions and test experimental data. However, due to their technical complexity, such models are often inaccessible to biological researchers.
Aims: To provide an interactive easy to use method to allow biological researchers to use these mathematical models.
Methods: We present a series of interactive Jupyter Notebooks, each dissecting a single mathematical model that describes a different aspect of platelet subcellular or functional changes. We demonstrate how to use this software, run and analyze the model. This allows a researcher with little theoretical knowledge to modify the levels of stimulants and compare outputs to experimental data.
Results: We provide the notebooks for six models crucial for understanding platelet activation. Firstly, we describe the initial steps of platelet activation by reviewing G protein-coupled receptor dynamics, receptor clustering, GPIV-induced activation and CLEC2-induced activation. Secondly, we provide the basic model for platelet calcium response. Finally, we show a model of platelet aggregation.
Summary/Conclusion: This is the first step in the development of a common approach to publishing models of biological systems and making it easy for researchers to access any model in a consistent and easy way.
Funding: The reported study was funded by RFBR and the Royal Society of London (RS), project number 21-51-10005

P-108

Protein disulfide isomerase-A1 Regulates Intraplatelet NADPH Oxidase-1–Reactive Oxygen Species–Thromboxane A2-dependent Pathway In Response to GPVI-mediated Platelet Activation

K. Przyborowski [1,*], A. Kurpinska [1], D. Wojkowska [2], P. Kaczara [1], J. Suraj-Prazmowska [1], K. Karolczak [2], A. Malinowska [3], A. Pelesz [1], I. Kalvins [4], C. Watala [2], S. Chlopicki [1 5]
[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, [2]Department of Haemostasis and Haemostatic Disorders, Medical University of Lodz, Lodz, [3]Mass Spectrometry Laboratory, Polish Academy of Sciences, Institute of Biochemistry and Biophysics, Warszawa, Poland, [4]Latvian Institute of Organic Synthesis, Riga, Latvia, [5]Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland

Background: Platelets contain several protein disulfide isomerases (PDIs), including PDIA1 and PDIA3, which control platelet function and thrombus formation *in vivo.* Although PDIA3 released from activated platelets has been suggested to regulate the platelet αIIbβ3 integrin activation directly on platelet surface, it seems that PDIA1 and PDIA3 regulate platelet activation through distinct mechanisms. However, a specific role of PDIA1 in the regulation of platelet function has not been thoroughly defined.
Aims: The aim of this study was to evaluate the content of PDIs in non-activated and activated human platelets, the expressions of major PDI isoforms on platelet surface, and specifically, to characterize the role of PDIA1 in platelets using a selective PDIA1 inhibitor – bepristat 2a.
Methods: Proteomic analysis of PDI isoforms in platelets was performed using LC–MS/MS and the expressions of PDIs on platelets in response to collagen, TRAP-14, or ADP were measured with flow cytometry. The effects of bepristat on platelet aggregation, expression of platelet surface activation markers, thromboxane A_2 (TxA_2) synthesis and reactive oxygen species (ROS) generation were evaluated by optical aggregometry, flow cytometry, enzyme-linked immunosorbent assay and dihydrodichlorofluorescein diacetate-based fluorescent assay, respectively.
Results: PDIA1 was less abundant compared to PDIA3 in resting platelets and platelets stimulated with TRAP-14, collagen or ADP. Collagen, but not ADP, induced a significant increase in PDIA1 expression on platelet surface. Bepristat strongly inhibited the aggregation of washed platelets induced by collagen or convulxin, which corresponded with the reduced activation of αIIbβ3 integrin, but bepristat induced only a weak inhibition of platelet aggregation triggered by TRAP-14 or thrombin, and had the negligible effect on platelet aggregation in response to arachidonic acid. Inhibition of PDIA1 by bepristat resulted in the profound reduction of thromboxane A_2 and ROS production in collagen- or thrombin-stimulated platelets. The effects of bepristat on collagen-induced intraplatelet ROS production were comparable to those observed for a selective NADPH oxidase-1 (Nox1) inhibitor, ML171, but they were different from the effects of apocynin, both a non-selective Nox inhibitor and a ROS scavenger, which inhibited ROS production to a lesser degree compared with ML171. Furthermore, bepristat reduced the expression of P-selectin, but did not affect the ristocetin-induced platelet agglutination mediated by GPIbα-vWF binding.
Summary/Conclusion: In the present work we have shown for the first time that PDIA1 can support platelet activation through regulating the intracellular Nox1-ROS-TxA_2 pathway upstream of COX-1, which plays an essential role in GPVI-mediated activation of the αIIbβ3 integrin on the platelet surface. This mechanism does not involve the platelet response to thrombin and is distinct from extracellular controlling αIIbβ3 integrin activation by PDIA3.
Funding: This work was supported by funding from The National Centre for Research and Development (grant STRATEGMED1/233226/11/NCBR/2015 to SCh), and partially by the Latvian National Fundamental and Applied Research Grant (grant No. Izp-2018/1-0143 to IK “Isoform selective PDI inhibitors: design, synthesis and SAR”).

P-109

Assessment of Platelet function and Inhibition by aspirin in patients Recovering from severe Infection (ASPIRIN-trial)

M. Schutte [1,*], M. Madsen [1], J. van Diemen [1], Y. Smulders [1], A. Thijs [1]
[1]Amsterdam UMC, VUmc, Amsterdam, Netherlands

Background: The risk of cardiovascular events is increased in patients hospitalized for a severe infection. One of the possible underlying mechanisms is platelet hyperaggregability induced by inflammation. Arterial prophylaxis may therefore be indicated in these patients. As platelet turn-over is increased during infection, a twice daily regimen of aspirin may be more effective than the usual regimen of once a day.
Aims: Our aims are to investigate platelet aggregability during infection and the efficacy of different dosage regimens of aspirin in inhibiting this aggregability.
Methods: This is a multi-center open label randomized trial. Patients hospitalized for a pneumonia, urinary tract infection or soft tissue infection, without a medical history of cardiovascular disease were included. They were randomized to either 80 mg aspirin once daily, 40 mg aspirin twice daily or no intervention, for 10 consecutive days. Measurements were performed <4 days after admission (T=1), at day 14 (one day after aspirin-treatment, T=2) and after more than 90 days (T=3). The primary outcome measure is platelet aggregability, measured by the closure time (CT) of the Platelet Function Analyzer (PFA).
Results: 60 patients were included. In the control group, the mean CT at T1 (98.5 seconds) was 20.5 seconds shorter than at T3 (119 seconds), indicating a higher platelet aggregability during infection ($p = 0.001$). After 10 days of aspirin (either 80 mg once daily or 40 mg twice daily), the mean difference in CT between T2 and T1 was 108 seconds, whereas in the control group the mean difference was 12 seconds ($p < 0.001$). The difference seemed larger in the twice daily group (126 seconds, 95% CI 59 ; 192) than in the once daily group (101 seconds, 95% CI 70 ; 132 seconds), although this was not statistically significant ($p = 0.42$). Also, the occurrence of aspirin resistance (CT < 194 seconds) was higher in the once daily group (45%) than in the twice daily group (25%).
Summary/Conclusion: Platelet aggregability seems to be increased during severe infection. Based on these results, aspirin on the one hand seems to be able to significantly reduce the level of platelet aggregability; however, we observed a high percentage of aspirin resistance. Doubling dose frequency (40 mg twice daily) may be more effective than 80 mg once daily, but further research has to confirm this hypothesis.

P-110

Efficacy of SANDOSTATIN in the treatment of haemorrhagic angiodysplasia of the digestive tract in Glanzmann's disease

D. Desprez [1,*], O. feugeas [2], A. C. gerout [3], P. chamouard [4]
[1]Hemophilia Teatment Centre, UNIVERSITY HOSPITAL, [2]hemophilia treatment centre, [3]Pharmacy, [4]gastroenterology, university hospital, strasbourg, France

Background: Les angiodysplasies peuvent dans certaines cas provoquer des hémorragies digestives récurrentes sévères chez les patients atteints de thrombasthénie de Glanzmann (GT). Leur incidence, estimée à 2,5 %, est comparable à celle observée dans la maladie de Von Willebrand.
Aims:
La prise en charge des angiodysplasies est délicate et nécessite l'association d'un traitement hémostatique et d'un traitement endoscopique des lésions
Methods: Nous rapportons le cas d'une patiente de 56 ans présentant un GT de type 1 et des angiodysplasies multiples du tube digestif. Alors que son phénotype hémorragique initial était relativement modéré, elle a connu de nombreux accidents hémorragiques au cours de la dernière décennie : AVC hémorragique frontal massif nécessitant un shunt ventriculopéritonéal et une craniotomie décompressive, une contracture de Volkmann suite à un hématome brachial, un choc hémorragique suite à un enclouage fémoral. À partir de 2017, des épisodes récurrents de melaena ont entraîné des baisses répétées des taux de globules rouges. La gastroscopie a révélé une angiodysplasie de l'artère du genu supérieur qui a été traitée avec un clip, et une angiodysplasie du cardia pour laquelle une électrocoagulation APC a été réalisée. Dans le même temps, les symptômes ont été traités par transfusion de concentré de globules rouges (RBC) et de fer injectable, combiné avec de l'acide tranexamique et un inhibiteur de la pompe à protons. Le patient a reçu 60 unités entre 2017 et 2020. Le tableau du traitement a été complété par une administration occasionnelle de concentrés plaquettaires (CP), tandis que NOVOSEVEN a été administré avec parcimonie en raison d'antécédents de thrombose de la veine cave et d'une mauvaise tolérance (spasticité pulmonaire après injection). En juin 2020, les besoins transfusionnels du patient sont passés de 2 à 3 RBCC par semaine. L'endoscopie vidéo par capsule a révélé de multiples angiodysplasies de l'intestin grêle En juin 2020, les besoins transfusionnels du patient sont passés de 2 à 3 RBCC par semaine. L'endoscopie vidéo par capsule a révélé de multiples angiodysplasies de l'intestin grêle En juin 2020, les besoins transfusionnels du patient sont passés de 2 à 3 RBCC par semaine. L'endoscopie vidéo par capsule a révélé de multiples angiodysplasies de l'intestin grêle
Results: L'utilisation de contraceptifs oraux estroprogestatifs étant contre-indiquée, un traitement par SANDOSTATIN LAR à 10 mg/mois a été instauré et a conduit à une stabilisation du taux d'hémoglobine pendant 6 mois. Face à un épisode de rupture, la posologie de SANDOSTATIN LAR a été augmentée jusqu'à 30 mg/mois, ce qui a de nouveau stabilisé la situation clinique après plus d'un an de suivi.
Summary/Conclusion:
En conclusion, SANDOSTATIN LAR semble efficace dans le traitement des angiodysplasies hémorragiques du tube digestif chez un patient avec GT après échec des thérapies usuelles. Cette efficacité doit cependant être confirmée dans un essai thérapeutique.

P-111

Platelet indices and coagulation markers in COVID-19 infected individuals

E. Petridou [1], A. Agorasti [1,*]
[1]General Hospital of Xanthi, Xanthi, Greece

Background: Coronavirus disease 2019 (COVID-19) patients are usually classified as “mild”, “severe” and “critical” types. Large platelets play a key role in the pathogenesis of thrombosis as they are more active metabolically compared to smaller platelets. COVID-19 is associated with thrombotic manifestations and related coagulation abnormalities.
Aims: The aim of our retrospective study is the evaluation of platelet indices and coagulation markers in COVID-19 infected individuals treated with the appropriate medical intervention as outpatients or inpatients.
Methods: Patients admitted to our hospital with COVID-19, diagnosed through real-time reverse transcription polymerase chain reaction, and had a complete blood count (on Sysmex XN-1000 automated hematology analyzer) and coagulation parameters determinations (on Sysmex CS-2100i System) within 24 hours of diagnosis were included. We retrospectively recorded the demographic data, and the laboratory findings upon admission: platelet count (PLT, x 10^9/L), mean platelet volume (MPV, fL), platelet-large cell ratio (P-LCR, %), prothombin time (PT, sec) and D-dimer (μg/L Fibrinogen Equivalent Units). We calculated the age-adjusted D-dimer threshold for patients over 50 years old as followed: patient’s age x 10 μg/L FEU. All study parameters were retrieved from the hospital electronic database system. Patients were divided in two groups: patients discharged and treated as outpatients (group A, N = 76) and patients hospitalized in COVID-19 inpatient wards (group B, N = 167). 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 genders (P = 0.183) but inpatients are older [70 (59-80) vs 60 (41-71) years, P = 0.000]. There is no statistically significant difference in platelet count between the two groups [group A: 190 (154-246) vs group B: 186 (146-264), P = 0.332)] but inpatients present statistically significant larger platelets [MPV group A: 10.7 (9.7-11.2) vs group B 11.0 (10.1-11.7), P = 0.021 and P-LCR group A: 30.7 (22.6-35.4) vs group B: 32.6 (25.2-38.8), P = 0.025]. Inpatients present prolongation of PT and elevated D-dimer in a statistically significant degree irrespective of the age [PT group A: 12.4 (11.8-13.1) vs group B: 13.1 (12.4-13.9), P = 0.000 and D-dimer group A: 0.52 (0.33-1.00) vs group B 0.95 (0.49-2.06), P = 0.000]. The AUC in discriminating inpatients is statistically significant for PT 0.692 (P = 0.000) and for D-dimer 0.631 (P = 0.003).
Summary/Conclusion: COVID-19 patients who need hospitalization present larger platelets, bigger prolongation of prothombin time and more elevated D-dimer as compared to outpatients. The predictive power of prothrombin time and D-dimer levels in discriminating hospitalization is regarded as good.

VESSEL WALL

P-112

Role of Syndecan-1 in Thoracic Aortic Aneurysm development

S. Zalghout [1,*], S. Vo [1], V. Arocas [1], E. Hamade [2], Y. boulaftali [1], B. Richard [1]
[1]INSERM U1148, PARIS, France, [2]Lebanese University, Beirut, Lebanon

Background: Glycosaminoglycans pooling has been considered since long as one of the histopathological characteristics defining thoracic aortic aneurysm (TAA) together with smooth muscle cells (SMCs) apoptosis and elastin fibers degradation. However, few information is provided about their potential implication or even composition in TAA pathology. Syndecan-1 (Sdc-1) is a heparan sulfate proteoglycan that is implicated in extracellular matrix interaction and assembly, regulation of SMCs phenotype and various aspects of inflammation in the vascular wall.
Aims: The aim of this work was to measure the level of expression of Sdc-1 in human TAA and to evaluate its role on the disease progression.
Methods: The regulation of Sdc-1 protein expression in human TAA has been examined by ELISA and immunohistochemistry. The role of Sdc-1 in TAA development has been assessed by the use of a pharmacological mouse model combining both aortic wall weakening by BAPN treatment, followed by hypertension induced by angiotensin II infusion.
Results: Our results showed that Sdc-1 protein expression is upregulated in human TAA aortas compared to healthy counterparts and that SMCs are a type of cells that increase this expression. However, in contrast to what has been observed in abdominal aneurysm, in our TAA model, Sdc-1 did not affect TAA incidence or rupture neither the extent of the dilatation of the aortic wall. In addition, Sdc-1 was not involved in elastin degradation (proteolytic activity) or collagen deposition; and immunostaining targeting leukocytes revealed that it is not involved in inflammatory cells recruitment in this model.
Summary/Conclusion: The possible role of Sdc-1 in human TAA is not established yet. Models of earlier or later stages of the disease may better explain the significance of the increased protein level observed in human TAA tissues.

P-113

Epigenetic regulation of endothelial dysfunction in thromboembolic venous disease

M. Pilard [1,*], V. Gourdou-Latyszenok [1], F. Couturaud [1], C. Lemarie [1]
[1]UFR Medecine, EA3878, GETBO,, Brest, France

Background: Venous thromboembolism (VTE), which encompasses pulmonary embolism and deep vein thrombosis, is a frequent disease that is associated with vein wall fibrosis. Endothelial cells undergo phenotypical changes named endothelial-to-mesenchymal transition (EndMT), characterized by the loss of endothelial markers and the acquisition of mesenchymal markers leading to fibrosis. Transforming growth factor (TGFβ) is the most potent inducer of EndMT. A recent study showed that in chronic thromboembolic pulmonary hypertension, TGFβ induces EndMT and impaired thrombus resolution. However, the molecular mechanisms implicated in TGFβ signaling in the context of venous thromboembolism and recurrent events are unknown. In addition, epigenetic mechanisms regulate EndMT. We hypothesized that epigenetic processes regulate the TGFβ signaling pathway in endothelial cells promoting EndMT and recurrent thrombosis.
Aims: The aim of this study was to test if EndMT is regulated by epigenetic mechanisms in recurrent thrombosis.
Methods: Endothelial cells were treated with TGFβ, thrombin or both during 5 days. To study the role of histone deacetylase (HDAC) in EndMT, endothelial cells were also incubated in presence of vorinostat, an HDAC inhibitor or TCS HDAC6 20b, a specific HDAC6 inhibitor. Real time PCR were performed to analyze endothelial and mesenchymal marker expression.
Results: Expression of the mesenchymal markers, calponin (CNN1) and α-smooth muscle actin (SM22), is increased by TGFβ and thrombin. CD146 expression, an endothelial marker, appears to be reduced by these treatments. Interestingly these changes appear to be inhibited in presence of vorinostat or TCS HDAC6 20b. Our results suggest that TGFβ and thrombin induce EndMT and that HDAC6 contribute to this mechanism.
Summary/Conclusion: We found that treatment of endothelial cells with TGFβ and thrombin is associated with EndMT. Our preliminary data suggest that HDAC6 contribute to EndMT. Deciphering the mechanisms by which epigenetic is regulating EndMT might lead to the discovery of biomarkers or new therapeutic targets would be instrumental in guiding decisions of treatment for patients with a high risk of recurrent VTE.

P-114

A rapid, sensitive and specific assay to measure TF activity based on chromogenic determination of thrombin generation

N. Latysheva [1,*], O. Snir [1], B. Jilma [2], B. Østerud [1], J.-B. Hansen [1 3]
[1]Faculty of Health Sciences, UiT/The Arctic University of Norway, Tromsø, Norway, [2]Department of Clinical Pharmacology, Medical University of Vienna, Vienna , Austria, [3]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Background: The majority of TF activity assays are based on measurement of FX activation by TF in the presence of FVII/FVIIa. This requires long incubation and thus results in TF-independent activity of FX and inaccurate measurement of TF activity
Aims: To develop a sensitive and specific TF activity assay, which does not register a non-specific TF activity, using commercial coagulation factors
Methods: TF activity was measured based on the ability of TF to accelerate the activation of FX by FVIIa and to rapidly convert prothrombin to thrombin in the presence of FV/Va, calcium, and phospholipids. Following 4 min incubation at 37°C, TF activity was quantified in test samples of different nature by thrombin generation using a thrombin chromogenic substrate
Results: The TF activity assay proved high sensitivity (low fM range) and specificity, assessed by neutralization of TF activity by anti-TF antibody and the use of FVIIai. TF activity was detected on extracellular vesicles (EVs) derived from HAP1-TF+ cells, while no activity was measured on EVs from HAP1-TF/KO cells. The assay was applicable for measurement of TF activity on the surface of live endothelial cells and monocytes activated in vitro as well as cell lysates. Treatment with low dose lipopolysaccharide (2 ng/kg bodyweight) caused a transient 8-fold increase (peaked at 4 hours) in TF activity on EVs isolated from plasma of healthy volunteers
Summary/Conclusion: Our assay provides a fast, sensitive and specific measurement of TF activity. It reliably quantifies TF activity on cell surface, in cell lysate and isolated EVs. The assay can be used for laboratory and clinical research

P-115

Endothelial P2X7 promotes venous thromboembolism

E. Olliver [1,*], V. Gourdou-Latyszenok [1], F. Couturaud [1], C. Lemarie [1]
[1]UFR Medecine, EA3878, GETBO,, Brest, France

Background: Venous thromboembolism (VTE) afflicts 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) chronic pulmonary hypertension resulting in significant chronic respiratory compromise. Inflammatory pathways are intricately involved in venous thromboembolism mechanisms. In pathological conditions, adenosine triphosphate (ATP) is released in the extracellular compartment and is recognized as a danger signal. Recent data indicated that the CD39/CD73 system involved in the metabolism of ATP into AMP might protect against thrombosis by downregulating the pro-inflammatory pathway of the inflammasome. ATP can also interact with the P2X7 receptor involved in inflammation causing a wide range of responses.
Aims: Determining how the endothelial P2X7 receptor contribute to venous thromboembolism.
Methods: HUVEC were incubated with BzATP, thrombin or both. In some experiment, HUVEC were primed with TNFaprior to stimulation. We used immunofluorescence, western blot and real-time quantitative PCR analyses to assess P2X7 expression by endothelial cells, activation of p38 and NFkB activation and expression of pro-inflammatory and pro-coagulant genes.
Results: We confirmed that endothelial cells expressed P2X7 in vitro in HUVECs and in vivo after induction of venous thrombosis by ligation of the inferior vena cava. Treatment of endothelial cells with BzATP and thrombin induced the activation of p38 and NFkB signaling pathways. This was associated with an increased expression of IL-1b and tissue factor and downregulation of thrombomodulin expression.
Summary/Conclusion: Our data suggest that ATP released in the extracellular space following cell damage or activation induced the inflammasome in endothelial through P2X7 activation. P2X7 might have a pro-thrombotic role exacerbating venous thromboembolism.

P-116

The effects of platelet factor 4 (CXCL4) and its non-allelic variant (CXCL4L1) on the phenotype of human vascular smooth muscle cells in vitro

D. Kaczor [1,*], R. Koenen [1], L. Schurgers [1]
[1]Biochemistry, Maastricht University, Maastricht, Netherlands

Background: Platelet factor 4 (PF4 or CXCL4) is a chemokine synthesized mainly (but not exclusively) by megakaryocytes and stored in platelets. PF4 is a ligand for various receptors, initiating diverse cell type-dependent downstream signalling pathways (de Sutter et al. 2012). Although the underlying molecular mechanisms and the main physiologic function of PF4 are still unknown, its interaction with a vessel wall is well established. Upon injury (Stemerman et al. 1981) or during the formation of an atherosclerotic plaque, PF4 is transported into deeper vessel layers by an unknown mechanism, modulating vascular smooth muscle cells (VSMCs), among others. Several studies in mice and human specimen studies have confirmed the presence of PF4 in the atherosclerotic plaques (Sachais et al. 2007) (Pitsilos et al. 2003)(Coppinger et al. 2004). Despite that PF4 and PF4alt differ only in 3 amino acids, PF4alt is highly distinct from PF4 (Dubrac et al. 2010). They share properties such as anti-angiogenic and anti-tumor effects in vivo (Struyf et al. 2007), but crucial differences also exist, e.g. binding to GAGs and interaction with receptors (Dubrac et al. 2010). While PF4 has mainly proinflammatory effects in atherogenesis, PF4alt may act as a competitive antagonist with a potential anti-inflammatory and plaque-stabilising effect (Domschke and Gleissner 2019), suggesting distinct roles for PF4 and PF4alt in vascular inflammation.
Aims: Given the versatile role of platelets in atherosclerosis, vascular remodeling and immune regulation, we aimed to elucidate the functional differences between PF4 and PF4alt, with relation to their ability to modulate VSMCs in vitro.
Methods: Primary human VSMCs were cultured in vitro at 37°C and treated with various concentrations of PF4 and PF4alt (0.5, 1 or 10 µg/ml), prior to performance of cell-based assays. Expression of selected genes was analysed by qRT-PCR, surface receptors and PF4-uptake was analysed by immunocytochemistry. Cell proliferation was investigated by impedance measurement, whereas calcification of cells was determined by O-Cresolphthalein method.
Results: PF4 was internalized by VSMCs at 37°C, but not at 4°C. Our results confirmed previous observations that PF4, unlike PF4alt, stimulated proliferation of VSMCs, however only at 10 µg/mL. Moreover, 24-hour incubation with both PF4 and PF4alt resulted in a decreased expression of selected contractile marker genes (CNN-1 and ACTA-2) and increased mRNA levels of KLF4 and NLRP3 transcription factors, measured by qRT-PCR. Further, heparin (and not PDGF) pre-treatment completely blocked PF4 uptake by VSMCs. Moreover, a role of candidate receptors (CXCR3 and DARC) in endocytosis of PF4 was excluded and observed a primary involvement of the LDL receptor family in this process. Lastly, PF4 appeared to promote calcification of VSMC at high concentrations, whereas this process was not affected by PF4alt.
Summary/Conclusion: PF4 and PF4alt interact differently with VSMCs, and both modulate the phenotype of VSMCs and may thus play a distinct role in the development of atherosclerosis.

P-117

Comprehensive transcriptomic analysis of aorta and aortic valve in hypercholesterolemia

N. Donis [1,*], Z. Jiang [1], C. D'Emal [1], A. Nchimi [1], P. Lancellotti [1 2], C. Oury [1]
[1]Laboratory of Cardiology, University of Liège - GIGA Institute, Liège, Belgium, [2]Gruppo Villa Maria Care and Research, Anthea Hospital, Bari, Italy

Background: Calcific aortic valve disease (CAVD), characterized by thickened and calcified aortic valve cusps with restricted movement, is a main cause of cardiac-related morbidity and mortality, and a steadily increasing public health problem that is anticipated to double by 2050. To date, there are no drugs approved to slow or halt disease progression. Particularly, while hypercholesterolemia is a risk factor for both atherosclerosis and CAVD, statins have shown no benefit in CAVD as opposed to atherosclerosis. It is, thus, still unclear how hypercholesterolemia promotes CAVD and if common mechanisms underlie the two diseases.
Aims: The aim of our study was to compare the histological and molecular changes occurring in aorta and aortic valves under conditions of hypercholesterolemia.
Methods: Twelve-week-old male New Zealand white rabbits were fed for 16 weeks with either chow diet (n=13, control group), 0.3% cholesterol-enriched diet including 2 weeks of vitamin D2 (VitD2) supplementation in drinking water (25kU/day/kg) (n=13, CHT group) or chow diet supplemented with Vit.D2 for 2 weeks (n=7). At the end of the 16 weeks, the heart and aorta from 7 rabbits were harvested and fixed for histological analyses. Calcification was detected by alizarin red staining of tissue sections. Total RNA was extracted from dissected aorta and aortic valve of 6 other rabbits from the control and CHT group. High throughput RNA sequencing was then performed. Genes that were differentially expressed (DEGs) between control and CHT conditions were considered when adjusted p-value was < 0.05 and fold-change > 1.5. Human orthologs were used for further gene ontology (GO) analysis of impacted biological processes.
Results: After 16 weeks of cholesterol-enriched diet, rabbits showed both atherosclerotic lesions associated with intimal calcification in the aorta and calcification in the aortic valve. Rabbits from the control group or those receiving VitD2 alone did not develop calcification, neither in aorta nor in aortic valve.
RNAseq identified 29.587 genes annotated in the rabbit genome, among which 15.135 genes were assigned to human genes. Hypercholesterolemia modified the expression of 1102 genes in the aorta and 612 genes in the aortic valve. Only 443 DEGs were common to the two tissues. Interestingly, the analysis of GO terms revealed that the aortic valve-specific DEGs were mostly involved in muscle system process, cardiac muscle contraction, regulation of actin filament-based process, cation transmembrane transport, glucan and creatine metabolic process. As for the biological processes affected by aorta-specific DEGs, we identified the regulation of inflammatory response, innate immune response, positive regulation of cytokine production, protein metabolic process, regulation of cell migration, and programmed cell death. Top 5 specific DEGs included *CXCR4* (log2FC=4.5), *DOCK10* (log2FC=4.6), *OAS2* (log2FC=4.9), *ABI3* (log2FC=4.4), and *CEMIP* (log2FC=4.9) in aorta, and *CKM*, (log2FC=-4.3), *TNNC1* (log2FC=-4), *MB* (log2FC=-4.3), *ARHGAP18* (log2FC=2.1), and *ABRACL* (log2FC=2.1) in aortic valve.
Summary/Conclusion: We provide a comprehensive analysis of changes in RNA expression in response to hypercholesterolemia in aorta and aortic valve. We identified specific biological processes that may contribute to distinct pathophysiological mechanisms underlying atherosclerosis and CAVD. Hence, our study may lead to the identification of new therapeutic targets of CAVD.
Fundings: Région Wallonne (DGO6) - Fondation Léon Frédéricq

P-118

Pathological vascular smooth muscle cell phenotype switching reduces expression of tissue factor pathway inhibitor

V. Bröker [1,*], L. Schurgers [1], T. Hackeng [1]
[1]Biochemistry, Maastricht University, Maastricht, Netherlands

Background: Vascular smooth muscle cell (SMC) phenotype switching from a contractile to a synthetic state is an established, but not well understood pathway contributing to initiation and progression of atherosclerosis. Previous studies have shown that control of the healthy, quiescent state of SMC is possibly governed by the local balance of coagulation factors and inhibitors such as tissue factor pathway inhibitor (TFPI).
Aims: The aim of this study is to investigate the expression of TFPI in contractile vs. synthetic SMC and to elucidate the modulating effects of TFPI and its various forms secreted by SMC on pathological SMC proliferation and subsequent calcification as a model for initiation and progression of atherosclerosis.
Methods: Human aorta-derived SMC were characterized on RNA levels of SMC-specific markers. Cells were then treated with low serum conditions vs. PDGF-BB to induce the phenotypic state of contractile and synthetic SMC, respectively. Conditioned culture medium was harvested and analysed for presence of TFPI using an in house-developed ELISA. The secretion was normalized to the total protein amount.
Results: SMC populations (n=3) showed expected SMC-specific contractile marker gene profiles. ELISA revealed that synthetic SMC have a more than two-fold increase in TFPI secretion compared to contractile SMC.
Summary/Conclusion: The increase in TFPI expression in pathological SMC phenotype-switching from contractile to synthetic will aid in understanding molecular mechanisms that lead to initiation and progression of atherosclerosis. Future studies will include the CRISPR-mediated knock-out of TFPI in induced pluripotent stem cells to generate TFPI-deficient SMC and endothelial cells in co-culture systems.

P-119

Cellular factor XIII in macrophage derived foam cells

L. Somodi [1,*], H. Bárdos [2], É. Katona [1], B. Baráth [1], G. Haramura [1], E. Horváth [3], L. Muszbek [1]
[1]Laboratory Medicine, Division of Clinical Laboratory Science, [2]Department of Public Health and Epidemiology, University of Debrecen, Debrecen, Hungary, [3]Department of Pathology, University of Medicine, Pharmacy, Science and Technology, Târgu Mures, Romania

Background: The dimer of potentially active A subunits of coagulation factor XIII (FXIII-A_2) also represents an intracellular component in a number of cells, including platelets, monocytes, monocyte derived macrophages, osteoblasts, osteocytes, chondroblasts, adipocytes and corneal keratocytes. In contrast to plasma FXIII, cFXIII (cFXIII) does not need proteolysis for activation, elevation of intracellular Ca^{2+} concentration is sufficient to transform it into an active transglutaminase that catalyzes the cross-linking of peptide chains though e(g-glutamyl)lysyl bonds. cFXIII of monocytes promotes the process of receptor mediated phagocytosis. Its role in monocyte derived macrophages is not clear. Macrophages recruited to the atherosclerotic plaque engulf oxidized low-density lipoprotein (oxLDL) particles and become transformed to foam cells. They undergo apoptosis and necrosis and play a major role in the maturation and structuralization of the plaque.
Aims: To evaluate if foam cells transformed from cultured human macrophages retain their cFXIII content, and if yes, how their intracellular localization relates to that of oxLDL particles. To monitor changes of the intracellular cFXIII content during the formation and maturation of foam cells.
Methods: Monocytes were differentiated into macrophages in the presence of 10 µg/mL granulocyte-macrophage colony-stimulating factor for three days. Foam cells were generated from macrophages by incubation with 50 µg/ml oxLDL. Intracellular localization of cFXIII and oxLDL particles within foam cells was investigated with confocal laser scanning fluorescence microscopy. Anti-human FXIII-A rabbit antibody prepared in our institute was combined with Dylight 488 secondary antibody, oxLDL particles were visualized by oil red staining. cFXIII content was measured from cell lysates daily by one-step sandwich ELISA (Katona et al. J Immunol Meth 2001; 258: 127-35) and Western blotting. cFXIII in the foam cells of atherosclerotic plaque was detected by immunoperoxidase staining. Rabbit anti-FXIII-A antibody and EnVision FLEX/HRP was used for visualization.
Results: Macrophages contained a considerable amount of cFXIII (127 ± 64 ng/10^6 cells). Macrophages transformed into foam cells by feeding them with oxLDL particles retained cFXIII. 24 hours after introduction of LDL particles into the medium cFXIII content of macrophages doubled (251 ± 79 ng/10^6, $p = 0.026$), then in the next two days only a slight, non-significant decrease could be observed. These results were supported by Western blotting analysis. cFXIII was of cytoplasmic localization and well separated from the oxLDL particles. The presence of cFXIII was also revealed in foam cells within the atherosclerotic plaque.
Summary/Conclusion: Transformation of cultured monocyte-derived macrophages by oxLDL treatment into foam cells results in a significant increase of cFXIII content. cFXIII of foam cells, if released from damaged cells or exposed to the surface it might stabilize protein structures.

P-120

Gender differences in the plasma concentration of the GAS6-TAM system in COVID-19 patients.

A. Morales [1], S. Rojo Rello [2], H. Cristóbal [1], A. Fiz-López [3], E. Arribas [3], M. Marí [1], A. Tutusaus [1], P. de la Cal-Sabater [3], N. Martínez-Bosch [4], M. L. Sorlí [5], J. Gomez-Junyent [5], P. Navarro [1], G. A. Nicolaes [6*], J. T. Ortiz-Pérez [7], D. Bernardo [3], P. Garcia De Frutos
[1]Cell Death and Proliferation, Institute of Biomedical Research of Barcelona (IIBB-CSIC), Barcelona, [2]Servicio de Microbiología, Hospital Clínico Universitario de Valladolid, [3]Mucosal Immunology Lab, IBGM-CSIC, Universidad de Valladolid, Valladolid, [4]Cancer Research Program, Hospital del Mar Research Institute (IMIM), [5]Infectious Diseases Department, Hospital del Mar-IMIM, Barcelona, [6]Department of Biochemistry, CARIM, Maastricht University, Maastricht, [7]Clinic Cardiovascular Institute, Hospiral Clinic Barcelona, Barcelona, Spain

Background: SARS-CoV-2 induces an immune response with potentially harmful effects for the patient due to an uncontrolled release of inflammatory factors, specially at the capillary wall. The vitamin K-dependent plasma protein GAS6 and the TAM (TYRO3, AXL, and MERTK) receptors play a relevant role among restorative mechanisms that counterbalance pro-inflammatory responses at the endothelial interface.
Aims: To study the influence of gender on the effects of SARS-CoV-2 infection in the GAS6/TAM system, as reflected by plasma concentration at patient admittance at the emergency ward.

Methods: The plasma content of GAS6, AXL, and MERTK was analyzed in a first group of 132 patients, 68 females and 64 males consecutively admitted to the emergency ward during the first peak of COVID-19. A confirmatory group was studied from the second wave of contagions. An analysis of gender differences in relation to the GAS6/TAM concentrations in plasma was performed on this population.
Results: In accordance with recently published GAS6 levels, significantly higher in the SARS-CoV-2 positive than in negative patients, increased progressively with the severity of the disease in SARS-CoV-2 positive individual irrespective of the gender of the patient. In contrast, while soluble AXL exhibited higher plasma concentration in deceased patients and no significant differences were observed in MERTK concentration, differential gender analysis suggest differences in soluble TAM receptors. While a COVID-19 related increase in sAXL was observed in men, this was not the case in women. Oppositely, MERTK differences due to COVID-19 infection were only significant in women.
Summary/Conclusion: GAS6-TAM system of ligands and receptors is implicated in the immune response to SARS-CoV-2 in patients from both genders. Plasma GAS6 levels paralleled COVID-19 severity being an early marker of disease prognosis in both sexes. In contrast, soluble TAM receptors presented a gender-specific behavior. Sex-related differences in sAXL and sMERTK expression in COVID-19 patients could affect therapy efficacy deserving further investigation.