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ABSTRACT BOOK

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1 SCIENCE, FAST AND FURIOUS

Science, fast and furious
SFF 1.1

Frequency and clinical impact of anti-PF4/Heparin antibodies in patients treated with Extra-Corporeal Membrane Oxygenation.

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Background: Extra-Corporeal Membrane Oxygenation (ECMO) provides circulatory support in case of severe cardiac failure, and systematic use of unfractionated heparin (UFH), aiming to prevent thrombotic complications, likely exposes patients treated with this procedure to a high risk of heparin-induced thrombocytopenia (HIT), which remains however poorly documented.
Aims: In this monocentric study, we prospectively evaluated the frequency of development of antibodies to platelet factor 4 modified by heparin (anti-PF4/H Abs) in patients who had underwent ECMO, and their clinical impact on platelet count (PC) and clinical course.
Methods: From February 2014 to January 2018, we enrolled, in the University Hospital of Tours, 57 adult patients with ECMO and treated with UFH for at least 5 days. Plasma samples were collected daily, and PF4-specific Abs were systematically detected using HAT45® (GTI), an ELISA assaying IgG, IgA and IgM isotypes, and another kit specific for IgG to modified PF4 (HAT45G®; GTI). Serotonin release assay (SRA) was also performed to detect pathogenic (platelet activating) Abs in plasma samples containing significant levels of anti-PF4/H IgG. Demographics, clinical and biological data, such as PC, clinical course, sepsis or thrombotic complications were collected until the end of ECMO.
Results: Significant titres of anti-PF4/H IgG/A/M Abs were detected before ECMO in 6 of 57 patients (10.5%), with anti-PF4/H IgG present in only 3 cases (5%). After ECMO was initiated, 28 patients (49%) developed IgG/A/M anti-PF4/H Abs, but anti-PF4/H IgG were present in only 17 of them (30%). Half of these patients were immunized within 5 days after ECMO initiation and 95% of them within 10 days, with a median delay of 8 days before Abs detection (ranges: 4-26 days). Only 3 patients (5%) exhibited pathogenic anti-PF4/H IgG with positive SRA. HIT was confirmed in only two of them, because of suggestive clinical manifestations at days 6 and 10 of ECMO. In the other cases, the development of anti-PF4/H IgG did not impact biological and clinical evolution, including thrombotic events or death. Therefore, patients who developed non-pathogenic anti-PF4/H IgG displayed the same PC pattern than non-immunized patients, with decreasing PC from ECMO initiation until day 5, followed by a slow PC recovery. In contrast, the 3 patients who developed pathogenic anti-PF4/H IgG displayed abnormal PC pattern after day 5, with a dramatic PC fall in two of them (of 49% and 31.5 % between days 9-10 and 5-6 respectively), or non-recovery of post-operative low PC in the third one.
Summary/Conclusion: Anti-PF4/H Abs are frequent during ECMO but are rarely pathogenic, and not associated with poorer prognosis. PC evolution analysis after day 5 of ECMO appears reliable for suspecting HIT, and a dramatic PC fall or a non-recovery of PC after this delay should incite to look for anti-PF4/H Abs. IgG specific assays must be preferred to polyspecific ones and combined with platelet activation tests in order to improve the specificity of HIT diagnosis in this particular clinical condition.

Science, fast and furious
SFF 1.2

Eltrombopag (EPAG) improved platelet counts with similar safety in patients with persistent or chronic immune thrombocytopenia (ITP): Subgroup analyses of a 2-year, Phase IV, open-label study

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Background: ITP, characterized by isolated platelet reduction, is classified as persistent (perITP) within 3–<12 months of diagnosis, and chronic (cITP) when continuing for ≥12 months. EPAG, an oral thrombopoietin receptor agonist, is approved for the treatment of previously treated (eg corticosteroids, immunoglobulins) cITP. However, few studies have evaluated EPAG specifically in subgroups of perITP patients. To gain better insight into the effects on platelet counts and long-term safety of EPAG in perITP, a subanalysis of the Phase IV, open-label safety study of EPAG in adults with ITP ≥6 months (Brynes *et al. Acta Haematol* 2017;137:66–72) was performed.
Aims: To evaluate the effects of EPAG on platelet counts and long-term safety, during 2 years' treatment in perITP or cITP patients.
Methods: Adults aged ≥18 years, with ITP duration ≥6 months, entered this 2-year, longitudinal, prospective study. Any prior treatment with EPAG or romiplostim must have been completed ≥6 months before screening; prior use of other TPO-RAs was not permitted. Patients with baseline marrow fibrosis (MF) reticulin grade MF-3, were not eligible. All patients started EPAG at 50 mg/day (25 mg for East Asian patients), titrated to 25–75 mg as required to maintain platelet counts within the clinically indicated range. This *post-hoc* subgroup analysis evaluated the effects of EPAG on platelet counts, MF, and safety during 2 years' treatment in perITP or cITP patients.
Results: At baseline, 37/161 (23%; mean±SD age 44±16 years) and 124/161 (77%; 43±16 years) had perITP or cITP, respectively. Median (range) exposure duration was 2.0 years (31 days to 2.1 years) and 2.0 years (21 days to 2.2 years); median (range) daily dose was 54.0 (11–75) and 49.6 (5–75) mg/day. In total, 31/37 (84%) perITP patients and 109/124 (88%) cITP patients achieved a post-baseline platelet count $\geq 50\times10^9$/L without rescue therapy. Overall, 16/37 (43%) perITP patients and 50/124 (40%) cITP patients received rescue therapy during the study.
Of patients with a BM assessment at 2 years, 17/18 perITP patients (94%) remained MF-0, and 1 had a 1-grade increase, while 62/75 cITP patients (83%) remained MF-0, 8 (11%) had 1-grade increase, 2 had 1-grade decrease, and 1 remained MF-1. No patient had symptoms or abnormalities typical of MF.
Adverse events (AEs) were reported in 32/37 (86%) perITP and 108/124 (87%) cITP patients. AEs more frequent (>10% difference) in perITP than cITP patients were arthralgia (22% vs 11%), dyspepsia (19% vs 7%), vertigo (16% vs 5%), and pain (14% vs 3%); none were >10% more frequent in cITP than perITP patients. Serious AEs occurred in 12 (32%) perITP patients, most frequently (≥5%) nausea (n=2, 5%) and fatigue (n=2, 5%), and in 29 (23%) cITP patients; none occurred at a frequency ≥5%.
Summary/Conclusion: The effects of EPAG on platelet counts in perITP patients were similar to those in cITP patients. Overall AE rates were similar in both subgroups and AEs were consistent with the known EPAG safety profile or underlying disease. Results in both subgroups are encouraging, with consistent outcomes following EPAG treatment in perITP and cITP patients.
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Science, fast and furious
SFF 1.3

Targeting Protease Nexin-1, a natural anticoagulant, to control bleeding in hemophilia A

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Background: Hemophilia A is a rare inherited bleeding disorders characterized by the deficiency of coagulation factor VIII (FVIII). Current hemophilia treatment requires clotting factor replacement therapy that is associated with a high risk of allo-antibody development. Treatment of patients who have developed these allo-antibodies therefore requires substitution agents. Unfortunately, these current substitutive agents present major limitations. Consequently, prevention and treatment of bleeding disorders in haemophilia remains a significant unmet medical need, requiring the development of alternative therapeutic approaches that do not rely on FVIII or FIX replacement. We propose an innovative approach consisting in targeting an inhibitor of coagulation expressed by platelets and called protease nexin-1 (PN-1) or serpinE2. Indeed, PN-1 is a natural negative regulator of thrombin, the final effector of the coagulation cascade. PN-1 is also an effective inhibitor of Factor XIa, a key protease in the initiation and amplification phases of coagulation, contributing to thrombin generation. Therefore, PN-1 can significantly inhibit both thrombin activity and thrombin generation.
Aims: Because haemophilia is characterized by the lack of FVIII or FIX leading to an insufficient thrombin production, and therefore uncontrolled bleeding, we hypothesize that PN-1 blockade could be a new strategy for haemophilia treatment.
Methods: We used the calibrated automated thrombogram (CAT) assay to test the effect of a PN-1 neutralizing antibody on thrombin generation in platelet rich plasma (PRP) of different types of hemophiliac patients (minor, moderate and severe, treated or not with recombinant FVIII). Different *in vivo* studies: i) measurement of total bleeding time, ii) measurement of venous bleeding time, iii) monitoring of mesenteric vessel occlusion after ferric chloride-induced thrombosis, were performed in haemophilia A (HA mice), wild-type, and double knockout mice for PN-1 and FVIII (dKO mice).
Results: The PN-1 neutralizing antibody increases thrombin generation in PRP from patients with minor, moderate or treated severe haemophilia and displaying circulating FVIII greater than 1%. No significant effect is observed in treated or not severe haemophilia patients, displaying plasma factor VIII of less than 1%. A minimum of FVIII is thus required to observe an enhancement of thrombin generation induced by the PN-1 neutralizing antibody.
In both bleeding time models, dKO mice display significant reduced blood loss and bleeding time compared to HA mice. Following ferric-chloride-induced injury of mesenteric vessels, platelet recruitment and fibrin(ogen) accumulation are significantly higher in DKO mice than in HA mice.
Summary/Conclusion: Our study provides proof-of-concept that PN-1 neutralizing can be a a novel approach for future clinical care in hemophilia and reinforces the interest in developing highly efficient agents blocking PN-1.

Science, fast and furious
SFF 1.4

Slc44a2 deficient mice exhibit less severity of thrombosis in a stenosis model of DVT

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Background: Recent genome wide association studies identified *SLC44A2* as a novel susceptibility locus for venous thromboembolism (VTE), a region encoding the solute carrier family 44 member 2 protein (SLC44A2). This is of interest as SLC44A2 has not been previously linked to hemostasis pathways and may therefore be a unique contributor to VTE.
Aims: To determine the importance of SLC44A2 in thrombotic disease by utilizing *Slc44a2* deficient mice (KO) in two different murine models of thrombosis
Methods: Mice lacking *Slc44a2* were included in two models of venous thrombosis: 1) a spontaneous thrombosis model using siRNA targeting anti-coagulants *Serpinc1* and *Proc* and 2) a model of deep vein thrombosis (DVT) induced by flow restriction (stenosis) of the inferior vena cava (IVC).
Results: In the model of spontaneous thrombosis, the onset of thrombotic phenotype after 32 hours within the *Slc44a2* deficient mice was enhanced with 11/12 KO mice displaying a bleeding phenotype as compared to 6/12 WT, however the difference was not statistically significant. Platelet counts and fibrin deposition in the liver was comparable. Remarkably, the levels of circulating neutrophils were significantly higher ($p=0.0017$) in KO mice along with substantially lower amounts of plasma VWF antigen ($p<0.0001$). As these findings suggested a link between SLC44A2 and neutrophil function, a DVT model previously determined to be neutrophil dependent, was also employed. After 48 hours of IVC stenosis, 100% of the WT mice developed an occlusive thrombus (10/10) whereas 80% of the mice lacking *Slc44a2* did (12/15). Thrombus length was significantly reduced in KO animals ($p=0.0184$), as was thrombus weight ($p=0.0413$). Immunohistochemical evaluation of the thrombi revealed no differences with regards to VWF or neutrophil content, as determined by Ly6G staining. However, a trend for less citrullinated histone H3, a marker of neutrophil activation of NETosis, was observed in the KO mice ($p=0.0953$). Interestingly, the percentage of area positive for platelet marker GPIb was significantly lowered in the KO animals ($p=0.0259$). In addition, the GPIb positive area of the thrombi from WT mice was significantly correlated with VWF staining ($r=0.9039$; $p=0.0052$) as could be expected, however no relationship between GPIb and VWF was observed in KO mice ($r=-0.3471$; $p=0.2956$).
Summary/Conclusion: These findings corroborate the original GWAS data, indicating a role for SLC44A2 in thrombotic disease. We have observed that SLC44A2 was less involved in a platelet-fibrin dependent model of thrombosis whereas it is more involved in a thrombosis model where platelet-neutrophil interactions have been shown to play a crucial role.

Science, fast and furious
SFF 2.1

New constitutional GATA1 variants study reveals the contribution of GATA1 in MYH10 silencing during megakaryopoiesis

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Background: Endomitosis is a key event in platelet formation, allowing megakaryocyte (MK) polyploidization. It requires *MYH10* gene expression silencing. We recently identified two new constitutional *GATA1* variants c.617A>T (p.Asn206Ile, family 1 - F1) and c.802C>A (p.Leu268Met, family 2 - F2) in unrelated French families using a 308-gene panel sequencing. Interestingly, the MKs of several *GATA1*-deficient murine models showed a polyploidization defect, which may indicate a role for GATA1 in *MYH10* silencing during megakaryopoiesis.
Aims: This study aimed at describing the phenotype of the new *GATA1* variants' carriers and to study the role of GATA1 in *MYH10* expression regulation.
Methods: 3D structure analysis and modeling using PyMOL software allowed to predict the functional effect of the new *GATA1* variants *in silico*. Platelet function and granule content were investigated using aggregation assay, mepacrine fluorescence assay and electron microscopy. Patients' MKs were derived from circulating $CD34^+$-cells. Ploidy was studied using flow cytometry after Hoechst staining. Platelet MYH10 expression was evaluated using western-blot. Transfected MSR cells were used to study GATA1 subcellular localization and GATA1-FOG1 interaction. *In silico* analysis of ChIP-seq data obtained in normal megakaryocytes (Tijssen, Dev Cell, 2011) investigated GATA1, FLI1 and RUNX1 binding on sequences corresponding to the *MYH10* gene or flanking sequences. Luciferase reporter assays were performed to evaluate *in vitro* the effect of GATA1 on the *MYH10* putative regulation sequences.
Results: One variant (F1) altered the N-terminal zinc-finger domain. The other variant (F2) altered the C-terminal zinc-finger domain, which had never been involved in the disease. A severe thrombocytopenia (15×10^9/l) was observed at diagnosis in the F1 patient, whereas the platelet count was initially normal in F2 and progressively decreased during the first years of life, remaining above 50×10^9/l. At the time when the platelet count was normal, F2 patients suffered from spontaneous bleeding associated with severe platelet aggregation defects in response to collagen and arachidonic acid. We identified both alpha and dense granule deficiencies in the carriers' platelets. Sixty-five to 72% of platelets lacked dense granules. Bone marrow smears showed hypolobular MKs. Accordingly, the patient's megakaryocytes were smaller compared to controls at day 11 of culture and showed a significant ploidy defect at day 9. MYH10 was strongly overexpressed in the *GATA1* variant carriers' platelets suggesting a defective *MYH10* silencing during megakaryopoiesis. *GATA1* variants did not alter the nuclear localization. As predicted by *in silico* structure analysis, only the F1 variant disrupted GATA1-FOG1 interaction in transfected MSR cells. ChIP-seq data analysis showed that GATA1, FLI1 and RUNX1 co-occupy the promoter and an intronic sequence in the intron 8 of *MYH10* gene. The two sequences were cloned in the pGL3 vector to obtain luciferase reporter constructs. Each sequences were associated with an increased luciferase activity in *GATA1*-transfected MSR cells, the strongest effect observed with the intronic sequence.
Summary/Conclusion: Among *GATA1* variants' carriers, thrombocytopenia severity varies with the variants and bleeding symptoms and platelet dysfunction can precede thrombocytopenia. MYH10 silencing is pivotal in the megakaryocyte polyploidization. We propose *MYH10* as a new GATA1 target involving both the promoter and an intronic regulatory element.

Science, fast and furious
SFF 2.2

Blockade of platelet glycoprotein VI induces intratumoral hemorrhage and increases efficacy of chemotherapy

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Background: Platelets are small anucleated cell fragments, which play a central role in the arrest of bleeding. Previous studies indicated that platelets are major regulators of the tumor vascular homeostasis by continuously preventing severe bleeding into solid tumors. Depletion of platelets rendered tumor vessels highly permeable and this leakiness enhances the access of chemotherapeutic agents to the tumor. Although, depletion of platelets may reduce tumor growth, this approach has no clinical application value due to the high risk of potentially fatal hemorrhages. Thus, it is imperative to identify specific targets on platelets to treat human cancers without risking bleeding complications.
Glycoprotein (GP) VI is a platelet-specific molecule regulating multiple platelet functions including adhesion, activation, aggregation and pro-coagulant activity. GPVI has been proposed as a potentially safe anti-thrombotic target based on the observation that its blockade reduces arterial thrombosis without impairing hemostasis. GPVI also contributes to the maintenance of vessel integrity under inflammatory circumstances. However, its role in the regulation of tumor vessel integrity remains elusive.
Aims: We aimed to elucidate the role of GPVI in the maintenance of vascular integrity in primary tumors and to study the potential effects of GPVI-blockade on the efficacy of chemotherapy.
Methods: Primary tumors were induced by heterotopic and orthotopic injection of prostate (Trampc1) and breast (AT-3) cancer cells, respectively. Tumor vascularisation and growth were analysed in wild-type and *Gp6*$^{-/-}$ mice and upon treatment with GPVI-blocking antibody and chemotherapeutic drugs.
Results: We show that inhibition of GPVI function induces tumor hemorrhage and reduces tumor growth, in a manner similar to the phenotype observed in platelet-depleted mice. Moreover, antibody-mediated blockade of GPVI in mice increased intratumoral accumulation of co-administered chemotherapeutic drugs. Consequently, combination of GPVI-blockade with chemotherapy resulted in a profound and prolonged anti-tumor effect.
Summary/Conclusion: Our study for the first time reveals a mechanism by which a platelet receptor regulates tumor vascular integrity and suggests that GPVI could represent a promising target for anticancer therapies.

Science, fast and furious
SFF 2.3

Seeking severe renal failure in patients anticoagulated for an acute venous thromboembolism: does the formula count?

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Background: In patients with acute venous thromboembolism, the evaluation of renal function is mandatory to choose and adapt the anticoagulant treatment. Patients with severe renal failure have a poor prognosis, with a high bleeding risk. Whereas the most accurate estimation of renal function is actually the CKD-EPI formula (Chronic Kidney Disease Epidemiology Collaboration), Cockcroft and Gault (CG) formula is the recommended formula for the adaptation of anticoagulation treatments.
Aims: However, it is unknown if CG and CKD-EPI formula individualized the same patients as having severe renal failure, in acute VTE.
Methods: The Registro Informatizado de Enfermadad ThromboEmbolica is a prospective international multicenter register including consecutive patients with acute, symptomatic and documented VTE. Renal function was assessed according to CG and CKD-EPI formula. Patients with severe renal failure were individualized according to CG formula (creatinin clearance –CrCl- less than 30mL/min), CKD-EPI formula (estimated glomerular filtration rate –eGFR- less than 30mL/min), and both formulas. Baseline characteristics and 3-month follow up were analyzed according to each sub-group. Our primary aim was to look if each formula individualizes the same patients as having severe renal failure.
Results: As of October 2017, 41 796 patients with acute VTE and available creatinin were included in RIETE. According to CG formula, 3999 patients had severe renal insufficiency (creatinin clearance –CrCl- less than 30mL/min), while they were 3451 according to CKD-EPI (estimated glomerular filtration rate –eGFR- less than 30mL/min). While 2772 patients had severe renal insufficiency according to both formulas, 1227 patients had severe renal insufficiency according only to CG and 677 only to CKD-EPI. Patients with severe renal failure according to CG were more frequently female, older and with a lower body weight. During the 3-month follow-up, the rate of recurrent VTE, major bleeding, and all-cause death were 1,5%, 9,9%, 22,5% and 1,7%, 10,4% and 21,5% in CG and CKD-EPI groups, respectively.
Summary/Conclusion: In patients with acute VTE, the CG and CKD-EPI formulas do not qualify the same patients as having severe renal failure. While the CKD-EPI formula may individualize patients with severe renal dysfunction which affect the pharmacology of major anticoagulants like DOACs, the CG formula seems to individualize frail patients who may benefit more strongly from safer treatment. The best treatment option for acute VTE in patients with severe renal failure is expected.

Science, fast and furious
SFF 2.4

Whole F8 and VWF gene sequencing using next-generation sequencing for mutation-negative French and Canadian hemophilia A patients

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Background: Identification of the molecular defect responsible for hemophilia A (HA) is an important component in diagnosis optimization. Despite *F8* exons and 5'/3' UTR regions screening as well as the search for copy number variants, a causative variant is still missing in about 5% of HA patients. In the last few years, next-generation sequencing (NGS) has provided significant improvements for complete *F8* and *VWF* genes analyses.
Aims: The aim of this study is to characterize intronic variants in *F8* gene of "mutation-negative" French and Canadian HA patients by using a whole gene sequencing method performed by NGS.
Methods: We developed a small panel containing whole *F8* (195kb) and *VWF* genes (188kb) including their upstream regions. Library preparation was performed using a capture-based enrichment strategy (Sure Select Target enrichment system, Agilent). We improved the probe design after an initial run by excluding repetitive elements, therefore significantly reducing the total number of probes in the second round of design. We analyzed 23 mutation-negative HA patients and 6 controls (3 HA and 3 VWD) with known pathogenic variants. NGS was performed on a MiSeq sequencer and raw data was analyzed according to Genome Analysis Toolkit best practices. Splicing site effect was predicted using Alamut® Visual. The functional effect of selected intronic variants was evaluated on patients' mRNA, by sequencing (Sanger method) cDNA after RNA extraction from leukocytes, reverse-transcription and nested PCR.
Results: The average number of reads (ANR) and global coverage performance were significantly improved with the second round of design (ANR = 40x in the first run versus ANR = 300x in the second one) with a higher global output. We had a complete lack of coverage on exon 26 of *VWF*, due to his 100% similarity with the pseudogene. NGS data revealed 4 new deep intronic candidate *F8* variants (including one recurrent in 2 related patients) in 5 moderate or mild HA patients. Patient 6, who had been diagnosed as a moderate HA patient, had two exonic variants in *VWF* described as VWD disease-causing. This patient has been reclassified as VWD type 2N/3. Two variants causing gene conversion in *VWF* have been found in patient 7 (mild HA). Some cases with the same variants have been described in VWD patients in France but they were considered as non-pathogenic because always associated with a more pathogenic variant causing the disease. The deleterious effect of this gene conversion between *VWF* and his pseudogene is not well understood. We found a recently described recurrent *F8* intronic deletion in 6 mild HA patients (including patient 7). No obvious candidate variant was found in 9 out of 23 patients, with low or absence of predicted effect on splicing. All the variants were confirmed with Sanger sequencing. Most importantly, the deleterious effect was confirmed by transcript analysis for 4 patients who had an aberrant *F8* transcript.
Summary/Conclusion: These results strongly corroborate the hypothesis that hemophilia A can be caused by deep intronic variants, by discovering 5 new candidate variants. It also shows the importance of sequencing *F8* and *VWF* at the same time, to identify misdiagnosed patients. RNA and minigene studies are still in progress to confirm the pathogenic effect of the intronic variants on *F8* mRNA splicing.

2 ORAL COMMUNICATIONS

Venous Thrombosis
OS.1.1

Validation of a profile of 6 miRNAs as predictor of early incidental post-surgical pulmonary embolism in glioma patients

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Background: Intracranial tumors increase the risk of thrombosis, and often may cause incidental pulmonary embolism (PE) after surgery. New biomarkers are needed to early identify brain cancer patients with high thrombotic risk. microRNAs (miRs) are small non-coding RNAs that regulate protein expression. miRs seem to regulate cancer progression and VTE.
Aims: To generate and validate a predictive model of miRNAs capable of predicting the risk of early incidental post-surgical PE in glioma patients.
Methods: We recruited and prospectively followed after surgery 50 glioma patients, who provided informed consent. All had negative baseline lung perfusion scan. PE was objectively diagnosed in 17 patients within 7 days after surgery. In plasma samples collected before surgery, we measured the expression level of 179 miRs with the Serum/plasma Focus microRNA PCR Panel V4 (Exiqon) in 10 selected patients with glioma (5 developed PE and 5 did not). Using a multivariable logistic regression model, the predictive ability for post-surgical PE was estimated as the area under the ROC curve (AUC) with R (v3.5.0). Next, we validated the predictive model in an independent subset of 40 glioma patients (12 developed PE and 28 did not). Additionally, we identified in the databases *miRWalk* and *KEGG* the predicted target proteins of these miRNAs, related to the coagulation cascade and complement.
Results: We adjusted an elastic net logistic regression model for PE risk in glioma patients using the expression levels of 6 miRs before surgery: miR-363-3p, miR-93-3p, miR-22-5p, miR-451a, miR-222-3p and miR-140-3p. We have validated this model in an independent cohort of glioma patients obtaining an AUC=0.78 (95% CI:0.63-0.94), which outperforms the predictive ability of the Khorana score in these patients (AUC=0.52).
Summary/Conclusion: Before surgery, the expression level of a profile of 6 miRs is a good predictor of early incidental post-surgical PE in glioma patients. Moreover, our predictive model outperforms the Khorana score, currently used in clinical practice. This predictive model could be useful to tailor post-surgical thromboprophylaxis in these patients. ISCIII-FEDER (PI12/00027, Red RIC RD12/0042/0029, PIE13/00046, PI14/00079, PI14/00512, FI14/00269, CPII15/00002, PI17/00495), Generalitat Valenciana (PrometeoII/2015/017, ACIF/2017/138), Sociedad Española de Trombosis y Hemostasia y GR-2011-02347854.

Venous Thrombosis
OS.1.2

The role of stroke as a trigger for incident venous thromboembolism: results from a case-crossover study

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Background: Stroke is associated with a short-term increased risk of subsequent venous thromboembolism (VTE). However, the contribution of acute stroke to the VTE risk after adjustment for some stroke-related complications that may also serve as transient risk factors for VTE, such as immobilization and infection, remains unclear.
Aims: To investigate the role of acute stroke as a trigger for incident VTE while taking other concomitant VTE triggers into account.
Methods: We conducted a population-based case-crossover study with 707 VTE patients. In the case-crossover study, participants serve as their own controls, and all potential fixed confounders are largely controlled for through the design. Stroke and other VTE triggers (i.e., immobilization, acute infection, major surgery, trauma, red blood cell transfusion and central venous catheter) were registered during the 90 days before a VTE event (hazard period) and in four preceding 90-day control periods. Conditional logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE according to triggers. Under the assumption that immobilization and infection were a consequence of acute stroke, we further examined to what extent both factors could mediate the effect of stroke on VTE risk. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: Stroke was registered in 30 of the 707 (4.2%) hazard periods and in 6 of the 2828 (0.2%) control periods, resulting in a high risk of VTE, with an OR of 20.0 (95% CI 8.3-48.1). However, after adjustments for immobilization and infection, ORs for VTE conferred by stroke were attenuated to 6.0 (95% CI 1.6-22.1), and further to 4.0 (95% CI 1.1-14.2) when the other VTE triggers were additionally introduced in the regression model. Subgroup analyses stratified by clinical presentation (i.e., deep vein thrombosis and pulmonary embolism) yielded similar results. A mediation analysis revealed that 67.8% of the total effect of stroke on the risk of overall VTE could be mediated through immobilization and infection. Similar results were obtained when analyses were restricted to ischemic stroke.
Summary/Conclusion: Acute stroke was a trigger for VTE in this case-crossover study, but our results suggest that the association between stroke and subsequent risk of VTE is largely mediated by immobilization and infection. The present findings suggest that strategies to improve prevention of not only immobilization but also infection may lower the risk of VTE after stroke. Our findings require confirmation by future investigation in a cohort of stroke patients.

Venous Thrombosis
OS.1.3

Association of Neutrophil Extracellular Traps Markers and Venous Thromboembolism. Results of RETROVE Project

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Background: Recently, immunothrombosis showed up as a mechanism involved in the pathophysiology of venous thromboembolism (VTE), and neutrophil extracellular traps (NETs) as the main actor. NETs are structures composed of DNA fibers, histones and antimicrobial proteins that are expelled to the extracellular space after neutrophil death (NETosis). It is possible to detect and quantify NET-related markers in plasma. In VTE, NETs act as a scaffold for platelets, red blood cells and coagulation factors that trigger the clotting cascade through several pathways.
Aims: The main objective is to identify the relationship between NET-plasmatic markers and VTE in a population of patients with VTE and healthy controls.
Methods: The group studied consists in 400 individuals with VTE and 400 healthy controls that belong to RETROVE project recruited between 2012 and 2016 in our center. Paraneoplasic thrombosis were excluded. The NET markers measured in all the individuals were: myeloperoxidase (MPO, ELISA kit, Cloud-Clone Corp.) and neutrophil elastase (NE, ELISA kit, Elabscience) quantified in poor platelet plasma using EDTA as anticoagulant, and extracellular DNA (Sytox Green, Life Technologies) in citrated plasma. Data are shown as mean ± standard error (SE). *Student* t test for unpaired samples was used and a p value less than 0.05 was considered statistically significant.
Results: Levels of extracellular DNA are significantly higher in patients compared to controls (311.76 ± 3.47 ng/mL *vs* 285.13 ± 2.46 ng/mL, respectively; $p = 4.98 \times 10^{-14}$). NE was also significantly elevated in patients (1653.67 ± 62.77 ng/mL) compared to controls (1263.35 ± 60.35 ng/mL) ($p = 8.45 \times 10^{-6}$). However, we don't observe significant differences in MPO between both groups (72.48 ± 2.76 ng/mL in patients *vs* 67.11 ± 2.64 ng/mL in controls; p=NS)
Summary/Conclusion: The significant differences in the main NET-related plasmatic markers (extracellular DNA and NE) observed between patients and controls in our study confirm the pathophysiological relationship between VTE and NETs. The lack of augmented levels of MPO in patients with VTE agrees with data reported by other groups, where the rest of the markers are elevated while MPO levels remain unchainged.
Measurement of NET-plasmatic markers might be useful to predict thrombotic events and/or to anticipate recurrent thrombosis.

Venous Thrombosis
OS.1.4

B lymphocytes and endothelial dysfunction promote DVT

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Background: Venous thromboembolism (VTE), consisting of deep venous thrombosis (DVT) and pulmonary embolism, 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, characterized by chronic leg pain, swelling, and ulceration, or 3) chronic pulmonary hypertension resulting in significant chronic respiratory compromise. DVT leads to post-thrombotic syndrome in 20–50% of patients and recurrent DVT is a significant risk factor for post-thrombotic syndrome, which occurs despite appropriate treatment of the initial thrombotic event. Our group and others demonstrated that the innate immune system promotes venous thrombosis through the activation of the coagulation cascade and platelets. Innate immunity also delays thrombus resolution through cytokine production. In addition, T cells were shown to participate in thrombus resolution through the production of IFNγ. Thus, it is possible that the adaptive immune system perpetuates an immuno-inflammatory response contributing to recurrent DVT. DVT is also characterized by vascular fibrosis leading to venous insufficiency and may be a second mechanism contributing to recurrent DVT. Studies have demonstrated that endothelial cells undergo endothelial-to-mesenchymal transition characterized by the expression of transgelin and collagen 1A1 that lead to fibrosis. We hypothesize that the adaptive immune system and endothelial dysfunction contribute to the recurrent nature of VTE.
Aims: In the present study, we investigated whether B lymphocytes promote DVT and if endothelial cells contribute to vein wall fibrosis.
Methods: DVT was induced in wild type and in μMT (mice do not develop B cells due to disruption of the membrane exon of the mu heavy chain gene) using a stasis model. At day 2, thrombus size was measured using high frequency ultrasound and thrombi, vein and lymph nodes were harvested for flow cytometry analysis. In parallel, HUVECs were incubated in vitro with thrombin and TGFβ2. After 72h of treatment, samples were collected and expression of transgelin (SM22), VE-cadherin and calponin 1 was quantified by real-time PCR.
Results: First, two days after DVT induction, we found that B cell number was increase in the vein wall and renal lymph nodes. More importantly, we showed that absence of B cells significantly reduces thrombus formation. In vitro, we found that thrombin enhances TGFβ2-dependent expression of transgelin in HUVECs, whereas VE-cadherin expression was significantly reduced.
Summary/Conclusion: The present study strongly suggests that B cells are activated during DVT. We found that a pro-thrombotic stimulus such as thrombin promotes endothelial dysfunction leading to the expression of pro-fibrotic factors. Future studies will determine if B cells and endothelial dysfunction contribute to recurrent DVT.

Platelets and Neutrophils
OS 2.1

HLA variant rs6903608 is associated with disease onset and relapse of immune-mediated thrombotic thrombocytopenic purpura

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Background: Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a rare, life-threatening, thrombotic microangiopathy caused by the autoimmune severe deficiency of ADAMTS13. A relapsing form of iTTP may develop in about one-third of patients surviving a first acute episode. In a previous case-control genetic association study using the Illumina Immunochip, we found that HLA variant rs6903608 increases the risk of having iTTP in Italians by 2.5 fold (C risk allele, frequency in controls 0.47, 95% confidence interval [CI] 2.05-3.05, P $1.01x10^{-19}$)[1]. Whether this genetic marker could predict iTTP relapse is currently unknown.
Aims: To confirm that HLA variant rs6903608 is associated with the occurrence of a first episode of iTTP and to understand whether it is associated with iTTP relapse.
Methods: We performed a case-control and cohort study of Italian patients enrolled in the Milan TTP registry for a first episode of iTTP occurred between January 2002 and March 2018. Diagnosis of iTTP was based on thrombocytopenia and microangiopathic hemolytic anemia, in absence of alternative causes, and evidence of ADAMTS13 severe deficiency and anti-ADAMTS13 antibodies. Controls were 456 Italian healthy subjects genotyped in the frame of our previous study[1]. Subjects were genotyped for rs6903608 using a TaqMan assay (Thermo Scientific). Logistic and Cox regression models were used to estimate the risk of having a first episode of iTTP or a relapse, respectively. The Kaplan-Meier method was used to estimate the cumulative incidence of relapse in patients with different genotypes. Patients were followed from the start date of remission to the date of relapse, death, cancer diagnosis, last contact or the 15st of March 2018, whichever came first.
Results: We enrolled 160 iTTP patients, 78% of whom were females with a median age at onset of 45 years (interquartile range 32-54). Twenty (13%) and 74 (46%) patients were homozygous for the reference (TT) and the risk (CC) allele, respectively, whereas 66 (41%) patients were heterozygotes (CT). To assess the association with the occurrence of a first episode of iTTP, genotype frequencies of patients were compared with those of controls (119 [26%] TT, 243 [53%] CT and 94 [21%] CC subjects). Variant rs6903608 was associated with the development of the first episode of iTTP, with the homozygous and heterozygous risk genotypes conferring an odds ratio of 4.68 (95% CI 2.67-8.23, P<0.0001) and 1.62 (95% CI 0.94-2.79, P=0.085), respectively. We then investigated the association between the rs6903608 genotype and relapse in 146 survivors of the first iTTP episode with available follow-up (15 TT, 63 CT, 68 CC patients). At the end of follow-up, cumulative incidence of relapse was 35% (95% CI 0-74), 30% (95% CI 7-53) and 48% (95% CI 32-64) for TT, CT and CC patients, respectively. Due to the low number of reference allele homozygotes and lack of power, we estimated the risk of relapse assuming a recessive model of inheritance (CC versus CT+TT patients). Homozygotes for the risk allele had a 2.30-fold higher risk of relapse than carriers of the reference allele (95% CI 1.20-4.42, P=0.012).
Summary/Conclusion: HLA variant rs6903608 is associated with both the occurrence of the first episode and relapse of iTTP. Although a larger sample size is needed to precisely estimate the effect of rs6903608 risk allele on iTTP relapse, this genetic marker could help identifying patients at a higher risk. [1]doi: 10.1111/jth.13548

Platelets and Neutrophils
OS 2.2

Neutrophils prothrombotic characteristics during myeloproliferative neoplasms

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Background: Thrombosis is the most frequent complication during evolution of myeloproliferative neoplasms (MPN) but the events causing these clotting abnormalities remain unclear. Recently, clinical studies have identified leukocytosis as a risk factor for thrombosis and neutrophils are now recognized as important actors of thrombosis, especially by their capacity to emit neutrophil extracellular traps (NETs) when activated.
Aims: To assess if *JAK2*V617F neutrophils are more prone to form NET, thus promoting thrombosis.
Methods: We first studied MPN patients and quantified: 1) NETosis markers, i.e plasma levels of free DNA and MPO-DNA complex 2) ex-vivo NET formation, 3) neutrophils reactive oxygen species (ROS) formation. In a second part, we studied two different mouse models. First, we used *PF4-iCre;JAK2*$^{V617/WT}$ mice with expression of *JAK2*V617F in hematopoietic cells. We quantified: 1) NET formation, 2) plasma levels of circulating DNA, 3) pulmonary thrombus formation before and after DNAse administration. Secondly, we used MRP8-iCre; *JAK2*$^{V617/WT}$ mice allowing expression of *JAK2*V617F in neutrophils and monocytes only. In this mouse model, we quantified NET formation.
Results: In MPN patients, we found: 1) increased plasma levels of free DNA in all patients and increased plasma levels of MPO-DNA complex in patients with history of thrombosis, 2) increased ex-vivo NETs formation, 3) increased neutrophils ROS production. In *PF4-iCre;JAK2*$^{V617F/WT}$ mice, we observed : 1) proliferation of all hematopoietic lineage, with *JAK2*V617F in neutrophils, platelets and red blood cells, 2) increased NET formation after neutrophils activation, 3) increased plasma level of free DNA and MMP9, 4) increased pulmonary thrombus formation, 5) inhibition of thrombus formation by DNAse administration. In *MRP8-iCre;JAK2*$^{V617F/WT}$ mice, we observed: 1) absence of MPN and presence of *JAK2*V617F in neutrophils and monocytes only, 2) increased NET formation after neutrophils activation.
Summary/Conclusion: Our results show that neutrophils are prone to form NETs during MPN, in a patient cohort, and in two different mouse model allowing *JAK2*V617F expression in neutrophils. Increased thrombus formation abolished by DNAse treatment in PF*4-iCre;JAK2*$^{V617F/WT}$ model indicate that NETs formation participate in thrombosis during MPN. To analyse precisely the role of *JAK2*V617F mutated neutrophils in pathogenesis of thrombosis during MPN, we will perform thrombosis experiments in our MRP8-*iCre;JAK2*$^{V617F/WT}$ model.

Platelets and Neutrophils
OS 2.3

Caplacizumab for acquired thrombotic thrombocytopenic purpura – results of the phase 3 HERCULES study

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Background: Acquired thrombotic thrombocytopenic purpura (aTTP) is a life-threatening thrombotic microangiopathy characterized by severe thrombocytopenia, microangiopathic hemolytic anemia and organ ischemia. Autoantibodies to ADAMTS13 lead to the persistence of ultra-large von Willebrand Factor (vWF) multimers that spontaneously capture platelets thereby causing microvascular thrombosis.
Aims: We report the results of a randomized, double blind, placebo controlled study of caplacizumab, a bivalent Nanobody, targeting the A1 domain of vWF.
Methods: Patients with an acute episode of aTTP who had received one PE treatment were randomized 1:1 to placebo or 10 mg caplacizumab, in addition to daily PE and corticosteroids. A single i.v. dose of study drug was given before the first on-study PE and a s.c. dose was given daily during the PE period and 30 days thereafter. If at the end of this period there was evidence of unresolved autoimmune disease (e.g., suppressed ADAMTS13 activity), investigators were encouraged to extend blinded treatment for a maximum of 4 weeks together with optimization of immunosuppression. All patients entered a 28-day treatment-free follow-up period.
Results: 145 patients were randomized (73 placebo, 72 caplacizumab). Compared to placebo, caplacizumab-treated patients were >50% more likely to achieve a platelet count response (platelet count normalization rate 1.55, 95% CI 1.10 – 2.20, $p<0.01$). Treatment with caplacizumab resulted in a 74% reduction in the composite endpoint of TTP-related death, recurrence, or a major thromboembolic event during the study drug treatment period, compared to placebo ($p <0.0001$). During the overall study period, patients administered caplacizumab had a 67% reduction in disease recurrence ($p<0.001$). Six caplacizumab-treated patients experienced a relapse during the follow up period; in all, ADAMTS13 activity was <10% at stop of study drug, reflecting ongoing autoimmune disease. Three patients on placebo were refractory to therapy versus none on caplacizumab ($p=0.057$). Treatment with caplacizumab was associated with a trend towards faster normalization of organ damage markers (cardiac troponin I, LDH and serum creatinine). Treatment with caplacizumab resulted in a 38% reduction in the mean number of days of PE, a 65% reduction in the mean length of ICU stay, and a 31% reduction in the mean length of hospitalization during the study drug treatment period. In the caplacizumab group, the most common drug-related adverse events were epistaxis, gingival bleeding and bruising, consistent with the mechanism of action of the drug. Three patients on placebo died during the study drug treatment period. One death occurred during the follow up period in the caplacizumab group and was assessed by the investigator as not related to study drug.
Summary/Conclusion: Treatment with caplacizumab reduced the time to platelet count response and resulted in a clinically meaningful reduction in aTTP-related death, recurrence of aTTP, or a major thromboembolic event during study drug treatment, as well as recurrences during the overall study period. Treatment with caplacizumab also resulted in meaningful reductions in healthcare resource utilization. The safety profile was favorable, with mucocutaneous bleeding the most frequently reported TEAE. Caplacizumab represents an important addition to the treatment armamentarium for patients with aTTP.

Platelets and Neutrophils
OS 2.4

Neutrophil-endothelial cell interactions determine the regulation of neutrophil activities by platelets

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Background: Platelets are important regulators of in immune responses and inflammatory reactions. Platelets can stimulate endothelial activation and regulate endothelial permeability, as well as leukocyte recruitment and effector functions. Notably, platelets have been shown to regulate neutrophil cytotoxic activities in various experimental models of inflammation. However, depending on the model considered, platelets either enhance or dampen neutrophil cytotoxic activities. How the regulatory action of platelets can be shifted towards stimulation or inhibition remains largely unknown.
Aims: Here, we investigated the determinants of the platelet regulatory activity towards neutrophil secretion and oxidative activity.
Methods: For this study, isolated human neutrophils and platelets were co-incubated in the absence or presence of endothelial cells, and stimulated with neutrophil and/or platelets agonists.
Results: We show that resting platelets limit the secretion of neutrophil elastase and the oxidative activity of neutrophils in suspension, and that this inhibitory action of platelets mostly relies on cell-cell contacts. Remarkably, collagen- and thrombin-activated platelets retained their inhibitory activity, indicating that platelet activation status is not a major determinant of the regulatory action of platelets towards cytotoxic activities of non-adherent neutrophils. When neutrophils were pre-adhered on endothelial cells (EC) prior to platelet addition, both resting and activated platelets enhanced neutrophil elastase secretion and oxidative activities. In contrast, platelets had no stimulatory effect on neutrophils pre-adhered to collagen and fibronectin-coated surfaces. Remarkably, neutrophils pre-incubated with endothelial cells were characterized by marked modifications in the expression levels of various ITIM receptors known to modulate neutrophil function.
Summary/Conclusion: Our results show that EC are major determinants of the regulatory action of platelets towards neutrophils. In particular, our results indicate that neutrophil/EC interactions prime neutrophils for subsequent activation by platelets, possibly by modifying the surface expression levels of neutrophil ITIM receptors.

Inflammation
OS 3.1

Complement Activation Assessed by Terminal Complement Complex and Future Risk of Venous Thromboembolism

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Background: Observational and animal studies suggest that the complement system is involved in the early steps in the pathogenesis of VTE. In a large population-based cohort study, subjects with plasma levels of complement C3 in the highest tertile had 31% higher risk of VTE compared to those in lowest tertile, and C3-deficient mice had lower incidence of venous thrombosis and reduced thrombus size compared to wild-type mice. Even though growing evidence support a role of C3 in the pathogenesis of VTE, it is not known to what extent complement activation, assessed by the terminal complement complex (TCC) - the final step of complement activation, is associated with VTE risk.
Aims: To assess the impact of plasma levels of TCC on the future risk of incident VTE in a nested case-control study recruited from the general population.
Methods: We conducted a nested case-control study derived from a population-based cohort (The Tromsø Study) with subjects included in 1994/95 and followed until the end of 2007. During the follow-up period (1994-2007), 417 individuals experienced a VTE event. For each case, two age- and sex-matched controls were sampled randomly from the parent cohort, who were alive at the index date of the VTE event (n=849). Physical measurements and blood samples were collected in 1994-95. Plasma TCC was measured by an in-house ELISA. Logistic regression models were used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE across quartiles of plasma levels of TCC determined in the control group. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: The OR of total VTE increased across quartiles of TCC (*p* for trend: 0.06). Subjects with plasma TCC in the highest quartile (>1.40 CAU/ml) had a 35% higher risk of VTE compared to those with TCC in the lowest quartile (<0.80 CAU/ml) (OR 1.35; 95% CI: 0.96-1.92). In analyses restricted to unprovoked VTE, the OR for VTE was 87% higher (OR 1.87; 95% CI: 1.14-3.08) among individuals with plasma TCC in the higher compared to the lower quartile of TCC, and there was a statistically significant linear trend for increased risk of unprovoked VTE across increasing quartiles of TCC (*p* for trend: 0.02).
Summary/Conclusion: We found that complement activation, assessed by plasma TCC, was associated with future risk of unprovoked VTE. Our findings suggest that complement activation may play an important role in the pathogenesis of VTE.

Inflammation
OS 3.2

Detection of different fibrinolysis patterns of plasminogen mutant proteins in patients with ligneous conjunctivitis associated to inherited plasminogen deficiency: impact on clinical outcome.

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Background: Ligneous conjunctivitis (LC) is a rare disease characterized by the development of recurrent woody-like pseudomembranous lesions on the palpebral conjunctivae. Corneal involvement occurs in 20-30% of the cases and may lead to visual impairment or blindness. LC is associated with a hypofibrinolysis state with decreased mucosal fibrin clearance and delayed wound healing. LC is the most common clinical manifestation of the type 1 inherited plasminogen (PLG) deficiency. However, all patients with PLG deficiency do not develop LC and the exact mechanism involved in the pathophysiology of LC remains unclear.
Aims: The aim of the study was to investigate whether global fibrinolytic activity evaluated by different methods is correlated with phenotype and/or genotype in patients suffering from LC and their related family members.
Methods: Ten patients with LC from 9 families and 17 healthy relative family members were included in the study. We determined PLG activity and antigen and PLG mutation in all patients by dideoxysequencing of all exons of the PLG gene. We evaluated plasma fibrinolytic potential on cultured corneal cell monolayers (HCLE) using a modified euglobulin clot lysis assay. Results were expressed as time to 50% euglobulin clot lysis (ECLT50). We also measured lysis front migration of plasma clots by laser scanning confocal microscopy.
Results: Median age of onset of LC was 7 years with a female/male ratio of 1.5/1. PLG activity and antigen in the plasma of patients varied from 5 to 40% (N >80%), and from 6 to 126 µg/mL (N 60-250 µg/mL), respectively. PLG activity varied from 36 to 105% and PLG antigen from 39 to 120 µg/mL in the plasma of relative family members. We identified 4 asymptomatic siblings with PLG deficiency.
The most frequent mutation was p.Lys38Glu missense mutation (9/10) which was identified in a homozygous (n=2) or in a compound-heterozygous (n=7) state. Homozygous p.Lys38Glu mutation was associated to normal antigen PLG level with a low ratio PLG: Act/Ag demonstrating that p.Lys38Glu mutation causes dysplasminogenemia. Unexpectedly, a normal fibrinolytic profile was observed in these patients. In contrast, patients with p.Lys38Glu/other mutation had a significantly increase of ECLT50% compared to controls (342±79 versus 204±64 min, p=0,005) and a decreased lysis-front velocity (2,1±0,8 versus 11,6±2,8 µm/min, p=0,0003) suggesting a hypofibrinolytic profile. Interestingly, patients and asymptomatic relatives with the same PLG level and genotype had similar ECLT50%.
Summary/Conclusion: In conclusion, we provide evidence that the homozygous mutant p.Lys38Glu leads to a PLG mutant protein with a normal fibrinolytic profile and is characterized by a dysplasminogenemia rather than a hypoplasminogenemia as previously described. We demonstrate that the expression and severity of LC are not strictly related to PLG deficiency, as normal fibrinolytic profile was observed in homozygous p.Lys38Glu mutants and as comparable fibrinolytic profile was observed in patients and their asymptomatic relatives with comparable levels of PLG and same mutations. An impact of external triggers as inflammation, modifier genes or epigenetics regulators is probably involved in the physopathology of the disease.

Inflammation
OS 3.3

The differential role of platelets and fibrin in adhesion on inflamed and damaged valves in a new mouse model of early staphylococcus aureus endocarditis

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Background: In no other disease the interplay between bacteria and coagulation is so crucial as in infective endocarditis (IE). Once the disease is established, IE with *S. aureus* has a mortality of 30%. Prevention of IE and early intervention thus remain crucial. Bacteria-platelet-interactions are thought to be an important factor in adhesion to the endothelium, but previous animal models did not allow sufficiently detailed insights in the early steps of this disease.
Aims: Therefore, this study aims to determine the role of platelets and the coagulation system in adherence of *S. aureus* to the cardiac valves.
Methods: By using our refined mouse model of early IE, we could directly visualize and quantify bacterial adhesion to the aortic valve. To this end, we intravenously injected Texas red labeled *S. aureus* in C57BL/6 mice after which a catheter was introduced in the carotid artery and advanced beyond the aortic valve. Subsequently, this catheter was used to locally stimulate or damage the endothelium. Afterwards, the catheter was removed and mice were immediately sacrificed. Adherence was determined by analyzing cryosections with confocal microscopy and quantifying adhesion by Imaris. To determine which coagulation specific factors are involved in adherence to damaged and inflamed valves, we conducted electron and fluorescent (A-546 fibrinogen, A-649 anti-GPIb antibody for platelets, isolectine B4 A-594 for endothelium) microscopy. The role of platelets in *S. aureus* adherence to damaged and inflamed valves was addressed by injecting mice with a platelet depletion antibody (anti-GPIb) or a control (isotope antibody) one hour before surgery. Finally, we injected a Srta mutant of *S. aureus* to investigate if cell-wall anchored proteins allow *S. aureus* to adhere to the valve.
Results: Electron microscopy images of inflamed aortic valves revealed the presence of platelets and fibrin in an early vegetation. Additionally, fluorescent images showed a layer of fibrin on damaged valves, whereas on inflamed valves platelets were more abundant. Furthermore, the role of platelets in the inflammatory model was confirmed by platelet depletion experiments. In platelet depleted mice, *S. aureus* adhered significantly ($p=0.0003$) less on inflamed valves (platelet depletion: $\log(1.514\pm0.5834)$, control: $\log(2.857\pm0.7357)$), whereas on damaged valves ($\log(2.672\pm0.4406)$) adhesion was not significantly reduced ($p=0.547$) in comparison with the control ($\log(2.861\pm0.6748)$). Injection of the Srta mutant revealed that adhesion on damaged valves is mediated by cell-wall anchored proteins ($\log(2.221\pm0.4785)$, control: $\log(2.792\pm0.1842)$, $p=0.0422$)), in contrast to inflamed valves ($\log(3.277\pm0.7867)$, control: $\log(3.365\pm0.3363)$, $p=0.7890$)).
Summary/Conclusion: Our model allows direct visualization of early bacterial adhesion. We found that adhesion on inflamed valves is mediated by platelets, whereas fibrin is essential for adhesion to damaged valves. In the inflamed model, adhesion to platelets and thereby to the endothelium is not dependent on sortase anchored cell-wall proteins. In conclusion, these results reveal that adhesion occurs differently on inflamed and damaged valves and highlight the differential role of platelets and fibrin in these conditions.

Inflammation
OS 3.4

Neutrophil extracellular traps are associated with the pathogenesis of diffuse intra-alveolar hemorrhage in murine lupus

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Background: Intra-alveolar hemorrhage (IAH) is a life-threatening complication of systemic lupus erythematosus (SLE) and vasculitides. Although initially described with antibacterial properties, increasing evidences suggest a detrimental role for neutrophil extracellular traps (NETS) in both autoimmune diseases and acute lung injury.
Aims: We investigate whether NETs could be detected in pristane-induced lupus IAH murine model, contribute to lung injury and may constitute a therapeutic target.
Methods: NETs were characterized by immunofluorescence staining of deoxyribonucleaic acid, neutrophil elastase and citrullinated histones. Evaluation of lung injury was performed with hematoxyline-eosine preparation using a quantification program. Clinical status of mice was assessed by the measure of arterial blood oxygenation and a survival curve after human deoxyribonuclease-1 (Rh-DNase-1, dornase alpha, Pulmozyme®) inhalations or polymorphonuclear neutrophils (PMN) depletion.
Results: We observed that pristane promoted NETS formation *in vitro* and *in vivo.* Treating mice with Rh-DNase-1 inhalations cleared NETs and reduced lung injury. Animal clinical status improved with a better arterial oxygenation, and increased survival. In agreement, following depletion of PMN, NETs were absent with subsequent reduction of lung injury and a better arterial oxygenation.
Summary/Conclusion: We showed a pathogenic role of NETs in excacerbating lung injury of pristane-induced IAH. Targeting NETs with Rh-Dnase-1 could be an interesting adjuvant therapy in human autoimmune IAH.

Von Willebrand Factor
OS 4.1

Von Willebrand factor deficiency does not influence angiotensin II-induced abdominal aortic aneurysm formation in mice

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Background: Abdominal aortic aneurysm (AAA) refers to a permanent, localized dilation of the abdominal aorta that exceeds its normal diameter by 50%. When left untreated, AAAs have a high risk of rupture, which is associated with a high mortality rate. AAA pathophysiology is characterized by progressive inflammation, proteolytic degradation, vessel wall destabilization and thrombus formation.
Von Willebrand factor (VWF) is a multimeric plasma glycoprotein, known for its role in hemostasis, thrombosis, vascular stability and inflammation. Clinical studies have reported elevated VWF levels in AAA patients and suggested VWF as a potential biomarker for AAA growth. However, the exact role of VWF in AAA pathophysiology is currently not clear.
Aims: To investigate the potential thromboinflammatory involvement of VWF in AAA pathophysiology using an angiotensin II continuous infusion-induced AAA mouse model.
Methods: AAA formation was induced in both wild type and VWF-deficient mice by subcutaneous implantation of an osmotic pump, which continuously released a dose of 1 µg/kg/min angiotensin II for 28 days. Survival in both groups was monitored for the duration of the experiment. After the infusion period, the surviving mice were sacrificed to harvest the abdominal aortas in order to determine AAA incidence and severity. Cryosections of the suprarenal segments were analyzed with different histological procedures to visualize the aneurysm and the associated intramural thrombus.
Results: After implantation of the angiotensin II-infusing osmotic pumps, 19% (3/16) of VWF-deficient mice did not reach the end of the 28 day-infusion period. In comparison, all wild type mice (n=15) survived; however, this difference was not statistically significant (p=0.08). After 28 days, both AAA incidence and severity were assessed from the isolated suprarenal segments. Overall AAA incidence was not statistically different between VWF-deficient mice (7/13; 54%) and wild type animals (5/15; 33%) (p=0.4). Also, overall severity was not significantly different between the two groups after classification of the mice based on the appearance of the abdominal aorta. Accordingly, the maximal abdominal aortic diameter in wild type animals (1.6 ± 0.7 mm) was comparable to the maximal diameter in VWF-deficient mice (1.9 ± 0.6 mm, p = 0.3). Despite increased VWF plasma levels (169 ± 45%) in the wild type mice after the angiotensin II infusion period, these levels were not correlated with AAA severity. Additionally, detailed histological analyses of important hallmarks of AAA, including elastic degradation, intramural thrombus formation and leukocyte infiltration were assessed. Four wild type mice (27%) developed an aneurysm with an intramural thrombus containing VWF. Interestingly, 4 VWF-deficient mice (31%) also formed an intramural thrombus in the absence of VWF. Measurement of both fibrin and red blood cell content in these thrombi did not reveal differences in thrombus composition between both groups. In addition, staining of leukocytes in the suprarenal aortic tissue revealed a similar extent of inflammation in both wild type and VWF-deficient mice.
Summary/Conclusion: Overall, no significant differences were observed between wild type and VWF-deficient mice in angiotensin II infusion-induced AAA. These data suggest that, at least in this mouse model, the role of VWF in AAA pathophysiology is limited.

Von Willebrand Factor
OS 4.2

HNA-3a Expressed by CTL-2 is a Crucial Epitope for Neutrophil Adhesion and Activation on VWF

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Background: *SLC44A2* has been recently identified as a new susceptibility gene for venous thrombosis (VT) through a genome wide association study (GWAS) approach. The single nucleotide polymorphism *SLC44A2* rs2288904 (461G>A) responsible for the expression of the HNA-3a (human neutrophil antigen) epitope on the choline transporter-like protein 2 (CTL-2) was identified as linked to the increased risk of VT of ~30% over the rs2288904-A allele coding for the HNA-3b epitope. CTL-2 has been recently described as a new receptor for Von Willebrand Factor (VWF).
Aims: Given the increasing evidence linking polymorphonuclear neutrophil (PMN) and VWF to VT physiopathology, the aims of this work are to understand how the *SLC44A2* rs2288904 polymorphism affects PMN adhesion and activation on VWF.
Methods: Human embryonic kidney cells (HEK-293 cell line) transfected to express similar levels of CTL-2 with either the HNA-3a or the HNA-3b epitope were perfused in flow chambers coated with VWF, in presence or not of VWF-A1 domain blocking antibodies. Shear rates of $100s^{-1}$ and $1000s^{-1}$ were used. PMNs purified from consenting healthy blood donors homozygous for HNA-3a or HNA-3b expression were perfused on VWF in the same conditions. Cell rolling and adhesion were quantified. Activation of rolling/adhering PMNs and plasmic membrane integrity were evaluated through real-time measures of extracellular neutrophil elastase activity and extracellular nucleic acid staining. The effect of VWF on NETosis was also evaluated in static experiments on TNF-alpha-primed PMNs.
Results: HEK293 cells have been found to adhere more to VWF when they over-express CTL-2/HNA-3a instead of CTL-2/HNA-3b at both $100s^{-1}$ and $1000s^{-1}$. However, the polymorphism did not drastically affect the rolling speed at both shear rates. Cell rolling was reduced and cell adhesion on VWF was abolished when cells were co-perfused with a VWF-A1 domain-blocking antibody previously described as blocking the CTL-2/VWF interaction. The same observations were made when PMNs purified from homozygous healthy donors were perfused on VWF, thus confirming the importance of the HNA-3a epitope on CTL-2 for cell adhesion to VWF under flow.
We have also observed that VWF interaction with CTL-2 can modulate PMN activation. VWF incubated with TNF-alpha-primed PMNs induced significant NETosis compared to control primed-PMNs. HNA-3a-expressing HEK293 cells and PMNs were found to adhere massively on VWF in presence of heterogeneous shear stress. This phenomenon was epitope-dependent and could be inhibited in presence of the VWF-A1 domain-blocking antibody. The adhered PMNs exposed elastase activity and were positive for extracellular DNA, showing NET-like structures. Platelet-activating factor-activated PMNs expressing CTL-2/HNA-3a were found to adhere with similar patterns on VWF whereas PMNs expressing CTL-2/HNA-3b did not.
Summary/Conclusion: Shear stress modulation in flow chambers has allowed us to show that CTL-2 is a crucial protein for adhesion and activation of PMNs on VWF under flow. HNA-3a could thus be essential for the recruitment and activation of PMNs at sites of vascular inflammation. These results could explain the increased risk for VT associated to HNA-3a expression.
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Von Willebrand Factor
OS 4.3

The role of von Willebrand factor in experimental malaria-associated acute respiratory distress syndrome

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Background: Malaria remains a global health problem, with 216 million documented cases resulting in 445,000 deaths in 2016. One of the most lethal symptoms is malaria-associated acute respiratory distress syndrome (MA-ARDS). Recent patient studies have demonstrated that severe malaria is associated with acute endothelial cell activation, accumulation of highly active von Willebrand factor (VWF) multimers, and decreased ADAMTS13 activity.
Aims: To investigate the role of VWF in malaria pathogenesis by using a murine model of MA-ARDS.
Methods: 10^4 *Plasmodium berghei* (*Pb*) NK65 parasites were injected intraperitoneally in wild-type (WT) C57BL/6 and VWF knockout ($Vwf^{-/-}$) mice. Blood samples were taken to assess plasma VWF and ADAMTS13 levels as well as platelet counts. Parasitemia levels were monitored by examination of Giemsa-stained blood smears. Total protein levels in bronchoalveolar lavage fluid were assessed as a measure of pulmonary edema. Quantitative RT-PCR technique was used to determine parasite accumulations in the lungs. Reticulocyte counts were evaluated using flow cytometry.
Results: In accordance with patient studies, infected mice had increased VWF levels and reduced ADAMTS13 levels ($p<0.0001$ and $p<0.01$, respectively). VWF multimer patterns were normal until the end-stage of disease (8/9 days-post infection (p.i.)), at which high molecular weight VWF multimers were significantly decreased ($p<0.0001$). Malaria-associated thrombocytopenia was VWF-independent, as thrombocytopenia was observed in both WT and $Vwf^{-/-}$ mice. Depletion of macrophages, however, significantly restored platelet counts in *Pb*NK65-infected WT mice. Alveolar permeability, as measured by protein leakage, was 2-fold lower in $Vwf^{-/-}$ mice compared to WT animals ($p=0.02$). This was accompanied by a significant reduction of leukocyte infiltration in the lungs ($p=0.03$). Despite reduced lung edema and inflammation, $Vwf^{-/-}$ mice died more rapidly compared with WT (9 days p.i. vs 10 days p.i., $P=0.003$), which possibly could be attributed to the significantly higher parasitemia levels and parasite accumulations in the lungs ($P<0.0001$ and $P=0.0003$, respectively). Interestingly, $Vwf^{-/-}$ mice showed increased numbers of reticulocytes compared to WT mice, which can account for the faster development of parasitemia in $Vwf^{-/-}$ mice.
Summary/Conclusion: Similar to patient studies, *Pb*NK65-mediated malaria infection in mice is associated with early elevated levels of plasma VWF, and decreased ADAMTS13 activity. Macrophages, but not VWF, contribute to malaria-associated thrombocytopenia. VWF contributes to alveolar leakage, potentially by recruiting leukocytes in the lungs. VWF deficiency is associated with increased numbers of reticulocytes, faster development of parasitemia, and shortened survival.

Von Willebrand Factor
OS 4.4

The VWF-GPIb interaction mediates thrombo-inflammation in experimental stroke via recruitment of monocytes, neutrophils and T-cells to the brain

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Background: Von Willebrand factor (VWF) is crucial for hemostasis by capturing platelets at sites of vascular damage and by serving as a carrier for FVIII. Recently, also an inflammatory role for VWF has emerged. Previously, we and others have shown that mice lacking VWF are protected from ischemic stroke. Intriguingly, mainly VWF-mediated platelet adhesion and not VWF-mediated platelet aggregation was found to be detrimental in the acute phase of ischemic stroke. Hence, both platelets and VWF contribute to stroke progression in a way that is not strictly related to thrombus formation, but most likely also involves an acute inflammatory component. However, how VWF mediates thrombo-inflammatory ischemic stroke brain damage is currently unclear.
Aims: To investigate the potential inflammatory role of the VWF A1-platelet GPIb interaction in ischemic stroke.
Methods: VWF knockout (KO) and wildtype (WT) mice were subjected to 1h of cerebral ischemia, followed by 23h of reperfusion. To study the role of the VWF - platelet GPIb interaction in VWF-mediated inflammation, mice were treated with a nanobody specifically blocking the GPIb-binding site in the VWF A1 domain (KB-VWF-006bi) or a control nanobody (KB-VWF-004bv), 1h after stroke onset. Twenty-four hours after stroke, mice were neurologically scored, cerebral infarct sizes were measured, and flow cytometric and immunohistological analysis of immune cell recruitment to the brain was performed.
Results: In a first set of experiments, the acute cerebral immune response after stroke was compared between VWF WT and KO mice by flow cytometric analysis. Twenty-four hours after stroke, the amount of recruited white blood cells in the ipsilesional hemisphere of VWF KO mice was 2-times less as compared to WT mice ($p=0.02$). Upon further analysis, we found two-fold less proinflammatory monocytes ($p=0.03$), five-fold less neutrophils ($p=0.01$) and four-fold less T-cells ($p=0.002$) in the ischemic brain of VWF KO mice compared to WT mice. Interestingly, immunohistological analysis revealed that most of the recruited neutrophils and T-cells were located within the microcirculation of the ipsilesional hemisphere.
Next, we investigated whether pharmacological inhibition of VWF-mediated platelet adhesion could reduce immune cell recruitment to the brain and thereby protect mice from ischemic stroke brain damage. To test this, we utilized a nanobody specifically inhibiting the interaction between the VWF A1 domain and the platelet receptor GPIb. Interestingly, inhibition of the VWF-A1–platelet GPIb interaction 1 hour after stroke onset led to a reduced recruitment of white blood cells to the ischemic brain compared to control treated mice. More specifically, recruitment of proinflammatory monocytes was two-fold less ($p=0.02$), neutrophil recruitment was five-fold less ($p=0.0003$) and T-cell recruitment was two-fold less ($p=0.02$) in VWF A1 nanobody treated mice compared to mice receiving a control nanobody. Importantly, this reduced recruitment of white blood cells to the ipsilesional hemisphere was accompanied by an improved neurological outcome ($p=0.02$) and reduced cerebral infarct sizes ($p=0.001$).
Summary/Conclusion: Inhibition of the interaction between VWF and platelet GPIb reduces the pro-inflammatory effects of VWF in experimental stroke, revealing an inflammatory component of the VWF-GPIb interaction in the ischemic brain. This puts forward inhibition of the VWF-GPIb interaction as a promising strategy to reduce thrombo-inflammation in ischemic stroke brain damage.

Novel Treatments
OS 5.1

Cleavage of anti-PF4/H IgG antibodies by IdeS, and potential benefit in the treatment of heparin-induced thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is a frequent drug-adverse event due to platelet-activating antibodies (Abs) directed against platelet factor 4 (PF4)/heparin (H) complexes. In most patients, HIT Abs are IgGs able to directly activate platelets and monocytes in the presence of heparin via FcgRIIA receptors. The interaction between the Fc fragment of anti-PF4/H IgG and FcgRIIA is thus a key step for cellular activation in HIT. Several bacterial proteases such as IdeS (IgG-degrading enzyme of Streptococcus pyogenes) are cleaving IgG in the lower hinge region of heavy chain leading to the formation of single cleaved IgG (scIgG) and then of Fab'2. Importantly, IgG cleavage can abolish their ability to bind FcgR including FcgRIIA.
Aims: The aim of this study was therefore to evaluate whether anti-PF4/H IgG cleavage by IdeS could suppress the cell activation induced by these antibodies, and their pathogenicity.
Methods: To achieve this objective we studied the effects of IdeS on platelet response induced by 5B9, a monoclonal chimeric anti-PF4/H IgG that we had recently developed and which fully mimics the effects of human HIT antibodies (Kizlik-Masson et al, J Thromb Haemost, 2017).
Results: IdeS was demonstrated to quickly (6 minutes) cleave purified 5B9 IgG, leading to the formation of sc5B9, without any reduction in its binding ability to PF4/H complex. However, the affinity of sc5B9 for FcgRIIA was dramatically reduced compared to those of uncleaved 5B9 and sc5B9 was unable to induce platelet activation and aggregation in the presence of heparin. In addition, incubation of IdeS (0.02 U/µg of IgG; 6 minutes) in whole blood containing 5B9 or HIT plasma samples also lead to the cleavage of anti-PF4/H Abs and fully abolished their capacity to induce heparin-dependent platelet aggregation. As expected, no effect of IdeS was observed on platelet aggregation induced by collagen (0.5µg/mL), TRAP (10µM) or ADP (5µM). In addition, tissue factor (TF) gene expression induced in monocytes by 5B9 in the presence of heparin was also fully inhibited after adding of Ides (0.02 U/µg of IgG; 6 minutes), whereas no impact of Ides on TF expression induced by LPS was evidenced. Finally, we showed that platelet aggregation and fibrin formation induced by 5B9 with heparin (0.5 UI/mL) when whole blood was perfused ($500s^{-1}$) in vWF-coated microfluidic channels was strongly reduced after IdeS treatment
Summary/Conclusion: In conclusion, the cleavage of anti-PF4/H IgG by IdeS prevents heparin-dependent cellular activation induced by HIT antibodies, thereby reducing their pathogenicity. Therefore, injection of IdeS could be considered as a potential treatment in patients with severe HIT, particularly in those who necessitate emergent cardiac surgery with cardiopulmonary bypass and thus anticoagulation with heparin, which remains the safest anticoagulant in this specific surgical procedure.

Novel Treatments
OS 5.2

Generation of llama-derived single-domain antibodies directed against anticoagulant protein S

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Background: Vitamin K-dependent protein S (ProS) is an anticoagulant protein which functions as a cofactor for activated protein C (APC) and tissue factor pathway inhibitor (TFPI), and has also been described to have direct anticoagulant functions independently of APC and TFPI.
Aims: To gain more insights into the anticoagulant functions of ProS *in vitro* and *in vivo*, we aimed at generating single-domain antibodies (sdAb) derived from llama heavy-chain-only antibodies and directed against ProS.
Methods: A llama was immunized with recombinant human ProS (rhProS) and a phagemid library displaying sdAbs was constructed from the animal's peripheral blood lymphocytes and transformed into *E. coli* TG1 cells, to generate a library of about 2.10^7 transformants. Phage particles were produced and selected in two rounds of biopanning on magnetic beads chemically coupled to rhProS. Periplasmic extracts of selected clones were screened by ELISA (PE-ELISA) on immobilized rhProS, and sdAbs that specifically bound to rhProS were sequenced and produced in the cytoplasm of *E. coli* T7 SHuffle cells. The ability of sdAbs to bind to rhProS was measured in ELISA by immobilizing either rhProS or sdAbs. Furthermore, the APC-cofactor activity of ProS was measured in a commercial plasma clotting assay and in a thrombin generation assay (TGA) in which soluble thrombomodulin was added to activate the protein C system.
Results: After two rounds of biopanning, two sdAbs (PS-003 and PS-004) were identified that bound to rhProS in PE-ELISA with high affinity and specificity. We sought to produce these sdAbs in T7 SHuffle cells but we were unable to detect PS-004 in the soluble fraction of cytoplasmic extracts. This might be due to a high content in hydrophobic acids within the paratopic CDR3 region of PS-004, and other expression strategies will be explored. In contrast, PS-003 was readily produced and purified to homogeneity with good yields, and was further characterized.
In ELISA, rhProS bound to immobilized PS-003 with a $K_{d\ app}$ value of 15 nM. Furthermore, PS-003 did not bind to immobilized albumin or to various immobilized vitamin K-dependent proteins, including Growth arrest-specific protein 6 (Gas6). In addition, PS-003 was found to bind to a recombinant form of the sex hormone-binding globulin (SHBG) domain of ProS, suggesting that PS-003 recognizes an epitope contained within this domain. However, PS-003 did not inhibit the binding of rhProS to immoblized C4b-binding protein (C4BP) in ELISA.
Intriguingly, PS-003 was found to potentiate the APC-cofactor activity of ProS in our plasma clotting assay, using either rhProS or a normal human plasma as a source of ProS, while having no effect in the absence of ProS. PS-003 also decreased the endogenous thrombin potential (ETP) of a normal human plasma only when soluble thrombomodulin was added.
Summary/Conclusion: The generation of an immune library of sdAbs directed against human ProS was validated with the identification of at least one sdAb (PS-003) which recognizes recombinant human ProS with high affinity and specificity. In addition, PS-003 unexpectedly exerts stimulating effects on the APC-cofactor activity and the effects of PS-003 on the TFPI-cofactor functions of ProS are being investigated. We also aim at using various selection and screening strategies for identifying a greater variety of sdAbs recognizing different regions of ProS and thus differentially modulating the anticoagulant activities of ProS.

Novel Treatments
OS 5.3

Factor VIII activity and anti-FVIII inhibitor titer determination on patients treated with Hemlibra® (emicizumab): which FVIII assay used?

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Background: The development of anti-Factor VIII (FVIII) inhibitors in severe haemophilia A (HA) patients renders standard FVIII treatment ineffective and complicates the patients' management. The introduction of the novel FVIII mimetic bispecific antibody, emicizumab, that permits the formation of an active tenase complex in the absence of endogenous FVIII by binding and bridging activated factor IX and factor X, offers a major advance in the treatment of HA patients with inhibitors. It is kwown that emicizumab significantly interferes with the conventional aPTT-based assays used for the FVIII activity (FVIII:C) and for the anti-FVIII inhibitors titers determination. However, the measurement of FVIII:C and anti-FVIII inhibitors titer during emicizumab prophylaxis may be needed in several situations such as severe major breakthrough bleeding or major surgery.
Aims: The aim of this study was to compare the FVIII:C and the anti-FVIII inhibitors determinations in patients with emicizumab prophylaxis using aPTT-based assay and two different chromogenic assays, using human or bovine reagents.
Methods: Thirty one plasma samples from nine severe haemophilia A patients were included. Five of these patients had developed an anti-FVIII inhibitor before the beginning of the emicizumab infusion. FVIII activity and inhibitor titer determination using Bethesda assay were performed using coagulometric aPTT-based assay (HemosIL® SynthASil and HemosIL® Factor VIII deficient plasma), chromogenic assay with human reagents (Hyphen® Biophen) and chromogenic assay with bovine reagents (Chromogenix® Coamatic) on ACL TOP® 750 (Werfen).
Results: Measurements of FVIII activity were significantly different between the three methods. The coagulometric assay gave aberrant FVIII activities (from 166 to 449%) for all samples, which shows an important interference of emicizumab. Moderated FVIII activities were obtained with chromogenic assay using human reagents (from 5 to 32%), suggesting recognition of human factors by the antibody. Then, undetectable activities were measured with bovine reagents, which confirm the absence of interference of emicizumab with bovine reagents. Patients without anti-FVIII inhibitor before treatment remained negatives (<0.6 UB/mL) with the 3 methods. Anti-FVIII inhibitor titers measured were significantly superior in 4 out of 5 patients (11 samples) with Chromogenix® Coamatic than both Hyphen® Biophen and aPTT-based assay ($p<0.001$). For one patient, important dilutions due to a very elevated titer (1800 UB/mL before treatment) showed a diminution of the interference caused by emicizumab, resulting in no significant difference between the three methods (3 samples).
Summary/Conclusion: Interference of emicizumab on both APTT-based assay and chromogenic method using human reagents seem to overestimate FVIII activity, resulting in an underestimation of anti-FVIII inhibitor titers. Thereby, the use of a chromogenic assay with bovine reagents, suppressing this interference, seems to enable a more accurate inhibitor titration.

Novel Treatments
OS 5.4

Search for antithrombin chemical inhibitors as an oral treatment for Hemophilia

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Background: Antithrombin (AT) is one of the major physiological anticoagulant protein, which binds covalently and inhibits most of the procoagulant enzymes in particular thrombin (FIIa) and FXa. The inhibition of AT is expected to increase procoagulant activity in plasma promoting the formation of a stable thrombus in patients suffering from hemorrhagic disorders.Indeed, a clinical trial using RNAi against AT reported important reduction in bleedings in hemophilia patients. However, RNAi strategies are associated with a delayed action and parental administration
Aims: Our project is to develop a small chemical inhibitor of AT, administrable orally for the treatment of patients with bleeding disorders such as hemophilia.
Methods: A screening assay measuring the apparent inhibition rate of FXa by AT in the presence of fondaparinux was designed for the screening of chemical libraries. Chemical compounds were tested at a final concentration of 50 µM.
The hit molecule was further characterized for its effect on AT activity in anti-FXa chromogenic assay in the presence or in the absence of saturating concentration of fondaparinux. Moreover, the formation of AT-FXa complexes was evaluated by semi-quantitative analysis on a SDS-PAGE after incubation of AT and FXa for 2 hours in the presence or in the absence of the chemical compound
Results: Among 10,200 molecules originating from 3 different chemical libraries, 4 inhibited anti-FXa activity of AT without affecting directly FXa activity. The most potent of them was P82H4, and was selected for further characterization on kinetic of inhibition of FXa by AT. In the presence of saturating concentrations of fondaparinux, P82H4 decreased by 5.6-fold the inhibition rate constant (kon) of FXa by AT from $2.09x10^{5}M^{-1}.s^{-1}$(in the absence of chemical compound) to $0.37x10^{5}M^{-1}.s^{-1}$(in the presence of chemical compound).
Interestingly, enzyme kinetics suggested that P82H4 promotes proteolysis of AT's reactive site loop by FXa in the absence of fondaparinux, thereby preventing formation of covalent. FXa/AT complexes. Indeed, while FXa was found fully inhibited by AT in the absence of chemical compound, FXa residual activity level off at 27% of its initial value after prolonged incubation with AT in the presence of P82H4. Moreover, SDS-PAGE analysis revealed decreased amounts of covalent FXa/AT complexes in the presence of P84H2.
Summary/Conclusion: By screening chemical libraries, we have identified a novel candidate small-molecule that interferes with AT anticoagulant activity *in vitro*. Although component P82H4 shows high druggability, it requires further optimisation to improve its inhibitory activity and develop a molecule able to impact coagulation in plasma. Indeed, the development of a small molecule suitable for oral administration would be a great improvement in the treatment of hemophilic patients.

Oral Anticoagulation
OS 6.1

Vitamin K antagonist (VKA) versus direct oral anticoagulants in patients with currently well controlled VKA for non-valvular atrial fibrillation: a randomised controlled pilot trial

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Background: Direct oral anticoagulants (DOAC) are non-inferior to vitamin K antagonists (VKA) in trial patients with atrial fibrillation. During VKA therapy clinical events occur predominantly in patients with a poor time within the therapeutic INR range (TTR). Overall non-inferiority of DOAC might therefore be the result of superiority over those with poor TTR, and inferiority compared with those with high TTR. The subgroup of patients with highest TTR might then not benefit from switching to DOAC.
Aims: To compare harms and benefits of switching to a DOAC with continuing VKA for patients with atrial fibrillation who are currently well-controlled on VKA. Collected data will be used to determine the effect size for, and feasibility of, a full-scale trial.
Methods: In this randomised controlled, open-label pilot study in a dedicated first-line thrombosis service in the Netherlands, we randomly assigned well-controlled (TTR in range 2.0-3.5 ≥ 70%, no thrombosis or major bleed while on VKA) patients who used VKA for atrial fibrillation to either continue VKA treatment as usual, or to switch to a DOAC. The primary endpoint during one year of follow-up was net clinical benefit: a composite of stroke, systemic embolism, myocardial infarction, vascular death and major bleed. Secondary endpoints were efficacy (composite of ischaemic or unspecified stroke, systemic embolism, myocardial infarction and vascular death) and safety (major and clinically relevant bleeding, all-cause mortality). Outcomes were adjudicated by an independent committee, blinded from allocated treatment.
Results: 241 patients gave informed consent, were included and analysed: 183 (76%) men; mean±SD age 72.3±6.89. 120 were randomised to VKA and 121 to DOAC. Of those, 4 in each group experienced a net clinical benefit endpoint (hazard ratio of VKA compared with DOAC 1.00, 95% CI 0.25 - 4.00). The efficacy endpoint occurred in 4 and 3 patients on VKA and DOAC, respectively (HR 1.33, 95% CI 0.30 - 5.96). The safety endpoint occurred in 12 and 14 patients (HR 0.85, 95% CI 0.40 - 1.85).
Summary/Conclusion: Our data do not support the hypothesis that continuing well-controlled VKA therapy is superior to switching to a DOAC in patients with non-valvular AF. We found no evidence that switching to DOAC harms these patients. A full-scale study is not warranted.

Oral Anticoagulation
OS 6.2

Perioperative management in patients using vitamin K antagonists: a cohort study

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Background: Observational research showed an elevated risk of major bleeding with an uncertain beneficial effect of the use of periprocedural bridging with low-molecular-weight heparin (LMWH) in patients with atrial fibrillation using vitamin K antagonists (VKA). Our local guideline therefore advises physicians to only bridge patients with atrial fibrillation and a CHA_2DS_2-VASc score ≥ 7. In support, the BRIDGE trial, published in 2015, showed a net clinical benefit in favour of a strategy without the use of bridging as compared to periprocedural bridging with LMWH.
Aims: To determine whether publication of the BRIDGE trial has led to less bridging procedures and better patient outcomes (i.e. a composite of thromboembolism, major bleeding and death) in patients from the Leiden Anticoagulation Clinic, the Netherlands, undergoing elective surgery or other invasive procedures.
Methods: At the Leiden Anticoagulation Clinic, we collected data of all procedures that required VKA interruption between April 1, 2014 and November 31, 2017. Procedures were divided in a period before (2014-2016; 22 months) and after the publication of the BRIDGE trial (2016-2017; 22 months). Cumulative incidences 30 days postprocedure and relative risks of thromboembolic events, major bleeding and mortality were calculated.
Results: 4892 And 4237 eligible procedures were performed in 2014-2016 and 2016-2017, respectively. The median age at time of the procedure was 74 years and 58% of procedures were performed in men. There was no difference in the cumulative incidence of thromboembolism (adjusted odds ratio [OR] 0.60, 95% confidence interval [CI] 0.30-1.21), major bleeding (adjusted OR 1.27, 95%CI 0.85-1.90) and all-cause mortality (adjusted OR 0.87, 95%CI 0.19-4.07), in 2016-2017 as compared to 2014-2016. Surprisingly, the frequency of bridging with LMWH (14.8% in 2014-2016 vs 16.6% in 2016-2017) did not decline, as did the mean CHA_2DS_2-VASc scores (3.0 (standard deviation [SD] 1.3) in 2014-2016 vs 3.1 (SD 1.4) in 2016-2017) in patients with atrial fibrillation who received bridging. Approximately 80% of those patients had a CHA_2DS_2-VASc score < 5. These findings indicate that physicians did not adhere to our guideline and did not change their policy and bridge less after publication of the BRIDGE trial.
Summary/Conclusion: We showed that despite publication of the BRIDGE trial, the frequency of bridging with LMWH aside of the protocol was high and thereby patient outcomes regarding bleeding complications did not change.

Oral Anticoagulation

OS 6.3

Neutralisation of non-vitamin K anticoagulants by α2Macroglobulin-trapped factor Xa

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Background: Alpha$_2$Macroglobulin (a_2M) is a broad-spectrum molecular trap inhibitor mainly targeting thrombin, factor Xa (FXa), and plasmin. When native α_2M is cleaved by such proteases or treated by primary amines such as methylamine, it is converted to an activated form. Alpha$_2$M activation results in a major conformational change, irreversibly trapping the activating protease within a cage-like quaternary structure where many functional groups are either formed or unmasked.
Aims: We investigated *in vitro* and *in vivo* the potential of a complex formed by Gla-domainless FXa (GDFXa) and a_2M as a universal antidote of direct oral anticoagulants (DOAC) targeting FXa (xabans) or thrombin (dabigatran) as well as heparinoid derivatives.
Methods: Human native α_2M was purified, incubated with GDFXa to form the GDFXa-α_2M complex, and characterized. Inhibition of GDFXa and GDFXa-α_2M by DOAC as well as by antithrombin or tissue factor pathway inhibitor (TFPI) was evaluated by chromogenic assays. *In vitro* effect of GDFXa-α_2M on plasma coagulation was tested in the presence or in the absence of xabans, dabigatran or heparinoids by clot waveform assay and on whole blood coagulation by rotational thromboelastometry. *In vivo* neutralization of the anticoagulants was evaluated in a mouse bleeding model. Procoagulant potential was assessed by Elisa quantification of D-dimers and thrombin-antithrombin complexes (TAT).
Results: Native α_2M alone had no detectable influence on coagulation in the presence or absence of DOAC. Contrary to GDFXa, GDFXa-a_2M was neutral with regard to coagulation and did not neutralise antithrombin or TFPI. Affinity of xabans for GDFXa-α_2M was comparable to that of FXa. In plasma as well as in whole blood, GDFXa-α_2M (1.7 µM) fully neutralised supratherapeutic amount of xabans (600 ng/mL) and, at least 80 % dabigatran (600 ng/mL) and 50 % heparinoids (1.5 IU anti-Xa/mL). *In vivo*, GDFXa-a_2M significantly decreased bleeding time and blood loss induced by rivaroxaban, dabigatran or enoxaparin. Half-life of GDFXa-α_2M in mice was 4.9 min but, due to saturation of the clearance mechanisms, GDFXa-α_2M persisted *in vivo* for hours when administered at high dose (0.5 mg/mouse), allowing anticoagulant neutralisation through a single IV bolus administration in mice. The absence of modification of coagulation tests as well as the absence of increase in D-dimers and TAT levels confirmed that GDFXa-α_2M was devoid of prothrombotic potential *in vivo*.
Summary/Conclusion: GDFXa-a_2M is an attractive antidote to DOAC and heparinoids that proved its efficacy *in vitro* as well as *in vivo* while being neither pro- nor anti-coagulant.

Oral Anticoagulation
OS 6.4

Targeted Substitution at the S4 Subsite of Human Factor X Infers Resistance to the Direct Factor Xa Inhibitors

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Background: The direct factor Xa (FXa) inhibitors effectively block its catalytic activity by reversible high affinity occupation of the FXa active site through stabilization in the S4 subsite. We previously reported that while substitution of the S4 pocket residues Tyr99 and Phe174 (chymotrypsinogen numbering) to alanine reduced the sensitivity to the direct FXa inhibitor apixaban by 10-100-fold, full factor X (FX) clotting activity was only maintained following Phe174 replacement (Verhoef et al. Nature Communications 2017). Substituting Phe174 could therefore be a promising target in the development of a bypassing agent for the FXa-inhibitors.
Aims: Here we further investigated whether FX variants carrying substitutions targeting the S4 subsite Phe174 infer resistance to the direct FXa inhibitors and, if so, evaluated their potential to bypass direct FXa inhibitor-mediated anticoagulation.
Methods: Phe174 was substituted for either an alanine or serine, thereby generating FX-F174A or FX-F174S, respectively, and these variants were stably expressed in HEK293 cells and purified to homogeneity using ion-exchange chromatography.
Results: Assessment of the kinetic parameters of prothrombin conversion and FVa-like cofactor FV-810 binding revealed that the FXa-F174 substituted variants displayed similar characteristics to human FXa. Furthermore, the rate of antithrombin inhibition, both uncatalyzed and catalyzed by unfractionated heparin, was comparable to human FXa, whereas the binding affinity for the inhibitor TFPIα was at most 1.5-fold reduced. We next evaluated the direct FXa sensitivity using the tissue factor-initiated (2 pM) calibrated automated thrombin generation assay in normal human plasma supplemented with FX-F174 zymogen variants (30 µg/ml). The FX-F174 variants exhibited a 10-20-fold increase in IC50 for apixaban inhibition, a 40-fold increase for rivaroxaban, and 10-fold increase for edoxaban. Confirmation of this induced inhibitor-resistance was obtained from Molecular Dynamics (MD) simulations of the FXa-apixaban complex. We observed partial unbinding of apixaban in at least one of the four individual FXa-F174A simulations, while the apixaban configuration remained stable in all the simulations for human FXa. The bypass activity of the FX-F174 variants was further evaluated in the setting of thrombin generation at physiological peak inhibitor concentrations (1 µM). Under these conditions, little, if any, thrombin was formed upon addition of 60 µg/ml human FX. In contrast, supplementation with 15-30 µg/ml FX-F174 fully restored the thrombin peak in the presence of peak inhibitor levels. Interestingly however, both the lag time and time to peak were 1.3- to 2-fold prolonged, whereas these parameters were not affected in the absence of inhibitor. This delay in thrombin generation was not caused by a defect in activation by the extrinsic (tissue factor-factor VIIa) or intrinsic (factor VIIIa-factor IXa) tenase complex, as the kinetic parameters for FX-F174 activation were unperturbed.
Summary/Conclusion: Collectively, our findings indicate that human FX variants comprising a single point mutation at position Phe174 are able to restore hemostasis in plasma inhibited by the direct FXa inhibitors. As such, these variants have the potential to serve as rescue therapeutic agents to overcome the effect of the direct FXa inhibitors in case of potential life-threatening bleeding events or emergency surgical interventions.

ADAMTS13
OS 7.1

Inhibiting ADAMTS13 activity prevents the loss of high molecular weight von Willebrand factor multimers in in vitro left ventricular assist devices

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Background: Gastrointestinal (GI) bleedings are one of the leading adverse events of left ventricular assist device (LVAD) therapy and have been associated with acquired von Willebrand syndrome (aVWS). All LVAD patients have a selective loss of high molecular weight (HMW) von Willebrand factor (VWF) multimers and hence loss of their haemostatic function. The loss of HMW VWF multimers might be explained by an increased shear-induced proteolysis of VWF by ADAMTS13 (A Disintegrin And Metalloprotease with Thrombospondin type 1 repeats, number 13) due to the high shear stress occurring in the LVAD device. Consequently, inhibiting ADAMTS13 would be an efficient way to rescue the loss of HMW VWF multimers in LVAD patients.
Aims: To investigate whether ADAMTS13 inhibition prevents the loss of both HMW VWF multimers and VWF activity, in two different in vitro LVAD circuits.
Methods: Two different in vitro LVAD circuits were used: one with a HeartMate II™ (HM II, long-term support, n=3) and one with an Impella CP® (short-term support, n=5) pump. Citrate-anticoagulated human blood was circulated to study ADAMTS13 mediated VWF proteolysis in the presence of the inhibitory anti-ADAMTS13 monoclonal antibody (mAb) 3H9 (20 µg/mL). A non-inhibitory anti-ADAMTS13 mAb 5C11 (20 µg/mL) or phosphate buffered saline (PBS) was used as a control. Blood samples were taken 5 minutes (min) before and 5, 30, 60 and 180 min after the onset of perfusion in the LVAD system. Plasma samples were analysed for VWF multimers, VWF antigen (VWF:Ag), VWF collagen binding activity (VWF:CB) and VWF ristocetin cofactor activity (VWF:RCo).
Results: A time-dependent decrease in HMW VWF multimers was observed in both LVAD systems in the presence of the non-inhibitory mAb 5C11 or PBS, leading to a 70% reduction of HMW VWF multimers, 180 min after the start of perfusion ($p=0.01$ for HM II and $p=0.0003$ for Impella). This was also reflected by a severely decreased VWF:CB/VWF:Ag ratio (0.59 ± 0.11 and 0.52 ± 0.10 at 180 min versus 1.00 ± 0.06 and 1.07 ± 0.09 before perfusion, for the HM II ($p=0.03$) and Impella ($p=0.001$) respectively) and moderately decreased VWF:RCo/VWF:Ag ratio (0.94 ± 0.07 and 0.81 ± 0.04 at 180 min versus 1.00 ± 0.11 and 1.03 ± 0.05 before perfusion, for the HM II ($p=0.34$) and Impella ($p=0.007$) respectively). Interestingly, blocking ADAMTS13 activity using the anti-ADAMTS13 mAb 3H9 at 20 µg/mL prevented the loss of HMW VWF multimers in both circuits: HMW VWF multimers were not decreased in function of time ($p=0.50$ for the HM II and $p=0.06$ for the Impella, 180 min after the start of perfusion). The preservation of HMW VWF multimers was also reflected by normal VWF:CB/VWF Ag (0.92 ± 0.16 and 0.97 ± 0.11 at 180 min versus 0.93 ± 0.09 and 1.19 ± 0.12 before perfusion, for the HM II ($p=0.75$) and Impella ($p=0.06$) respectively) and VWF:RCo/VWF:Ag ratios (0.98 ± 0.21 and 0.98 ± 0.07 at 180 min versus 1.03 ± 0.20 and 0.90 ± 0.05 before perfusion, for the HM II ($p=0.25$) and Impella ($p=0.25$) respectively).
Summary/Conclusion: Using an inhibitory anti-ADAMTS13 antibody, we were able to prevent the loss of HMW VWF multimers in both the in vitro HeartMate II™ and Impella CP® device. Our data unequivocally show that the loss of HMW VWF multimers is due to shear-induced ADAMTS13 proteolysis. Hence, inhibiting ADAMTS13 activity might be an effective way to treat aVWS in LVAD patients.

ADAMTS13
OS 7.2

Crystal structure and allosteric activation of ADAMTS13

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Background: The multimeric size and platelet-tethering function of von Willebrand factor (VWF) is proteolytically regulated by ADAMTS13. Highly unusually for a plasma protease, ADAMTS13 circulates in an apparently active form. Despite this, it exhibits proteolytic specificity for VWF alone. Furthermore, ADAMTS13 is resistant to the effects of plasma inhibitors, and exhibits a very long plasma half-life for an active protease (3-7 days). How these characteristics are manifest remains unclear. Physiologically, ADAMTS13 proteolysis only occurs when VWF unravels in response to elevated shear forces, which exposes the cleavage site and four cryptic binding sites in its central A2 domain. Recognition of the unfolded A2 domain involves four ADAMTS13 exosites located in the Spacer, Cysteine-rich (Cys), Disintegrin-like (Dis) and Metalloprotease (MP) domains.
Aims: To determine the contribution of each ADAMTS13 exosite to VWF proteolysis and understand ADAMTS13 function at a molecular level.
Methods: To determine the role of each exosite interaction, we generated a panel of composite VWF A2 domain fragment (VWF96) variants in which all amino acids involved in each exosite binding site were mutated and thereafter monitored proteolysis by ADAMTS13 kinetically. We used crystallography to resolve the structure of the N-terminal domains of ADAMTS13 (MDTCS).
Results: Kinetic analysis revealed proteolysis of the VWF96-Spacer variant was ~15-fold reduced due to an increase in K_m (i.e. reduced substrate binding). The VWF96-Cys variant was proteolysed ~20-fold slower, again due an increase in K_m. Conversely, the VWF96-MP variant was cleaved ~200-fold slower, due to a reduction in k_{cat} (i.e. functionality of the active-site), consistent with the proximity of the mutation to the VWF cleavage site. Interestingly, the VWF96-Dis variant was proteolysed ~750-fold slower due to a combination of a 14-fold increase in K_m, and a 55-fold decrease in k_{cat}. Together, these data demonstrates that the Cys and Spacer exosites are primarily involved in substrate binding, and the MP domain primarily influences the proteolytic event. However, the Dis domain contains the most important exosite for VWF proteolysis as it influences both substrate binding and the functionality of the active-site. We hypothesised that ADAMTS13 specificity is dictated by an allosteric mechanism in which free/circulating ADAMTS13 adopts a latent conformation preventing off-target proteolysis and conferring resistance to inhibition. Only when the Dis domain exosite binds the VWF A2 domain does the adjacent MP domain become allosterically activated (by 55-fold) enabling proteolysis to proceed efficiently. In this model therefore, VWF functions as both co-factor and substrate for ADAMTS13. To corroborate this, we resolved the crystal structure of the 70kDa ADAMTS13 MP-Dis-TSP1-Cys-Spacer domain fragment in complex with an anti-MP domain Fab (3H9).
Summary/Conclusion: This is the first structure of this fragment for any ADAMTS-family member. Moreover, the structure (in the absence of VWF binding) was entirely consistent with our hypothesised allosteric mechanism as the MP domain revealed an apparently latent conformation in which a loop in the MP domain occludes the active site cleft. This occluding loop must be removed to facilitate access of the substrate to the active-site, which we propose occurs allosterically through engagement of the Dis exosite with VWF. These results reveal a novel mode of action for ADAMTS13 in its regulation of VWF function.

ADAMTS13
OS 7.3

Open ADAMTS13 conformation in immune-mediated thrombotic thrombocytopenic purpura is induced by anti-ADAMTS13 autoantibodies and corresponds with an ongoing ADAMTS13 pathology

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Background: Deficient ADAMTS13 activity (TS13:act <10%) induced by anti-ADAMTS13 autoantibodies (autoAbs) causes immune-mediated thrombotic thrombocytopenic purpura (iTTP). Recently we showed that an open ADAMTS13 conformation is characteristic for acute iTTP patients, while folded ADAMTS13 was found in 78% of iTTP patients in remission with an TS13:act >50%. However, also iTTP patients in remission with a persistent (<10%) or moderately restored (10-50%) TS13:act have been described, but their ADAMTS13 conformation is unknown. Intriguingly, the factor responsible for inducing open ADAMTS13 in iTTP patients remains elusive. Identifying the cause of open ADAMTS13 in iTTP will help better understand the pathophysiology of iTTP and could help appreciate the prognosis and better manage the prevention of subsequent relapses.
Aims: Determine ADAMTS13 conformation in plasma of iTTP patients during acute TTP and remission when TS13:act is <10%, moderately restored (10-50%) or >50% and investigate if anti-ADAMTS13 autoAbs induce conformational changes in ADAMTS13.
Methods: TS13:act was determined in 120 iTTP plasma samples from 4 different centers (Marseille, Milan, Budapest, Utrecht). Samples were categorized according to the presence of clinical symptoms (acute *versus* remission) and their TS13:act in remission (>50%, 10-50%,<10%). Next, ADAMTS13 conformation was determined in all samples using our ADAMTS13 conformation ELISA. Additionally, presence of anti-ADAMTS13 autoAbs was also determined via ELISA. Finally, IgG's from 18 acute iTTP plasma samples were purified and added to folded ADAMTS13 from healthy donor (HD) plasma to test whether iTTP IgG's are able to induce the open HD ADAMTS13 conformation.
Results: Of the 120 iTTP plasma samples, 46 were obtained during the acute (clinical signs present) and 74 during the remission phase (clinical signs absent). Further subdividing remission samples showed that TS13:act was >50% in 41, 10-50% in 14 and <10% in 19 samples. ADAMTS13 was open in 98% (45/46) of the acute samples and folded in 71% (29/41) of the remission samples with TS13:act >50%, confirming our previous results. Interestingly, ADAMTS13 was open in 93% and 89% of remission samples with TS13:act 10-50% and <10%, respectively (chi square, P<0.0001). Since anti-ADAMTS13 autoAbs influence TS13:act in iTTP patients, we next could demonstrate that open ADAMTS13 conformation was linked with presence of anti-ADAMTS13 autoAbs (chi square, P<0.0001) suggesting that anti-ADAMTS13 autoAbs could be a factor able to induce an open ADAMTS13 conformation in iTTP patients. To further test this hypothesis, we purified IgG's from 18 acute iTTP plasma's with open ADAMTS13 and added them to HD plasma containing closed ADAMTS13, where 14 of the 18 patient IgG pools (78%) did induce the open conformation in HD ADAMTS13, indicating that patient anti-ADAMTS13 autoAbs indeed can induce conformational changes in ADAMTS13.
Summary/Conclusion: We show that ADAMTS13 is not only in the open conformation in iTTP patient plasma during the acute phase but also in remission when TS13:act is <10% or 10-50%. Hence, the presence of open ADAMTS13 in those remission patients indicates that the underlying pathophysiology is still ongoing, emphasizing the need for a close monitoring of those patients. In addition, anti-ADAMTS13 autoAbs were identified as a factor responsible for the change in conformation in ADAMTS13 in iTTP.

ADAMTS13
OS 7.4

ADAMTS13 peptide presentation in acquired thrombotic thrombocytopenic purpura

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Background: Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a devastating disease resulting from ADAMTS13 autoantibody formation. In healthy individuals, ADAMTS13 is responsible for regulation of blood clotting by decreasing the size of von Willebrand factor multimers. The etiology of iTTP is not yet fully understood, however, previous studies reported the presence of ADAMTS13 autoreactive CD4 T cells in patients with iTTP. The ADAMTS13 peptide repertoire presented on MHC-II has already been investigated in the context of HLA-DR, but no such study has been performed for HLA-DQ. Additionally, it is yet unknown which epitopes of ADAMTS13 are immunodominant and therefore involved in the onset of iTTP.
Aims: The aim of this study was to determine whether ADAMTS13 peptides can be presented on HLA-DQ molecules of MHC-II and if so, whether this peptide repertoire differs from the one on HLA-DR. Furthermore, we wished to use immunoinformatics analysis to determine which of these MHC-II presented peptides are likely to result in autoreactive CD4 T cell response and thus potentially playing a role in the onset of iTTP.
Methods: Monocyte-derived dendritic cells from nine HLA-typed healthy individuals were pulsed with 100 nM ADAMTS13. After cell maturation and lysis, peptide/MHC-II complexes were purified over a column, using HLA-DR- and HLA-DQ-specific antibodies (L243 and SPV-L3, respectively). HLA-bound peptides were eluted and identified using mass spectrometry. These newly and previously identified peptides were subjected to *in silico* analysis using EpiMatrix and JanusMatrix tools (Moise *et al.*, 2013) to evaluate for their putative effector or regulatory T-cell responses at the population level, in the context of HLA-DR.
Results: In total, 12 ADAMTS13 core-peptide sequences were identified on HLA-DR and 8 on HLA-DQ. Immunoinformatics analysis of the identified peptides was performed using EpiMatrix and JanusMatrix to evaluate their potential for putative effector or regulatory T cell responses at the population level. Interestingly, a majority (70 %) of peptides with a significant EpiMatrix score (significant immunogenic potential) originated from the CUB1/2 domains. Six of the eluted peptides shared low cross-conservation with the human proteome (based on TCR-facing residue analysis with JanusMatrix) and thus were considered to be more likely to elicit an effector T cell response. In contrast, T cell receptor contact residues of four predicted and eluted peptides were highly conserved with multiple predicted epitopes found in the human proteome, and, therefore, were considered likely to be tolerated or actively tolerogenic in patients with iTTP.
Summary/Conclusion: The results from this study provide a basis for the identification of immunodominant, immunogenic or potentially tolerogenic epitopes on ADAMTS13 that may be involved in the onset of iTTP.

Cancer and Thrombosis
OS 8.1

Biological pro-coagulant and pro-inflammatory effect of interferon alpha in myeloproliferative neoplasms.

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Background: Myeloproliferative neoplasms (MPN) are associated with an increased risk of arterial and venous thrombosis. Pegylated-interferon alpha (IFN) and hydroxyurea (HU) have obviously a great therapeutic efficacy on hematopoietic cell proliferation but their effects on hemostasis and inflammation have not been studied.
Aims: The aim of the study is to determine whether treatment impacts the biological profile of MPN patients comparing endothelial, platelet and coagulation parameters in IFN or hydroxyurea (HU) treated and non treated (NT) patients.
Methods: 85 patients were included: 28 treated by IFN 20 polycythemia vera (PV) and 8 essential thrombocythemia (ET), 38 treated by HU (27 PV and 11 ET) and 22 NT (6 PV and 16 ET). All patients were treated with aspirin 75 or 100 mg/day. Incidence of previous arterial and venous thrombotic events was similar between the 3 groups according to treatment: 7 in the NT group, 12 in HU- and 5 in IFN-treated group, respectively, without statistical difference. During the follow up (> 6months), 2 patients with PV had distal venous limb thrombosis, 2 years after the initiation of the HU treatment.
Results: We observed significant effects of HU on von Willebrand factor (vWF) levels and a more pronounced effect of IFN: vWF antigen increased from 111.7±9.5 % in NT to 162.9±10.3% in HU and 227±17.1 % in IFN-patients ($p< 0.01$). VWF activity increased to a similar extends. We observed only in IFN patients decreased protein S activity compared to NT patients (62.2±2.5% vs 88.7±3.9%, $p< 0.01$) and also significant increased levels of factor VIII:C and fibrinogen. We determine if treatment could have an impact on thrombin generation evaluated by a global test (Calibrated Automated Thrombogram assay*).* Thrombin generation was not different between the 3 groups of patients whatever the criteria observed. We had the opportunity to test again 10 patients at least 6 months after IFN discontinuation. IFN was stopped for side effects in 6 patients, for complete molecular response in 2 and for normalization of hematological parameters in 2 others. VWF activity and antigen, protein S activity, FVIIIC and fibrinogen returned to levels similar to those of NT patients and were significantly different from levels observed in IFN-treated patients.
Summary/Conclusion: We observed an increase of procoagulant and inflammatory markers in IFN-treated MPN patients that could be considered as a part of IFN biological effects. Though minimal, these alterations could be associated with an increased thrombotic risk and require more intensive preventive antithrombotic therapy than aspirin in patients with associated minor thrombotic risk factors such as FV Leiden or FII G20210A.

Cancer and Thrombosis
OS 8.2

Analysis of microvesicle-mediated thrombosis in CRISPR/CAS9-mediated tissue factor knockout breast cancer cells

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Background: Cancer-associated thrombosis is the second leading cause of death in cancer patients. Nonetheless, the mechanisms involved in the prothrombotic state of cancer patients are not completely understood. Expression of Tissue Factor (TF) by tumor cells is associated with aggressiveness, tumor progression and increased propensity to thrombosis in certain cancer types. Reports from clinical studies have shown increased TF-positive microvesicles (MVs) in cancer patients, which are able to initiate blood coagulation, and are correlated with higher risk of thrombosis in some tumor types, such as pancreatic cancer.
Aims: In this study, we investigated the impact of TF-positive MVs derived from breast cancer cells in the promotion of coagulation activation and pulmonary embolism *in vivo*.
Methods: TF silencing was achieved using the CRISPR/Cas9 system in the human breast cancer cell line MDA-MB-231, MVs were isolated from cell culture supernatant by centrifugation. TF expression and activity on MVs was analyzed by flow cytometry and clotting assays, respectively. Mice were injected intravenously with 1-10 µg/100 µL (n=6/group) of MVs and observed for 30 min.
Results: CRISPR editing in MDA-MB-231 cells efficiently reduced TF expression and coagulant activity on MVs. TF-bearing MVs promoted fatal pulmonary embolism (PE) in mice and this was dependent on the concentration of MVs. After injection of 10 µg of TF-positive MVs, 100% of mice died within 5 minutes; after injection of 5 µg, 50% of animals died in 30 min; and after injection of 1 µg, no animals died. In contrast, TF-negative MVs were unable to promote PE or death even at the highest dose used (10 µg). Blocking TF using the monoclonal antibody, 5G9, led to an efficient deceleration of the clotting time (from 137± 27 sec to 246 ± 18 sec) and also inhibited death and PE in 100% of the mice injected with 10 µg of MVs in combination with 5G9 antibody.
Summary/Conclusion: Taken together, our results suggest that TF-positive MVs from breast cancer cells are able to promote acceleration of the clotting time and promotes thrombosis in a dose-dependent manner. Using 5G9 antibody, it is possible to decelerate the clotting time and prevent thrombosis.

Cancer and Thrombosis
OS 8.3
Paradoxical roles of platelets in colorectal cancer

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Background: Increasing evidences demonstrated that platelets actively participate to the progression of metastasis. However, their roles in tumor growth are still subject to controversy.
Aims: In this study, we investigated the interaction of platelets with colorectal tumor cells within the tumor microenvironment and we assessed their roles in tumor growth and metastasis.
Methods: The presence of extravasated intratumoral platelets was assessed by immunofluorescence in murin and human colorectal tumors. We characterized the interactions between platelets and colorectal tumor cells in vitro and in vivo using human and mice colorectal tumor cell lines HT29 and CT26 respectively. The iMPs generated by platelets-tumor cells interactions were purified from the interaction supernatant, characterized by flow cytometry and their roles in (i) the recruitment of monocytes to the tumor microenvironment were assessed by transwell assay and cytokines array; and (ii) in the formation of metastasis by investigating the abilities of colorectal tumor cells to interact with the endothelium in dynamic conditions.
Results: We showed that platelets extravasate in the tumor microenvironment and interact with the tumor cells in a cadherin-6 dependent manner. This interaction induces the spreading of platelets, the release of their granules content and the generation of three types of microparticles named iMPs expressing makers of platelets, tumor and both. The presence of iMPs was confirmed in patients suffering from a colorectal cancer. In the microenvironment, platelets induce a significant diminution of the tumor growth and a significant increase in the number of intratumoral macrophages. iMPs participate in the recruitment of macrophages by expressing the cytokines RANTES, MIF and CXCL12 and activate their tumor cell killing capacity through IFN-gamma and IL-4. This lead to the cell cycle arrest of the tumor in a P21 dependent pathway. In contrast, in the bloodstream, platelets and production of iMPs induce the adhesion of tumor cells to the endothelium mainly via the transfer of ß3—integrins from platelets to tumor and the activation of endothelial cells.
Summary/Conclusion: Altogether, our results indicate that, in the tumor microenvironment, Cadherin 6 -dependent platelet-tumor cells interactions induce the generation of iMPs leading to the recruitment and the activation of tumoricidal macrophages whereas, in bloodstream, platelets and circulating iMPs favor the interaction of tumor cells with the endothelium, promoting the formation of metastasis.

Cancer and Thrombosis
OS 8.4

Increased incidence of cancer in the follow-up of obstetric antiphospholipid syndrome: the NOHA-K observational study.

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Background: Antiphosphospholipid antibodies (aPL Abs) are often described in patients with cancer, cancer is one of the leading causes of death in the antiphospholipid syndrome (APS), but the incidence of cancer in APS is uncertain.
Aims: To comparatively assess the rate of incident cancers during the long-term follow-up of patients with the purely obstetric form of APS.
Methods: We performed a 17-year observational study (range: 12-22 years) of 1,592 nonthrombotic women with 3 consecutive spontaneous abortions before the 10^{th} week of gestation or 1 fetal death at or beyond the 10^{th} week of gestation. We compared the incidence of cancer diagnosis during follow-up among women positive for antiphospholipid antibodies (APS group; n=517), women carrying the *F5* rs6025 or *F2* rs1799963 polymorphism (Thrombophilia group; n=279) and women with negative thrombophilia screening results (Control group; n=796).
Results: The patients collectively contributed data for a total of 26,588 person.years. A diagnosis of cancer was made in 52 women, the annualized rate of cancer being 0.136% (0.09% - 0.21%) in the Control group, 0.172% (0.09% - 0.34%) in the Thrombophilia group and 0.300% (0.200% - 0.440%) in the APS group, hazard ratio HR: 2.224 (1.219 - 4.057), p=0.0092 between the APS and the Control groups, Kaplan-Meier estimates of cancer-free survival, log-rang test: p=0.0239. Among aPL Abs, lupus anticoagulant (HR 2.124 (1.189 - 3.796), p= 0.0109) and anticardiolipin IgM (HR: 1.881(1.069-3.308), p=0.0284) were associated with incident cancers.
Summary/Conclusion: Despite long-term primary thromboprophylaxis using low-dose aspirin, obstetrical APS women are exposed to an excess of incident cancers.Some more fundamental studies trying to elucidate the underlying pathophysiological mechanisms are warranted.

Renal Disease and Thrombosis
OS 9.1

Frequency and prognostic impact of acute kidney injury in patients with pulmonary embolism. Data from the RIETE registry.

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Background: Acute kidney injury (AKI) is associated with a poor prognosis. Although pulmonary embolism (PE) may promote AKI through renal congestion or hemodynamic instability, its frequency and impact on the prognosis of patients with acute PE have been poorly studied.
Aims: To assess the influence of PE severity on the risk for acute kidney injury (AKI). Secondary objectives were to assess the incidence and prognostic impact of AKI in this setting.
Methods: The frequency of AKI (defined according to the "Kidney Disease: Improving Global Outcomes" definition) at baseline and its influence on the 30-day mortality was evaluated in patients with acute PE from the RIETE (Registro Informatizado Enfermedad TromboEmbolica) registry. We used multivariate analysis to assess whether the presence of AKI influenced the risk for 30-day death.
Results: The study included 21,131 patients, of whom 6,222 (29.5%) had AKI at baseline: 4,385 patients (21%) in stage 1, 1,385 (6.5%) in stage 2 and 452 (2%) in stage 3. The proportion of patients with high-risk PE in those with no AKI, AKI stage 1, AKI stage 2 and AKI stage 3 was: 2.8%, 5.3%, 8.8% and 12%, respectively ($p<0.001$).After 30 days, 1,236 patients (5.9%) died. Overall mortality was 4% in patients with no AKI, 8.4% in AKI stage 1, 14% in AKI stage 2, 17% in AKI stage 3 (all $p<0.001$). AKI was independently associated with an increased risk of death at 30 days (odds ratio=1.25; 95%CI: 1.02-1.54).
Summary/Conclusion: One in every 3-4 patients with acute PE had AKI at baseline. The presence of AKI independently predicted 30-day mortality. This study suggests that AKI may deserve to be evaluated as a prognostic factor in patients with acute PE.

Renal Disease and Thrombosis
OS 9.2

Comparison of two different prophylactic regimens for the prevention of arteriovenous fistula thrombosis in patients with end stage renal disease

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Background: Hemodialysis requires vascular access with capacity to provide adequate extracorporeal blood flow, with arteriovenous fistula (AVF) currently being the one with most advantages. AVF failure may be result of complex pathophysiological changes, common complications include failure to mature, stenosis and thrombosis. There are no widely accepted pharmacological strategies to prevent AVF thrombosis. AVF failure highly affects patients quality of life and increases morbidity. Whether the improvement in the reduction of early thrombotic events may be achieved by pharmacological thromboprophylaxis has not been established so far.
Aims: To investigate the efficacy and safety of two different antithrombotic regimens: low molecular weight heparine (nadroparine) and antiplatelet drug ticlopidine, for the prevention of AVF thrombosis/dysfunction during the period of its maturation – within the six weeks.
Methods: The study included 121 patients (pts) with end stage renal disease (ESRD) who were assigned into 3 groups after the AVF was created: 40 pts who did not receive antithrombotic therapy, 42 pts who were given ticlopidine 2x125mg daily for 6 weeks and 39 pts who received 0.3ml of nadroparine daily for 6 weaks. The efficacy outcome was AVF thrombosis/dysfunction diagnosed by clinical finding and ultrasonographic imaging; safety outcome was any bleeding during the treatment.Signed informed consent was obtained from all participants and the study was conducted according to Helsinki declaration.
Results: The incidence of AVF thrombosis/dysfunction in study group was 21 out of 121 (17.3%). Among pts who received no thromboprophylaxis 12 cases of thrombosis/dysfunctional AVF occurred, in ticlopidine group 4 and in nadroparine group 5, the difference being statistically significant (p=.033). There was no statistically significant difference in AVF thrombosis occurrence between ticlopidine and nadroparine group (4/42 vs 5/39, p=.009). In total, 9 out of 81 pts who received any thromboprophylaxis were diagnosed with AVF thrombosis, compared to 12 out of 40 wthout prophylaxis. Univariant analysis revealed that the implementation of thromboprophylaxis lowers the risk for AVF thrombosis/dysfunction occurrence (OR 3.5, 95%CI 1.30-9.03, p=0.013). There was no major bleeding in study groups, in treatment groups only one case of minor bleeding was recorded in patient receiving nadroparine.
Summary/Conclusion: Prophylactic use of either antiplatelet or anticoagulant drug (ticlopidine and nadroparine) significantly reduced the incidence of early thrombosis of primary AVF for hemodialysis during its maturation.The risk of thrombosis/ dysfunction of the AVF during its maturation was threefold reduced by implementation of antithrombotic therapy. Results of our study showed no evidence of superiority of nadroparine as compared to antiplatelet treatment, both regimens were equally effective. There was no increased risk in major or clinically relevant non major bleeding in patients who received thromboprophylaxis.

Renal Disease and Thrombosis
OS 9.3

ARYL HYDROCARBON RECEPTOR-RELATED MECHANISMS OF ENDOTHELIAL TISSUE FACTOR INDUCTION BY THE UREMIC TOXIN INDOLE-3 ACETIC ACID

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Background: Chronic kidney disease (CKD) is associated with high risk of thrombosis. Indole-3 acetic acid (IAA), a uremic toxin predictive of cardiovascular events in CKD patients, induces the expression of tissue factor (TF) in human umbilical vein endothelial cells (HUVEC) via the transcription factor aryl hydrocarbon receptor (AhR).
Aims: This study aimed to understand the signaling pathways involved in AhR-mediated TF induction by IAA.
Methods: Human endothelial cells were in vitro stimulated with IAA at 50µM, the maximal concentration found in patients with CKD. Circulating TF and serum IAA levels were measured in a cohort of 92 CKD patients on hemodialysis.
Results: In vitro, IAA induced TF expression in different types of human endothelial cells: umbilical vein (HUVEC), aortic (HAoEC), and cardiac-derived microvascular (HMVEC-C). Using AhR inhibition and chromatin immunoprecipitation experiments, we showed that TF induction by IAA in HUVEC was controlled by AhR, and that AhR did not bind to the TF promoter. The analysis of TF promoter activity using luciferase reporter plasmids showed that the NF-kB site was essential in TF induction by IAA. In addition, TF induction by IAA was drastically decreased by an inhibitor of the NF-kB pathway. IAA induced the nuclear translocation of NF-kB p50 subunit, which was decreased by AhR and p38MAPK inhibition. Finally, in CKD patients on hemodialysis, circulating TF was independently related to serum IAA in multivariate analysis.
Summary/Conclusion: In conclusion, TF up-regulation by IAA in human endothelial cells involves a non-genomic AhR/p38 MAPK/NF-kB pathway. The understanding of signal transduction pathways related to AhR thrombotic/inflammatory pathway is of interest to find therapeutic targets to reduce TF expression and thrombotic risk in patients with CKD.

NET's
OS 10.1

MiR-146a contributes to arterial thrombosis development through regulating neutrophils extracellular traps (NETs) formation

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Background: Neutrophils extracellular traps (NETs) release (or NETosis) can be triggered by a great variety of infectious or sterile inflammatory stimuli. This process induces a complex response linking inflammation and immunity with thrombosis. Thus, NET perpetuation is associated with several thrombo-inflammatory diseases although the underlying mechanisms are still largely unknown. MiR-146a is an important brake of immune and inflammatory diseases and it is involved in risk for cardiovascular events.
Aims: To investigate the role of miR-146a in NETosis and arterial thrombosis.
Methods: For evaluating NET release in vivo a sepsis model was generated by injecting lipopolysaccharide (LPS) (1 mg/kg) to miR-146a -/- and wild-type (wt) mice (n=9/group). In addition, mice lacking LDL receptor (Ldlr -/-) were transplanted with bone marrow (BM) from miR-146a-/- (n=22) or wt (n=24) mice and fed with a fatty diet (HFD) for generating an atherosclerosis model. Blood was drawn and plasma was isolated before and after each procedure (4 and 24h after LPS, 8 and 20 weeks of HFD). For quantifying NET release in vitro neutrophils isolated from BM of miR-146a -/- or wt mice (n=11/group) were stimulated with PMA (20 nM, 2h). Cell-free DNA (cfDNA) was quantified by SYTOX Green. Neutrophil elastase (NE), reactive oxygen species (ROS) and thrombin-antithrombin complexes (TAT) were measured by ELISA. Citrullinated Histone H3 (citH3) was assessed by western blot and immunofluorescence (IF). Arterial thrombus formation was evaluated, after inducing a FeCl3-vascular injury in carotid arteries of miR-146a -/- (n=17) or wt mice (n=24), by measuring blood flow occlusion time.
Results: After LPS treatment, cfDNA, NE and citH3 levels in plasma were significantly higher in miR-146a -/- mice than in wt at 4h (1653.0±216.5 vs 845.6±294.4 ng/ml $p<0.01$; 1671.3±95.6 vs 1206.1±99.2 ng/ml $p<0.01$ and 1.5x107±4.6x106 vs 7.8x107±1.3x106 densitometric area $p<0.001$, respectively) and 24h (267.7±51.7 vs 76.2±12.4 ng/ml $p<0.05$; 2458.0±57.1 vs 1524.6± 61.2 ng/ml $p<0.001$ and 4.2x107±1.3x106 vs 1.7x106± 1.3x106 densitometric area $p<0.05$, respectively). In addition, ROS and TAT plasma levels increased significantly after LPS stimulation only in miR-146a -/- mice. Moreover, Ldlr -/- transplanted mice with miR-146a -/- BM showed higher levels of cfDNA and NE than wt (348.1±73.0 vs 177.3±39.4 ng/ml, $p<0.05$ and 228.6±32.6 vs 113.0±14.2 ng/ml, $p<0.05$, respectively) but only after 8 weeks of HFD. These data are being verified in atherosclerotic plaques by IF (DNA- NE). In turn, PMA treated miR-146 -/- neutrophils showed higher cfDNA and citH3 levels than wt (3671±337 vs 2841±284 ng/ml, $p<0.05$ and 5.7±0.4 vs 3.7±0.3 % ratio positive cells, $p<0.01$, respectively). Finally, in FeCl3-induced thrombosis carotid flow occlusion time was significantly shorter in miR-146a-/- mice than in wt littermates (374±21 vs 452±26 s, $p<0.05$).
Summary/Conclusion: MiR-146a deficiency significantly affects NETosis in vitro and in vivo in two inflammatory murine models (sepsis and atherosclerosis). In addition, in FeCl3-induced vascular injury model, miR-146a deficient mice accelerated thrombus formation in carotid arteries. Our data suggest that miR-146a could act as a thrombosis modulator due, in part, to the regulation of NET release.
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NET's
OS 10.2

Novel platelet-neutrophil interaction via activated αIIbß3 mediates NETosis under flow

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Background: Platelet-leukocyte interactions are important for our normal innate immune responses, but also contribute to the pathogenesis of thrombotic disorders, such as deep vein thrombosis, through the excessive production of intravascular neutrophil extracellular traps (NETs), which are highly thrombotic. In many cases the ability of platelets to bind leukocytes is dependent on their initial tethering under flow by von Willebrand factor (VWF). Precisely how platelet-leukocyte interactions are manifest, and how they influence leukocyte function remains poorly understood.
Aims: We aimed to explore the role of VWF in promoting platelet-leukocyte interactions.
Methods: We examined the influence of platelet binding to VWF under defined shear rates to mediate leukocyte interactions using Cellix microchannels coupled to a Mirus nanopump. Whole blood, plasma-free blood and isolated cells were analysed in this system. Platelet binding, leukocyte binding, changes in intracellular Ca^{2+}-release and NETosis were all analysed in real time using video fluorescence microscopy.
Results: We demonstrate that binding of glycoprotein Iba on platelets to von Willebrand factor, or the isolated recombinant VWF A1 domain, under flow 'primes' platelets resulting in intracellular Ca^{2+} release and αIIbß3 activation. This 'priming' does not induce appreciable P-selectin exposure, or lead to classical platelet activation. VWF/flow-mediated 'priming' enables platelets to bind leukocytes via a mechanism that is largely independent of P-selectin, but that is inhibited by blocking αIIbß3. We show that neutrophils and T-cells (but not monocytes or B cells) bind directly to activated αIIbß3 under flow, identifying this platelet integrin as a novel leukocyte receptor. Binding to αIIbß3 under flow causes rapid intracellular Ca^{2+} release in neutrophils, and initiates Ca^{2+}- and NADPH oxidase-dependent signaling leading to production of NETs after ~60 mins. NET production is itself dependent upon a mechanosensitive mechanism, as NETosis is markedly diminished if neutrophils are captured by αIIbß3 under static conditions.
Summary/Conclusion: Taken together, these data demonstrate a novel mechanism for platelet-neutrophil cross-talk and mechanosensitive NET production. These mechanisms may represent some of the early VWF- and platelet-dependent events leading to intravascular NET-production that can occur in deep vein thrombosis. Identification of the counter receptor on neutrophils, may provide novel therapeutic strategies that may assist in the prevention of venous thrombosis.

NET's
OS 10.3

Inhibition of protein Z-dependent protease inhibitor by neutrophil elastase may promote procoagulant activity of neutrophil extracellular traps.

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Background: Protein Z-dependent protease inhibitor (ZPI) is a physiological regulator of coagulation. It is a serpin that targets factor Xa (FXa) and factor XIa (FXIa). ZPI is one of the faster inhibitors of FXIa in plasma and is consumed by FXIa-catalyzed cleavage when coagulation is initiated by contact system activation. The contact system activation is one of the mechanisms involved in the procoagulant response induced by neutrophil extracellular traps (NETs) expelled by stimulated neutrophils. NETs provide a scaffold of DNA activating factor XII, decorated with histones that activate platelets, and neutrophil enzymes, such as elastase, that inactivate tissue factor pathway inhibitor (TFPI).
Aims: This project aims at evaluating the effect of NETs on anticoagulant activity of ZPI and at establishing a link between contact system and regulation of this pathway in the context of NETs-induced immunothrombosis.
Methods: Neutrophils were isolated from human whole blood. NETosis was induced with Phorbol Myristate Acetate (PMA, 50 nM), and stimulated cells were incubated with ZPI (2 µM). Binding of ZPI to NETs was revealed by immunofluorescence, shape of ZPI after incubation with NETs was studied by western-blotting and anti-FXIa activity of ZPI was evaluated in a chromogenic assay. Effect of purified neutrophil elastase (0.4 U/mL) on ZPI was also analyzed by electrophoresis on polyacrylamide gel (SDS-PAGE) and in an anti-FXIa chromogenic assay.
Results: After incubation on NETs, ZPI (revealed by immunofluorescence) was found specifically localized on extracellular DNA fibres (visualized by DAPI staining). ZPI remaining in the supernatant was collected and NETs-bound ZPI was recovered by treatment with DNase. Both fractions were analyzed by western-blot revealing the presence of two bands of ZPI in NETs supernatant as well as in NETs shed with DNase. The upper band (apparent molecular weight ~70 kDa) migrated at the same level than unreacted ZPI. The lower band (~63 kDa) resulted from a proteolytic cleavage of ZPI probably catalyzed by neutrophil elastase, since it was completely inhibited by concomitant treatment with alpha-1-antitrypsin. Surprisingly, no anti-FXIa activity was found in both fractions, as assessed in chromogenic assay, whereas ZPI, prior incubation with NETs, inhibited FXIa with an inhibition rate constant of $3.7x10^5$ $M^{-1}.s^{-1}$. This suggested that ZPI may undergo another inactivating cleavage not evidenced on western-blot.
Cleavage of ZPI by purified neutrophil elastase was analyzed on 4-12% SDS-PAGE. An upper band (~70 kDa), present prior addition of elastase, remained until 20 minutes, while two more bands (~63 kDa, and ~52 kDa) appeared after 5 minutes incubation. However, a complete loss of anti-FXIa activity was observed within the first minute of the reaction. Although, no significant change on electro-mobility of ZPI was observed, it was further evidenced that this loss of anti-FXIa activity was accompanied by a cleavage of a small peptide (~4 kDa) only revealed on 10-20% tricine SDS-PAGE.
Summary/Conclusion: This study shows that ZPI is sensitive to elastase-catalyzed proteolytic cleavage that inactivates its anti-FXIa activity. This cleavage likely occurs on NETs scaffold inducing a loss of ZPI anticoagulant activity. This ZPI inactivation on NETs may emphasize their procoagulant activity by impairing the regulation of contact pathway activation of coagulation.

Platelets
OS. 11.1

Platelet lamellipodia formation is not required for thrombus formation and stability

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Background: During platelet spreading, the actin cytoskeleton undergoes rapid rearrangement, forming filopodia and lamellipodia. The latter structure displays a circumferential zone with orthogonally arrayed short actin filaments. Controversial data have been published on the role of lamellipodia in thrombus formation and stability, mainly based on inhibitor studies or analyzing platelets with additional functional defects. One study showed that Rac1 deficiency leads to defective lamellipodia formation and a specific ITAM signaling defect, resulting in impaired platelet adhesion and defective thrombus formation on collagen under flow. This defect could be fully rescued by supplementation with ADP and a thromboxane A2 analogue (Pleines *et al.*, 2009), thus providing indirect evidence that lamellipodia formation might be dispensable for thrombus formation and stability.
The WAVE regulatory complex, which has been shown in other cell types to drive lamellipodia formation by enhancing actin nucleation via the Arp2/3 complex, is activated by Rac1 interaction with the WAVE complex subunit Cyfip1 (Cytoplasmic FMR1-interacting protein, also known as Sra-1). Based on this, we suggest that Cyfip1 might function as an important regulator of platelet lamellipodia formation. However, the role of Cyfip1 in platelet function is unknown.
Aims: The aim of this study was to investigate the impact of Cyfip1 deficiency on platelet physiology, and the role of platelet lamellipodia in thrombus formation and stability.
Methods: We generated megakaryocyte- and platelet-specific Cyfip1-deficient mice (*Cyfip1*$^{fl/fl}$ *Pf4-Cre*), and performed flow cytometry, immunoblotting, confocal and electron microscopy as well as *in vitro* and *in vivo* thrombus formation studies.
Results: *Cyfip1*$^{-/-}$ mice displayed normal platelet counts and a slight reduction in platelet volume, but unaltered glycoprotein surface expression. Activation of mutant platelets was only moderately reduced to all tested agonists as measured by αIIbβ3 integrin activation and P-Selectin surface exposure. Cyfip1-deficient platelets adhered to multiple adhesive surfaces including fibrinogen under static conditions but, strikingly, lamellipodia formation was completely abolished and the platelets rather exhibited an increased length and number of filopodia. Despite defective lamellipodia formation under static conditions, *Cyfip1*$^{-/-}$ platelets formed stable thrombi, when perfused *ex vivo* over collagen fibers at intermediate shear rates and *in vivo* in two models of occlusive arterial thrombosis. Similarly, the hemostatic function was unaltered in the mutant mice. Investigation of control and *Cyfip1*$^{-/-}$ platelet morphology in an induced thrombus under flow by scanning electron microscopy revealed that platelets rather form filopodia in the thrombus shell, and are flattened with filopodia-like structures when in direct contact to collagen fibers at the bottom of the thrombus.
Summary/Conclusion: Here, we provide for the first time direct evidence by analyzing Cyfip1-deficient mice that surprisingly platelet lamellipodia formation is not required for stable thrombus formation, and that morphological changes of platelets differ between a static spreading assay and thrombus formation under flow. We are currently investigating, whether platelet lamellipodia formation plays a role in other platelet-dependent processes *in vivo*.

Platelets
OS. 11.2

Critical role of platelets during lung fibrosis

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Background: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and irreversible disease leading to death between 2 to 5 years after diagnosis. Despite the significant morbidity and mortality associated with IPF its pathogenesis remains poorly understood and there is no curative treatment. Recent evidence showed that platelets are in a preactivated state in IPF patients. However, the role of platelets and activation mechanisms during the development of IPF have not been established yet.
Aims: To investigate the role of platelets during lung fibrosis
Methods: We used intratracheal instillation of bleomycin to induce pulmonary fibrosis in mice and collected their bronchoalveolar lavages (BAL) fluids, blood and lungs. Mice treated with intratracheal saline were used as controls.
Results: The mortality rate was of 60% 14 days after bleomycin instillation in Wild-Type (WT) mice compared with the control group that displayed no mortality. Platelets and white blood cells counts increased in BAL 3 days after bleomycin instillation and peaked at day 6 compared to the control group. Lung collagen content assessed by hydroxyproline levels plateaued 6 days after bleomycin administration compared to control mice. Thrombocytopenic (TP) mice challenged with bleomycin died prematurely 6 days after instillation. Remarkably, hemoglobin level was significantly higher in the BAL of bleomycin-induced TP mice compared to bleomycin-induced mice with normal platelet counts. To further identify platelet mechanisms in bleomycin-induced lung fibrosis, transgenic mice lacking the GPIbα subunit of the platelet receptor for von Willebrand factor, hIL4R/GPIbα mice, died 6 days after bleomycin administration. Similarly, mice deficient for the glycoprotein VI, the collagen receptor, died also prematurely 7 days after bleomycin instillation. However, bleomycin-challenged mice lacking the thrombin receptor PAR4 showed a similar survival rate than WT mice. Unlike the hIL4R/GPIbα and GPVI-/-mice, Par4 deficient mice do not show any sign of lung bleeding.
Summary/Conclusion: We here showed that platelets play a protective role during lung fibrosis. We uncovered that GPVI and GPIbα, but not PAR4 are critical in lung fibrosis. Our studies require further experiments to explore other platelets signaling molecules and highlight a potential clinical complication of future anti-platelet agents.

Platelets
OS. 11.3

Effect of platelet-specific protein S depletion in murine haemostasis.

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Background: Anticoagulant plasma protein S (PROS) is essential for haemostasis. About 2.5% of PROS in blood is stored in platelet α-granules and released upon platelet stimulation.[12] Although platelet PROS is cleaved by proteases in platelets upon its release, it has been shown to maintain part of its anticoagulant activity.
Aims: The aim of this study is to elucidate the role of the GAS6/PROS-TAM system in platelet plug stabilization and to ascertain its therapeutic potential for new antithrombotic therapies, using *Gas6* knockout mice and a conditional floxed knockout allele for the *Pros1* gene deleted selectively in platelets. We studied both the haemostatic phenotype and the functionality of their platelets.
Methods: Platelets were isolated from platelet-specific ProS-targeted mice; Gas6-knockout mice and wild types. Presence of platelet PROS was tested by immunoblotting and immunofluorescent confocal microspcopy. In vivo models were used to asses the effect of PROS depeletion in platelets, including tail bleeding, carotid artery thrombosis; pulmonary thromboembolism and venous thrombosis through inferior vena cava ligation model. Further, isolated platelets were obtained and compared using thromboelastography, adhesion assays and biochemical techniques.
Results: *Pf4-Cre*/*Pros1*$^{fl/fl}$ mice showed no detectable expression of *Pros1* at mRNA and protein level in platelets, while PROS concentration was similar in plasma. In a tail bleeding model, *Pf4*-Cre/*Pros1*$^{fl/fl}$ mice showed higher bleeding time than WT mice (345.8±31.8 s vs 145.0±23.9 s; P<0.0005). Similarly, GAS6-deficient animals showed increased bleeding time (580.1±7.7 s). Interestingly, *Pf4-Cre*/*Pros1*$^{fl/fl}$ mice showed a similar behaviour to WT mice in a carotid artery thrombosis model and in a model of collagen and epinephrine induced pulmonary embolism. However, an inferior vena cava ligation model of venous thromboembolism resulted in larger thrombi in *Pf4-Cre*/*Pros1*$^{fl/fl}$ mice compared to WT animals (12.3±0.5 vs 6.4±0.3 mg; P<0.001). In all models, *Gas6*-/- mice showed less thrombosis, in line with results on record. Isolated PROS or GAS6-deficient platelets aggregated less than WT ones in response to ADP and collagen, but not in response to thrombin.
Summary/Conclusion: Our data suggest that PROS acts locally as a modulator of platelet activation, reinforcing a pro-aggregant response. However, lack of platelet ProS also increases thrombus size, indicating that platelet PROS provides a regulatory anticoagulant mechanism inside the growing thrombus.

Acquired Platelet Impairment
OS. 12.1

Shedding of platelet adhesion receptors GpIb and GpVI under continuous-flow mechanical circulatory support

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Background: Continuous-flow mechanical circulatory support (CF-MCS) including Extra corporeal Membrane Oxygenator (ECMO) expose blood to high shear stress promoting Von Willebrand factor proteolysis by ADAMTS13. High shear stress was also reported to induce a proteolytic shedding of platelet adhesion receptors GpIb and GpVI in a non physiologic Cone and Plate viscometer model but was not explored in CF-MCS.
Aims: To assess if CF-MCS promotes GpIb and GpVI shedding *in vitro* and *in vivo*
Methods: Platelet shedding was first investigated *in vitro* using a CF-MCS (Impella-CP®, Abiomed) loop model. CTAD-anticoagulated plasma with normal platelet count (plasma-NPC) was obtained by dilution of platelet-rich plasma collected from healthy donors in fresh frozen plasma. Sampling was performed before and after 5, 30 and 180 min perfusion of plasma-NPC in the loop model (n=4 runs). Platelet shedding was also investigated in EDTA blood samples collected from 13 ECMO patients after informed consent. GpIbα and GpVI shedding were analyzed by flow cytometry and expressed as a mean fluorescence intensity (MFI) versus baseline ratio.
Results: A significant time-dependent decrease of GpIbα ($p<0.01$) and GpVI ($p<0.001$) MFIs were observed after 180 min in the Impella®-CP loop model. *In vivo* baseline GpIbα and GpVI MFIs were significantly decreased before ECMO implantation compared to healthy subjects ($p<0,001$). After ECMO implantation, no significant variation of GpIbα and GpVI MFIs were observed.
Summary/Conclusion: CF-MCS induces platelet GpIb and GpVI shedding *in vitro.* Increased GpIb and GpVI shedding are already present before ECMO implantation and remain significantly elevated after implantation.

Acquired Platelet Impairment
OS. 12.2

Rivaroxaban and Apixaban Reduce Thrombin-dependent Platelet Aggregation

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Background: Rivaroxaban and apixaban are direct oral anticoagulants whose target specificity is to activate factor X (FXa). It is still not fully understood how xabans impact platelet function.
Aims: This single-center observational study aimed to assess in vitro platelet function in patients with atrial fibrillation receiving rivaroxaban or apixaban.
Methods: It examined quantification of platelet aggregation assessed by light transmission aggregometry in thirty-four patients treated with apixaban or rivaroxaban. LTA was tested on the Chrono-Log model 700 (Chronolog Corp, Havertown, Pennsylvania, USA). Platelet aggregability (%) was assessed on the basis of the change in plasma turbidity after the addition of the γ-thrombin in final concentration 100 nmol/L (Mybiosource Inc., San Diego, USA).
Results: The thrombin-induced platelet aggregation was significantly lower two hours after taking selected xabans compared to baseline value (69.55% ± 32.15% vs. 44.79% ± 34.97.9%; p<0.0001). This effect was only observed in patients who received rivaroxaban or apixaban for more than one week.
Summary/Conclusion: The thrombin-induced platelet aggregation is reduced in cardiovascular patients receiving rivaroxaban or apixaban. This reduction is likely to depend on the duration of the treatment. Duration of treatment should be considered in future studies focusing on DOACs and platelet aggregation. Acknowledgements: The study was supported by the VEGA 1/0187/17 grant.

Acquired Platelet Impairment
OS. 12.3

Platelet function during extracorporeal membrane oxygenation (ECMO) in adult patients

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Background: Extracorporeal membrane oxygenation (ECMO) is used to provide respiratory and/or cardiac support to critically ill patients suffering from acute pulmonary and/or cardiac failure. Bleeding and thrombosis are frequent complications, predominantly due to the coagulopathy that arises during ECMO treatment.
Aims: Firstly, to investigate platelet function in adult patients treated with ECMO. Secondly, to investigate the association between platelet function and the incidence of bleeding and thrombosis during ECMO treatment.
Methods: We plan to include 25 patients undergoing ECMO treatment at the Intensive Care Unit, Aarhus University Hospital, Denmark. The first blood sample is collected on the first morning following ECMO initiation. Subsequently, blood samples are obtained on the 3rd, 14th and 21st day, if the patient is still receiving ECMO treatment. Platelet function is evaluated by whole blood impedance aggregometry (Multiplate®Analyzer) using adenosine diphosphate (ADPtest, 6.5 μM), arachidonic acid (ASPItest, 0.5 mM), and thrombin-receptor-agonist-peptide-6 (TRAPtest, 32 μM) as agonists. On the 1st and 3rd day, platelet function is also assessed by flow cytometry (Navios) using collagen-related peptide (0.12 μg/ml), ADP (10.8 μM), TRAP (28.5 μM), and arachidonic acid (0.58 mM) as agonists to induce platelet activation. Moreover, we employ a recently published model implying adjustment for platelet count in impedance aggregometry. The study compiles with the Declaration of Helsinki, and the Danish Data Protection Agency approved the study (Ref. no. 1-16-02-712-17).
Results: Blood samples are currently being collected and analyzed. To date, impedance aggregometry and flow cytometry analyses have been conducted on 17 patients in total; Blood samples from all 17 patients were analyzed on day 1 of ECMO treatment; 11 patients were still on ECMO and analyzed on day 3; A single patient was still receiving ECMO treatment and analyzed on days 14 and 21. We expect the inclusion to be completed September 2019. Preliminary results will be ready for presentation at the congress October 2018.
Summary/Conclusion: The study contributes with new knowledge regarding platelet function in adult patients treated with ECMO. By clarifying the role of platelets in the coagulopathy occurring in these patients, we aim to improve their treatment and reduce the incidence of bleeding and thrombotic complications.

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Inherited Bleeding Disorders
OS. 13.1

Improved survival of patients with haemophilia in the Netherlands in the 21st century: preliminary results

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Background: In the Netherlands, health-related outcomes of patients with haemophilia, such as mortality, have been studied closely for more than 45 years. This research showed an increased overall mortality, especially in patients with severe haemophilia and patients infected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV).[1] The prevalence of HIV and HCV has decreased since safe products became available 25 years ago. However, information on mortality since the start of this millennium is lacking.
Aims: To assess all-cause mortality and life expectancy among patients with haemophilia in the Netherlands between 2001 and 2018.
Methods: We performed a cohort study between 2001-2018 among patients with haemophilia in the Netherlands. Baseline information on HIV, HCV and inhibitor status was obtained from the 2001 nationwide questionnaire that was filled in by 1066 patients, 70% of the haemophilia population at that time. Information on type and severity of haemophilia and all-cause and cause-specific mortality was provided by Dutch haemophilia treatment centres. The rate of all-cause mortality among patients with haemophilia was compared to that of the Dutch general male population by calculating standardized mortality ratios (SMR). In addition, median life expectancy was calculated using left truncated survival analysis. The current preliminary results were compared with previous cohorts.[1]
Results: Currently, follow-up data are available for 940 patients (86%). Between 2001 and 2018, 126 patients (13%) died. Median age at death was 68.9 years (range 16-98 years). Overall, mortality was 1.5 times higher in patients with haemophilia than in the general population (SMR: 1.5, 95%CI: 1.3-1.8). The SMR was 2.6 (95%CI: 2.0-3.3) for severe haemophilia, 1.2 (95%CI: 0.7-1.9) for moderate haemophilia and 1.1 (95%CI: 0.8-1.4) for mild haemophilia. Restricting the analysis to patients without HIV or current/previous HCV infections yielded an overall SMR of 1.1 (95%CI: 0.9-1.5). For severe, moderate and mild haemophilia, this was 2.2 (95%CI: 1.2 – 3.7), 1.2 (95%CI: 0.6 – 2.1) and 1.0 (95%CI: 0.7 – 1.3) respectively.
Life expectancy was 77 years for all patients in comparison to 80 years for the Dutch male population. Median life expectancy was 73 years for severe and 78 years for moderate and mild haemophilia. Overall life expectancy after exclusion of patients with HIV and HCV was 78 years. For severe, moderate and mild haemophilia life expectancy was 73, 78 and 79 years respectively after exclusion of patients with HIV and HCV.
Mortality has improved in comparison to previous cohorts.[1] An improvement in SMR was seen in the overall population (SMR 2.3 to 1.5) and particularly in severe and moderate haemophilia (SMR 5.1 to 2.6 and 2.6 to 1.2 respectively). Life expectancy increased in the overall population (67 to 77 years), especially in severe haemophilia (59 to 73 years).
Summary/Conclusion: Mortality and life expectancy have improved since 2001 and are similar to the general Dutch male population when patients with HIV and HCV are excluded. However, in patients with severe haemophilia without HIV and HCV, mortality is still increased in comparison to the general population.

Reference

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Inherited Bleeding Disorders
OS. 13.2

Molecular defects in the F7 gene of patients with severe factor VII deficiency in Iran

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Background: Inherited factor VII (F7) deficiency is known as a rare autosomal recessive disorder with an estimated prevalence of 1:500 000 in the developed countries. According to the WFH annual survey in 2016, 690 cases has been reported from Iran, i.e. five times higher in prevalence compared with Europe, mainly due to the frequent first cousin marriages. Plasma levels of FVII can reach to low or undetectable levels resulting in severe bleeding which can be lethal in some patients. Management of inherited F7 deficiency becomes more complicated and life-threatening when patients develop alloantibodies in response to replacement therapy using rFVIIa.
Aims: The present study was aimed to identify pathogenic genome variations in patients suffering from bleeding complications linked to the low level or absence of circulating F7 with severe phenotype of F7 deficiency that might be related to their clinical manifestation. Additionally, we intended to investigate the relation between genetic profile of *F7 gene* in the studied patients and development of inhibitors.
Methods: After obtaining informed consent from all participants in accordance with the Helsinki Declaration, genomic DNA was isolated from peripheral blood of 47 unrelated patients with severe F7 deficiency (FVII: C<2%) and 42 of their close relatives. The nine exons, their intronic boundaries and untranslated region of *F7* gene were directly sequenced by Sanger sequencing.
Results: Sequence alteration were identified in 43 probands in homozygous state. Mutations occurred in the signal peptide and the pro peptide sequence were five M1V and a G22S. A splice mutation, IVS3+1G>T in the EGF1 domain was characterized. Mutations occurred in the EFG2 domain were two C151S and three G156S. In the heavy chain containing the activation peptide and the catalytic domain identified mutations were three R212X, two R212Q, two G239R, nine Leu264X (c.790delC), two G343S, a P363T, three C370F, three H408R, a W416X, three T419M, a deletion/insertion mutation W424F (c.1271-1272delGG and InsTT), a A429T and a novel A deletion in position c.1346. All mentioned mutations where detected in heterozygous state in parents or siblings of the index patient whenever possible.
All nine individuals carrying deletion C in c.790 suffered from severe bleeding symptoms in early infancy (FVII: C<1%). Spontaneous cerebral hemorrhage occurred in four patients with C deletion (c.790) shortly after birth that in one case has caused irreversible brain damage. Two patients with the same C deletion developed inhibitors to recombinant F7 at age 3 and 8 years old (191 and 170 BU mL^{-1} respectively). In the remaining four index cases in our group no pathogenic mutations could be detected in the F7 coding, splice site and promoter regions.
Summary/Conclusion: Presence of founder effect is suggested for identical *F7 gene* alterations according to common geographical origin. The single nucleotide deletion C at c.790 leading to a premature stop codon was present in both alleles of nine probands and in one allele of 17 close relatives. The origin of these familes was from southwest of Iran and mainly with Lor ethnicity. Another common mutation, M1V, was seen in families originated from northeast of Iran. The presence of so many founder mutations not only facilitates genetic counseling and carrier detection but also could be helpful in better understanding of the correlation between genetic defects and bleeding symptoms and anticipating development of inhibitory antibodies.

Inherited Bleeding Disorders
OS. 13.3

AAV-mediated gene transfer of engineered factor IX fusion proteins does not increase factor IX plasma level overtime in mice

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Background: The variety of treatment for Haemophilia B (HB), an X-linked bleeding disorder characterised by coagulation factor IX (FIX) deficiency, has recently improved with the emergence of both AAV-based gene therapy (1, 2) and engineered human FIX (hFIX) molecules with enhanced half-life due to genetic fusion to either albumin (Alb) or immunoglobulin Fc fragment (Fc).
Aims: To explore if the prolonged half-life of hFIX-Alb and hFIX-Fc fusion proteins translates to higher plasma levels of hFIX in the context of AAV-based gene therapy for HB.
Methods: Single-stranded cross-packaged AAV2/8 vectors expressing hFIX-Alb, hFIX-Fc and hFIX were evaluated *in-vitro* after transduction of Huh-7 cells, and *in vivo* in C57BL/6J mice. The expression levels of hFIX-Alb, hFIX-Fc and hFIX proteins were evaluated up to 8 weeks after injection of of 5 x 10^{10} vg per mouse of these vectors.
Results: *In vitro*, both hFIX-Alb and hFIX-Fc fusion proteins were synthesised and expressed as single chains of expected size following AAV mediated gene transfer in Huh-7 cells. The procoagulant properties of these hFIX-fusion proteins were comparable to wild-type hFIX. However, their expression levels were 3-fold lower than wild-type hFIX *in vivo* and did not increase overtime. Additional experiments, including the study of the cell trafficking, suggested that the lower level of hFIX-fusion proteins was most likely due to inefficient secretion from transduced liver cells.
Summary/Conclusion: We present here the first evaluation of hFIX-fusion proteins in the context of AAV gene transfer. Our results suggest that the hFIX-fusion proteins are secreted inefficiently from the liver, thus preventing their optimal use in gene therapy approaches.

Inherited Bleeding Disorders
OS. 13.4

Severe postpartum hemorrhage as a first presenting symptom of a bleeding disorder

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Background: Postpartum hemorrhage (PPH) is one of the major causes of maternal death worldwide. It is not yet known how frequent postpartum hemorrhage is a first presenting symptom of an underlying bleeding disorder.
Aims: To investigate the prevalence of bleeding disorders in women with excessive PPH, not previously diagnosed with a bleeding disorder.
Methods: Women with excessive primary PPH, defined as ≥2L blood loss in the first 24 hours after delivery, without a pre-existing bleeding disorder, referred to the Hematology clinic for hemostatic evaluation between 2011 and 2017, were retrospectively investigated. Data about obstetric risk factors for PPH (uterus atony, retained placenta, preeclampsia, and several others), bleeding symptoms (ISTH-BAT), and hemostatic variables (at least three months postpartum) were collected. A bleeding disorder was diagnosed based on (inter)national guidelines. A bleeding disorder of unknown cause (BUC) was defined as bleeding in medical history with normal laboratory results and necessity for treatment during hemostatic challenges in the future, based on the severity of the bleeding tendency. A group of women without PPH (<0.5L blood loss in the first 24 hours after delivery) giving birth between 2009 and 2010, was included as a control group. This study was not subject to the Medical Research Involving Human Subjects Act and approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam.
Results: In total, 85 women with excessive PPH were included, with a median blood loss of 3L (IQR 2,5-4L). Hemostatic variables were available of 79 women. In 16 (20%) of these women a bleeding disorder was diagnosed. Mild Von Willebrand Disease type 1 (VWF:Ag 30-60 U/ml) was most prevalent (9/16), followed by a platelet function disorder (5/16), and BUC (2/16). In total, 45 women without PPH were included. Four women (9%) in the control group had VWF:Ag levels below 60 U/ml. No significant differences in hemostatic variables (platelet count, PT, aPTT, fibrinogen, VWF:Ag, and FVIII:C) between women with excessive and without PPH were found. Women with excessive PPH had significantly more uterus atony ($p<0.01$), retained placenta ($p<0.01$) and pre-eclampsia ($p<0.01$) as obstetric risk factors for PPH. Women without PPH had significantly more augmented labors ($p<0.05$), instrumental deliveries ($p<0.05$) and episiotomies ($p<0.05$). No significant associations, however, were found between mode of partus (OR 1.5, 95%CI 0.98-1.01), retained placenta (OR 1.0, 95%CI 0.97-1.04), uterus atony (OR 1.0, 95%CI 0.98-1.01) or ruptures/ (OR 1.0, 95%CI 0.95-1.04) in women with and without a bleeding disorder. In total 25/85 women gave birth again after hemostatic evaluation. Of these women, 60% (15/25) had recurrent PPH, of whom 9/15 had recurrent excessive PPH.
Summary/Conclusion: In 20% of women with excessive blood loss a bleeding disorder was diagnosed. This is more than previously described. This high number of hemostatic diagnosis found, although mild, does implies that excessive PPH can be a first clinical relevant symptom of a bleeding disorder. This can guide clinical management during hemostatic challenges, hereby preventing bleeding complications later in life. Based on the high number of obstetric risk factors present, PPH most probably has a multifactorial cause. Therefore, proactive anticipation of (recurrent) PPH in women with risk factors and prior PPH is recommended.

Thrombosis Biomarkers
OS. 14.1

Identification of endothelial derived plasma biomarkers associated with cardiovascular disease risk factors

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Background: Cardiovascular disease is a major health problem in the western world, causing over 4 million deaths per year in Europe, mostly related to complications from atherosclerotic disease such as myocardial infarction (MI) and stroke. The vascular endothelium, composed by the endothelial cells (EC) that line the inside of all vessels, has a key role in the maintenance of cardiovascular homeostasis. Its structural integrity is crucial for normal function, and injury or inappropriate activation ('endothelial dysfunction') is central to atherosclerotic cardiovascular disease (CVD) and is present in several CVD risk conditions (e.g. diabetes, smoking). Expression specificity of a protein is a prerequisite for it to be used as marker of injury/disease of a particular tissue or cell type. Previously, our group identified the core EC enriched transcriptome (Butler et al, 2016), using unfractionated RNAseq data generated as part of the Human Protein Atlas (HPA) project. The identified EC-specific/enriched proteins are interesting candidates to pursue as potential markers specific for vascular injury/dysfunction, as no such marker exists in routine clinical use today. Such markers would hold potential clinical value in identifying asymptomatic individuals with advanced atherosclerosis at high risk of future CVD events such as MI and stroke, to select patients for primary prevention and optimised risk intervention.
Aims: To identify and validate EC specific or enriched proteins for which plasma levels are associated with acquired CVD risk factors
Methods: The study population comprised the Swedish CArdioPulmonary bioImage Study (SCAPIS) pilot study that recruited and investigated 1111 men and women aged 50 to 65 years with detailed medical examination including blood values (e.g. lipids, hsCRP), diagnosis of diabetes, blood pressure and body-mass-index (BMI) as well as imaging and functional analyses of cardiovascular and pulmonary systems (e.g. CT scan). Additionally, information from self-reported lifestyle questionnaire (e.g. smoking) was collected. Plasma samples of all participants were analysed using affinity proteomics; suspension bead arrays, based on multiplexed single binder immunoassays, were used for screening of 295 EC specific/enriched protein candidates, using antibodies from the Human Protein Atlas resource
Results: We identified distinct protein profiles associated with acquired CVD risk factors (e.g. smoking, diabetes, BMI/obesity, hypertension). Protein levels of several candidates showed strong associations with age or gender. 55 of the 295 candidates were associated ($p<0.01$) with two or more cardiovascular risk factors, after adjusting for age and gender. With a more stringent Bonferroni corrected P-value 1.69E-04, 17 candidates were associated with 2 or more cardiovascular risk factors. Higher plasma levels of Von Willebrand factor (vWF) were associated with obesity/BMI ($p=2.89E-09$), hypertension ($p=1.74E-04$) and diabetes ($p=0.001$). Several of the identified proteins are previously unknown to be associated with cardiovascular disease or cardiovascular risk factors.
Summary/Conclusion: We found associations between plasma levels of EC enriched proteins and acquired cardiovascular risk factors (e.g. smoking, diabetes, BMI/obesity, hypertension), which could represent new biomarkers for endothelial dysfunction and CVD. Additional studies will include replication in new clinical materials, representing both symptomatic and asymptomatic atherosclerosis, to validate our findings.

Thrombosis Biomarkers
OS. 14.2

Plasma levels of growth differentiation factor 15 are associated with future risk of venous thromboembolism

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Background: Growth differentiation factor 15 (GDF-15), a marker of inflammation and oxidative stress, is a well-established biomarker for arterial cardiovascular diseases. However, the association between GDF-15 and future risk of incident venous thromboembolism (VTE) remains uncertain.
Aims: To investigate the association between plasma levels of GDF-15 and future risk of incident VTE in a nested case-control study.
Methods: The study population consisted of 416 subjects with incident VTE, and 848 age- and sex-matched controls who were randomly selected from the fourth survey of the Tromsø study. Baseline information was collected by physical examination and non-fasting blood samples in 1994-95. Unconditional logistic regression models were used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE across quartiles of plasma levels of GDF-15. The quartile cut-offs of GDF-15 were derived from the control group. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: The risk of VTE increased linearly across quartiles of GDF-15 plasma levels (p for trend 0.002) in models adjusted for age, sex and body mass index. Similar results were obtained for subgroup analyses of provoked and unprovoked events, and deep vein thrombosis (DVT) and pulmonary embolism (PE). Participants with GDF-15 values in the highest quartile (≥359 pg/mL) had an OR for VTE of 2.05 (95% CI 1.36-3.10) compared to those with GDF-15 in the lowest quartile (<200 pg/mL). In analyses restricted to unprovoked events, the OR was 1.8-fold higher for subjects with GDF-15 levels in the highest compared to the lowest quartile (OR 1.83, 95% CI 1.04-3.19). Subjects with plasma GDF-15 levels in the highest quartile had a 2.2-fold higher OR for provoked events compared to those with plasma GDF-15 levels in the lowest quartile (OR 2.24, 95% CI 1.34-3.75). The ORs for DVT and PE were 1.99 (95% CI 1.21-3.24) and 2.16 (95% CI 1.19-3.91), respectively, when comparing GDF-15 values ≥359 pg/mL to values <200 pg/mL. Further adjustment for C-reactive protein (CRP) yielded essentially similar ORs for VTE, both overall and in subgroup analyses.
Summary/Conclusion: In the present study, we found a dose-response relationship between plasma GDF-15 levels and risk of future VTE, which was independent of chronic inflammation assessed by CRP. Our findings may suggest that oxidative stress is associated with increased risk of VTE.

Thrombosis Biomarkers
OS. 14.3

The Role of Thrombin Generation in Cardiovascular Disease and Mortality – Results from the Population-based Gutenberg Health Study

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Background: Thrombin formation is one of the key enzymatic processes that direct the activity of the hemostatic system. Thrombin generation (TG), a method addressing the overall potential of a given plasma sample to form thrombin, may be a potential tool to improve risk stratification for cardiovascular diseases (CVD).
Aims: This study was set up to evaluate the association between TG parameters and demographic characteristics such as age and sex. Furthermore, it assesses the relation between TG parameters and traditional cardiovascular risk factors. Lastly, it aims to explore the association between TG parameters and total mortality.
Methods: For this study, N=5000 subjects from the population-based Gutenberg Health Study were analyzed in a highly standardized setting. TG was measured by the Calibrated Automated Thrombogram method at 1 and 5 pM tissue factor (TF) trigger in platelet poor plasma. Lag time, endogenous thrombin potential (ETP), and peak height were derived from the TG curve. Sex-specific multivariable linear regression analysis adjusted for age, CVRFs, CVD and therapy (vitamin K antagonists, oral contraceptives and hormonal replacement therapy), was used to analyze the determinants of TG. Cox regression models adjusted for age, sex, CVRFs and vitamin K antagonists investigated the association between TG parameters and total mortality.
Results: Lag time (at 1 and 5 pM TF) was positively associated with obesity and dyslipidemia for both sexes ($p<0.0001$). Additionally, obesity was a positive determinant of ETP (at 1pM and 5 pM TF) in both sexes ($p<0.0001$) and peak height in males (1 pM TF, $p=0.0048$) and females (1 pM TF and 5 pM TF, $p<0.0001$). Cox regression models showed an increased mortality in individuals with a lag time (1 pM TF, HR=1.46, [95% CI: 1.07; 2.00], p=0.018) and ETP (5 pM TF, HR = 1.50, [1.06; 2.13], p=0.023) above the 95th percentile of the reference group, independent of the cardiovascular risk profile. Kaplan Meier survival curves showed a decreased survival in individuals with a lag time above the 90th percentile of the reference (1 and 5 pM TF, $p<0.0001$) and ETP above the 97.5th percentile of the reference (1 pM TF, $p=0.00097$).
Summary/Conclusion: This large-scale study provides important insights in the effects of traditional CVRFs, particularly obesity, on TG in males and females. Lag time and ETP were found as potentially relevant predictors of increased mortality in the general population, which deserves further investigation.

Thrombosis Biomarkers
OS. 14.4

Validation of plasma marker candidates for VTE using affinity and mass spectrometry-based proteomics indicate a link to alternative complement pathway

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Background: Venous thromboembolism (VTE), has an incidence of 1-2/1,000 year, high mortality and 25% recurrence rate. Better clinical tools are needed for diagnosis and risk prediction of recurrence.
Aims: To verify and validate novel plasma markers for diagnosis and risk prediction of VTE and to study their relevance to disease development.

Methods: This work is based on a first discovery proteomics screen in the Swedish Venous ThromboEmbolism BIOmarker Study (VEBIOS) study, using multiplexed suspension bead arrays with antibodies from the Human Protein Atlas project, targeting 408 proteins. The discovery sample set (n=270) contained both patients sampled when presenting with acute VTE and patients sampled after discontinuation of anticoagulant treatment following a first-time thrombosis. Antibody target verification was performed by immunoprecipitation-mass spectrometry (IP-MS) analysis and quantitative bead based dual binder immunoassays were developed for validation and replication in the French FARIVE study (n=1200). pQTL analysis was performed using genetic data from the FARIVE and the MARTHA studies. Functional analysis with recombinant protein in platelet activation assays were performed using platelets and plasma from healthy donors.
Results: From a panel of proteins significantly associated with VTE in VEBIOS, we selected one biomarker candidate that was significantly associated with acute VTE after Bonferroni correction ($p=1.1e-05$) and also associated to risk of VTE in patients sampled after discontinuation of anticoagulants ($P<0.01$). IP-MS revealed a target involved in complement regulation. In house developed dual binder quantitative assays verified an association between increased risk of VTE and plasma levels in both the acute set and the follow up set of the VEBIOS study ($p<0.001$) and was replicated the FARIVE ($p<0.001$). pQTL analysis in revealed cis-regulatory SNPs. Functional studies indicated enhanced agonist-induced platelet activation in presence of recombinant protein.
Summary/Conclusion: Our results suggest that increased levels of a biomarker candidate involved in complement activation is associated with both acute VTE and VTE risk. Preliminary analysis indicates a possible causal effect through the potentiation of platelet activation.

Vessel Wall
OS. 15.1

Role of LIGHT deficiency in the development of atherosclerosis and atherothrombosis in mice.

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Background: Cardiovascular disease usually stems from vascular dysfunction, as a result of thrombosis developing on ruptured/eroded atherosclerotic plaque and its ischemic complications. Body of evidence is suggesting the involvement of the costimulatory molecule LIGHT/TNFSF14 in cardiovascular pathologies, in particular atherosclerosis and thrombosis. LIGHT is present in human atherosclerotic lesions and accumulates in thrombi formed on disrupted plaques. Besides, platelet-derived soluble LIGHT activates the endothelium and conditions platelet adhesion.
Aims: Thus we aimed to evaluate the role of LIGHT in atherosclerosis development and thrombosis in the inflammatory setting of atherosclerosis.
Methods: We generated mice lacking LIGHT that are prone to atherosclerosis ($ApoE^{-/-}/LIGHT^{-/-}$) and compared them with $ApoE^{-/-}$ mice. Atherosclerosis development (from 15 to 80 weeks of age) was studied in aortic sinus plaques. *In-vivo* thrombosis was evaluated on a model of carotid atherosclerotic plaque rupture and in FeCl3-injured mesenteric arteries. Hemostasis was analyzed measuring tail-bleeding time and platelet aggregation studied in response to ADP and collagen.
Results: Immunohistochemical characterization of aortic sinus lesions shows that LIGHT deficiency leads to increased size of atheromatous plaques (2-3 fold increase at 50 and 80 weeks, $p<0.005$), without affecting their lipid compositions. This was accompanied by a significant increase in inflammatory cell infiltration i.e. macrophages, neutrophiles and T-lymphocytes at advanced time points (50 and 80 weeks). Furthemore, we observed enhanced smooth muscle cells proliferation and collagen deposits at 30, 50 and 80 weeks old $ApoE^{-/-}/LIGHT^{-/-}$ mice as compared to $ApoE^{-/-}$.
To further evaluate the implication of LIGHT in pathological complications of atherosclerosis, we examined thrombosis on injured atherosclerotic plaques in 50 weeks old $ApoE^{-/-}$ and $ApoE^{-/-}/LIGHT^{-/-}$ mice. In absence of LIGHT, a 2-fold increase in thrombus area ($p<0.03$) was observed on ruptured plaques. In contrast, LIGHT deficiency had no effect on the occlusion time of FeCl3-injured arteries in mice devoided of atherosclerosis.
Evaluation of haemostasis reveals that atherosclerosis development results in prolongation of the tail-bleeding time which is prevented in the absence of LIGHT ($ApoE^{-/-}$ vs $ApoE^{-/-}/LIGHT^{-/-}$: 10 weeks: 98±1 vs 111±17 s; 30 weeks: 145±18 vs 94±7 s, p±0.01). To test whether these effects of LIGHT on thrombosis and haemostasis can originate from platelet hyperactivity, we measured platelet aggregation in $ApoE^{-/-}$ and $ApoE^{-/-}/LIGHT^{-/-}$ mice. Evaluation of platelet aggregation in response to ADP and collagen revealed that LIGHT deficiency increased platelet reactivity in response to low dose agonists (ADP 2.5 μM: 43.3±6.2 vs. 70.8±3.1% and 1.25 μM: 29.7±4.6 vs. 60.6±4.2% in $ApoE^{-/-}$ vs. $ApoE^{-/-}/LIGHT^{-/-}$ respectively) in the presence of advanced atherosclerotic lesions.
Summary/Conclusion: In summary, LIGHT plays a protective role on atherogenesis and modulates thrombosis and haemostasis during atherosclerosis at least through platelet aggregation modulation.
Knowing the cellular and molecular mechanisms linking LIGHT to atherothrombosis will pave the way to the development of new strategies for the prevention and treatment of cardiovascular diseases.

Vessel Wall
OS. 15.2

Microvesicles from T cells overexpress miR-146b-5p in HIV-1 infection and repress endothelial activation

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Background: Human immunodeficiency virus type 1 (HIV-1) promotes a generalized activation of host responses that involves not only CD4 T cells, but also cells of the microenvironment, which are not directly infected, such as endothelial cells. The mechanisms triggering HIV-1-associated vascular alterations remain poorly understood. Microvesicles (MVs), implicated in cell-to-cell communication, have been recently described as carriers of microRNAs (miRNAs).
Aims: In this study, we hypothesized that HIV-1 infection induces cellular miRNA expression in CD4 T cells, which may be carried by MVs and transferred in a paracrine manner to endothelial cells to regulate vascular homeostasis.
Methods: To test the hypothesis that HIV-1 infection can induce miRNA expression in CD4 T cells, which may be transferred to endothelial cells by MVs, we first developed an *ex vivo* model to obtain CD4 T cell-derived MVs from HIV-1-infected patients. To identify miRNAs of interest in CD4 T cell-derived MVs from ART-naive HIV-1-infected patients, we evaluated the differentially expressed miRNAs in both CD4 T cells and CD4 T cell-derived MVs from ART-naive HIV-1-infected patients in comparison with CD4 T cells and CD4 T cell-derived MVs from healthy subjects. To investigate the ability of MVs to protect miRNA content from degradation by RNase, we used MVs generated from CEM cells (CEM-MVs).We next generated an in vitro model system using CEM cells and human umbilical vein endothelial cells (HUVEC) to determine whether CEM-MVs can transfer RNAs to endothelial cells. We next investigated whether the systemic administration of miR-146b-MVs can repress endothelial activation in lungs. To this end, miR-146b-MVs were injected into mice 24 hours prior to the injection of vehicle or TNFα for 4 h.
Results: Here, we show that miR-146b-5p is upregulated in both CD4 T cells, CD4 T cell-derived MVs and circulating MVs obtained from antiretroviral therapy-naive HIV-1-infected patients. We further demonstrate that MVs from T cell line overexpressing miR-146b-5p mimics (miR-146b-MVs): 1) protect their miRNA cargo from RNase A degradation, 2) transfer miR-146b-5p mimics into endothelial cells and 3) reduce endothelial inflammatory responses in vitro and in vivo in the lungs of mice through the downregulation of nuclear factor-kB-responsive molecules.
Summary/Conclusion: These data advance our understanding on chronic inflammatory responses affecting endothelial homeostasis, in infectious and non-infectious diseases and pave the way for potential new anti-inflammatory strategies.

Vessel Wall
OS. 15.3

Sphingomyelin phosphodiesterase in targeted treatment of vascular calcification

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Background: Vascular calcification is an independent risk factor for development of cardiovascular disorders, with currently no therapy to treat or prevent it. In patients with vascular calcification vessel wall undergoes remodeling supported via extracellular vesicle(EV) release. Sphingomyelin phosphodiesterase-3 (SMPD3) is recognized as a key signaling molecule in extracellular vesicle release and calcification. Therefore the objectives are to develop targeted therapeutic strategies that limit EV formation by inhibiting SMPD3, with the focus on drug discovery and drug testing (High Content Analysis and preclinical evaluation), by combining in silico approaches utilizing structural bioinformatics methods (identification and prioritization of druggable pockets in target protein SMPD3) and Structure-Based Virtual Screening of small molecule inhibitors of calcification. Cell-based assay are used as a readout to follow with confocal imaging SMPD3-GFP trafficking and determine the effect of small molecules.
Aims: We aim to resolve the molecular machinery that modulates vascular calcification with a focus on modulation of molecular targets that drive vesicle release, and thus suggest potential small molecules that could inhibit vascular calcification progression.
Methods: To identify and prioritize druggable pockets in target protein SMPD3 we used WHATIF package and run molecular dynamics using AMBER to optimize the structure and asses rigidity.
Structure-Based Virtual Screening of small molecules was done following GLIDE protocols (HTVS, SP, XP). *In vitro* experiments were performed using in Biohybrid system.
Results: Complete structure of catalytic part of human SMPD3 was generated using YASARA, and optimized while running molecular dynamics simulation for 100ns. Binding pocket with Mg^{2+} as a cofactor stayed rigid for 100ns. Any deviation that was observed after each 10ns was selected and used as a separate target structure for Structure Based Vrtual Screening. This way we derived 7 structures and 1 original structure. After the structures of the target protein with druggable pocket had been resolved, first 450000 compound were docked using High throughput virtual screening, standard precision, and extra precision (XP) protocol in GLIDE. According to structure/score performance we are testing the best 1000 molecules on vascular smooth muscle cells switched into pathological phenotype observed in patients with remodeling in the vessel wall. For this purpose we utilized Biohybrid and confocal imaging optimized in house.
Summary/Conclusion: Our in-depth research combining computational modeling, virtual ligand screening and molecular, cellular biology is propelling our understanding of vascular calcification and revealing potential molecular targets that will be combated using pharmacological interventions.

Vessel Wall
OS. 15.4

The effect of factor XIII on the proliferation and migration of vascular smooth muscle cells

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Background: Plasma factor XIII (pFXIII) is a heterotetramer of FXIII-A and FXIII-B subunits. The cellular form (cFXIII), present in platelets, monocytes, macrophages, osteoblasts, chondrocytes and adipocytes is a dimer of FXIII-A. pFXIII plays an important role in blood coagulation, wound healing, angiogenesis and in maintaining pregnancy. cFXIII has been implicated in phagocytosis, cell differentiation, and extracellular matrix formation. Its role in mineralization has also been suggested. In atherosclerotic plaque vascular smooth muscle cells go through osteoblastic transformation in which de novo cFXIII expression might play a role. Another aspect of the involvement of FXIII in the development of atherosclerotic plaque is its possible effect on vascular smooth muscle cells.
Aims: 1/ To investigate if cFXIII is expressed in human aorta derived smooth muscle cells (HAoSMC) during osteoblastic transformation. 2/ To investigate the effect of extracellular FXIII on HAoSMC.
Methods: Osteoblastic transformation of HAoSMC was induced by Pi and Ca^{2+}. FXIII-A in cell lysate was measured by ELISA and Western Blot. Cell proliferation and migration were measured by EZ4U, CCK-8 cell proliferation assay kits and CytoSelect 24-Well Wound Healing Assay. Cell migration was monitored by Juli Stage Real Time Cell History Recorder. Thrombospondin-1 (TSP-1) levels in the medium and the amount of cell-associated TSP-1 were measured by ELISA. TSP-1, c-Jun and Egr-1 transcription factors mRNA levels were estimated by RT-qPCR.
Results: FXIII-A could not be detected in differentiated HAoSMCs. Activated recombinant cFXIII (rFXIIIa), but not the non-activated form, increased cell proliferation in concentration dependent manner. Its effect in the in vitro wound healing assay was even more considerable. The time to reach 30% and 80% confluence was decreased to less than 1/7th and 1/3rd in the presence of 20 µg/mL cFXIIIa. In parallel a highly significant (67%) decrease of TSP-1 concentration in the medium and a 2.5-fold increase of cell associated TSP-1 were observed. Neither TSP-1 nor c-Jun and Egr-1 mRNA changed significantly. In HAoSMC culture FXIIIa induced the appearance of cell-associated granular precipitates, which contained highly cross-linked TSP-1.
Summary/Conclusion: As opposed to a few other cell types, osteoblastic transformation HAoSMCs does not induce de novo cFXIII expression. However, activated extracellular FXIII considerably promotes the proliferation, in vitro wound closure and collagen production. It also induces the formation of cell associated cross-linked TSP-1 precipitate. It is proposed that during the course of plaque hemorrhage FXIII becomes activated and exerts its effect on vascular smooth muscle cells. This effect might be important in the pathogenesis of atherosclerotic plaques.

Paedriatic Haemostasis
OS. 16.1

Fibrinogen in Pediatric Craniosynostosis Surgery: a double-blind, placebo-controlled trial
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Background: Craniosynostosis is the premature fusion of one or more sutures in either the cranial vault or anterior skull base, resulting in an abnormal head shape and sometimes increasing intracranial pressure. Primary non-syndromic craniosynostosis occurs in 1 in 2000 births. Conventional treatment involves surgical correction at an early age to release increased intracranial pressure and to normalize cranial shape. Pediatric craniofacial surgery is associated with high perioperative blood loss. Some studies advocate the administration of fibrinogen concentrate to prevent bleeding and reduce the amount of transfusions in these patients. But no placebo controlled, randomized trial exists to support this hypothesis.
Aims: To investigate whether the administration of fibrinogen concentrate during craniofacial surgery in children with non-syndromic craniosynostosis will decrease intra-operative and intensive care unit (ICU) blood loss and transfusion of red blood cells (RBC), plasma and platelets.
Methods: This is a double-blind, placebo-controlled, randomized trial. The day before surgery fibrinogen was determined in all children. Patients were randomized into two groups. The fibrinogen-group received fibrinogen after induction of anaesthesia to achieve a fibrinogen level of 3 g/l. In addition, we infused 60 mg/kg bodyweight of fibrinogen during the first hour of surgery. The placebo-group received the same amount of NaCl in ml at the same time points. Endpoints included estimated blood loss during surgery, exact blood loss on ICU and transfused blood products.
Results: After informed consent, 110 children were included in the study, 55 in the fibrinogen group and 55 in the placebo group. Baseline characteristics of the patients in both groups were not statistically different. A median of 79 mg/kg of fibrinogen was given in de fibrinogen-group and 81 mg/kg placebo in the placebo-group. No differences were found in time of surgery or anesthesia, length of ICU stay and length of stay in hospital between both groups. No differences were found between both groups neither in the median intra-operative blood loss (Fibrinogen: 44.7 ml/kg vs. Placebo: 45.9 ml/kg, p=0.536) and ICU blood loss (Fibrinogen: 23.9 ml/kg vs. Placebo: 25.1 ml/kg, p=0.491), nor in the amount of transfusions of RBC, plasma and platelets.
Summary/Conclusion: Supplementation of fibrinogen of 79 ml/kg during craniofacial surgery in children with primary non-syndromic craniosynostosis has no effect on intra-operative and ICU blood loss and transfusion requirement.

Paedriatic Haemostasis
OS. 16.2

Diagnostic markers and predictors of neonatal immune thrombocytopenia. Russian experience.

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Background: Neonatal immune thrombocytopenia is caused by transplacental passage of maternal antiplatelet allo- or autoantibodies into fetal circulation. Neonatal alloimmune thrombocytopenia (NAIT) results from maternal-fetal incompatibility for platelet alloantigens (HPA, Human Platelet Alloantigens). Neonatal autoimmune thrombocytopenia (defined here as neonatal trans-immune thrombocytopenia, NTIT) is revealed in some children born to mothers with immune thrombocytopenic purpura (ITP).
Aims: (1) Investigation of HPA conflicts causing NAIT in Russian population. (2) Search for potential predictors of NTIT in pregnant women with ITP.
Methods: Twenty-seven families to which children with NAIT were born and 100 pregnant women with ITP were included in the study. Thirty-seven and 63 women with ITP gave birth to babies with and without NTIT, respectively (NITP+ and NITP- groups). Platelet-associated IgG (PA-IgG) were measured using ^{125}I-labeled antibodies against human IgG. Antiplatelet circulating antibodies (cAB) in maternal sera were evaluated by ELISA by reaction with paternal or donor platelets in NAIT and NTIT studies, respectively. Antigenic specificity of cAB was assessed using MAIPA (Monoclonal Antibody Immobilization of Platelet Antigens) assay. Genotyping of HPA-1, -2, -3, -4, -5 and -15 polymorphisms was performed in newborns with NAIT and their parents.
Results: In all 27 mothers having newborns with NAIT we observed no decrease in platelet count, no increase in PA-IgG but detected cAB reacting with paternal platelets. Maternal-fetal incompatibility was revealed by HPA genotyping in 23 out of 27 families. HPA-1 conflicts were identified in 16 out of these 23 families (70%). Eight mothers were homozygotes for rare HPA-1b allele and other eight, for HPA-1a allele (incompatibility with fetal HPA-1a and HPA-1b, respectively). Incompatibility with fetal HPA-15a or -15b was detected in 5 out of 23 families (22%); with HPA-5b, in 1 family (4%); with HPA-3b, in one family (4%). The specificity of cAB against HPA-1a and -1b was confirmed by MAIPA.
All pregnant women with ITP had decreased platelet count and increased PA-IgG. There were no differences in platelet count, PA-IgG level, time of ITP onset (before or during pregnancy), and frequency of corticosteroid treatment between NTIT+ and NTIT- groups. The only difference was the presence of antiplatelet cAB which were detected in 33 out of 37 and in 2 out of 63 mothers in NITP+ and NITP- groups, respectively ($p < 0.001$). Thus, the sensitivity of this test was 89% and the specificity was 97%. A strong reverse correlation ($r = -0.749$, $p < 0.001$) was established between maternal cAB titer and platelet count of the newborns. Antibodies against glycoproteins IIb-IIIa and/or Ib were identified by MAIPA only in 10 out of 19 (53%) tested sera containing platelet-reactive cAB.
Summary/Conclusion: (1) In Russian population HPA-1a and -1b conflicts are the most frequent and HPA-15 conflicts are the second frequent cause of NAIT. (2) The presence of antiplatelet cAB in pregnant women with ITP can serve as a reliable predictor of NTIT in their babies.

Paedriatic Haemostasis
OS. 16.3

Genetic and Immune Heterogeneity in Pediatric Immune Thrombocytopenia is Associated with Treatment Responses to IVIG

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Background: Thirty percent of children with immune thrombocytopenia (ITP) show no response to intravenous immunoglobulins (IVIG) and a predisposition to chronic ITP. It is not known why patients do not respond to treatment.
Aims: We systematically profiled patients with newly diagnosed ITP to identify predictors of IVIG resistance.
Methods: We conducted a nested case-control study in the treatment arm of the TIKI randomized controlled trial (N=100). This multicenter trial evaluated the effect of IVIG on prevention of chronic ITP in children with newly diagnosed ITP. Patients were systematically profiled at diagnosis, before treatment with IVIG, including targeted genotyping, immunophenotyping and determination of antigen-specific platelet autoantibodies. Binary penalized regression regression was used to select predictors of response after IVIG administration.
Results: One week after IVIG, 9 patients showed a partial and 21 showed no resolution of thrombocytopenia. Principal component analysis indicated a profound influence of age on overall variance. Compared to older children, patients <7 years (75th age percentile) were clearly distinct in multiple variables and variable selection was first restricted on them (N=80). IVIG resistance was associated with a large deletion in the Fc-gamma receptor locus (copy number region 1), Buchanan bleeding Score > 1, lower hemoglobin levels, insidious disease onset and absence of anti-platelet IgG autoantibodies. These five variables achieved a reasonable discrimination between responders and non-responders with a receiver-operator-characteristic AUC of 0.81. Non-responders were classified with a sensitivity of 0.98 and a low specificity of 0.54. All children that were predicted to have a complete sustained response were also observed to have a complete response without relapses. Misclassifications occurred mostly in patients who showed a partial response to IVIG. Furthermore, we found that in the untreated observation arm of the trial (N=100), the same variables distinguished children with persistent thrombocytopenia during follow-up (6-month non-response, AUC 0.78; 12-month non-response, AUC 0.85). This indicated that the selected variables are associated with unfavorable disease outcomes irrespective of IVIG treatment.
Summary/Conclusion: We identified several markers associated with resistance to IVIG and a predisposition to chronic disease. This potentially allows the design of a model to predict responses to IVIG at diagnosis.

Paedriatic Haemostasis
OS. 16.4

Incidence and features of thrombotic events in children with inherited antithrombin deficiency: retrospective analysis of 968 subjects with antithrombin deficiency from 441 unrelated families

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Background: Pediatric thromboembolism (<18 years) is very rare (0.07-0.14/10,000/year) but may be more prevalent in children with severe thrombophilia (protein C, protein S or antithrombin deficiency). Moreover, there is controversy on the benefit of thrombophilia screening among children because of the little information on this field due to the low incidence of both pediatric thrombosis and thrombophilia. The aim of the study is to define the prevalence and clinical characteristics of pediatric thrombosis in subjects with inherited antithrombin deficiency, the strongest thrombophilia.
Aims: To define the prevalence and clinical characteristics of pediatric thrombosis in subjects with inherited antithrombin deficiency.
Methods: An observational retrospective multicentric study from two countries was performed. 968 patients of any age with antithrombin deficiency were recruited from 441 unrelated families during more than 20 years. Antithrombin
Results: 73 subjects (7.5%) developed pediatric thrombosis. Two periods with high risk of thrombosis were identified: adolescence (12-18 years, N= 49) with thrombus localization (deep venous thrombosis or pulmonary embolism) and triggering factors common to adults (oral contraceptives, surgery or pregnancy); and newborns (<30 days, N= 14) with idiopathic thrombosis at unusual sites. The clinical evaluation of pediatric thrombosis in subjects with antithrombin deficiency revealed i) a high incidence of cerebral sinovenous thrombosis (N= 14), mainly at early ages (8 neonates and 5 children <6 years); ii) severe outcome; with fatality in 6 cases (3 neonates, two of them homozygous for p. Leu131Phe), invalidating sequels (psychomotor retardation or limb amputation) or recurrent thrombotic event (N= 18). A slightly higher incidence of pediatric thrombosis was observed in males than females (54.8% *vs* 45.2%, respectively). This difference was even more pronounced when considering thrombosis at early age: 10 out of 14 neonates with thrombosis (71.4%) were male. However, most thrombosis in adolescence was reported in girls under estrogen-related conditions: oral contraceptive pill, pregnancy and puerperium (N= X). Quantitative type I deficiency was predominant among children with thrombosis (76.7%). In contrast, among the remaining 14 children with thrombosis having a type II deficiency, additional genetic risk factors were detected in 43%, all with heparin binding site defect (Type II HBS).
Summary/Conclusion: Our study provides strong evidence for the high risk of pediatric thrombosis associated with antithrombin deficiency (300-fold compared with the general population: 0.41%/year *vs* 0.0014%/year, respectively). This and the severe clinical phenotype of symptomatic children strongly encourage the screening of antithrombin deficiency in children of affected families, in order to reduce the exposition of risk factors to carriers. The screening might be particularly beneficial for girls from families with type I deficiency, as they might receive antithrombotic prophylaxis under risk situations, and avoid triggering factors. In contrast, screening is not recommended in children of families with type II HBS unless additional risk factors are present. Finally, our study suggests reducing risk factors associated to cerebral vein thrombosis, like assisted delivery, in carriers or potential carriers of antithrombin deficiency.

Vessel Wall
PS 1.1
Board No. 1

NETs can help to estimate the thrombotic risk in biliopancreatic cancer patients at diagnosis

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Background: Venous thromboembolism (VTE) is a common complication of cancer that increases mortality and morbidity in these patients. However, limited tools are available to identify the patients at need for anticoagulation due to a high thrombotic risk. Neutrophil extracellular traps (NETs) are highly prothrombotic networks released by neutrophils upon activation, made up of DNA, myeloperoxidase (MPO), calprotectin and nucleosomes, among other elements.
Aims: To evaluate the ability of NETs to identify biliopancreatic cancer patients at high risk of developing VTE during follow-up.
Methods: 125 biliopancreatic cancer patients were prospectively recruited and followed for two years. Blood was drawn at diagnosis and every three months. Written informed consent was obtained from all participants. In every plasma sample obtained from patients, we measured NETs markers: DNA (PicoGreen), nucleosomes, MPO and calprotectin (specific ELISAs). We estimated the predictive potential of VTE of NETs using R (v3.5.0).
Results: We conducted a Cox regression survival model with a time-dependent covariate, including the NETs variables measured in each sample of the patients in rank form. We obtained that, for each unit that the logarithm of the calprotectin concentration increases, the VTE risk in biliopancreatic cancer patients increases 6 times (P = 0.009). The other markers of NETs showed no predictive capacity of VTE. Next, we adjusted an Elastic Net logistic regression model and obtained a predictive model of VTE with calprotectin as a predictor (AUC = 0.77).
Summary/Conclusion: The concentration of calprotectin at diagnosis allows us to estimate the risk of VTE in biliopancreatic cancer patients. This information could be very useful to prevent thrombotic events in these patients through closer monitoring and personalized thromboprophylaxis. ISCIII-FEDER (PI12/00027, Red RIC RD12/0042/0029, PI14/00079, PI14/00512, FI14/00269, CPII15/00002, PI17/00495), Generalitat Valenciana (PrometeoII/2015/017, ACIF/2017/138), Sociedad Española de Trombosis y Hemostasia and OBEL Family Foundation (26145).

Vessel Wall
PS 1.2
Board No. 2

Plasma Levels Of Proteins With Endothelium Enriched Expression And Risk Of Venous Thromboembolism - A Nested Case-Control Study.

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Background: Venous thromboembolism (VTE) has an incidence of 1-2/1,000 year, which increasewith age. It carries a high risk of mortality and recurrence. We currently lack tools to accurately identify individuals at high risk of VTE, and our understanding of the mechanisms and risk factors predisposing to VTE is incomplete. Endothelial cells (EC) line the inside of all vessels and has an important protective role to maintain vascular health and prevent thrombus formation. Injury or inappropriate activation, termed 'endothelial dysfunction' has a central role in pathogenesis and development of venous thrombosis.A blood test for EC dysfunction could potentially be of value to identify individuals at higher risk of future incident VTE. Previously, Butler et al identified the core EC enriched transcriptome (Cell Systems 2016), providing novel potential biomarker candidates for EC dysfunction for targeted plasma proteomics studies
Aims: To investigate the association between plasma levels of EC enriched proteins and future risk of incident VTE, in a nested case control study using antibody based affinity proteomics
Methods: The prospective study consisted of 417 subjects with incident VTE, and 850 age- and sex-matched controls randomly selected from the fourth survey of the Norwegian Tromsø study. Plasma samples from non-fasting blood for all subjects were collected in 1994-95 in two independent phases. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants. We performed a multiplexed proteomic profiling of the Tromsø nested case control set of samples (n=1267) using suspension bead arrays with antibodies targeting a candidate panel of proteins with EC enriched expression. Statistical analysis was performed with the R package to identify proteomic profiles associated with risk of future VTE and with phenotypes associated with VTE risk.
Results: After adjusting for differences in sex and age we identified significant associations between plasma level of several endothelium enriched proteins and risk incident VTE. As an example, plasma levels of vWF were associated with increased risk of VTE (p=1.7E-04). When this was stratified for sex, the association was stronger in females (p=2.4E-03) than in males (p=0.02). Overall plasma profiles were significantly affected by sex, and clear sex differences were observed in the proteomic profiles that were significantly associated with risk of VTE
Summary/Conclusion: Our preliminary results show that proteins with specific or enriched expression in endothelial cells constitute potential plasma biomarkers for risk of incident VTE. Furthermore, the risk associated plasma protein profiles vary between the sexes

Vessel Wall
PS 1.3
Board No. 3

The role of coagulation factors and platelets on microvascular integrity in atrial fibrillation: impact of NOACs

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Background: Atrial fibrillation (AF) patients are at risk for heart failure due to atrial remodeling. AF is associated with a hypercoagulable state, activated platelets and low grade inflammation. Our central hypothesis links AF remodeling to microvascular loss via hypercoagulability. Today, AF patients are treated with anticoagulants primarily to prevent stroke, however the effects on microvascular integrity are not yet investigated.
Aims: Assess the mechanistic role of platelets, coagulation factors and novel oral anticoagulants (NOACs) on microvascular integrity (*in vitro*).
Methods: To study the role of the extrinsic-mediated pathway of coagulation, an endothelial cell (EC)-monolayer was treated with TNFα, leading to the expression of tissue factor. The intrinsic pathway of coagulation was blocked using corn trypsin inhibitor. Loss of endothelial cell-cell contact, initiating microvascular destabilization, was measured using Electric Cell-Substrate Impedance Sensing (ECIS). To study the role of coagulation and platelets, platelet-free plasma (PFP) or platelet-rich plasma (PRP) was incubated in the absence or presence of the NOACs Rivaroxaban (anti-FXa) or Dabigatran (anti-thrombin) on an EC-monolayer, while monitoring the anticoagulant effect of the NOACs with a thrombin-activity assay.
Results: Under pro-inflammatory conditions (TNFα) and complete inhibition of coagulation (Rivaroxaban+Dabigatran), platelets (PRP versus PFP) supported the EC-barrier function. However, when activated, coagulation overruled the platelet-supporting effect, leading to the loss of barrier integrity, which coincided with thrombin-activity. Inhibiting the coagulation cascade at the Xa-level (Rivaroxaban) established the EC barrier, but inhibition at the IIa-level (Dabigatran) resulted in loss of barrier function, while thrombin-activity was completely blocked with either Rivaroxaban or Dabigatran.
Summary/Conclusion: Inhibition of coagulation at Xa-level may not only prevent stroke but also protect for microvascular complications in AF patients.

Vessel Wall
PS 1.4
Board No. 4

Endothelial cells and neutrophils dependent, platelets and NETs-independent are involved in activation of the blood coagulation cascade leading to thrombus formation following a laser-induced injury

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Background: Since decades thrombus formation is studied in different preclinical models. One of the stat of the art model consist in a laser -induced injury in arterioles of living mice. In this model, neutrophils were described as the first cells recruited at the site of laser injury and induced the tissue factor dependent activation of the blood coagulation cascade. This leads to the accumulation and activation of platelets and the generation of fibrin at the site of the laser-induced injury. Recently, however it was described that Neutrophils extracellular traps (NETs) may be involved in thrombus formation.
Aims: The goal of this study was to investigate the consequences of the interaction between endothelial cells, neutrophils, NETs and platelets on the activation of the blood coagulation cascade in living mice.
Methods: We studied the accumulation of fibrin following a laser-induced injury in presence and in absence of platelets. Colocalization of fibrin, platelets, neutrophils and endothelial cells in a developing thrombus was performed by confocal intravital microscopy. We investigated the presence of negative phospholipids as contact phase necessary for the activation of the coagulation cascade. Finally, we search for NETs formation in vitro and in vivo in two different models of thrombosis by electron microscopy.
Results: Most of the Fibrin detected was colocalized with the endothelial cells (97%) and neutrophils (87%). At the opposite, less than 10% of fibrin colocalized with platelets participating in the growing thrombus. Consistently, depletion of circulating platelets did not affect the generation of fibrin in vivo. Furthermore, negative phospholipids were detected colocalized on the endothelial cells and not platelets at the site of injury. Last, the interaction of neutrophils with endothelial cells was sufficient enough to activate in few seconds the coagulation cascade detected by the accumulation of fibrin at the site of injury. Since the formation of NETs required 2 to 3 hours *in vitro*, we next determined the ultrastructure of neutrophils present at the site of thrombosis by electronical microscopy. Our results indicate that neutrophils implicated in the developing thrombus were intact and did not formed NETs.
Summary/Conclusion: Endothelial cells and neutrophils but not NETs nor platelets are implicated in the activation of the blood coagulation cascade leading to thrombus formation following a laser induced injury in living mice.

Vessel Wall
PS 1.5
Board No. 5

Frequency and characteristics of atrial appendages thrombi in atrial fibrillation patients treated with warfarin or dabigatran

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Background: It's known, that in patients with atrial fibrillation (AF) thrombus most commonly develops in the left atrial appendage (LAA) and current guidelines recommend transoesophageal echocardiography (TOE) to ensure thrombus resolution in LAA prior to early cardioversion. However, thrombi may originate from right atrial appendage (RAA) as well. Little is known about thrombi characteristics in patients treated with oral anticoagulants before cardioversion.
Aims: To determine the frequency, predictors and characteristics of LAA and RAA thrombi in non-valvular AF patients after 4 weeks of anticoagulant treatment with warfarin or dabigatran before electrical cardioversion.
Methods: 192 patients with persistent non-valvular AF were enrolled in the study after informed consent had been obtained. Among them 133 patients (69,3%) were treated with warfarin and 59 patients (30,7%) received dabigatran. TOE was done in all patients after 4 weeks of anticoagulant treatment with assessment of thrombus absence or degree of thrombus burden and localization. Parietal thrombus with clear contours and a homogeneous structure was classified as organized. Thrombus was considered unorganized if it was mobile or fixed with clear contours and a homogeneous or unhomogeneous structure. Electrical cardioversion was performed in patients without thrombi or only after clot organization confirmed by TOE.
Results: The mean age of the patients was 61,5±0,9 years and 62,3% were men. The mean duration of last AF episode was 6,5±0,6 months. The prevalence of thrombi in both LAA and RAA in warfarin group was 47,4% (63 patients), in dabigatran group - 44% (26 patients). The groups didn't differ in number of patients with thrombosis of only LAA (40 patients - 30,1% vs 20 patients - 33,8%) or only RAA (10 patients - 7,5% vs 4 patients - 6,7%). In dabigatran group there were significantly more patients with organized thrombi in comparison with warfarin group: among patients with thrombosis of both appendages 86,5% vs 39,7% ($p<0,001$), with thrombosis of only LAA - 65,0% vs 94,8% ($p<0,001$); with thrombosis of only RAA - 70,0% vs 100% ($p<0,001$), respectively. There was found an assotiation between thrombus formation in atrial appendages and obesity with an 3,4-fold increased risk (OR=3,39, 95% CI: 1,25-9,18; $p<0,05$), GFR≤59 ml/min/1,75 m^2 with an 3,9-fold increased risk (OR=3,89, 95% CI: 1,08-14,04; $p<0,05$) and left ventricle ejection fraction (LVEF) ≤ 45% with 2,4-fold increased risk. AF duration less than 1 year resulted in a 3,2-fold increased risk of unorganized thrombus formation (OR=3,22, 95% CI: 1,22-8,47; $p<0,05$). The mean duration of thrombus organization on warfarin therapy was 12±6 weeks, on dabigatran - 4±2 weeks.
Summary/Conclusion: Our study found high prevalence of LAA and RAA thrombi in non-valvular persistent AF patients. Obesity, GFR≤59 ml/min/1,75 m^2 and LVEF≤45% were independent predictors of thrombus formation in atrial appendages. Patients with AF duration less than 1 year have a risk of unorganized thrombus formation. Thrombus organization rates after 4 weeks of anticoagulant treatment were higher in patients receiving dabigatran in comparison with patients treated with warfarin.

Vessel Wall
PS 1.6
Board No. 6

Direct inhibition of factor Xa by rivaroxaban is cardioprotective in rats via a cytoprotective effect on cardiomyocytes

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Background: Acute myocardial infarction is a leading cause of death worldwide. Although highly beneficial, reperfusion of myocardium is associated with reperfusion injury. Indirect pharmacological inhibition of factor Xa by fondaparinux has been shown to attenuate myocardial ischemia-reperfusion (I/R) injury *via* the activation of the SAFE pathway. The link between the inhibition of factor Xa and the activation of this cardioprotective pathway remains unclear.
Aims: Our aims were to study the effect of a direct inhibitor of factor Xa, rivaroxaban (RIV), on myocardial I/R injury and to determine its cellular targets.
Methods: We investigated the ability of RIV to prevent I/R injury in a model of transient coronary ligation in rats. A 40-min myocardial ischemia was followed by 120-min of reperfusion. RIV (3 mg/Kg) was given *per os* 1-hour before reperfusion. Infarct size was assessed after 120-min of reperfusion. RIV concentrations were measured after administration. Myocardial tissues were collected at 30-min reperfusion for western-blots analysis of RISK and SAFE pathways proteins (Akt, ERK 1/2, STAT3, GSK-3β). Cellular effects of RIV were studied with hypoxia-reoxygenation (H/R) models on human umbilical vein endothelial cells (HUVEC) and on rat cardiomyocytes cell line H9C2. mRNA expression of endothelial markers (ICAM-1, VCAM-1, EPCR, thrombomodulin) was assessed on HUVEC. Cellular viability of H9C2 cells was determined using a tetrazolium salt-based assay (MTT assay).
Results: RIV decreased infarct size by 21% (42.9% *vs.*54.2% in RIV-treated rats and controls respectively, $p<0.05$). After *per os* administration, RIV concentrations were similar to human therapeutic concentrations (387.7 +/- 152.3 ng/mL respectively). RIV cardioprotective effect was not related to the activation of RISK and SAFE pathways. RIV had no effect on H/R-induced modulation of endothelial phenotype but had a cytoprotective effect on H9C2 cells submitted to H/R.
Summary/Conclusion: RIV decreased myocardial I/R injury in rats at concentrations similar to those known to be antithrombotic in human. Unlike FDX, this protective effect was not mediated through the activation of the cardioprotective pathways RISK and SAFE. Rivaroxaban-induced cardioprotection appeared to be related a direct protective effect on cardiomyocytes.

Bleeding 1
PS 2.1
Board No. 7

The Pharmacokinetic Properties Of Factor IX Can Be Improved Using Genetic Fusion To Factor XIII-B Subunit

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Background: Haemophilia B is an X-linked bleeding disorder characterised by coagulation factor IX (FIX) deficiency. Prophylaxis, using regular infusions of FIX concentrates, is currently considered as the gold standard of care for the prevention of bleeding episodes in patients with severe haemophilia B, who have FIX clotting activity (FIX:C) < 1 IU/dl. Considering the half-life of standard FIX concentrates, of approximately 20h, 2 to 3 intravenous infusions per week are required to achieve bleeding prevention. This is very demanding for patients, and alters their quality of life. Recently, extended half-life FIX concentrates have been developed in order to reduce the frequency of infusions. Two major strategies are used: biochemical modification such as (glyco)PEGylation, and genetic fusion to either immunoglobulin Fc fragments, or albumin. These technologies may have some potential disadvantages including reduced specific activity, unknown long-term adverse effects of PEG exposure and immunological reactivity, which are currently under investigation.
Aims: To develop a new strategy to prolong half-life of recombinant human FIX molecule (rFIX) using an innovative approach of genetic fusion, to factor XIII-B subunit (FXIII-B). FXIII-B subunit was chosen because it is responsible for the long half-life of FXIII of approximately 10 to 12 days, has no catalytic activity in contrast to FXIII-A subunit, and is present in excess in plasma, bound to fibrinogen.
Methods: rFIX molecule was fused to FXIII-B subunit through a short linker sequence containing an activated Factor X-cleavable site (rFIX-LXa-FXIII$_B$). The rFIX-LXa-FXIII$_B$ and the control, wild-type rFIX (rFIX-WT), molecules were expressed by stable clones of Huh-7 and CHO cells, and purified via a two-step protocol prior to *in vitro* and *in vivo* biochemical and pharmacokinetic characterisations.
Results: rFIX-LXa-FXIII$_B$ was correctly expressed by Huh-7 cells at the expected molecular weight of 150 kDa and presented a similar specific activity compared to rFIX-WT. The LXa linker was cleavable by FXa, releasing free rFIX molecule before its activation. After cleavage, rFIX was fully activable by activated FXIa. We also showed that rFIX-LXa-FXIII$_B$ bound to fibrinogen *in vitro*. *In vivo*, the half-life of rFIX-LXa-FXIII$_B$ molecule was determined in wild-type mice and rats. The improvement of the half-life was 3.9-fold longer in C57BL/6J mice, and 2.2-fold longer in rats compared to rFIX-WT ($p=0.0286$ and $p=0.0007$, respectively). The thrombin generation capacity in FIX$^{-/-}$ mice was similar 2 min after injection of rFIX-LXa-FXIII$_B$ or rFIX-WT, with a mean endogenous thrombin potential (ETP) ±SEM at 306.1nM.min±10.52 and 303.6nM.min±29.7 ($p>0.6905$), respectively. 24 h after injection, it was significantly higher for rFIX-LXa-FXIII$_B$ (251.8mM.min±19.31) compared to rFIX-WT (183.5±14.48; $p=0.0152$), thus confirming the prolonged clotting efficacy. rFIX-LXa-FXIII$_B$ also corrected the bleeding phenotype of FIX$^{-/-}$ mice, which was evaluated using tail-clip assay.
Summary/Conclusion: Here, we report a new fusion protein strategy using FXIII-B. The rFIX-LXa-FXIII$_B$ molecule exhibited normal procoagulant efficacy associated with improved half-life in small animal models.

Bleeding 1
PS 2.2
Board No. 8

Emicizumab prophylaxis administered once-weekly or every two weeks provides effective bleed prevention in persons with hemophilia A (PwHA) without inhibitors – Results from the phase III HAVEN 3 study

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Background: Regular prophylactic intravenous factor VIII (FVIII) infusions are the optimal treatment approach for severe haemophilia A. However, clinical and subclinical bleeds may occur despite prophylaxis and high treatment burden leading to suboptimal care for those unable to adhere. Therefore, there's an unmet need for highly effective treatment options with reduced treatment burden for PwHA without inhibitors.
Aims: HAVEN 3 (NCT02847637) is a randomized, global, multicenter, open-label, Phase 3 clinical study. This study assessed the efficacy, safety, and pharmacokinetics (PK) of emicizumab prophylaxis QW and every 2 weeks (Q2W) in adolescent/adult PwHA without inhibitors.
Methods: Severe haemophilia A patients without inhibitors aged ≥12 years were enrolled. Patients on prior episodic FVIII, with ≥5 bleeds over the previous 24 weeks, were randomized (2:2:1) to emicizumab prophylaxis: 3 mg/kg QW for 4 weeks, followed by 1.5 mg/kg QW (Arm A) or 3 mg/kg Q2W (Arm B) maintenance; or to no prophylaxis (Arm C). Primary efficacy objective compared treated bleed rates (Arm A vs C; Arm B vs C). Patients previously on FVIII prophylaxis received 1.5 mg/kg QW emicizumab maintenance in Arm D; those from a non-interventional study (NIS; NCT02476942) were included in intra-individual comparisons.
Results: 152 patients, aged 13–77 years (median: 38) were enrolled including 36, 35, 18, and 63 patients in the arms A, B, C and D respectively. Statistically significant and clinically meaningful reductions of ≥94% were observed in treated, all, treated spontaneous, joint and target joint bleeds with emicizumab QW or Q2W versus no prophylaxis. For treated bleeds, ABR (95% CI) was 1.5 (0.9; 2.5) in Arm A, 1.3 (0.8; 2.3) in Arm B and 38.2 (22.9; 63.8) in Arm C. For all bleeds, ABR (95% CI) was 2,5 (1.6; 3.9) in Arm A, 2.6 (1.6; 4.3) in Arm B and 47.6 (28.5; 79.6) in Arm C. For treated spontaneous bleeds ABR (95% CI) was 1.0 (0.5; 1.9) in Arm A, 0.3 (0.1; 0.8) in Arm B and 15.6 (7.6; 31.9) in Arm C. >55% of patients receiving emicizumab QW (Arm A) or Q2W (Arm B) had zero treated bleeds and >91% had ≤3 treated bleeds. An intra-individual comparison showed a 68% reduction in treated bleed rate with QW emicizumab versus prior FVIII prophylaxis observed during NIS, ABR (95% CI) was 4,8 (3.2; 7.1) in NIS: FVIII prophylaxis versus 1.5 (1.0; 2.3) in the Emicizumab QW. Emicizumab was well tolerated; most common AE was injection site reaction (25%). No thrombotic events, antidrug antibodies, or de novo FVIII inhibitors occurred. Sustained emicizumab trough concentrations were achieved with both regimens.
Summary/Conclusion: Emicizumab prophylaxis QW or Q2W significantly reduced bleed rates in PwHA without inhibitors, with a favorable safety profile. Most notably, an intra-individual comparison demonstrated superiority of emicizumab over previous FVIII prophylaxis. Subcutaneous emicizumab prophylaxis may provide a highly efficacious and flexible management option, with reduced burden for PwHA without inhibitors.

Bleeding 1
PS 2.3
Board No. 9

Revised pharmacokinetic of Benefix

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Background: Pharmacokinetic (PK) studies performed during product development defined the half-life of Benefix as being 16 and 24 h in pediatric and adult patients respectively. However, in these studies, sampling stopped 72 h after Benefix infusion. In a study in which the PK of Benefix was compared to that of factor IX concentrate (IX-Fc), sampling was unfortunately stopped at 96 h in patients receiving Benefix; at that time, factor IX (fIX) level differed by only 2.5% between patients treated with Benefix and IX-Fc, respectively (1). Moreover, in our clinical practice, we observed residual fIX levels above 2% even 170 h after Benefix infusion in some patients. We therefore suspected that the half-life of Benefix had been largely underestimated.
Ref 1: Powell and al. N Engl J Med 2013; 369: 2313-2323.
Aims: To evaluate the half life of Benefix on factor IX values measured 30 minutes to 10 days after Benefix infusion.
Methods: We evaluated 430 values (short PK evaluations or residual fIX levels measured at a routine visit) in 63 haemophilic patients treated with Benefix or IX-Fc in 6 haemophilic centres. FIX activities were analysed using a non-linear mixed model and Monolix software. A covariate analysis including body weight, age, dose (IU/kg), and infusion frequency was performed to identify factors implicated in inter-patient PK variability. Differences in reagent/instrument use between the six centres were taken into account. Individual PK parameters, e.g. clearance and half-life, were estimated using a Bayesian approach.
Results: The half-life of Benefix was found to be 2 to 2.5 times longer (depending on the age group) and Benefix clearance was from to be 2 times lower (at least) than previously reported.
Summary/Conclusion: The long half-life of Benefix could be related to extravascular storage of this product. These results are particularly interesting from a financial point of view considering that Benefix is 30% cheaper than IX-Fc, at least in France. A PK study comparing Benefix and IX-Fc, with the same late sampling points, is warranted to evaluate differences in PK between these two products.

Bleeding 1
PS 2.4
Board No. 10

MULTICENTER PHARMACOKINETIC EVALUATION OF RFVIII-FC (ELOCTA®) IN 'REAL LIFE' AND COMPARISON WITH NON-EXTENDED HALF-LIFE FVIII CONCENTRATES.

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Background: The use of extending half-life ($T_{1/2}$) FVIII or FIX products is today a current strategy in Hemophilia A (HA) patients for improving prophylaxis and reducing the number of IV injections. FVIII-Fc fusion technology allows prolonging the $T_{1/2}$ of rFVIII by utilizing the neonatal Fc receptor and endogenous Fc recycling pathway. A single dose phase 1/2 pharmacokinetic (PK) study performed in 16 severe HA patients demonstrated a prolonged $T_{½}$ of rFVIII-Fc (equal to 18.8 hours (mean) compared to 12.2 hours with one conventional rFVIII (Malhangu et al. Blood 2014).
Aims: To analyze PK data collected with Elocta® in "real life" i.e. in a large cohort of patients treated in 12 different French hemophilia care centers, and results were compared to those obtained with conventional FVIII, when available
Methods: 100 severe HA patients with the following characteristics were included: mean age 30 years (range 2 – 70); weight 64 Kg (17-125); total FVIII-Fc dose injected 2700 IU (500-5750); FVIII-Fc IU/Kg: 41 (25 – 59); VWF Ag 95% (41-223). The FVIII recovery (R) was calculated as follows: (body weight (Kg) x observed increased in FVIII (%))/administered dose (IU/Kg). The $T_{1/2}$ was calculated with the following formula: Ln2/((Ln FVIII% T1 – Ln FVIII%T2)/T2 – T1)), with T1 > 4 hours and T2 > 24 hours. Results were compared to those performed with conventional FVIII (non EHL-FVIII) in 48 patients (Advate® n = 14, Refacto® n = 2, Helixate®/Kogenate®/Kovaltry® n = 29, Factane® n = 3)
Results: rFVIII-Fc activity was measured by clotting (OSA) and chromogenic assays (CSA) and levels were 20% lower in OSA compared to CSA whatever the FVIII:C levels. Therefore, rFVIII-Fc recovery (R) always appeared lower when measured with OSA (Mean 2.41, range 1.33 – 5.7) than with CSA (mean 2.83, ranges 1.35 – 5.5) ($p < 0.0001$). No correlation was found between R and age, weight or injected doses. Mean $T_{1/2}$ measured with rFVIII-Fc equaled 15 hours whatever the measurement method used (OSA or CSA) and was strongly correlated with vWFAg levels ($r^2 = 0.6$). Significantly lower recovery (2.28 vs. 2.92, $p = 0.006$) and $T_{1/2}$ values (12 vs. 15.7 hours, $p = 0.005$) were measured in children (< 10 years, n = 17) compared to adults.
The PK parameters of FVIII-Fc were compared to those obtained with conventional rFVIII or pdFVIII in 48 patients (mean $T_{1/2}$ equal to 10.06 hours; range 5.3 – 21.2). The apparent benefit with FVIII-Fc was variable from one patient to another with a mean $T_{1/2}$ rFVIII Fc / $T_{1/2}$ FVIII ratio equal to 1.4 (range 0.6 – 2.4). Interestingly, the increase in $T_{1/2}$ with FVIII-Fc was lower than 20% only in patients previously treated with BHK-derived rFVIII i.e. Helixate®/Kogenate®/ Kowaltry® (n=10). Whatever the FVIII injected (FVIII-Fc or other non EHL-FVIII), the $T_{1/2}$ measured was also strongly correlated to vWF levels, and these levels were significantly lower in patients for whom the mean $T_{1/2}$ rFVIII Fc / $T_{1/2}$ FVIII ratio was > 1.3 ($p=0.005$).
Summary/Conclusion: This study is the first to report PK data with EHL rFVIII-Fc (Elocta) in a large group of HA patients. Our results confirm the benefit of rFVIII-Fc in most HA patients, adults or children, but also emphasize the impact of vWF on half-life of FVIII, rFVIII-Fc or conventional non EHL-FVIII. Indeed, the benefit of rFVIII-Fc clearly appears higher in patients with lower vWF levels, with a more significant prolongation of $T_{1/2}$.

Bleeding 1
PS 2.5
Board No. 11

Efficacy and safety of hFVIII/VWF concentrate (Voncento®) in patients with von Willebrand disease (VWD) requiring surgical procedures: the French experience

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Background: OPALE is an observational study describing the use of human coagulation FVIII/VWF concentrate (hFVIII/VWF, Voncento®) to treat and prevent bleeding episodes in a French cohort of patients with inherited VWD in a real life setting.
Aims: The aim of the study is to describe the efficacy and safety of this human coagulation FVIII/VWF concentrate in the prophylaxis and treatment of haemorrhage or surgical bleeding.
Methods: National French cohort study of patients with inherited VWD receiving treatment with hFVIII/VWF(Voncento®)
Results: Between May 2016 and May 2018, sixty-eight patients were enrolled by 14 haemophilia treatment centres. The average age was 41.6 ± 20.1 years and 60.3% were female. VWD types were distributed as follows: 26.5% were type 1, 20.6% type 3, 19.1% type 2M, 13.2% type 2B, 8.8% type 2A, 8.8% type 2N and 1.5% type 2B/2N.
Out of these patients 47 received hFVIII/VWF concentrate during 71 surgical procedures: 36 were minor surgeries (ms) and 35 were major surgeries (Ms).
The average age was 46.0 ± 20.1 years and 70.2% were female. Thirty six percent were type 1, 21.3% type 2M, 12.8% type 2N, 10.6%) type 2A, 8.5% type 2B and 8.5% type 3. The 71 surgical procedures were divided into 36 minor surgeries (ms) and 35 major surgeries (Ms).
Patients undergoing a major surgery received an average dose of Voncento® of 42.2 ± 10.7 (VWF UI/kg.d) during 4 to 6 days, IC 95[2.4 - 6.8] days. The FVIII:C level before surgery was 39.8 ± 19.8 IU/dL and the average FVIII:C trough level during their hospital stay including the day of surgery was 120.9 ± 32.3 IU/dL. The VWF:RCo level after surgery was 69.1 ± 19.7 IU/dL.
Patients undergoing minor surgeries received an average dose of Voncento® 36.9 ±11.6 (VWF UI/kg) during 1 day, IC 95[0.4 - 1.5] days. The FVIII:C level before surgery was 37.3% ± 19.3 IU/dL and the average FVIII:C trough level during their hospital stay including surgery was 93.3% ± 32.5 IU/dL. The VWF:RCo level after surgery was 65.7 ± 25.6 IU/dL.
The overall clinical efficacy was qualified as excellent in 61.1% of cases (ms: 63.6% and Ms: 58.8%), good in 37.3% (ms: 33.3%; Ms:41.1%) and moderate in 1.5% (ms: 3.0%; Ms:0.0%).
Regarding safety, 3 adverse events were reported (4.2% of surgeries): 2 cases of headache and 1 case of deep vein thrombosis following orthopaedic surgery. This event occurred on day 10 after the last infusion of hFVIII/VWF and was evaluated as unrelated to the hFVIII/VWF treatment by the investigator.
Summary/Conclusion: hFVIII/VWF (Voncento®) was effective and well-tolerated in the prevention and treatment of surgical bleeding in a French cohort of patients with inherited VWD.

Bleeding 1
PS 2.6
Board No. 12

Management and outcome of bleeding complications in patients treated with direct oral anticoagulants

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Background: The initial lack of a specific reversal agent for direct oral anticoagulants (DOACs) has created concern about the outcome and optimal management of bleeding. In previous studies in healthy volunteers we showed that a high 50 IU/kg dose of prothrombin complex concentrate (PCC) can restore thrombin generation to pre-DOAC levels. Based on such studies, international guidelines suggest PCC in patients treated with a direct factor Xa inhibitor who present with bleeding or the need for an acute emergency intervention. To what extent PCC is used in clinical practice, as well as the efficacy and safety in bleeding patients has not been established.
Aims: The primary objective is to assess the management and outcomes of DOAC-related bleeding or emergency intervention and to evaluate the efficacy and safety of PCC for reversal.
Methods: This is an ongoing cohort study in patients with bleeding or the need for an emergency procedure while on DOACs. Patients were enrolled in 4 university hospitals and 1 regional hospital in the Netherlands in two different ways. In prospectively enrolled patients we collected additional blood samples at presentation and, if applicable, immediately after PCC and 4-8 hours after presentation. Patients in whom no additional blood samples could be collected were still enrolled for their clinical data. The institutional ethics committee waived the need for consent for patients enrolled retrospectively. The primary outcome for bleeding emergencies is haemostatic efficacy, assessed by a modification of the Sarode criteria. The primary outcome for patients needing an emergency intervention is occurrence of post-procedural major bleeding <7 days. Secondary outcomes were thromboembolism and 30 day mortality. In patients treated with PCC in whom additional samples were collected, we will determine the effects on thrombin generation and clot lysis.
Results: Ninety-seven subjects were included (54% prospectively) up until June 2018; 83 patients with major bleeding, 14 patients with the need to undergo an emergency procedure. Rivaroxaban was the most frequently prescribed DOAC (52%), followed by dabigatran (27%). Intracranial haemorrhage was most common within the bleeding group (59%). PCC was administered in 46 bleeding patients (51%), idarucizumab in 14 (15%), and in 37% no reversal agent was administered. The median administered PCC dosage was 3500 IU (50IU/kg). Effective haemostasis was achieved in 66% (95% confidence interval [CI 95%] 51-78), 83% (CI 95%, 55-95), and 52% (CI 95%, 34-70) after administration of PCC, idarucizumab and no reversal agent, respectively. Of the patients with the need for an emergency intervention, 93% received a reversal agent and one (7%) had a major bleed. Of the patients who received PCC, one (2% CI 95% 0-10) developed a thromboembolic event, more specifically a pulmonary embolism 8 days after administration of reversal agent.
30 day mortality was 21%. At the meeting we will present the effects of PCC on thrombin generation and clot lysis, which are being performed right now.
Summary/Conclusion: Effective haemostasis was achieved in the majority of patients treated with PCC and in almost all patients treated with idarucizumab with a low observed thromboembolic complication rate.

Bleeding 2
PS 3.1
Board No. 13

Inhibiting ADAMTS13 in a preclinical ovine left ventricular assist device model does not rescue the loss of high molecular weight von Willebrand factor multimers

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Background: Acquired von Willebrand syndrome has been linked to the bleeding diathesis observed in patients treated with an LVAD as these patients have a loss of high molecular weight (HMW) von Willebrand factor (VWF) multimers. At this meeting we show for the first time that by specifically blocking ADAMTS13, using the inhibitory anti-ADAMTS13 monoclonal antibody (mAb) 3H9, the loss of HMW VWF multimers was prevented in an in vitro Heartmate II™ and Impella CP® LVAD circuit with human blood. However, it remains to be determined if blocking ADAMTS13 is an effective novel therapy to rescue the loss of HMW VWF multimers in a preclinical animal model.
Aims: To investigate if an inhibitory anti-ADAMTS13 mAb can (1) prevent the loss of HMW VWF multimers in an in vitro Impella CP® system with ovine blood and (2) can rescue the loss of HMW VWF multimers in an in vivo preclinical ovine Impella CP® model.
Methods: Since our mAb 3H9 does not cross-react with sheep ADAMTS13, we used a novel inhibitory anti-ADAMTS13 mAb 17C7 (30 µg/mL), which potently inhibits both human and sheep ADAMTS13 and a non-inhibitory anti-ADAMTS13 mAb 5C11. Ovine blood was circulated through an in vitro Impella CP® system (n=4) and blood was sampled 5 minutes (min) before and 5, 30 and 60 min after start of the pump. Plasma samples were analysed for VWF multimers. For the in vivo study, Impella CP® pumps were implanted in sheep (n=8). One dose of 600 µg/kg of the inhibitory mAb 17C7 (n=4) or phosphate buffered saline (PBS) (n=4) was injected 1 day after Impella implantation. Blood was sampled before implantation, 30 minutes and 1 day after pump implantation and 1 and 2 days after injection of mAb 17C7 or PBS. ADAMTS13 inhibition and the loss of HMW VWF multimers were determined.
Results: In vitro, control experiments (using mAb 5C11) led to a 54% reduction of HMW VWF multimers in the Impella circuit, 60 min after pump initiation ($p=0.003$). In contrast to our previous in vitro data with human blood and the mAb 3H9, blocking ovine ADAMTS13 using the mAb 17C7, did not prevent the loss of HMW VWF multimers in the in vitro Impella circuit as HMW VWF multimers were still 42% decreased, 60 min after start of perfusion ($p=0.003$). The efficacy of mAb 17C7 was also tested in a preclinical Impella ovine model. As expected, HMW VWF multimers significantly decreased one day after Impella implantation (n=8, 55% decrease, $p<0.0001$) and HMW multimers remained decreased when PBS was injected (n=4, 53% decrease). However, in agreement with the in vitro experiments, injection of mAb 17C7 did not rescue the HMW VWF multimers (n=4, 57% decrease) although ADAMTS13 activity was blocked in all sheep (n=4, 8.2 ± 4.7% ADAMTS13 activity, 1 day after injection of 17C7 compared to 81.0 ± 53.6% before injection). To prove that the mechanism of shear induced VWF proteolysis is different between sheep and humans, we performed an in vitro Impella experiment with human blood and showed that addition of mAb 17C7 (as observed with mAb 3H9) did prevent the loss of HMW VWF multimers (13% decrease versus 70% with mAb 5C11).
Summary/Conclusion: In contrast to human blood, blocking ADAMTS13 activity in an in vitro Impella CP® circuit with ovine blood, could not prevent the loss of HMW VWF multimers. Although ADAMT13 activity could be inhibited in a preclinical ovine Impella model, the shear-induced proteolysis of VWF could not be rescued. Taken together, these data indicate that in sheep other mechanisms than ADAMTS13 are responsible for the loss of HMW VWF mulitmers.

Bleeding 2
PS 3.2
Board No. 14

Novel approaches to the diagnosis and classification of anti-FXIII allo-, and autoantibodies

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Background: Factor XIII (FXIII) consists of two catalytic A subunits and two inhibitory/protective B subunits. Alloantibodies against FXIII subunits rarely develop in inherited FXIII deficiency, but make its management very difficult. Acquired FXIII deficiency due to anti-FXIII autoantibodies is also a rare bleeding diathesis usually with severe, sometimes life-threatening soft tissue bleedings. Neutralizing anti-FXIII-A antibodies may interfere with FXIII activation and/or inhibit activated FXIII (FXIIIa), while non-neutralizing antibodies form complex with either of the FXIII subunit and accelerate their clearance. The traditional method for detecting neutralizing antibodies is the mixing study and its semi-quantitative variant, the Bethesda-Nijmegen assay. There is no established method for the diagnosis of non-neutralizing antibodies.
Aims: Our aim was to introduce and evaluate new methods in the diagnosis and classification of anti-FXIII antibodies.
Methods: For quantitatively assessing the inhibitory capacity of an antibody we determined the 50% inhibitory concentration (IC50). For the determination of the antibody's binding affinity to FXIII and its subunits surface plasmon resonance measurement was introduced. For targeting the effect of neutralizing antibody a three-step assay system was designed. The cleavage of FXIII-A by thrombin was investigated by Western blotting, the effect on Ca^{2+} induced activation and FXIIIa was investigated by modified FXIII activity assays. The clearance of FXIII was assessed by activity measurement following the administration of FXIII concentrate.
Results: Anti-FXIII-A antibodies from three patients were characterized using the above methods. The IC50 for IgGs varied between 0.05 and 0.34 mg/mL. The antibodies bound with the same high affinity (Ka: in the range of 10^8-10^9 M^{-1}) to FXIII-A_2 and FXIII-A_2B_2, but not to FXIII-B_2. The three-step activity assay and the clearance study revealed different types of anti-FXIII-A antibodies: the alloantibody and one autoantibody exerted a combined effect; they inhibited FXIII activation and FXIIIa activity and accelerated the clearance of FXIII. The main effect of one autoantibody was the inhibition of FXIIIa.
Summary/Conclusion: Introduction of new methods in assessing anti-FXIII antibodies improved their functional characterization and allowed us to design a new classification, which could contribute to novel therapeutic approaches.

Bleeding 2
PS 3.3
Board No. 15

High soluble thrombomodulin is associated with an increased risk for major bleeding during treatment with oral anticoagulants: a case-cohort study

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Background: Bleeding is the most common adverse event of treatment with vitamin K antagonists (VKA), with major bleedings occurring in 1-3% of patients per year. It is assumed that bleeding is in part mediated by endothelial damage. Elevated plasma levels of soluble thrombomodulin (sTM) are associated with various vascular diseases (diabetes, ischemic heart disease) and are considered to be a marker for chronic endothelial damage. High levels of sTM may therefore predispose for bleeding.
Aims: To better understand the susceptibly for major bleeding while using VKA, we assessed whether sTM plasma levels are associated with an increased risk for major bleeding in patients starting VKA.
Methods: Plasma was collected from a cohort of 16,570 patients starting VKA treatment between January 2012 and December 2014. Patients were followed until a major bleeding event, the end of VKA treatment, death, or December 31st 2014, whichever came first. From the cohort, we assembled all 326 cases who developed a major bleeding during follow-up and a random sample of 652 patients selected at baseline (case-cohort study). Plasma sTM levels were measured by ELISA 3 weeks after start of VKA treatment and stratified by the 25th, 50th, 70th, and 85th percentile. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by means of weighted Cox regression and adjusted for age, sex, diabetes mellitus, and hypertension. To account for possible different causes of major bleeding we performed a subgroup analysis by stratifying patients into "provoked" (INR >3.5 at time of major bleeding) or "unprovoked" (INR <3.5 at major bleeding) and into "labile" or "stable" INR using their time in therapeutic range (TTR) 6 weeks before a major bleeding event (TTR cut-off value 60%).
Results: Plasma was available from 263 cases (mean age 75, 56% men) and 538 subcohort patients (mean age 70, 54% men). Adjusted HRs for major bleeding increased with increasing sTM levels, from 1.2 (95%CI 0.8-2.0) in the 25th to 50th percentile to 1.9 (95%CI 1.1-3.1) above the 85th percentile as compared with the lowest 25th percentile. The adjusted HR for provoked major bleeding increased from the 70th percentile onward (70th to 85th percentile adjusted HR 1.9, 95%CI 1.0-3.9; above 85th percentile adjusted HR 1.8, 95%CI 0.9-3.7). In contrast, the risk of unprovoked major bleeding was increased in the upper 85th percentile only (adjusted HR 1.8, 95%CI 1.0-3.2). Similar results were obtained after stratification by TTR in the stable (adjusted HR1.9, 95%CI 1.0-3.6 above the 85th percentile) and labile group (adjusted HRs 2.2, 95%CI 1.2-4.1 and adjusted HR 1.8, 95%CI 0.9-3.4 in the 70th to 85th percentile and above the 85th percentile respectively).
Summary/Conclusion: In this study, elevated sTM levels were associated with major bleeding during VKA treatment, indicating that chronic endothelial injury contributes to major bleeding in these patients. This association was most evident at a lower sTM plasma level threshold in provoked major bleeding and labile TTR, which suggests that chronic endothelial damage combined with a high or fluctuating INR can increase the risk for major bleeding.

Bleeding 2
PS 3.4
Board No. 16

The effect of different prophylaxis regimens on fibrin gel structure in patients with severe hemophilia A

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Background: Prophylaxis with Factor VIII (FVIII) concentrate prevents recurrent bleeding and hemophilic arhtropathy in patients with severe hemophilia A (hA) but it is not widely available due to the high costs.
Aims: We investigated the effect of different prophylactic regimens in comparison with on-demand treatment on fibrin structure in patient with severe hA.
Methods: The study included 17 adults patients with severe hA. Informed concent was obtained. The informed consent was obtained. Five patients received FVIII concentrate in standard dose (20 IU/kg three times per week), five received intermediate dose (10-15 IU/kg three times per week), while seven patients received FVIII concentrate only on-demand. Blood samples were collected before the start of prophylaxis and after 3 months, before receiving next dose. Fibrin permeability, expressed as permeability coefficient (Ks), was assessed by a flow measurement technique. The results were correlated with FVIII. Also, electron microscopy (E
Results: Ks was significantly decreased after 3 months of standard dose prophylaxis (11.032 vs 7.308, p=0.043) as well intermediate dose (8.774 vs 5.748, p=0.043) while no change was observed in on-demand treated patients (9.334 vs 9.090). Initial Ks before the treatment did not differ between 3 groups (p=0.509). Ks correlated significantly with FVIII concentration (r^2=-0.643, p=0.002). EM images showed significant improvement of fiber's thickness and decreased porosity of fibrin network in patients after 3 months of treatment with both prophylaxis regimens (standard and intermediate dose).
Summary/Conclusion: The results of this study showed that fibrin clot permeability was significantly improved after prophylactic treatment with FVIII concentrate and this improvement was not directly and only associated with FVIII level. Intermediary dose was as good as standard dose prophylaxis in this context confirming that as it was previously described frequency rather than intensity is the most important for the treatment efficiency. This also indicates that sufficient and safe hemostasis may be achieved with lower cost of the treatment what is very important not only for developing but even countries with large healthcare budgets

Bleeding 2
PS 3.5
Board No. 17

Reduced clot firmness may contribute to bleeding phenotype in patients with mild bleeding disorders

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Background: Currently, around 50% of patients with a bleeding tendency does not fulfill diagnostic criteria, using the available diagnostic tools. Also, it is unclear if bleeding phenotype can be satisfactory explained by mild abnormalities, such as low Von Willebrand factor-levels or mild factor deficiencies. We hypothesize that the bleeding phenotype in these patients can be explained by an impaired interplay of several hemostatic factors. As formation of thrombin is the endpoint of most diagnostic coagulation tests, global coagulation assays might give more insight into the pathogenesis of bleeding of unknown cause.
Aims: To investigate the role of global coagulation assays in patients with a bleeding tendency not or incompletely explained by current diagnostic laboratory tests.
Methods: Patients aged ≥12 years with an increased bleeding tendency not explained by current diagnostic tests were included from July 2016 until March 2018. Medical history was taken by a (pediatric) hematologist. The ISTH-BAT was used to objectify bleeding. After routine hemostatic testing, patients were divided in three subgroups: 1) No bleeding disorder (BD-); 2) Bleeding of Unknown Cause (BUC); 3) Bleeding disorder with mild hemostatic abnormalities (BD+), e.g. Von Willebrand factor levels between 30-50% (low-VWF) or abnormal but nonspecific aggregometry pattern. To investigate global coagulation, rotational thromboelastometry and thrombin generation was used. The results were compared with healthy age- and sex matched controls. This study was subject to the Medical Research Involving Human Subjects Act and approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam.
Results: In total 185 patients (BD- n=59; BUC n=50; BD+ n=71) and 76 controls were included. Mean age was similar in patients and controls (33.7 and 35.8 years). An abnormal bleeding score was found in 65% of patients and 1% of controls. Overall, no differences in ROTEM variables were found between patients and controls. When comparing subgroups, BUC patients had a higher clot firmness after 1 hour (ML) than controls, in the EXTEM and INTEM assay (EXTEM ML 10.1±12.5% versus 6.9±3.3%, $p<0.05$; INTEM ML 12.6±16.1% versus 8.2±3.35%, $p<0.05$). BD+ had a lower maximum clot firmness (MCF) and lower clot firmness after 10 (A10) and 20 (A20) minutes than BD- in the FIBTEM assay (FIBTEM MCF: BD+ 14.1±3.7mm, BD- 17.3±5.3mm, $p<0.01$; FIBTEM A10: BD+ 13.7±3.6mm, BD- 16.4±4.9mm, $p<0.05$; FIBTEM A20: BD+ 14.3±3.5mm, BD- 17.2±5.1mm, $p<0.05$). For thrombin generation (data available of 68 patients and 30 controls), patients had lower, but not significantly different, median peak height (patients: Mdn 128nM, IQR 100–165; controls: Mdn 135nM, IQR 106–205, p=n.s.) and ETP (patients: Mdn 981nM.min, IQR 815–1228; controls: Mdn 1086nM.min, IQR 872–1368, p=n.s.).
Summary/Conclusion: Remarkably, patients with BUC had significantly higher clot firmness after one hour (ML) than controls, possible caused by differences in clot contraction or fibrinolysis. Also, patients with mild hemostatic abnormalities (BD+) had significantly lower clot firmness (MCF, A10 and A20), specifically in the FIBTEM assay, possible caused by disturbed clot polymerization. As the majority of these patients (BD+) had abnormal laboratory values suggestive of a disorder of primary hemostasis (e.g. low-VWF), this may confirm the hypothesis that the severity of bleeding can be caused by a second hemostatic abnormality impairing global hemostasis.

Bleeding 2
PS 3.6
Board No. 18

Genetics and hemostatic potential in hemophilia A patients with a discrepancy between one-stage and chromogenic assays: A cross-sectional study from Hemophilia centers in Stockholm and Belgrad

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Background: FVIII activity (FVIII:C) can be measured with several methods, including one-stage clotting assays (OSA) and chromogenic assay (CSA). Discrepancy in FVIII:C among the assays is well known and found associated with a number of genetic variations causing mild and moderate HA.
Aims: The objective of this study was to investigate the prevalence of discrepancy between OSA and CSA, identify associated genetic variations and to determine the usefulness of global hemostatic assays in HA
Methods: This is a cross-sectional study on HA patients with mild and moderate phenotype from the Hemophilia Center at Karolinska University Hospital, Stockholm Sweden and Hemostasis Department and Hemophilia center at Blood Transfusion Institute of Serbia, Belgrade, Serbia during 2013-2016. No patient had received any FVIII concentrate at least 30 days prior to the blood collection. An informed consent was obtained from all patients.
All laboratory measurements were performed at Karolinska Univeristy Hospital. FVIII activity was measured in citrated plasma using OSA (Factor VIII Deficient Plasma and Actin FSL, Siemens Healthcare Diagnostics, Marburg, Germany) and CSA (Coamatic Factor FVIII assay, Chromogenix, Milano, Italy). Both assays were analyzed on a BCS XP Instrument (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). We considered a discrepancy between OSA and CSA if the ratio was ≥ 2.0 between estimated FVIII:C. The coding regions of the *F8* gene were sequenced (Sanger sequencing, Macrogen, Seoul, Korea). Two global hemostatic assays; Endogenous Thrombin Potential (Innovance ETP test kit, Siemens Healthcare Diagnostics, Marburg, Germany) and Overall Hemostatic Potential (in-house method), were performed in patients from Stockholm.
Results: We detected discrepancies in 22 of 50 patients. All presented higher levels of FVIII:C with the one-stage clotting assay. The prevalence of discrepant patients was higher in the patients from Stockholm, 18 of 31, compared to the group from Belgrade, 4 of 18. A causal mutation was detected in all patients sequenced. In the discrepant group, ten different mutations were found. Three of them have previously been reported to be associated with higher OSA than CSA. One mutation was found in both groups and has previously been found in a non-discrepant patient. The remaining six mutations have not previously been connected with FVIII:C discrepancy. These novel mutations include Ile1194Phefs, Ile1679Thr, Phe679Leu, Tyr6Cys, Arg1696Gly and Met680Leu and they cluster in the A1, A2, a3 and A3 domains of FVIII. We found no difference between discrepant and non-discrepant patients with either of the global assays of hemostasis.
Summary/Conclusion: The frequency of discrepancy was three times higher in Sweden compared to Serbia. Six novel mutations not previously associated with FVIII:C discrepancy were identified in discrepant patients. Global hemostasis assays did not contribute to recognizing discrepancy. We suggest that both FVIII:C assays should be used in the diagnosis of patients with possible hemophilia A.

Bleeding 3
PS 4.1
Board No. 19

Sports participation and physical activity in patients with von Willebrand disease

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Background: Sports participation and physical activity may put patients with bleeding disorders at an increased risk of bleeding. On the other hand, these activities may lead to improvement of physical health and quality of life. Several studies on sports participation have been performed in hemophilia patients, but studies in patients with Von Willebrand disease (VWD) are lacking.
Aims: To study the sports participation and physical activity of a large cohort of VWD patients, and to identify subgroups of VWD patients that experience difficulties in sports participation and physical activity.
Methods: We included patients from the 'Willebrand in the Netherlands study'; a nationwide cross-sectional study in VWD patients in the Netherlands. All patients completed a questionnaire on sports participation, physical activity, quality of life (Short Form-36) and bleeding symptoms (Tosetto bleeding score). The study was approved by the Medical Ethical Committees of all participating centers. All patients signed informed consent.
In the statistical analysis, binomial outcomes (sports vs no sport, etcetera) were adjusted for age and sex using logistic regression analysis.
Results: In total 798 VWD patients were included, of whom 474 with type 1, 301 type 2 and 23 type 3 VWD. The mean age was 39 ± 20 (standard deviation) years. Five hundred fifty two patients (69.3%) participated in sports. Sports participation and physical activity were associated with a better quality of life ($p<0.001$). There was no difference in sports participation among the three types of VWD. Most VWD patients participated in cycling (42%), followed by walking (36%) and swimming (24%). Type 3 VWD patients more often did not participate in sports due to fear of bleeding and physical impairment, respectively OR=13.24 (95% CI:2.45-71.53) and OR=5.90 (95% CI:1.77-19.72), corrected for age and sex. Patients that did not participate in sports due to physical limitations had a higher total bleeding score and bleeding score item for joint bleeds, respectively OR=1.05 (95% CI: 1.01-1.09) and OR=1.31 (95% CI: 1.07-1.61), corrected for age and sex. Furthermore, severe limitations to walk 1000m were associated with type 3 VWD and bleeding score item for joint bleeds, respectively OR=4.31 (95% CI: 1.07-17.37) and OR=1.24 (95% CI: 1.01-1.53), corrected for age and sex. Patients with type 3 VWD had more often severe limitations to lift or to carry groceries; and to bow, to kneel or to bend, respectively OR=5.80 (95% CI: 1.37-24.56) and OR=9.84 (95% CI: 2.83-34.24), corrected for age and sex. A higher total bleeding score and bleeding score item for joint bleeds were associated with severe limitations to climb up stairs, respectively OR=1.07 (95% CI: 1.03-1.12) and OR=1.36 (95% CI: 1.09-1.69) corrected for age and sex.
Summary/Conclusion: The majority of VWD patients participated in sports. Compared to earlier studies, patients with VWD participated as much and in the same types of sports as the general population. Patients with type 3 VWD, a more severe bleeding phenotype, and a history of joint bleeds frequently experienced limitations in sports participation and physical activities during daily life. Therefore, the sports participation and physical activity of these patients requires special attention.

Bleeding 3
PS 4.2
Board No. 20

Platelet count and risk of major bleeding at venous thromboembolism diagnosis

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Background: Accurate stratification of major bleeding (MB) risk may optimize the management of venous thromboembolism (VTE) during anticoagulant therapy. Platelet count is a readily available biomarker that may predict the future risk of MB. However, conflicting results have been reported regarding the association between platelet count and future risk of MB in patients with VTE.
Aims: To investigate the association between platelet count, measured at VTE diagnosis, and risk of MB during the first year after VTE.
Methods: The study population comprised 777 patients with a first lifetime symptomatic VTE confirmed by objective tests, who had platelet count measured at VTE diagnosis (Tromsø Study). Potential cases of MB were identified through review of medical records during the following year after incident VTE. MB was defined according to the International Society on Thrombosis and Haemostasis recommendation. Cox proportional hazards regression models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for MB across quartiles of platelet count. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: There were 56 MBs within the first year of follow-up (incidence rate 9.3/100 person-years, 95% CI 7.1-12.0). The risk of MB increased across quartiles of platelet count, where subjects in the highest quartile ($\geq$303 x 10^9/L) had a 3-fold higher risk of MB (HR 3.2, 95% CI 1.3-7.7) compared to those in the lowest quartile (<191 x 10^9/L [reference]) in an age- and sex-adjusted model. The association between high platelet count ($\geq$303 x 10^9/L) and risk of MB was particularly pronounced for subjects with pulmonary embolism, with a HR of 12.7 (95% CI 1.5-105.5). Further adjustments for active cancer at VTE diagnosis and competing risk by death yielded essentially similar risk estimates for MB. The 3- and 12-month cumulative incidences for the highest quartile of platelet count were substantially higher (5.4% and 8.1%, respectively) than the corresponding cumulative incidences for the lowest quartile (2.0% and 2.9%, respectively) in competing risk by death analyses.
Summary/Conclusion: We found that a high platelet count ($\geq$303 x 10^9/L), measured at VTE diagnosis, was associated with an increased risk of MB during the following year. Our findings suggest that the upper spectrum of a platelet count has the potential to identify patients at risk of MB, aiding clinical decision on VTE management.

Bleeding 3
PS 4.3
Board No. 21

Discrepancy between one-stage clotting and chromogenic substrate Factor IX assays in hemophilia B patients: A cross-sectional study from Stockholm hemophilia center

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Background: Chromogenic substrate assay (CSA) has recently become available to use in routine laboratories for measuring FIX. One-stage clotting assay (OSA) is still predominantly used in laboratories around the world. Discrepancy between OSA and CSA in hemophilia A is a well-known phenomenon for Factor VIII assays. However, little is known if there is a discrepancy between OSA and CSA for FIX.
Aims: We aimed to study if there was any discrepancy in measured FIX levels between OSA and CSA in HB patients and if this correlated to causative mutation in *FIX*.
Methods: Citrated plasma samples, sent to Karolinska University Laboratory with request for FIX levels during 2015-2018, where analyzed with OSA and CSA. Actin FSL reagent (Siemens Healthcare, Marburg, Germany) with ellagic acid as activator and immunodepleted Factor IX deficient plasma from Stago (Diagnostica Stago, Asnières, France) was used as OSA. For CSA, two different assays were evaluated; Biophen Factor IX (Hyphen BioMed, Neuville-sur-Oise, France) and Rox Factor IX kit (Rossix AB, Mölndal, Sweden). All samples were measured on the instrument BCS XP (Siemens Healthcare, Marburg, Germany). At the laboratory the samples were analyzed blinded (the disease status was unknown). The samples were cross-checked with medical records, only patients with confirmed HB were included. We considered a discrepancy between OSA and CSA if the ratio was ≥ 2.0 between estimated FIX-levels. HB patients have previously been genotyped.
Results: FIX levels in plasma were evaluated in fifty-five samples from patients with known HB. 32 samples were excluded for further analysis because of influence of FIX-concentrate (<7 days since last exposure) or presence of auto antibodies against FIX. The remaining 23 samples represented 21 individual patients, whereas 6 were female carriers. A discrepancy ratio of ≥ 2.0 between OSA and CSA was observed in 13 of these 21 patients. CSA misclassified six HB patients as severe despite both mild to moderate clinical phenotype and FIX levels determined by OSA. Six additional patients were classified as moderate by CSA and mild by OSA. The genotype was known in 12 of these 13 patients. They all had missense mutations; one in the pro domain (p.Arg43Trp), next in GLA (p.Glu53Gly) and further 6 mutations in the serine protease domain whereas three in p.Ala380Asp, three in p.Gly402Glu, and further one in each p.Thr342Met, p.Ser384Cys, p.Tyr444Cys and p.Ser430Ile.
Summary/Conclusion: In more than half of the patients with mild and moderate HB, a discrepancy was found with lower FIX levels using two different CSA assays compared to OSA. Causative mutations were in the pro/GLA and serine protease regions. For HB diagnostic, both CSA and OSA should be applied to avoid misclassification of HB severity.

Bleeding 3
PS 4.4
Board No. 22

A novel, cost effective lateral flow immunoassay (LFIA) technique for the diagnosis of severe von Willebrand disease (VWD)

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Background: Diagnosis of VWD requires a series of laboratory investigations, most of which are not available in many of the laboratories in India. Coagulation factor assays are being performed in few laboratories; however exclusion of VWD which needs VWF antigen assays at least for the diagnosis of severe VWD, is lacking in most of the laboratories in the country. A rapid and accurate diagnosis is critical in severe VWD patients, as early therapy can be life-saving. The existing methods for the detection of VWF:Ag have several limitations i.e. time consuming, needs expert personnel and are expensive. Except PT, rapid test kit is not available for diagnosis of any of the common bleeding disorders. LFIA based Point of care (POC) testing possess the advantages of rapid, low cost, high specificity and sensitivity, reproducibility, very simple to operate, compactness, portability, on-site detection and no requirement of expert personnel or sophisticated instruments.
Aims: To develop a rapid, specific, user friendly and cost effective lateral flow immunoassay based technique for diagnosis of severe VWD.
Methods: Blood samples were collected from both type 3 and type 1VWD patients and were analyzed for FVIII:C, platelet aggregation using ristocetin, VWF- RiCof and VWF:Ag using conventional techniques. The most reliable, widely used gold nanoparticles (GNPs) were synthesized in the laboratory using different citrate reduction methods and were characterized by Visual Inspection, UV-Vis Spectra Analysis, Dynamic Light Scattering Analysis and Transmission Electron Microscopy. GNP-Ab conjugation was achieved by passive adsorption and covalent bonding techniques. Different combinations of lateral flow membranes were assembled manually in the laboratory according to the GNP size and mobility on the membrane.
Results: Smaller (15 ± 2nm) and stable GNPs were synthesized using the citrate reduction method (Turkevich method). All possible permutations and combinations of membranes and VWF antibodies (primary as well as secondary from different sources) were used. Sensitivity of the strips was tested on the standard plasma dilutions with known concentration of antigen measured by ELISA. LFIA designed for the detection of the VWF:Ag was 100% specific and did not show cross reactivity against any of the coagulation factors. The assay was standardized and made sensitive to detect all VWD cases with >10% VWF:Ag, in order to diagnose clinically severe VWD patients
Summary/Conclusion:

- The citrate reduction process was a simple and reproducible way to produce mono-disperse, quasi-spherical, smaller, stable GNPs.
- Electron microscopy with high resolution was required for the estimation of shape and exact size of the smaller GNP synthesis.
- Different LFIA membranes showed differential migration of the sample on the strips. Immediate visualization of signals or bands was generated within 10 minutes after the application of the sample.
- A simple POC semi-quantitative test for diagnosis type 3 von Willebrand disease established.
- The cost per test by LFIA VWF strip is approximately **€0.7**

Bleeding 3
PS 4.5
Board No. 23

Bleeding In Subjects With Congenital Fxi Deficiency Exposed To Risk Situations: Anticoagulant Treatments And Gynecological And Obstetrical Complications. Results From The Real World.

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Background: FXI, the second element of the contact pathway, is activated by FXIIa and thrombin. Then, FXI is involved in the amplification of thrombin generation. However, there are contradictory data on the bleeding phenotype of patients with congenital FXI deficiency, an underestimated disorder. Spontaneous bleeding is rare and usually mild, but haemorrhages can occur at sites of injury, particularly in highly fibrinolytic tissues. In contrast, recent results support a protective role of FXI deficiency against thrombosis. Of note, most studies in this field have been done in a specific population, Ashkenazy Jews, with a homogeneous genetic background.
Aims: To retrospectively evaluate bleeding in subjects with congenital FXI deficiency exposed to two risk situations: anticoagulant treatments and gynecological/obstetrical complications.
Methods: The study was done in a large and molecularly heterogeneous cohort of Caucasian subjects with congenital FXI deficiency (N= 214, with 13 different *F11* gene defects, mostly heterozygous) identified by prolonged aPTT (> 1.3) or family studies, not by clinical events, therefore preventing clinical selection bias.
Results: Nine cases received anticoagulant therapy (acenocoumarol) mainly due to atrial fibrillation. Not a single bleeding event was recorded over the course of more than 428 months of treatment, despite the fact that 2 individuals had severe FXI deficiency and 3 simultaneously received antiplatelet therapy (one case with double antiaggregant therapy). Likewise, no bleeding was recorded in a patient treated with low molecular weight heparin during an acute thrombotic event. INR adjustment and acenocoumarol doses required to achieve the target INR were unaltered by FXI deficiency and the time these individuals remained in therapeutic range was standard.
Ninety-five women had FXI deficiency. Forty haemorrhagic events were reported in 26 women, 52% after dental, surgical or obstetrical procedures. Only 19 events were spontaneous. Abnormal uterine bleeding was the event most frequently found (N= 12), but it was mild as all episodes turned self-limited and no one required from hospitalization or antihaemorrhagic interventions. Nine postpartum haemorrhages were recorded from 136 deliveries, 6 requiring from intervention (red blood cell transfusion; fresh frozen plasma administration and/or endometrial curettage). Four postsurgical bleeding complications were registered among 25 gynaecological surgeries. Prophylaxis with fresh frozen plasma, used in 12 gynaecological surgeries, did not prevent from postoperative bleeding in 3 cases, but 2 developed severe adverse reactions (TRALI). Abnormal uterine bleeding, postpartum and postsurgical haemorrhages were related to both a positive bleeding history and FXI:C$\leq$ 43.5%.
Summary/Conclusion: The clinical observations of a large cohort of Caucasian subjects with congenital FXI deficiency that might represent the *real world*, as they were not selected based on clinical features and have a wide molecular base, suggest that FXI deficiency does not entail a severe bleeding phenotype, even in high-risk scenarios. This disorder might not increase the risk of severe gynecological/obstetrical haemorrhages and our results question the effectiveness and safety of prophylaxis with fresh frozen plasma in this setting. Finally, congenital FXI deficiency does not interfere with anti-vitamin K treatment management or bleeding risk. All these results support the safety of novel antithrombotic treatments targeting FXI.

Bleeding 3
PS 4.6
Board No. 24

Development of the first Point-of-Care Test selective of the direct oral anticoagulant Apixaban for emergency assessment of coagulation.

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Background: Direct oral anticoagulants (DOACs) are being increasingly prescribed. Consequently, more DOAC-treated patients are admitted to emergency units across all clinical specialities. Importantly, fast and reliable emergency coagulation testing has not been solved yet because specific point-of-care test (POCT) are not currently available.
Aims: The challenge was to develop a specific and semi-quantitative POCT on whole blood within 5 to 15 minutes using an optical reader. The proof of concept was first applied on targeting Apixaban a selective factor Xa inhibitor.
Methods: The POCT was based on a lateral flow immunoassay (LFIA) using monoclonal antibodies (MAbs) against the Apixaban molecules which were recently developed in our lab. Three different clones (8C9, 9D9, 3C1), ranging affinity (K_D) respectively from 2 to 140 nM, were tested in two models (competition and inhibition). The selectivity and the sensitivity were evaluated on spiked solutions (saline, plasma, whole blood) with different concentrations of Apixaban (0, 10, 30, 250 ng/mL) and on plasma from patients treated with Apixaban, Rivaroxaban, Heparins (UNF, LMWH) and Fondaparinux. After 15 min running, results were obtained using an optical reader (Skannex, Norway).
Results: The sensitivity obtained on spiked solutions (whole blood and plasma) has shown a nice linear correlation between the Apixaban concentration from 0 to 250 ng/mL and the optical density (OD) in a range from 6.5 to 3.5 respectively. These results were confirmed in plasma from Apixaban treated-patient (n=55). The selectivity was also checked on plasma from patients (n=9 for each) treated with Rivaroxaban, UNF, LMWH and Fondaparinux and gave the same response in OD than the control, which clearly show no cross-reactivity.
Summary/Conclusion: These first results confirmed that the POCT targeting Apixaban is selective with a good tendency for semi-quantitative diagnostic and could be a fast and reliable alternative for guiding emergency treatment during Apixaban therapy.

Clotting 1
PS 5.1
Board No. 25

Prevalence and clinical features of a delayed or missed diagnosis of pulmonary embolism: A Swedish cross-sectional study from emergency department visits

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Background: Acute pulmonary embolism (PE) is the most severe presentation of venous thromboembolism due to its potentially fatal outcome. In the emergency department (ED), PE poses a diagnostic challenge and is assumed to be one of the most frequently missed diagnoses.
Aims: We aimed to assess the prevalence of a delayed or missed PE diagnosis in patients admitted to the ED and compare the clinical features and emboli location in both groups to those diagnosed without delay.
Methods: This is a cross-sectional study based on extracted electronic medical record data from the ED at Karolinska University Hospital between January 2012 and April 2016. PE cases were identified from the data based on main discharge diagnosis and diagnostic imaging (computed tomography pulmonary angiography or pulmonary scintigraphy).The patients were categorised into three groups: 1) delayed diagnosis; diagnosed within 72 hours after being admitted, 2) missed diagnosis; previous visit within 20 days with a chief complaint typical for PE but discharged with a different diagnosis, and 3) controls; diagnosed without delay in the ED.Categorical variables were compared using chi-squared test. Tests were two tailed and p-values of <0.05 were considered statistically significant.
Results: 1280 PE-related patient visits where identified in the register, from which 761 patients met the inclusion criteria. Of these, 512 patients (67%) were diagnosed without delay (controls), 118 patients (16%) had a delayed diagnosis and 48 (6%) an initial missed diagnosis (the remaining 11% not fitting into any category). Compared to the controls, patients in the delayed group were significantly older (75 vs 68 years) and had higher levels of Troponin T and NT-proBNP as well as more comorbidities. Those with a missed diagnosis had a trend towards a younger age (61 vs 68 years), had significantly higher CRP and presented more often with abdominal pain compared to the controls. The most common initial diagnoses at the first visit were pneumonia or respiratory tract infection (RTI), followed by abdominal pain.A higher prevalence of peripheral PE was observed in the delayed group compared to the controls (54% vs 43%), whereas no difference was found between the controls and those with missed diagnosis.
Summary/Conclusion: A delayed and missed diagnosis of PE were found common and accounted for more than one fifth of all PE cases. Diagnosis was more often delayed in the elderly, possibly attributed to comorbidities that share clinical features with PE. In contrast, atypical clinical presentation may explain why younger patients more often tended to be misdiagnosed.

Clotting 1
PS 5.2
Board No. 26

Dietary intake of marine n-3 fatty acids is associated with lowered risk of incident VTE

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Background: Marine polyunsaturated fatty acids (n-3 PUFAs) have demonstrated antithrombotic effects in experimental studies, suggesting that dietary intake of n-3 PUFAs may be associated with a lower risk of venous thromboembolism (VTE). However, results from epidemiological studies have so far been conflicting, potentially due to methodological challenges related to the assessment of n-3 PUFAs-intake and changes in dietary habits during a long follow-up (regression dilution).
Aims: To investigate whether dietary intake of n-3 PUFAs was associated with the risk of incident VTE in a population-based cohort with repeated assessments of intake of n-3 PUFAs-intake.
Methods: We recruited 21,941 participants from the fourth (1994-95) and the sixth (2007-08) surveys of the Tromsø Study, and recorded incident VTE events up to 31.12.2016. Those attending both surveys had their exposure data updated and contributed with two observation periods. Total weekly intake of n-3 PUFAs was computed from self-reported intake of fat and lean fish, fish as spread and fish oil supplements, and participants were divided into quartiles according to intake in grams per week (Q1: <4.7, Q2: 4.7-13.4, Q3: 13.4-29.1, Q4: >29.1). Hazard ratios (HRs) for VTE adjusted for age (as time scale), sex and body mass index were calculated across quartiles using time-varying Cox-regression models with Q1 as the reference. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: There were 541 incident VTE events during follow-up, and median duration of the observation periods was 11.6 years. Compared to Q1, subjects in Q2-4 had 22-26% lower risk of VTE (HR Q2 0.74, 95% CI 0.57-0.96; HR Q3 0.77, 95% CI 0.59-0.99; HR Q4 0.78, 95% CI 0.61-1.00). The association was most pronounced in relation to provoked VTE, particularly provoked pulmonary embolism (PE), where the corresponding risk estimates were 0.42 (95% CI 0.25-0.72), 0.40 (95% CI 0.23-0.68) and 0.61 (95% CI 0.38-0.96) for Q2-4, respectively.
Summary/Conclusion: By applying time-varying analyses and a comprehensive evaluation of dietary n-3 PUFAs-intake, we found that a moderate weekly consumption was associated with a lower risk of VTE, and particularly provoked PE. The association displayed a threshold pattern, and suggests that a protective effect is achieved with intakes exceeding 4.7 grams of n-3 PUFAs per week.

Clotting 1
PS 5.3
Board No. 27

Characterization of disease presentation and treatment outcomes in aTTP patients with initial or recurrent disease during the Phase III HERCULES trial of caplacizumab

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Background: Acquired thrombotic thrombocytopenic purpura (aTTP) is a life-threatening autoimmune blood clotting disorder. Patients are at risk for significant morbidity and death during each episode. The efficacy results of the Phase III HERCULES study in patients with aTTP showed that caplacizumab significantly reduced time to platelet count response, the incidence of a composite of TTP-related death, recurrence, or major thromboembolic event during study drug treatment, recurrences during the study, and healthcare resource utilization. The safety profile of caplacizumab was favorable. Consistent with its mechanism of action, and in line with previous results, there was an increase in mucocutaneous bleeding events (Scully *et al*., Blood 2017 130:LBA-1).
Aims: Characterization of disease presentation and evaluation of treatment outcomes in patients enrolled in the HERCULES study with an initial or recurrent aTTP episode.
Methods: Demographics, baseline disease characteristics, and treatment outcomes (time to platelet count response, mortality, recurrence, major thromboembolic (TE) events and refractoriness) were evaluated for both subgroups using descriptive summaries.
Results: 145 patients were randomized, 82 with an initial aTTP episode and 63 with recurrent disease. Demographics were generally balanced between groups, whereas baseline disease characteristics were more severe in initial vs. recurrent episodes: mean platelet count ($28.8x10^9$/L vs. $44.4x10^9$/L), mean LDH (598U/L vs. 523U/L) and median cardiac Troponin-I (0.119µg/L vs. 0.036µg/L). The time from first symptoms until diagnosis was also longer in those experiencing an initial episode (6.5 days) vs. a recurrent one (3.9 days). More patients in the caplacizumab group had their first aTTP episode (66.7%, n=48) vs. the placebo group (46.6%, n=34). Treatment with caplacizumab improved outcomes in both subgroups compared to placebo. Specifically, treatment with caplacizumab resulted in:

- A faster time to platelet count response compared to placebo, for both subgroups: the platelet count normalization rate was 1.67 (95% CI 1.03 to 2.72) and 1.64 (95% CI 0.95 to 2.82), in patients with an initial versus recurrent episode, respectively.
- A lower proportion of patients with TTP-related death, recurrence of TTP (i.e., exacerbation), or a major thromboembolic event during the treatment period compared to placebo, in both sugroups: 6 (12.8%) vs. 19 (55.9%) in patients with an initial episode; 3 (12.5%) vs. 17 (43.6%) in patients with a recurrent episode
- A reduction in disease recurrence (i.e., exacerbation and relapse) during the overall study period compared to placebo, for both subgroups: 6 (12.8%) vs. 15 (44.1%) in patients with an initial episode; 3 (12.5%) vs. 13 (33.3%) in patients with a recurrent episode
- A reduction of refractory disease. No caplacizumab-treated patient was refractory to therapy compared to 3 placebo-treated patients (1 patient with an initial episode and 2 patients with a recurrent episode)

Summary/Conclusion: Patients with an initial aTTP episode have a delayed presentation and more severe baseline disease characteristics than those with recurrent disease. Treatment with caplacizumab improves outcomes in both subgroups, i.e., a faster time to platelet count response, lower proportion of patients with either death, recurrence or a major TE event during the treatment period, lower recurrence rate during the overall study period, and prevention of refractoriness.

Clotting 1
PS 5.4
Board No. 28

Obese patients treated with apixaban and rivaroxaban for venous thromboembolism: a pilot study

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Background: Obesity is an independent risk factor for venous thromboembolism (VTE) and its prevalence is steadily increasing. As direct oral anticoagulants (DOAC) are given in fixed dose, uncertainties have arisen on their pharmacokinetics in obese patients and specific studies on their efficacy and safety in this population are limited.
Aims: The main goal of this prospective pilot two-center study is to assess DOAC concentrations in obese patients, treated with apixaban or rivaroxaban for VTE, and to compare them to values available for non-obese patients, as suggested by the ISTH guidelines (Martin et al, JTH 2016). We also recorded the clinical events during the follow-up.
Methods: Since July 2017 in one center and March 2018 in the other one, consecutive obese patients (> 30 kg/m^2) with VTE and treated with rivaroxaban or apixaban, seen in the outpatient thrombosis clinics of Rennes and Brest University Hospitals, were included in this study. DOAC plasma concentrations were systematically measured using STA-Liquid-anti-Xa®, specific controls and calibrators on a STA-R Evolution® analyser (Stago). The lower limit of quantification was 20 ng/mL. The exact time between DOAC intake and blood sampling was recorded for each patient. DOAC values were compared to those from PK studies with rivaroban and apixaban. Any thrombotic or hemorrhagic adverse event was registered.
Results: Fifty nine patients (34 women, 25 men) were included in this study with 73 DOAC measurements. The mean±sd BMI was 35±5 kg/m^2 with 10 patients having a BMI > 40 kg/m^2. The mean±sd age was 56±16 years and the mean±sd creatinine clearance (Cockcroft) 84±24 mL/min. The treatment was: rivaroxaban 20mg (n=33), apixaban 5mgx2 (n=28) and apixaban 2.5 mgx2 (n=12). The concentration ranged from <20 to 381 ng/mL and the mean delay between DOAC intake and blood sampling was 8±7h. Whatever the delay between drug intake and sampling, all but one DOAC concentrations were within the expected range. One patient receiving apixaban 2.5 mgx2 had a peak concentration < 20 ng/mL; but she had likely not taken the drug.
The median (min-max) delay since the thrombotic event was 9 months (min-max: 1-84) with no thrombotic or hemorrhagic event reported during this period.
Summary/Conclusion: In this pilot study that is on-going, we found that in obese patients treated for VTE, apixaban and rivaroxaban concentrations are comparable to those in non-obese patients. Further data and larger studies with a longer follow-up are required to define the pharmacokinetics profile of DOAC and assess their efficacy and safety, in this special population.

Clotting 1
PS 5.5
Board No. 29

Red cell distribution width and risk of atrial fibrillation and subsequent thromboembolism. The Tromsø Study

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Background: Red cell distribution width (RDW) is associated with future risk of cardiovascular diseases, including atrial fibrillation (AF) and venous thromboembolism (VTE). Whether RDW is associated with thromboembolic events in AF patients is scarcely known.
Aims: We aimed to assess the impact of RDW on the risk of incident AF, and subsequent AF-related VTE and ischemic stroke, in a cohort recruited from a general population.
Methods: RDW was measured in 26,111 participants from the fourth survey of the Tromsø study, and incident AF cases were registered through December 31, 2013. Among participants with AF, first-ever VTE events and ischemic strokes were registered from the date of AF diagnosis through the end of follow-up. Cox proportional hazards regression models were used to estimate hazard ratios (HR) with 95% confidence intervals (CI) for AF across quartiles of RDW or RDW above the 95th percentile, using the lowest quartile as the reference. In addition, cause-specific HRs for VTE and ischemic stroke by tertiles of RDW were calculated for participants with AF. The regression models were also adjusted for potential confounders. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: There were 2081 incident AF cases during a median of 18.8 years of follow-up, yielding a crude incidence rate of 4.7 per 1000 person-years. Participants with RDW in the highest quartile (RDW ≥ 13.3%) had 27% higher risk of AF than those in the lowest quartile (RDW ≤ 12.3%) (HR 1.27, 95% CI 1.09-1.47) in the multivariable adjusted model). Among those with AF, subjects with RDW in the upper tertile had a 2-fold higher risk of ischemic stroke (HR 2.07, 95% CI 1.20-3.57). In contrast, RDW was not associated with incident VTE in subjects with AF.
Summary/Conclusion: RDW was significantly associated with incident AF in a general population. Among subjects with AF, high RDW was associated with risk of ischemic stroke, but not VTE.

Clotting 1
PS 5.6
Board No. 30

HIV infection and risk of recurrent venous thromboembolism: a community-based cohort study

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Background: People living with HIV (PLWH) are at higher risk of a first venous thrombotic event (VTE). Whether this translates into a higher risk of recurrent VTE is undetermined.
Aims: To estimate VTE recurrence rates in PLWH and compare this to HIV-negative VTE patients.
Methods: PLWH with a first lower extremity deep vein thrombosis (DVT) and/or a pulmonary embolism (PE) were identified in a subset of the ATHENA cohort, which represents 70% of consenting PLWH in care in the Netherlands. First VTEs had occurred from 2003 to 2014. Patients with first VTE from the MEGA-follow-up study (followed from 1999-2009) without HIV acted as a comparator group.
Rates of recurrent VTE were evaluated after anticoagulant therapy discontinuation. Recurrent VTE were adjudicated as definite or probable according to a predefined rule that was similar for ATHENA and MEGA. The primary outcome was definite recurrent lower extremity DVT and/or PE. Unprovoked VTE were defined as not being associated with cancer (in the past 6 months in ATHENA, in the past 5 years in MEGA) or major surgery, use of estrogen, pregnancy, being bedridden >3 days and/or wearing a plaster cast due to a fracture (in the past 90 days).
Cumulative incidences were estimated with Kaplan-Meier analyses accounting for death as competing risk and were stratified by type of event (provoked/unprovoked). Cox proportional hazards regression was used to estimate hazard ratios (HR), adjusted for age and sex.
Results: Of 201 PLWH with eligible index events, 153 had observations after withdrawal of anticoagulation. Of these events 126 (95 unprovoked) were in males and 27 events (13 unprovoked) were in females. In MEGA, 4005 patients with VTE stopped anticoagulation; 1813 (998 unprovoked) were male and 2192 (363 unprovoked) were female.
Overall, 27 definite events occurred during 541 years FU (median 3.2 years) in PLWH (rate: 5.0/100 person years [PY]) whilst 635 definite events occurred during 20,215 PY (median 6.1 years) in MEGA, (rate: 3.1/100 PY). Kaplan-Meier estimates (KMEs) were higher for PLWH after the first year FU (12 vs 6%), with absolute differences slightly declining thereafter (2 & 5-year KMEs: 15 vs 8% & 18 vs 15%). In participants with an unprovoked index event, the KMEs for PLWH vs controls were: 15 vs 9% at 1 year and 21 vs 17% at 3 years.
The KMEs suggested a Cox model assessing all observations might violate proportionality assumptions, which was confirmed in analysis of Schoenfeld residuals (p associated with HIV as a covariate=0.01). Evaluation of the KMEs suggested a maximum risk difference between PLWH and controls at one year FU. Consequently, the Cox model was split into two models at one year FU, which did not violate the proportionality assumption. The model considering first FU year yielded an adjusted HR of 1.72 (95%CI: 1.03-2.87) for PLWH versus controls. The model considering observations after the first FU year yielded an adjusted HR of 0.65 (95%CI 0.31-1.39).
Summary/Conclusion: PLWH had a 70% higher risk than controls of recurrent VTE in the first year after anticoagulant withdrawal, which was not sustained in later FU. A possible explanation for this is that PLWH may have a temporarily higher risk due to immune deficiency, which subsides as immune reconstitution takes place with antiretroviral therapy. However, significant imprecision remains in our estimates. Therefore, further research is needed to make these estimates more precise and identify whether any HIV-specific factors (i.e. immune status) predict VTE risk.

Clotting 2
PS 6.1
Board No. 31

The Pseudonaja textilis factor Va A2 domain retains functional integrity following activated protein C-mediated proteolysis

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Background: Coagulation factor Va (FVa) is proteolytically inactivated by activated protein C (APC), which is key to downregulate the procoagulant response. APC cleaves FVa at several positions throughout the A2-domain, with Arg306 and Arg506 as major cleavage sites. These cleavages result in A2-domain dissociation and complete loss of cofactor activity. We previously reported the functional resistance of snake venom *Pseudonaja textilis* factor V (ptFV) to human APC, despite proteolysis within the A2-domain. Sequence analysis revealed the absence of the 306 site and surrounding residues in ptFV. In addition, ptFV was observed to comprise a unique disulfide bond covalently linking the A2- and A3-domains.
Aims: Here we aim to assess the role of ptFV's unique disulfide bond and the sequence that replaces the region surrounding cleavage site Arg306 in relation to its functional APC-resistance.
Methods: The non-conserved ptFV 306 region (GNPDTLT) was exchanged for the homologous human Arg306 region (PKKTRNL), thereby generating ptFV-h306 and hFV-pt306. Furthermore, ptFV's unique disulfide bond was recombinantly removed by replacing the cysteines for serine residues, thereby generating ptFV-h306-SS. All variants were stably expressed in BHK cells and purified to homogeneity employing ion-exchange chromatography.
Results: Incubation with human APC revealed that while ptFVa was not proteolyzed at the 306 position, introduction of the human 306 region resulted in Arg306 cleavage of ptFVa-h306 and ptFVa-h306-SS. Conversely, this cleavage was absent in hFVa-pt306. Full proteolysis of human FVa (500 nM) was achieved following treatment with 10nM APC, while a 75-fold higher APC concentration (750 nM) was required to obtain fully proteolyzed ptFVa-h306 and ptFVa-h306-SS, similar to ptFVa. Functional analysis of APC-cleaved FVa variants employing a purified prothrombinase system revealed that hFVa-pt306 retained procoagulant activity, in a similar manner to hFVa-R306T. Surprisingly, all ptFVa variants maintained full cofactor activity, despite extensive APC-mediated proteolysis. In contrast, while treatment with plasmin (400 nM) resulted in full proteolysis of the ptFVa variants at 5 min. of incubation, functional inactivation profiles showed a gradual loss of cofactor activity over time, retaining 20% cofactor activity at 15 min. of plasmin inactivation.
Summary/Conclusion: Collectively, these findings indicate that, conversely to human FVa, APC-dependent cleavage of ptFVa at Arg306 or deletion of the unique disulfide bond does not abrogate FVa cofactor function. This may suggest that even following APC cleavage at the positions homologous to human 306 and 506, the functional integrity of the ptFVa A2-domain is stabilized such that it is able to form productive interactions. This is further supported by plasmin cleavage, suggesting an enhanced stability of the regions essential to cofactor function in ptFVa. As such, ptFVa provides a biological model to further study the unique structural elements that prevent A2-domain dissociation.

Clotting 2
PS 6.2
Board No. 32

A novel ADAMTS13 conformation ELISA shows conformational activation of rat ADAMTS13 with exposure of cryptic epitopes in vitro

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Background: Thrombotic thrombocytopenic purpura (TTP) is characterized by a severe deficiency in the multidomain metalloprotease ADAMTS13, consisting of a metalloprotease (M), disintegrin-like (D), cysteine-rich (C), spacer (S), 8 thrombospondin type 1 repeats (T1-8) and 2 CUB domains. Human ADAMTS13 can adopt different conformations and several monoclonal anti-T2-CUB2 antibodies (mAbs) are able to induce an increased proteolytic activity (FRETS-VWF73 assay) by opening ADAMTS13, and subsequently revealing cryptic epitopes in human ADAMTS13. Recently, our group has shown that ADAMTS13 adopts an open conformation in acute immune-mediated TTP. Therefore, animal models mimicking conformational changes in ADAMTS13 would be of great importance to further elucidate the pathophysiology of TTP.
Aims: We aimed at inducing conformational changes in rat ADAMTS13 using anti-ADAMTS13 mAbs and to develop an ELISA to discriminate between open and folded rat plasma ADAMTS13.
Methods: First, cross-reactivity of the activating anti-human ADAMTS13 mAbs 17G2 (anti-CUB1) and 19H4 (anti-T8) with rat ADAMTS13 was tested in ELISA. Next**,** rat plasma ADAMTS13 activity was determined in the absence/presence of the activating mAb 19H4, using the FRETS-VWF73 assay. Thereafter, a panel of anti-ADAMTS13 mAbs recognizing cryptic epitopes in human ADAMTS13 were screened for cross-reactivity with cryptic epitopes in rat ADAMTS13 (pre-incubated with 19H4) using ELISA. Finally, an ELISA was developed to discriminate between rat plasma ADAMTS13 in the absence (**folded**) and presence (**open**) of the activating mAb 19H4.
Results: Of the activating anti-human ADAMTS13 mAbs, only 19H4 bound to rat ADAMTS13 in ELISA. In addition, mAb 19H4 induced a 2.3 fold increase in rat plasma ADAMTS13 activity in the FRETS-VWF73 assay. The cloned patient anti-S Ab I-9, which recognizes a cryptic epitope in human ADAMTS13, cross-reacted with rat ADAMTS13 but only after pre-incubation of rat ADAMTS13 with mAb 19H4. Finally, a novel rat ADAMTS13 conformation ELISA was developed, in which only open rat ADAMTS13 is captured by Ab I-9 e.g. upon pre-incubation of rat ADAMTS13 with mAb19H4, and detected by biotinylated cross-reacting polyclonal rabbit-anti-mouse ADAMTS13 Abs.
Summary/Conclusion: Activating conformational changes can be induced in rat ADAMTS13 upon addition of the anti-T8 mAb 19H4. The folded vs open rat ADAMTS13 conformation can be discriminated in our novel in-house ELISA by using the anti-S Ab I-9, which recognizes a cryptic epitope in ADAMTS13. This unique tool can now be used to further investigate conformational changes in ADAMTS13 *in vivo* and to elucidate whether exposure of cryptic epitopes in ADAMTS13 can evoke an anti-ADAMTS13 immune response in the rat.

Clotting 2
PS 6.3
Board No. 33

Cytosolic localization of small antithrombins caused by a new mutation affecting the initiation codon in a large thrombophilic family. Bioinformatic analysis of mutations affecting initiation codons

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Background: Gene variations in *SERPINC1* may cause antithrombin deficiency through different mechanisms. The analysis of families with extraordinary clinical phenotype and antithrombin deficiency may help to identify new mechanisms involved, which may also be extrapolated to other disorders and similar gene defects.
Aims: To characterize the relevance of a mutation in the initiation codon of *SERPINC1* and to analyze the effect of mutations affecting initiation codons all over the genome through a bioinformatic approach.
Methods: A large Norwegian thrombophilic family with antithrombin deficiency was studied. Wild type and 19 variant antithrombins were expressed in HEK293-EBNA cells. Recombinant proteins were evaluated by biochemical, functional and proteomic methods. Intracellular localization of antithrombin was explored by electron microscopy.
A bioinformatic search of mutations affecting initiation codons in whole-exome of 5 subjects and in ENSEMBL was done. The consequences of alternative initiation codons of mutations with low (<0.01) and high (>0.01) minor allele frequency (MAF) were compared.
Results: The severe thrombosis (early, recurrent and/or fatal) of 17 members of the family was associated to a type I antithrombin deficiency (anti-FXa 52% and antigen levels 56%) caused by a c.3G>T heterozygous mutation in exon 1, affecting the translation-initiation codon of *SERPINC1*. Expression of this variant in a eukaryotic cell produced three different antithrombins. Two downstream methionines located after the signal peptide were used as alternative initiation codons, generating highly expressed small aglycosylated antithrombins with cytoplasmic localization. Wild-type antithrombin was explained by the use of the mutated ATT as initiation codon. Actually, any codon except for the three stop-codons might be used as translation initiation in this strong Kozak context.
Whole-exome analysis revealed the mean of 12 gene variations per person affecting initiation codons, 88% with high MAF. Analysis of ENSEMBL revealed 11,261 genetic variations affecting the initiation codon of 7,205 genes, 99.5% with low or unknown MAF. Genetic variations with high MAF had closer alternative ATG downstream codons than did those with low MAF (29bp *vs* 49bp, p=5:42e-6). Besides, the high-MAF group better maintained both the signal peptide and reading frame.
Summary/Conclusion: Nature is always the best inspiration for basic research. The analysis of a family with an exorbitant clinical thrombosis has revealed unexpected consequences of natural mutations affecting translation-start codons. The small antithrombin generated by downstream initiation codons has cytoplasmic localization, explaining the type I deficiency. However, this aberrant localization, together with the different biochemical properties may render unexplored consequences that could contribute to the dramatic clinical phenotype of carriers. The pathogenic consequences of initiation mutations may not be forecasted by current prediction tools, as they calculate the effects of a missense change that never occurs. Instead, downstream alternative initiation ATG codons may be used, generating smaller or different molecules. We have identified differentiating elements (location of alternative downstream ATG codons; conserved reading frame or signal peptide) that could help to determine the pathogenicity of these variations. Finally, our data further support the use of other codons apart from ATG for initiation of translation in eukaryotes.

Clotting 2
PS 6.4
Board No. 34

Mannose-binding lectin-associated serine protease-1 (MASP-1) of the complement system is associated with disseminated intravascular coagulation in septic shock patients

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Background: Disseminated intravascular coagulation (DIC) is a serious complication to sepsis with a mortality up to 40% despite intensive care treatment. Activation of the complement system is part of the dysregulated host response in severe sepsis. The enzymes mannose-binding lectin-associated serine proteases -1 and -2 (MASP-1 and -2) of the complement system activate prothrombin and induce fibrin formation *in vitro*, but their role in the development of sepsis-related DIC is not well investigated.
Aims: To explore the association between MASPs and other proteins of the complement system's lectin pathway and DIC in septic shock.
Methods: We included patients from the Intensive Care Unit, Aarhus University Hospital, Aarhus, Denmark (n=36). Inclusion criteria were age above 18 years and septic shock defined as a clinical diagnosis of sepsis and need for vasopressor treatment to maintain mean arterial blood pressure above 65 mmHg. Exclusion criteria were pregnancy, cancer or chemotherapy within 3 months, major trauma or surgery within 48 hours, known inherited bleeding disorder, plasma transfusion within 3 days and treatment with oral anticoagulants within 3 days. Sequential Organ Failure Assessment (SOFA) score, total fluid administration, medication at blood sampling, comorbidities, microbiological results and 30-day mortality were registered. Plasma levels of lectin pathway proteins (mannose-binding lectin (MBL), H- and M-ficolin, collectin liver-1 (CL-L1), MASP-1, -2 and -3, MAp19, MAp44) were analysed with time-resolved immunofluorometric assay (TRIFMA). Furthermore, coagulation parameters (platelet count, international normalised ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen, fibrin d-dimer, antithrombin), infection markers (C-reactive protein, leukocyte count) and organ dysfunction markers (alanine aminotransferase (ALAT), bilirubin, creatinine, urea, lactate) were analysed. DIC score was calculated according to the International Society of Thrombosis and Haemostasis. The study was conducted in accordance with the Helsinki Declaration and the Danish Health Care Act, including rules for informed consent, but it was regarded as a quality assurance study by the regional health ethics committee and was as such exempt from notification to the ethics committee system under Danish law.
Results: MASP-1 plasma levels were significantly lower in patients with high DIC score (≥5, n=12) than patients with low DIC score (<5, n=24), median (interquartile range (IQR)): 6168 (5255-7374) vs 9279 (6860-12733) ng/ml, p=0.02. MASP-1 plasma levels also correlated negatively with aPTT (Spearman's rho -0.43, p=0.008) and positively with antithrombin plasma level (rho=0.38, p=0.02). There was a numerically, though non-significantly, lower H-ficolin (median (IQR): 17228 (13905-25955) vs 4957 (19893-30993) ng/ml, p=0.07) and MAp44 (median (IQR): 1868 (1427-3159) vs 2569 (1995-3245) ng/ml, p=0.29) in the high DIC score group. Patients with high DIC score had higher median SOFA score (p=0.01), plasma-bilirubin (p=0.02) and -lactate (p=0.01) than patients with low DIC score. Administered fluids and ALAT did not differ significantly between groups (p>0.05).
Summary/Conclusion: Low MASP-1 plasma levels are associated with high DIC score, prolonged aPTT and low antithrombin levels. This may indicate that increased MASP-1 activation and consumption is associated with more severe coagulation disturbances in septic shock. Thus, our findings point to a possible role for MASP-1 in sepsis-related DIC.

Clotting 2
PS 6.5
Board No. 35

Anti-CUB1 or anti-spacer antibodies that increase ADAMTS13 activity act by allosterically enhancing metalloprotease domain function

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Background: ADAMTS13 circulates in a folded conformation, which is mediated by interactions between the C-terminal CUB domains and its central Spacer domain. Binding of ADAMTS13 to the VWF D4-CK domains disrupts the CUB-Spacer interaction, inducing a structural change that extends ADAMTS13 into an open conformation that enhances catalytic efficiency ~2-fold. This mechanism supports a model in which ADAMTS13 unfolding induces exposure of an exosite in the Spacer domain that interacts with the VWF A2 domain, increasing the affinity between the two molecules, and, therefore, the rate of proteolysis. The D4-CK-mediated conformational activation of ADAMTS13 can be mimicked *in vitro* with the use of antibodies (Abs) that disrupt the CUB-Spacer interaction, such as the previously published anti-CUB antibody, Ab17G2. We recently generated a novel, activating antibody against the Spacer domain (Ab3E4).
Aims: To characterize the mechanism by which the Ab17G2 and Ab3E4 enhance the catalytic efficiency of ADAMTS13.
Methods: The effects of the Ab17G2 and Ab3E4 on the activity of ADAMTS13 were studied using FRETS-VWF73. The effects of the Ab17G2 and Ab3E4 on the kinetics of VWF96 (VWF G1573-R1668) proteolysis were characterized using an in-house assay. ELISA was used to investigate conformational changes in ADAMTS13 induced by the Ab17G2 and Ab3E4.
Results: Both Ab17G2 and Ab3E4 enhanced FRETS-VWF73 proteolysis by ~1.7-fold. This result was reproduced using the VWF96 substrate; the Ab17G2 and Ab3E4 enhanced the catalytic efficiency (k_{cat}/K_m) of ADAMTS13 by ~1.8- and ~2.0-fold, respectively. The activation was dependent on the conformational extension of ADAMTS13, since the Abs could not enhance the activity of an ADAMTS13 variant that lacks the TSP2-CUB2 domains (MDTCS). Surprisingly, ADAMTS13 activation was not mediated through exposure of the Spacer or Cys-rich domain exosites as previously proposed, as the Ab17G2 and Ab3E4 efficiently enhanced proteolysis of VWF96 variants in which the Spacer/Cys-rich exosite binding sites were disrupted. Kinetic analysis of VWF96 proteolysis showed that the Ab17G2- and Ab3E4-induced activation of ADAMTS13 is primarily manifest through a ~1.5- to ~2-fold increase in enzyme turnover (k_{cat}). Thus, contrary to the current model, this suggests that the conformational extension of ADAMTS13 influences the functionality of the active site, and not substrate binding affinity (K_m). Incubating ADAMTS13 with either Ab17G2 or Ab3E4 exposed a cryptic epitope in the metalloprotease domain that was specifically detected by an Ab binding to this cryptic site in ELISA, further corroborating that the Abs induce a conformational change in ADAMTS13 affecting the M domain.
Summary/Conclusion: Abs can be used as tools for understanding the structure/function of enzymes. Using activating Abs against the Spacer and CUB1 domains of ADAMTS13, we show for the first time that the activation of ADAMTS13 following its unfolding is not a result of exposure of a functional exosite in Spacer/Cys-rich domain that increases affinity to VWF. Rather, our data are consistent with an allosteric activation mechanism upon the metalloprotease domain. We propose that ADAMTS13 unfolding causes a conformational change in the active site that further activates the enzyme. We are currently investigating whether the D4-CK-induced enhancement of ADAMTS13 proteolytic activity is also mediated by conformational changes in the active site.

Clotting 2
PS 6.6
Board No. 36

Structural features of acute ischemic stroke thrombi

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Background: Acute ischemic stroke is one of the leading causes of death and disability worldwide. Despite the huge clinical, economical and social burden associated with ischemic stroke, only two strategies are currently available to remove the occluding thrombus and restore blood flow to the brain: (i) pharmacological thrombolysis using tissue plasminogen activator (t-PA) and (ii) mechanical removal of the thrombus via endovascular thrombectomy. However, more than half of the patients receiving t-PA do not respond to the therapy due to so called 't-PA resistance'. In addition, the thrombectomy procedure is not successful in 10-20 % of patients. The factors contributing to such therapy resistance are not known but are most likely linked to specific characteristics of the thrombus that is occluding the blood vessel in stroke patients.
Aims: The aim of this study was to analyze ischemic stroke thrombi retrieved from patients treated with thrombectomy to better understand thrombus characteristics and structure.
Methods: A total of 176 thrombi were collected from endovascular treated ischemic stroke patients at Groeninge Hospital (Kortrijk, Belgium). Fresh thrombi were fixed in 4 % paraformaldehyde, embedded in paraffin and cut into 5 µm sections. Histological analysis was performed using hematoxylin and eosin, martius scarlet blue staining, Feulgen staining, and both immunohistochemical and immunofluorescence analysis of von Willebrand factor (VWF), platelets (GPIb), fibrin, DNA and white blood cells (CD45). Multicolor immunofluorescent analysis was performed on a randomly selected subset of thrombi (n = 8).
Results: Stroke thrombi are heterogenous. Nevertheless, histological analysis reveals common structural features. In general, stroke thrombi are composed of red blood cell (RBC)-rich areas, interspersed with platelet-rich areas. On a microstructural level, RBC-rich areas consist of red blood cells that are trapped within a relatively loose fibrin network in a honeycomb structure in which platelets and VWF are absent. In contrast to RBC-rich areas, platelet-rich zones consist of much denser fibrin structures that in addition are lined with VWF. These fibrin-VWF organizations are filled with platelets. White blood cells tend to accumulate on the boundaries between RBC-rich and platelet-rich areas. DNA networks are seen throughout the thrombus in some but not all thrombi and are particularly present in the platelet-rich areas and in the boundary zones.
Summary/Conclusion: We describe the complex microstructure of patient stroke thrombi. Fibrin and VWF align in dense platelet-rich structures, together with DNA networks that span through the thrombus. These observations corroborate our previous findings that VWF and DNA can contribute to t-PA resistance. Whether the dense platelet-rich zones that are interspersed within loosely arranged RBC areas influence efficiency of mechanical thrombus retrieval during thrombectomy is currently being investigated. The identification of these defined thrombus characteristics is crucial for further research to improve acute ischemic stroke therapy.

Clotting 3
PS 7.1
Board No. 37

Phenotypic and genotypic analysis of a cohort of 114 patients with acquired thrombotic thrombocytopenic purpura

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Background: ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type I repeats, member 13) specifically regulates the adhesive activity of von Willebrand factor (VWF) by cleaving its high-molecular-weight multimers (HMWM). A severe functional deficiency of ADAMTS13 leads to thrombotic thrombocytopenic purpura (TTP), a thrombotic microangiopathy (TMA) that can be either hereditary due to recessively inherited mutations of the *ADAMTS13* gene (Upshaw-Schulman Syndrome, USS) or acquired mostly due to autoantibodies to ADAMTS13, thus termed acquired autoimmune TTP. The deficiency of ADAMTS13 causes the accumulation of hyperadhesive VWF HMWM which spontaneously bind to platelets and form microthrombi in the circulation. Acquired TTP is a medical emergency requiring rapid management, but clinical presentation is widely heterogeneous and could partially depend on the percentage of VWF HMWM. Electrophoretic analysis is the reference method for the study of VWF multimers, but it is time-consuming and complex to handle. Moreover, a specific mutation of the *ADAMTS13* gene (R1060W), well established in USS patients, was unexpectedly previously described in 3 patients with acquired autoimmune TTP.
Aims: The first aim of this work was to compare VWF multimeric distribution assessments using an ELISA measuring the capacity of VWF to bind to collagen with electrophoretic analysis, in order to detect an excess of VWF HMWM in a cohort of acquired TTP patients. The second aim was to screen this cohort for the mutation R1060W of the *ADAMTS13* gene.
Methods: We gathered clinical and biological data of a cohort of 114 patients with acquired TTP and 9 patients with USS used as controls. Informed consent was obtained from each patient according to the Declaration of Helsinki. Plasma VWF antigen (VWF:Ag) and VWF collagen-binding capacity (VWF:CB) were assessed in every patient and VWF multimers gel electrophoresis was performed in 25 patients (16 acquired TTP and 9 USS). We expressed the results as ratios (VWF: CB/VWF:Ag) and compared them to VWF HMWM percentages assessed by electrophoresis. Finally, the mutation R1060W of the *ADAMTS13* gene was screened in 98/114 acquired TTP patients using Sanger sequencing.
Results: In our cohort of 114 acquired TTP, the median VWF:Ag level was 146 UI/dL and 81 patients (71%) had increased VWF:Ag levels (N: 50 – 100 UI/dL). The median VWF: CB/VWF:Ag ratio was 0.53, markedly below reference values (0.7 – 1.0), including ratios <0,7 in 73 patients (64%), within reference values in 27 patients (23.7%) and >1.0 in 14 patients (12.3%). In the 9 USS patients, the median VWF: CB/VWF:Ag ratio was 1.17. The correlational study between VWF: CB/VWF:Ag ratio and VWF HMWM percentage assessed by electrophoretic analysis (25 patients) is statistically significant ($p< 10^{-5}$). However, no R1060W mutation was found in our cohort of acquired TTP patients.
Summary/Conclusion: VWF: CB/VWF:Ag ratio calculated through an easy-to-perform ELISA is a satisfactory approach to estimate VWF HMWM. The potential clinical relevance of this ratio will be further studied in our cohort. The absence of R1060W mutation in our cohort most likely rules out its implication in the development of anti-ADAMTS13 autoimmunity.

Clotting 3
PS 7.2
Board No.38

Cumulative meta-analysis of Low Molecular Weight Heparin's antitumoral effect: toward the disappearance of the treatment effect

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Background: LMWH corresponds to the standard of care for the treatment and prevention of venous thromboembolism in cancer patients. An unexpected reduction of mortality was observed in early studies evaluating LMWH in post-hoc subgroup analysis with no clear pharmacological explanation. First randomized clinical trial conducted to confirm this effect were promising, but the most recent ones were rather contradictory
Aims: To investigate the evolution of LMWH's treatment effect estimates over time by a cumulative meta-analysis of randomized clinical trials.
Methods: We conducted a systematic review of randomized clinical trials that compared LMWH for at least 1 month with placebo or standard therapy in cancer patient. The computer-assisted search was performed on electronic database (MEDLINE, The Cochrane Library databases, Google Scholar) up to July 2017. The cumulative meta-analysis was performed by sequentially including studies one by one from the oldest to the most recent one. Each time the treatment effect on overall survival was estimated using the sum of hazard ratio (HR) weighted by the inverse of the variance.
Results: When considering only early studies (up to 2005), treatment with LMWH is associated with an improvement of overall survival in cancer patients. Since 2005, this treatment effect continuously decreases toward an HR of 1. In 2017, 17 studies are included in the meta-analysis (10 567 patients) with an HR for overall survival estimated to HR=0.99 [95% CI, 0.94-1.05].
Summary/Conclusion: This meta-analysis is not in favor of an improvement of survival in cancer patients treated with LWH. First studies (up to 2005) seems to correspond to false positive results with a regression toward the absence of survival benefit since 2005.

Clotting 3
PS 7.3
Board No. 39

Estimated cardiorespiratory fitness and future risk of venous thromboembolism in the general population

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Background: Cardiorespiratory fitness (CRF) is an integrative measure of whole-body functional capacity, and a predictor of arterial cardiovascular disease, cancer and mortality. Although CRF largely reflects habitual physical activity, inherited factors also contribute to the inter-individual variation in CRF. In general, CRF is found to be a superior health predictor compared to physical activity. Current evidence suggests that physical activity may lower the risk of incident venous thromboembolism (VTE), but limited data exists on the association between CRF and VTE risk.
Aims: To investigate whether estimated CRF (eCRF) was associated with the risk of incident VTE in a cohort recruited from the general population.
Methods: A total of 10 393 individuals participating in the sixth survey of the Tromsø Study (2007-08) were included, and incident VTE was recorded up to December 31, 2016. eCRF was estimated from sex-specific algorithms based on age, waist circumference, resting heart rate and self-reported physical activity. Age- and sex-specific quintiles (Q) were used to categorize participants into low (Q1+2), moderate (Q3+4) and high (Q5) eCRF. Hazard ratios (HRs) adjusted for age (as time scale) and sex were calculated using Cox regression models with low eCRF as the reference. Analyses were also performed in subgroups stratified by weight status (i.e. normal weight: body mass index (BMI) 18.5-24.9 kg/m^2 and overweight/obese: BMI ≥25 kg/m^2). The Regional Committee for Medical and Health Research Ethics approved the study, and written informed consent was obtained from all study participants.
Results: There were 176 incident VTE events during a median follow-up of 8.5 years (incidence rate 2.1 per 1000 person-years). In the total study population, those with moderate and high eCRF had 41% (HR 0.59; 95% CI 0.43-0.82) and 54% (HR 0.46; 95% CI 0.28-0.73) lower risk of VTE, respectively, compared to individuals with low eCRF. The effect was essentially similar for provoked and unprovoked events. In analyses restricted to overweight and obese individuals, moderate and high eCRF was associated with 39% (HR 0.61; 95% CI 0.41-0.89) and 41% (HR 0.59; 95% CI 0.26-1.36) lower risk, respectively. The corresponding effect sizes in normal weight individuals were 49% (HR 0.51; 95% CI 0.24-1.08) and 65% (HR 0.35; 95% CI 0.15-0.78).
Summary/Conclusion: We found that moderate and high CRF, estimated from easily available clinical variables, were associated with lower risk of incident VTE. The effect remained in subgroups stratified by weight status, suggesting that CRF may also lower the elevated VTE risk associated with obesity.

Clotting 3
PS 7.4
Board No. 40

Syncope Is Significant Predictor Of 30-day Mortality In Female Pulmonary Embolism Patients

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Background: Syncope is common symptom in pulmonary embolism (PE). Although recent studies indicated that syncope is not significant predictor of 30-day mortality, it is still not clear does that finding apply for male and female PE patients when observed separately.
Aims: To explore whether syncope is significant predictor of 30-day mortality in male and female PE patients observed separately.
Methods: This is a multi-center retrospective observational study on consecutive PE patients. Patients were divided into two cohorts, males and females, and each cohort was evaluated for the presence of differences between patients with syncope and without syncope regarding basic admission characteristics, clinical characteristics and laboratory values. Thirty-day mortality was evaluated separately for male and female PE patients.
Results: Five hundred eighty eight patients were enrolled in the study, 294 (50%) were males and 294 (50%) were females. In a male cohort, patients with syncope were older, had significantly more often high-risk PE, higher PESI score, and higher values of admission glycaemia, troponin T and B-type natriuretic peptide, as compared with patients without syncope. However, male patients with syncope did not have significantly different 30-day mortality, as compared with those without syncope (log rank p=0.942). In contrast to male cohort, fewer differences were noticed between female PE patients with and without syncope, but those with syncope had significantly increased 30-day mortality rate (log rank p=0.025). Multivariable cox regression analysis has shown that syncope was significant predictor of 30-day mortality in female patients (HR=3.18, 95%CI 1.05-9.58).
Summary/Conclusion: Syncope is significant predictor of 30-day mortality only in female pulmonary embolism patients.

Clotting 3
PS 7.5
Board No. 41

Pulmonary emboli localization - impact on clinical presentation and case fatality rate: A Swedish cross-sectional study from emergency department visits

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Background: The clinical presentation of pulmonary embolism (PE) at the Emergency Department (ED) varies and therfore poses a diagnostic challenge. Studies on the relationship between emboli localization and clinical presentation as well as case fatality rate (CFR) are still few and small in sample size.
Aims: We aimed to investigate the association between emboli localization and clinical presentation in PE at the ED. We further studied how the emboli localization affected the CFR at different time points.
Methods: This was a cross-sectional study based on extracted electronic medical record data from the ED at Karolinska University Hospital between January 2012 and April 2016. PE patients (≥18 years old), were identified from the data through main discharge diagnosis based on findings from computed tomography pulmonary angiography performed within 24 hours of admission. If a patient had multiple visits, only the first was included in the study. Emboli localization was categorized to central; pulmonary trunk and arteries or peripheral; lobar, segmental and subsegmental arteries. Patients were divided into two groups based on the most proximal localized embolus; central (with or without peripheral emboli) or peripheral. Categorical variables were compared using Chi-square test, i.e. sex, chief complaint, triage level at the ED; priority group 1-2 vs 3-5 and CFR at 30 days and 1 year. Numerical variables, i.e. age, CRP, Troponin T, NT-proBNP and vital signs were compared using Mann-Whitney U Test and presented as median values. P-values of < 0.05 were considered statistically significant.
Results: Out of 1280 recorded ED visits with PE, 607 patients met the inclusion criteria. Of these, 182 had centrally and 425 peripherally localized PE, without any difference in sex and age between the groups. Patients with central PE more frequently reported shortness of breath as chief complaint (58 vs 48%) whereas abdominal/flank pain was more common in peripheral PE (7,3 vs 2,8%). For vital signs, there were significant differences for all variables except systolic blood pressure. Most prominent was the difference in heart rate, being higher (102 vs 92 bpm) in centrally localized PE. Cardiac biomarker levels were higher in patients with central PE compared to peripheral, Troponin T (38 vs 14 ng/L) and NT-proBNP (758 vs 254 ng/L). The triage level at the ED significantly differed, patients with central PE were more often classified as priority 1-2. There was no significant difference in CFR at either 30 days nor 1 year, nonetheless a higher rate was observed in peripheral PE compared to central (5,7 vs 2,8% and 24 vs 18% respectively).
Summary/Conclusion: Patients admitted to the ED with central PE had a more alarming clinical presentation with shortness of breath, impaired vital signs and increased cardiac biomarker levels compared to those with peripheral. This was successfully identified in the triage, resulting in a higher priority for these patients. No statistical difference was found in CFR, suggesting that emboli localization has subsidiary influence on fatality from one month to one year after the ED visit.

Clotting 3
PS 7.6
Board No. 42

Venous thromboembolism risk assessment in 936 women of reproductive age from families with thrombophilia: subanalysis of the MARFAST cohort

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Background: The thrombophilia testing in relatives from families with known thrombophilia has not demonstrated its utility. Indeed, the result of the testing does not allow to discriminate at risk/low risk individuals for venous thromboembolism (VTE). Although the identification of at risk individuals is of major importance in order to handle at risk situations among which combined oral contraceptives (COC) and pregnancy are challenging because of their great frequency and duration of exposition.
Aims: The aim of this study is to assess the risk associated with inherited thrombophilia and additional genetic and environmental factors in women of reproductive age from families with known thrombophilia.
Methods: All women of reproductive age were selected from MARFAST, a large family study (2214 relatives from 651 families with thrombophilia). A thrombophilia testing, ABO blood group determination and the genotyping of 11 polymorphisms known to associate with VTE in the general population were performed in all included patients. Thrombophilia was classified as mild (factor V Leiden (FVL) heterozygous (HTZ) or G20210A prothrombin mutation (PTG) HTZ) or severe (antithrombin, protein C, protein S deficiencies, FVL homozygous, PTG homozygous, combined defects). In the whole cohort of women, hazard ratios (HR) were calculated taking into account the family structure (frailty term) in a multivariate survival analysis. Then a subgroup analysis was performed. A first group was defined as all women with a history of COC use. Incidences of COC-related VTE were calculated with duration of COC use as time of follow-up. The second group was composed of women with pregnancy history.
Results: A total of 936 women were included among whom 113 (12%) had a personal history of VTE. 22 VTE (19%) occurred during COC use and 30 (27%) were pregnancy/post-partum related. 369 (39%), 388 (41%) and 179 (19%) relatives had no defect, mild or severe thrombophilia respectively. In the multivariate survival analysis, compared to women with no defect, relatives with severe thrombophilia displayed a 3.78 HR (95% confidence interval 2.17-6.60). Of note, mild thrombophilia wasn't associated with VTE risk (HR= 1.28; 0.75-2.21). ABO blood group was strongly associated with VTE, the highest risk being observed for AB blood group (HR= 3.40; 1.69-6.86, as compared to O blood group). FGG_rs2066865 CT/TT genotypes and F11_rs2036914 CT/TT genotypes were associated with VTE: HR= 1.75 (1.15-2.66) and 1.65 (0.99-2.77) respectively. Very interestingly, slightly different results were obtained within the subgroups. In the COC subgroup F11_rs2036914 wasn't associated with VTE (p= 0.25) whereas another polymorphism located on *F11* was, F11_rs2289252: HR= 8.33 (1.11-62.43) for CT/TT genotypes. Of note, all VTE patients but one harbored the at-risk allele (T). In the pregnancy/post-partum subgroup AB blood group was particularly prevalent in VTE patients (27%) and polymorphisms located on *F11* did not demonstrate association with VTE (p= 0.91 for both polymorphisms).
Summary/Conclusion: In conclusion, in families with thrombophilia, women harboring a severe defect present an increased risk of VTE compared to relatives without defect. This difference does not hold for mild thrombophilia. ABO blood group and *F11* polymorphisms are significant modifiers of VTE risk in women. A better understanding of the association between VTE and hormonal situations is mandatory.

Clotting 4
PS 8.1
Board No. 43

Development of a prothrombinase-based assay to measure the susceptibility of factor V variants to inhibition by TFPIα

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Background: Coagulation factor V (FV) is the precursor of activated FV (FVa), which assembles with factor Xa (FXa) on phospholipid surfaces to form the prothrombinase complex, accelerating prothrombin activation >1000-fold. FV activation occurs *via* three proteolytic cleavages, which generate activation intermediates with increasing affinities for FXa. Recently, it has been shown that tissue factor pathway inhibitor α (TFPIα) inhibits both the activation of FV and the ability of partially activated FV(a) species to enhance prothrombin activation. These effects are mediated by interactions of the C-terminus of TFPIα with an acidic region in the B-domain of FV as well as with the FV(a) heavy chain. The pathophysiological relevance of these novel anticoagulant activities of TFPIα is still unexplored, but evidence has been provided that the FV Leiden (FVL) mutant is less susceptible to TFPIα inhibition. Moreover, FV splicing variants (FV-short) with increased affinity for TFPIα have been described.
Aims: To develop an assay that measures the susceptibility of FV variants to TFPIα inhibition in plasma.
Methods: FV in 1/1000 diluted plasma was activated for 3 minutes with a suboptimal FXa concentration (20 pM) on 20/60/20 DOPS/DOPC/DOPE lipids (30 μM) in the presence or absence of a peptide mimicking the C-terminus of TFPIα (TFPIα C-term, 100 nM). Purified prothrombin (0.5 μM) and a chromogenic substrate for thrombin (500 μM) were then added, and the activity of the prothrombinase complex was monitored continuously up to 30 minutes. Absorbance curves were fitted to parabolas and the coefficient of the x^2-term was taken as the rate of prothrombin activation. The assay outcome was expressed as residual prothrombinase ratio (RP-ratio), defined as the ratio between the rates of prothrombin activation obtained in the presence and absence of TFPIα C-term. The assay was validated using plasma from 4 FVL homozygotes and FV-depleted plasma reconstituted with recombinant FV-short or full-length FV.
Results: The rate of prothrombin activation in the absence of peptide was a function of plasma FV level and pre-incubation time, and was inhibited by TFPIα C-term in a dose-dependent manner. A pre-incubation time of 3 minutes and a peptide concentration of 100 nM, yielding an RP-ratio of 0.30 in normal pooled plasma, were chosen. The RP-ratio was independent of the plasma FV level in the 75-150% range. Moreover, control experiments indicated that, at this high dilution, the plasma background did not influence the assay outcome. The intra- and inter-assay coefficients of variation of the RP-ratio were 5.4% and 12%, respectively. FVL homozygotes had higher RP-ratios than normal controls (0.45 ± 0.04 vs. 0.30 ± 0.03, $p=0.002$), indicating resistance to inhibition by TFPIα C-term. Differently, FV-short yielded a markedly reduced RP-ratio compared to full-length FV (0.18 vs. 0.35), as expected from their respective affinities for TFPIα.
Summary/Conclusion: We have developed and validated an assay that measures the susceptibility of (plasma) FV(a) to inhibition by TFPIα. This assay can be used to test whether TFPIα-mediated inhibition of FV activation and prothrombinase activity differs for (genetically) different FV variants and whether it correlates with the risk of thrombosis or bleeding.
Supported by grant 2014-1 from the Dutch Thrombosis Foundation.

Clotting 4
PS 8.2
Board No. 44

Clinical and laboratory characteristics of type IIHBS Antithrombin deficiency; prevalence of Antithrombin Budapest 3 mutation among different patients groups and investigation of its origin

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Background: Antithrombin (AT) is an important circulating inhibitor of blood coagulation proteases. Hereditary AT deficiency is classified as type I, type IIRS (reactive site defect), type IIHBS (heparin-binding site defect) and type IIPE (pleiotropic effect). The mutation profile of AT gene (*SERPINC1)* is heterogeneous, the most prevalent mutations are AT Cambridge II (IIRS), AT Budapest 3 (ATBp3, IIHBS), AT Padua (IIHBS) and AT Basel (IIHBS). The ATBp3 mutation underlies the vast majority of AT deficiencies in the Hungarian population due to a founder effect.
Aims: The aim was to describe the clinical and laboratory characteristics of type IIHBS AT deficiency in a large cohort of Hungarian patients (n=124). Our goal was to determine the prevalence of ATBp3 mutation among different patients groups; in general Hungarian (n=1000) and Roma population (n=1185); in patients with venous thrombosis (n=304), in young myocardial infarction (MI) (n=88) and in stroke (n=119) patients. We also aimed to investigate the age and origin of the most recent common ancestor of the ATBp3 mutation-bearing chromosomes.
Methods: Clinical and laboratory data of IIHBS patients were collected, the IIHBS mutations were detected by Sanger sequencing. Presence of ATBp3 was investigated with a LightCycler480 instrument by using real-time PCR and melting curve analysis. Analysis of eight short tandem repeat sequences (STRs) was executed on an ABI3130 Genetic Analyzer. The decay of linkage disequilibrium (LD) over generations was modeled by DMLE+ method. Informed consent was obtained from all participants.
Results: Among the selected patients with known IIHBS AT deficiency the frequency of ATBp3, AT Padua I and AT Basel was 86%, 9% and 4%, respectively. Clinical and laboratory phenotypes of IIHBS were heterogeneous and dependent on the specific mutation. While venous thrombosis was the most severe in ATBp3 homozygotes, arterial thrombosis and pregnancy complications were the most frequent in AT Basel and AT Padua I, respectively. The ATBp3 mutation did not occur in the general Hungarian population, in contrast, the frequency of this mutation was surprisingly high, 2.8% in the general Roma population. The mutant “T” allele was associated with one single STR haplotype in ATBp3 mutation carrier Roma patients, which was identical to that of Hungarian ATBp3 individuals. The occurrence of ATBp3 in patients with venous thrombosis was 1.6%, in young patients with MI it was 2.3% and in stroke it was 0.8%. Assuming an average of 25 years per generation, the results of LD decay modeling suggests the most recent common ancestor of ATBp3 in the XVII century.
Summary/Conclusion: Type IIHBS AT deficiency is a heterogeneous disease. Among IIHBS subtypes the ATBp3 is most common among Hungarian AT deficient patients. It is especially interesting that the prevalence of ATBp3 mutation in the general Roma population is so common. This knowledge draws the attention to the possibility to improve the health status of the Roma population by including the screening for ATBp3 mutation in their risk assessment for thrombotic diseases. The estimated age of the ATBp3 mutation, the geographic distribution of families with ATBp3 and the history of the modern Hungarian population are consistent with the hypothesis that the mutation originated (or was originally introduced) to Hungary.
Funding: NKFI-OTKA K116228 (Hungarian National Research Fund) GINOP-2.3.2-15-2016-00039 (Ministry of National Economy, Hungary)

Clotting 4
PS 8.3
Board No. 45

ABO blood group and venous thromboembolism risk: input of the measurements of plasmatic A and B glycosyltransferase activities.

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Background: ABO blood group is one of the major risk factor for venous thromboembolism (VT). The ABO locus encodes A and B glycosyltransferases (AGT and BGT) defining A and B blood groups. The most commonly accepted hypothesis is that ABO blood group influences the risk of VT by modifying GT activity that further modulates Factor VIII (FVIII) and von Willebrand Factor (VWF) plasma levels.
Aims: The main objective of this work was to evaluate the association of GT activities in plasma and VT risk in a case-control study for VT.
Methods: The case-control study included 420 cases with personal history of VT (deep vein thrombosis and or pulmonary embolism) from the MARTHA population (MARseille THrombosis Association study) and 587 healthy controls recruited from the French blood transfusion center. Cases and controls were matched for age, sex and blood group. GT activities in plasma were measured using the quantitative transfer of tritiated N-acetylgalactosamine or galactose to the 2'-fucosyl-lactose as acceptor and expressed in terms of disintegration per minute / 30µL of plasma (dpm/30µL). FVIII and VWF plasma levels were respectively measured using human FVIII-deficient plasma in a 1-stage factor assay and STA LIATEST VWF (Diagnostica Stago) on a STAR automate.
Results: ABO A1 was associated with increased A GT activity in an additive mode ($p<10^{-4}$), consistently in cases and controls. Similarly, individuals homozygotes for ABO B group were at higher BGT activity than individuals heterozygotes ($p=0.003$). Interestingly, we found that AGT (7962±3992 vs 9235±3621 dpm/30µL, $p<10^{-4}$) and GTB (5055±2415 vs 5843±2726 dpm/30µL, $p=0.0004$) activities were lower in cases than in controls. These differences remained significant after adjusting for VWF ($p<10^{-4}$) and ABO blood group ($p= 0.0006$).
Summary/Conclusion: This work showed, for the first time, that in individuals with the same ABO blood group, GT activities were decreased in patients with VT. The biological mechanisms responsible for this association remained to be determined.

Clotting 4
PS 8.4
Board No. 46

Plasma miRNAs expression as markers for cardiovascular events in patients with atrial fibrillation

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Background: Atrial fibrillation (AF) is the most common arrhythmia and it is associated to increased mortality and morbidity. The use of oral anticoagulation (OAC) reduces stroke risk; but even using risk stratification schemes such as CHA_2DS_2-VASc score for stroke, and more recently 2MACE for major adverse cardiovascular events (MACE: including acute coronary syndrome/revascularization and cardiac death), AF patients with OAC suffer from undesirable complications. Thus, the search for new biomarkers is needed to improve the clinical management of these patients. In recent years microRNAs (miRNAs) have become important players in cardiovascular biology.
Aims: To investigate if plasma miRNA expression forecasts the risk for ACE in anticoagulated AF patients and to evaluate whether the predictive performance of CHA_2DS_2-VASc or 2MACE scores improves with the addition of plasma miRNA levels.
Methods: From our cohort of 789 AF patients stable on OAC with vitamin K antagonist (INR=2-3) (median follow-up: 2639 days), we selected 19 patients (discovery cohort) for a pilot study (9 with and 10 without ischemic stroke, similar age and sex) and additional 164 for validation. In the validation cohort, patients were compared considering ischemic stroke (33 stroke and 131 non-stroke) but also ACE (82 ACE and 84 non-ACE), and MACE (49 MACE and 117 non-MACE). All samples were obtained at diagnosis. Plasma levels of 178 miRNAs were quantified by qRT-PCR with the Serum/Plasma focus miRNAs PCR panel V4 (Exiqon). We included miRNA values for testing the improvement of both scores, the CHA_2DS_2-VASc in the stroke group and the 2MACE in the MACE group. The data were analyzed with R software (Stats, pROC and survIDINRI), SPSS and GraphPadPrism 5.
Results: In the discovery cohort, miR-107 and miR-22-3p levels were higher in plasma from stroke patients compared to non-stroke patients (p-value<0.1; fold>1.5) using t-test Sidak-Bonferroni. These results were confirmed in the validation cohort showing that both miR-107 and miR-22-3p had elevated levels in plasma from patients with ACE (p=0.032 and p=0.009, respectively). Only miR-107 remained statistically elevated in stroke cohort (p=0.026). In addition, we investigated miR-146a-5p, associated with the development of ACE. The levels of miR-146a-5p were lower in plasma from patients with ACE (p=0.002). Receiver operating characteristic curves showed that miR-107 significantly discriminate for stroke (AUC=0.619) and miR-146a discriminate for ACE, stroke, and MACE (0.689, 0.618, and 0.655, respectively). Interestingly, the addition of miR-22-3p statistically improved AUC values for ACE (0.736, p=0.001). Interestingly, we observed an improvement in MACE discrimination with the 2MACE score when adding the levels of these miRNAs (c-index: 0.694; 95% CI, 0.617-0.764 vs 0.762; 95% CI, 0.689-0.825, p=0.012; integrated discrimination improvement: 0.056, p=0.015).
Summary/Conclusion: In this study, the levels of miR-107, miR-22-3p, and miR-146a-5p improve the MACE discrimination of the 2MACE clinical score. Further validation of these results in an external and larger cohort would support the use in clinical practice of these miRNAs as biomarkers of risk for ACE in patients with AF.
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Clotting 4
PS 8.5
Board No. 47

Plasma levels of Procoagulant phospholipids (PPL) are associated with future risk of incident venous thromboembolism

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Background: Extracellular vesicles are believed to play an important role in the pathogenesis of venous thromboembolism (VTE). EVs are formed by activated or apoptotic cells and express negatively charged phospholipids, mainly phosphatidylserine – PS, on the external leaflet of their bi-layer membrane. PS facilitates coagulation activation and the levels of procoagulant phospholipids (PPL) in plasma are mainly caused by PS expression at the surface of EVs. Plasma PPL reflect both the degree of PS expression and the total concentration of EVs in plasma. It is not known whether the procoagulant activity of EVs, assessed by plasma PPL, is associated with future risk of VTE.
Aims: To investigate the association between plasma levels of PPL and the risk of incident VTE in a nested case-control study.
Methods: The study population consisted of 296 subjects with incident VTE during 12 yrs of follow-up, and 673 age- and sex-matched controls who were randomly selected from the fourth survey of the Tromsø study. Baseline information was collected by physical examination and non-fasting blood samples in 1994-95. Unconditional logistic regression models were used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE across quartiles and in dichotomized analyses (> 95% percentile versus ≤ 95% percentile) of the plasma PPL activity. The analyses were adjusted for age, sex and BMI. The cut-off levels of PPL were derived from the control group. Plasma levels of PPL were measured using a modified factor Xa-dependent clotting assay. The PPL assay displayed low CV (< 2%) and variations between runs were adjusted for by an internal standard. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: The risk of VTE did not increase across quartiles of plasma PPL with 12 years of follow-up. The OR for VTE was 1.17 (95% CI 0.86-1.60) for individuals in the quartile with shortest clotting times compared to the other quartiles. When the follow-up time after blood collection was restricted to 6.5 years, subjects in the quartile with shortest clotting times (26.5-51.7 sec) had a 1.5-fold higher OR for VTE (OR 1.53, 95 % CI 0.97-2.37) than those in the other quartiles. Further, the OR for VTE was 2.6-fold higher (OR 2.63, 95% CI 1.17-7.07) for those with clotting times below (26.5-88.1 sec) compared to those above (88.2-112.1 sec) the 95% percentile of clotting times.
Summary/Conclusion: Our findings suggest that plasma levels of PPL are associated with future risk of incident VTE. The association revealed a threshold effect attenuated by increasing time between blood sampling and follow-up for VTE events to occur.

Clotting 4
PS 8.6
Board No. 48

Measuring circulating FXIa to assess elevated FXIa as risk factor for thrombosis.

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Background: Reduced *in vivo* levels of FXI have been associated with a lower thrombosis risk while interestingly at the same time do not lead to an increased bleeding risk. For this reason, the anticoagulant therapy field is shifting toward FXIa inhibition. It is, however, not known if increased FXIa levels lead to a higher risk on thrombosis. Previous studies were focused on the indirect measurement of FXIa by quantification of FXIa-inhibitor complexes via sandwich ELISA's. However, detected levels of FXIa by this assay were outside of the physiological range due to contact activation with blood drawing, and thus an improved assay for circulating FXIa quantification is required.
Aims: The development of an assay to directly measure the amount of FXIa present in blood by isolation of circulating FXIa homodimer by complexation - in the blood drawing tube- with a multimeric form of natural FXIa inhibitor Fasxiator from the banded krait snake (*Bugarus Fasciatus*). Upon isolation, the multimeric complex is disrupted resulting in a decrease in affinity of the inhibitor and release of FXIa. In subsequent steps, FXIa is quantified using the natural amplification cascade (FIX/FVIIIa/FX) as present in the coagulation system.
Methods: Fasxiator (62 amino acids; 3 disulfide bonds) was synthesized using tBoc-solid phase peptide synthesis protocols in two parts, subsequently ligated using native chemical ligation (NCL) and folded to obtain the native protein containing 3 disulfide bonds. The inhibitor was modified with a desbiotin tag to enable tetramer formation of Fasxiator with avidin. In a proof of concept study desbiotin-Fasxiator was coupled to avidin and subsequently used to inhibit FXIa. To subsequently release FXIa, the multivalent enzyme-inhibitor complex was incubated with free biotin. To study the kinetic parameters of FXIa inhibition, Lineweaver Burk plots were made and Biacore assays were performed.
Results: The Lineweaver Burk plot showed competitive inhibition of FXIa by the monomeric-Fasxiator with a K_i of 1 nM, which was 10-fold decreased to 0.1 nM by tetramerization through avidin coupling. Subsequently, disruption of the tetrameric Fasxiator-FXIa complex lead to maximal dissociation of FXIa and regain of its catalytic activity.
Summary/Conclusion: Multivalent association of the natural FXIa inhibitor Fasxiator increased the affinity 10-fold by a decrease in K_i from 1 nM to 0.1 nM, which makes the tetrameric Fasxiator a suitable candidate for FXIa capture. Upon disruption of the tetrameric Fasxiator complex, active FXIa was regained. The so designed "Catch and Release" FXIa assay is a promising approach to measure FXIa in circulation.

Platelets 1
PS 9.1
Board No. 49

Immune thrombocytopenia (ITP) is associated with a high disease burden and has a negative impact on patient-reported quality of life and productivity: Results from the ITP World Impact Survey (I-WISh)

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Background: ITP is defined by isolated thrombocytopenia and has an incidence of 10–70 cases/1,000,000/year. While fatigue is an important, recognized morbidity in ITP patients, there are limited data regarding the overall impact of ITP on patients' quality of life (QoL), energy, or productivity.
Aims: I-WISh aims to understand the burden of ITP and its impact on QoL using a global patient and physician sampling frame. We present interim data from the patient survey.
Methods: I-WISh is a cross-sectional survey of ITP patients and physicians; patients were recruited via physicians and patient support groups. Patients completed a 30-minute online survey covering demographics, diagnosis experience, symptoms, impact on daily living and emotional well-being, QoL, and ITP treatment and management. Survey materials were designed and endorsed by a steering committee, including expert clinicians and patient advocacy leads specializing in ITP.
Results: 1400 patients from 12 countries, 65% female, with a mean age (SD) of 47.1 (16.0) years, completed the survey. Mean (SD) time since ITP diagnosis was 110 (131.4) days.
Using a 7-point Likert scale (7 = excellent health), most patients (63%) reported good current health state (score ≥5); 15% reported a decreased state of health (score ≤3). Mean time (SD) from initial presentation to a healthcare professional and ITP diagnosis was 113 (568) days. Of all patients, 22% felt they experienced a delay in their ITP diagnosis and 66% wanted more support during their diagnosis, most frequently from a physician (61%) or patient support group (50%). Most (53%) patients initially presented to a general practitioner, and diagnosis was commonly made by a hematologist (86%).
The most frequently reported signs and symptoms at diagnosis were petechiae (65%), bruising (of the skin/mucous membranes; 64%), and fatigue (60%). Signs and symptoms reported at time of survey completion included fatigue (52%), bruising (31%), and petechiae (31%). At survey completion, patients reported thrombosis, anxiety surrounding unstable platelet count, and fatigue as the most severe symptoms (scored ≥5 on 7-point Likert scale, where 7 = worst imaginable).
Overall, 44% of patients reported that ITP impacted their energy levels more than half the time and 36% said ITP had a negative impact on their normal capacity to exercise more than half the time. Half of all patients stated that ITP had a high impact on their emotional well-being (score ≥5 on 7-point Likert scale, where 7 = great deal). 37% of patients reported that they reduced their hours at work because of ITP.
The three main treatment goals reported by patients were to achieve a "healthy blood count" (65%), followed by "preventing episodes of worsening of my ITP" (43%), and "increasing my energy levels" (42%).
Summary/Conclusion: Results of I-WISh demonstrate the multifaceted burden of ITP, especially its high symptom burden and its negative impact on emotional well-being and ability to work. Patients were primarily concerned with the impact of ITP on their QoL. These results underscore the importance of exploring and defining the overall disease burden of ITP and of considering how these findings may influence ITP management.

Platelets 1
PS 9.2
Board No. 50

Hypersensitive platelet subpopulations determine collective behaviour: Results from a study of single platelet function using droplet microfluidics

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Background: Platelets have long since been known to be heterogeneous in size, volume and density. Functional heterogeneity has been suggested in several studies, however, there are no methods currently available that can study platelets on a single cell level. Research on a single cell level is needed to study pure intrinsic heterogeneity without the influence of adjacent cells and associated amplification mechanisms. Such a method should provide a high throughput way of studying platelets in isolation, to be able to detect potentially rare phenotypes, without interfering with normal platelet function. To this effect, this study implements a droplet microfluidics approach to study single platelet functionality.
Aims: To develop a droplet microfluidic technique to study platelet function and to use this to study single platelet function and intrinsic functional diversity.
Methods: The innovative droplet microfluidic protocol involves compartmentalising platelets in monodisperse droplets with agonists while excluding paracrine signalling. High frequency encapsulation is coupled with flow cytometry for high throughput quantification of platelet responses to multiple agonists (such as convulxin, TRAP-14 and ADP). Platelet response to an agonist is quantified using three endpoints, integrin $\alpha_{IIb}\beta_3$ activation, degranulation and membrane inversion. This study design allows for the high throughput examination of single platelet response and the presence of intrinsic heterogeneity. All platelets used in this study were donated by healthy volunteers who gave informed consent.
Results: Platelets are individually encapsulated in monodisperse (CV of 1-4%) water-in-oil droplets with a mean diameter of 25 μm. Droplets are produced with a throughput of 4 kHz, with droplets containing a single platelet produced at a rate of 0.25 kHz (following a Poisson distribution). In droplets, the absence of paracrine signalling and the presence of autocrine signalling produces a binary-like response. Single platelet responses to convulxin identify a small hypersensitive subpopulation of platelets that is 10-fold more sensitive than the main population. In platelet collective experiments, this hypersensitive subpopulation is sufficient to direct activation of the entire population via paracrine signalling. The hypersensitive subpopulation varies in number and sensitivity between agonists and individuals. This platform was also used to confirm procoagulant states as suggested in previous studies.
Summary/Conclusion: This study demonstrates the value of a high throughput droplet microfluidics and flow cytometry workflow for measuring system heterogeneity. Procoagulant states as described it literature were shown to be intrinsic in origin. Intrinsic, pre-programmed states were observed, with hypersensitive platelets driving global platelet activation in health and potentially in disease.
This study is kindly sponsored by the British Heart Foundation and a Marie Curie research fund.

Platelets 1
PS 9.3
Board No. 51

Aspirin therapy reduces embolic events in patients with infective endocarditis

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Background: Embolic events are major complications of infective endocarditis (IE). Animal models have demonstrated the benefit of aspirin in prevention of embolic events. However, previous clinical studies have given conflicting results.
Aims: To evaluate the benefit of prior aspirin therapy on embolic risk in patients with IE.
Methods: We conducted a retrospective monocentric study of patients who presented in our endocarditis reference center with a diagnosis of IE from 2010 to 2016. In this cohort, outcome of patients with or without prior aspirin therapy on admission were compared. The primary end point was embolic event occurring before or during hospitalization. The secondary end point was the occurrence of a hemorrhagic event. Multivariable logistic regression was used to assess the impact of daily aspirin therapy on the risk of embolic events associated with IE.
Results: The cohort included 529 patients, among them 135 (25%) were treated by aspirin daily at the time of hospitalization for IE. Embolic events occurred in 264 (50%) patients including 242 (46%) before and 61 (11%) after initiation of antibiotic therapy
As compared with the 394 patients without previous aspirin therapy, the 135 patients treated by aspirin were significantly older (67.8 ±11 vs 64.4 ±16 p=0.02) and had more frequent history of acute coronary syndrome and renal failure (p=0.000 and p=0.0008, respectively) and presented with less frequent embolic events (53 (39%) vs 211 (53%) p= 0,01 and similar hemorrhagic complications (8 (5,9%) vs 45 (11%) p=0,06)
By univariate analysis, factors associated with increased embolic events were IVDA (OR 2.68 [1.45;5.22]), vegetation length (OR 1.07 [1.05;1.09]), and staphylococcus aureus (OR 1.51 [1.05;2.18]). By univariate and multivariate analysis, aspirin therapy (OR respectively: 0.56 [0.38;0.83] and 0.6 [0.40;0.92]) was protective
Prevalence of cerebral hemorrhage was comparable whatever the group of patients (11,5% without aspirin and 5,9% with previous aspirin).
Summary/Conclusion: Prior aspirin therapy reduces embolic events in patients with IE without increasing hemorrhagic events.

Platelets 1
PS 9.4
Board No. 52

Predictive factors for successful splenectomy outcome – systematic review

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Background: Splenectomy may lead to a good response in 60-80% of adults with primary immune thrombocytopenia (ITP). However, in the era of novel drugs the appropriate selection of patients for splenectomy is essential for optimization of the treatment outcome. Accordingly, it is important to identify both pre- and post-operative parameters predictable for the splenectomy outcome.
Aims: We analysed the large number of publications addressing this issues, in order to make systematic review of predictive factors for outcome of splenectomy in corticosteroid refractory ITP
Methods: The Medline database was searched from January 1,1966, to January 1, 2018. using keywords: “splenectomy,” “spleen and remov,” “spleen and extract”, “thrombocytopenia,” “thrombocytopenic purpura,” “ITP” or “AITP” as well as “predictor “. The search was limited to English-language articles. The bibliographies of all retrieved articles were searched for additional relevant articles. Retrieved articles were selected for review if they reported ≥15 consecutive splenectomized ITP patients. Articles that reported data on children were included only if it could be determined that 75% or more of the patients were ≥14 years old.
Results: The literature search identified 410 articles; 335 articles did not meet our selection criteria and were not reviewed. We selected 75 articles with 6151 patients analysing three groups according to the type of response to the splenectomy: predictors of initial response (50 articles), predictors of sustained remission (35 articles) and predictors of relapse (11 articles).
Predictors of initial response: Age, duration of illness before splenectomy, platelet sequestration site, response to steroids and intravenous immunoglobulin’s (IVIg), preoperative and postoperative platelet count (PC), spleen size/weight were predictive in 62.5%(20/32), 14% (3/21), 63.3% (7/11), 42.9% (9/21), 20% (2/10), 50% (4/8), 100% (17/17), 20% (1/5) articles, respectively. Neither sex nor antiplatelet antibodies were predictive in analysed articles (19/19 and 11/11 articles).
Predictors of sustained remission: Age, duration of illness before splenectomy, platelet sequestration site, response to steroides and IVIg, preoperative and postoperative PC were predictive in 34.8% (8/15), 5.9% (1/17), 62.5% (5/8), 21.7% (5/23), 25% (1/4),71.4%(5/7), 70.6% (12/17) articles, respectively. Sex, spleen size/weight and antiplatelet antibodies were non-predictive in analysed articles (18/18, 2/2 and 6/6 articles).
Predictors of relapse: Age, response to steroides and IVIg, postoperative PC, spleen size/weight were predictive in 28.5% (2/7), 50% (2/4), 100% (1/1), 92.3% (12/13) and 2/6 (33.3%) articles, respectively. Sex, duration of illness before splenectomy, preoperative PC and antiplatelet antibodies were non-predictive in analysed articles (8/8, 5/5, preoperative PC 3/3 and 3/3 articles, respectively).
Summary/Conclusion: The reviewed articles used diverse criteria to evaluate patients' characteristics and to report outcomes. However, a comprehensive analysis of all published reports for predictive parameters for the outcome of splenectomy showed that the most reliable predictor is postoperative PC. On the other hand, among preoperative variables, younger age and platelet sequestrationsite were related with response.

Platelets 1
PS 9.5
Board No. 53

Sticky Platelet Syndrome - epidemiological and clinical data from Slovak National Registry of Thrombophilia States

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Background: Sticky platelet syndrome (SPS) is inherited thrombocytopathy considered to be the second most common hereditary thrombophilia after resistance to activated protein C (APC-R) and the most common thrombophilia associated with arterial thrombosis. The SPS is also the second most common thrombophilia after antiphospholipid syndrome that causes recurrent spontaneous abortions or fetal loss syndrome. The clinical symptoms of SPS are similar to thromboembolic events (TE) from other causes. However, certain distinct features could be identified: • Young adults (< 40 years), usually without known risk factors, • Pregnant woman often affected; association with fetal loss syndrome, • Often atypical localization of thrombosis (retinal veins, cerebral sinuses), • Both arterial (more often) and venous thrombosis presented, • Recurrent/new thrombosis during adequate anticoagulation therapy (e.g. VKA), • Often positive family history for TE with both genders affected.
Aims: The aim of the work is to summarize the epidemiological and clinical data from Slovak National Registry of Thrombophilia States (NRTS) on SPS.
Methods: SPS is defined by the increased platelet aggregation in response to low concentrations of two platelet agonists - adenosine diphosphate (ADP) and / or epinephrine (EPI) [1]. According to laboratory findings, three SPS types (hyperaggregability to ADP and EPI - type I, to EPI alone - type II, to ADP alone - type III) can be identified.
Results: There has been investigated 2930 subjects on presence of SPS (2201 symptomatic patients and 729 asymptomatic individuals, usually blood relatives of affected patients) in National Centre of Thrombophilic States (NCTS). In total the SPS positivity was found in 727 subjects (24%). Among the 2201 symptomatic patients there were 541 carriers of SPS (23%). The most of cases with SPS were females (67%) and the average age of SPS carriers was 40,4 year. SPS type II was found the most common SPS type (72,60%). The most frequent clinical manifestation of SPS was venous thromboembolism (VTE) in 238 patients (46,4%). Arterial thrombosis was verified in 195 patients (38%) and fetal loss in 77 females (14,6%).
Summary/Conclusion: Family studies of patients with SPS showed that some of their relatives fulfilled laboratory criteria for SPS but remained clinically asymptomatic. It seems, that acquired risk factors for thrombosis may be crucial for the clinical manifestation of SPS. The relation between the onset of TE and stressful situation in SPS is in favor of this idea. Acknowledgement: the work was supported by EU projects CEPV II (ITMS 26220120036), CEVYPET (ITMS 26220120053) and Slovak projects Vega 1/0168/16, APVV 16-0020.

Platelets 1
PS 9.6
Board No. 54

Platelet aggregation varies by microenvironment in women with myeloproliferative neoplasms

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Background: We hypothesized that bioactive microenvironment may appreciably to vary platelet response.
Aims: To determine distinct and interacting roles of platelet microenvironment impacting to ADP- and collagen-induced aggregation.
Methods: ADP- and collagen-induced aggregation (%) was examined with laser transmission aggregometry in 57 women with myeloproliferative neoplasms in remission (MPNs; mean age 36 years; Group I) and in 76 women with chronic cerebrovascular diseases (CCVD) comorbided with MPNs (mean age 65 years; Group II). The microenvironment was considered with 98 biomarkers reflecting coagulation, anticoagulation, platelets, vascular wall, angiogenesis, inflammation, etc. Multivariate analysis was performed for obtained data.
Results: Patients in Group I had lower median cell counts and creatinine clearance than Group II, higher fibrinogen with supressed fibrinolysis, and higher vWB associated with low ADAMTS-13, and higer thrombomodulin, and higher VEGF-A opposited with higher thrombospondin, and higher IL-10. No differences were found in clotting factors, TF, PS and PC.
The ADP aggregation was significantly lower in Group I than in Group II (Median 15.5% vs. 24.5%; p=0,049), collagen-induced aggregation was the same in both groups.
Significant mean force correlations of ADP-aggregation were found positively with PC, TF, sVCAM, and TNF and negatively with sVEGF-R1 and sVEGF-R2 in Group I whereas only negatively with Age in the Group II. Collagen-induced aggregation has presented correlations with PC, PS, TF in Group I, and no correlations were determined in Group II.
Considering the microenvironment as a matrix of multidimensional interplays, the modelling analysis showed in Group I for ADP-aggregation managing trigger consists of Protein C and VEGF-A as a opposing (68.8% of cumulative power) together with sVCAM (22.9% in residual power). In Group II managing trigger consisted of PC jointly with fVII and creatinine and VEGF-A and sVCAM (87.5% of cumulative power), and Protein S with sVEGF-R1 as a residual.
For collagen-induced aggregation the managing trigger includes PC with paradoxically oppositing thrombomodulin (53.2% of cumulative power) together with the composition of fXII, TF, sICAM and VEGF-A+sVEGF-R2 (46.8% in residual power) in Group I. In Group II 65.2% of controlled power belonged to PC balanced with PS, TNF and thrombospondin, and residual management was a concert of sVCAM, sICAM, vWB, fV, fXII and sVEGFR2.
Summary/Conclusion: In responce to ADP the microenvironmental activation of platelets is explained through the composition of mechanisms requiring platelet ApoER2 receptors with VEGF as a vascular protective factor in the presense of high leukocyte-platelet aggregation. FVII and renal function in trigger composition reflect the specifics of CCVD pathophysiology. Whereas the same components are composited in the managing triggers for both groups that demonstrates leadership influence of myeloproliferative process of one as well in comorbidity.
For responce to collagen protein C only is common component of both controlled triggers. This finding presumes distinct roles of platelet microenvironment in collagen-induced aggregation in women with MPNs compared in women with CCVD comorbied with MPNs. In first great value belongs to residual control that has managed by endothelium-leukocyte-platelet interactions with TF. In second there are added proinflammatory with antiangiogenic effects adjusted with clotting factors and endothelial dysfunction level.

Platelets 2
PS 10.1
Board No. 55

Immature platelet fraction (IPF): a surrogate marker of Mean Platelet Volume (MPV) in hereditary macrothrombocytopenia?

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Background: The immature platelet fraction (IPF) represents a population of newly formed platelets containing a greater amount of residual RNA. This parameter correlates well with the reticulated platelet count obtained from CD61 flow cytometry. IPF is also influenced by platelet size and may be a useful parameter for macrothrombocytopenia screening when Mean Platelet Volume (MPV) is not measurable in samples with very low platelet counts and/ or very large platelets. In these cases, assessment of platelet size is based on complicated and non-standardized methods.
Aims: Evaluate the IPF contribution to identify hereditary macrothrombocytopenia (MPV>12 fl).
Methods: Platelet count, MPV and IPF values were measured with Sysmex XN haematology analyser in a healthy local population (n=307) and in 30 patients from 23 families with a genetic diagnosis of hereditary macrothrombocytopenia (16 males and 14 females, aged from 5 to 74 years, platelet count ranged from 17 to 154 G/L): MYH9 disorders (n=10, 7 families), ACTN1-related thrombocytopenia (n=6, 3 families), biallelic Bernard-Soulier Syndrome (bBSS) (n=4, 4 families), monoallelic BSS (mBSS: Bolzano mutation) (n=5, 4 families), Di George syndrome (n=2, 2 families), Jacobsen syndrome (n=2, 2 families) and pseudo-von Willebrand disease (n=1).
Results: The mean IPF is higher in patients with hereditary macrothrombocytopenia than healthy subjects (41.77 $\pm$ 17.92%, range [12.8-74%] vs 4 $\pm$ 1.1%, range [0.7 – 8%] ($p < 0.001$). MYH9 and bBSS have high and comparable IPF (56.60 $\pm$ 13.02% and 59.58 $\pm$ 3.76% respectively) and are almost twice as high in ACTN1 (IPF 31.08 $\pm$ 6.12%), mBSS (IPF 31.34 $\pm$ 10.87%) and pseudo-von Willebrand disease (IPF 32.8% n=1) ($p <0.001$). Di George syndrome is associated with mean IPF 22.6 $\pm$ 0.84% and Jacobsen syndrome with mean IPF 13.75 $\pm$ 1.34%. MPV is measurable for 15 patients, mean MPV is 14.66 $\pm$ 1.25 fl, range [12-15.9]. We analyzed the relationship between MPV and IPF (pearson r =0.701; $p = 0.004$). Fifteen MPV are missing and concern 7 MYH9, 4 bBSS, 3 mBSS, and 1 Di George (low platelet count). For 14 patients, the missing MPV concern the highest expected platelet sizes, and correspond to the highest IPF values (54.04 $\pm$ 15.07% for missing MPV vs 31.55 $\pm$ 12.93 % for measured MPV) ($p < 0.001$).
Summary/Conclusion: We confirm that IPF increases with MPV, and could be a surrogate marker of MPV. We suggest using IPF as a marker of platelet size in hereditary thrombocytopenia. Standardisation of IPF cut-off according to genetic abnormalities responsible for thrombocytopenia is a major need to rapidly help identify hereditary macrothrombocytopenia as MPV is not always available.

Platelets 2
PS 10.2
Board No. 56

Flow cytometry-based platelet function testing for assessing platelet reactivity in inherited and acquired platelet dysfunction

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Background: The ability of platelets to carry out their hemostatic function can be impaired in a wide range of inherited and acquired conditions: genetic deficiences or defects of major functional proteins and developmental regulators, trauma, surgery, inflammation, pre-term birth, sepsis, hematological malignancies, solid tumors, chemotherapy, autoimmune disorders, and many others. Evaluation of this impairment is vitally important for research and clinical purposes.
Aims: Here we describe a whole blood flow cytometry screening method of comprehensive clinical platelet function screening.
Methods: Venous blood was collected into vacuum plastic tubes with sodium citrate, final concentration 3.8%. 20 µL of blood was diluted 1:20 in HEPES-buffered Tyrode buffer. For platelet activation, a mixture of collagen related peptide (20 µg/mL), PAR-1 activating peptide SFLLRN (25 µM) and 5 mM $CaCl_2$ was used. We determined forward (FSC-H) and side scatter (SSC-H), CD42b-PE, CD61-PE, CD62P-Alexa Fluor 647, PAC1-FITC, annexin V-Alexa Fluor 647 binding and mepacrine levels. Investigations were performed in accordance with the Declaration of Helsinki under protocols approved by the respective institutions' Ethical Committees, and written informed consent was obtained from all donors and patients, or their parents.
Results: Pediatric patients with inherited platelet disorders: type I Glanzmann's thrombasthenia (GT, n=4), Bernard-Soulier syndrome (BSS, n=3), Hermansky-Pudlak syndrome (HP, n=2), MYH-9 type thrombocytopenia (n=3) and Wiscott-Aldrich syndrome (WAS, n=3) were analyzed. In BSS all parameters were increased, even more so in MYH-9 in accordance with the macrothrombocytopenic nature of these disorders and similarly greatly decreased in microthrombocytopenic WAS. In resting and activated state CD42b was normal in type I GT and naturally absent in BSS, which made it clearly different from MYH-9. In HP syndrome there was a decrease of mepacrine uptake in dense granules. In type I GT all markers were within normal range with the exception for PAC1 and CD61. A direct relationship between the clinical index of hemorrhage and the integrin activation degree and dense granules release ($p < 0.05$) was revealed for pediatric patients with hemorrhagic syndrome of unclear origin and excluded coagulopathy (n=32). All platelet functions without exception (specifically, lack of all granules, of their release and integrin activation) were profoundly (by 50-70%) impaired in newborn (>37 gestation weeks, n=10), with greater defects in pre-term newborn (33-34 gestation weeks, n=10). Adult patients with chronic lymphocytic leukaemia (n=17) had initially impaired response of platelets and ibrutinib additionally inhibited it. In adult patients with chronic immune thrombocytopenia (n=32), our data indicate that platelets are pre-activated, large and slowly reacting; these changes are associated with bleeding independently of platelet count.
Summary/Conclusion: Whole blood flow cytometry-based screening assay provides valuable information about all major functions (adhesion, aggregation, granule secretion, procoagulant activity) for circulating and stimulated platelets, is sensitive to both classic inherited disorders and various acquired conditions, and has a potential for practical applications.

Platelets 2
PS 10.3
Board No. 57

Disagregin: a platelet aggregation inhibitor from the tick Ornithodoros Moubata

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Background: Ticks, hematophagous parasites, obtain blood by puncturing the skin of humans and animals. When a blood vessel is intruded, the extrinsic coagulation pathway is activated by excretion of tissue factor (TF) from damaged endothelial cells and the blood coagulation cascade starts to repair the vessel wall. TF forms a complex with Factor VIIa (FVIIa) that activates FX into FXa. Then, FXa, together with calcium, phospholipids and FV, converts prothrombin into thrombin. Subsequently, thrombin cleaves fibrinogen into fibrin and a stable clot is formed. Ticks secrete saliva containing several anticoagulant proteins to continue feeding on the host. Here, we studied a protein consisting of 60 amino acids with proposed anticoagulant properties: Disagregin[1], derived from salivary glands of the *Ornithodoros moubata*, a soft tick living in parts of Africa.
It is assumed that Disagregin blocks signaling of GPIIb/IIIa integrin receptor on platelets, resulting in less platelet aggregation and reduced fibrin levels. Commonly, GPIIb/IIIa antagonists contain an Arg-Gly-Asp (RGD) sequence for specific binding. In contrast, Disagregin contains an Arg-Glu-Asp (RED) sequence, suggesting a different mode of inhibitory action in platelet aggregation.
Aims:

- Investigate the inhibitory effect of Disagregin on platelet aggregation
- Investigate the possible role of the RED sequence in Disagregin

Methods: Disagregin was synthesized by solid-phase peptide synthesis (SPPS) using tert-butyloxycarbonyl (Boc) chemistry and native chemical ligation (NCL). Light Transmission Aggregation (LTA) was performed in platelet-rich plasma (PRP) from healthy volunteers to assess the inhibitory effect of Disagregin.
To investigate the possible role of the RED sequence, an E15G Disagregin analogue (RGD) was synthesized and both proteins were studied by NMR experiments. Next, flow cytometry was performed using native Disagregin and the RGD analogue.
Results: Disagregin inhibited adenosinediphosphate (ADP)- and collagen-activated platelet aggregation in plasma with an IC50 = 99.1 nM and 63.8 nM, respectively. Remarkably, from a platelet extracellular vesicle (EV) release assay it appeared that Disagregin also reduced extracellular vesicle release from Convulxin-activated platelets.
NMR data showed that E15G substitution does not lead to a substantial difference in overall protein folding compared to native Disagregin. Both proteins adopted a Bovine Pancreatic Trypsin Inhibitor (BPTI)-type structural fold in solution. Interestingly, native Disagregin as well as the E15G analogue displayed two major different conformations in fast exchange (milliseconds), which internal conformational changes are located relatively distant from the RGD loop. At the local structural level of the RGD loop, differences in chemical shift values suggest the presence of a salt-bridge between the end group of R41 and the side chain carboxylic group E15 in native Disagregin, while this interaction appears not present in the E15G analogue.
Summary/Conclusion: Tick Disagregin seems to have evolved passed classical RGD binding and now uses an antagonistic style of binding.
References
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Platelets 2
PS 10.4
Board No. 58

Platelet PN-1 regulates clot structure and retraction

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Background: Serpin E2 or Protease Nexin-1 (PN-1), is a strong inhibitor of serine proteases. We recently established platelet PN-1 as a negative regulator of thrombin generation and thrombosis. The mechanical properties of clot are dependent of thrombin-induced platelet activation and fibrin formation. Altered clot architecture and retraction are known to be associated with abnormal thrombin generation. We hypothesize that platelet PN-1 can regulate clot architecture.
Aims: To evaluate the role of platelet PN-1 on clot structure and retraction.
Methods: Clot retraction was assessed after recalcification in glass tubes of human platelet-rich plasma (PRP) incubated or not with a PN-1-blocking IgG, or of PRP obtained from wild-type (WT) or PN-1 knock-out (PN-$1^{-/-}$) mice. Clot architecture was evaluated with confocal microscopy of human or mice platelet-rich clots (PRC) supplemented with Alexa647-fibrinogen and Alexa488 anti-CD41, a platelet marker. Platelet adhesion and spreading were assessed on immobilized fibrinogen at two reaction times (5 or 30 min) at 37°C. Clot viscoelasticity properties were analysed with rotational thromboelastometry test (ROTEM). Platelet ADAM17, a sheddase for GPIbα the vWF receptor, was quantified by western blot and fluorometric assay.
Results: Surprisingly**,** clot weight was increased by 66% in human PRC incubated with a blocking anti-PN-1 IgG and by 40% in PN-$1^{-/-}$ PRC compared to their respective controls, indicating a positive effect of PN-1 on clot retraction. Confocal microscopy images showed that the fibrin network structure was more porous in PRC from PN-$1^{-/-}$ mice or in human PRC incubated with a blocking anti-PN-1 IgG. TEMograms showed that the blocking anti-PN-1 IgG induced a 26% decrease of the maximum clot elasticity, a clot strength parameter. In our experimental conditions, no difference was observed in PRC incubated with tranexamic acid or with cytochalasin and in platelet-poor clots indicating a direct role of PN-1 in platelet contractile force. No difference was observed between WT or PN-$1^{-/-}$, regarding platelet adhesion and spreading to fibrinogen in resting condition. In contrast, adhesion and spreading to fibrinogen was markedly decreased (P<0,001) in TRAP-4 (Thrombin receptor-activating peptide)- or convulxin-activated platelets from PN-$1^{-/-}$ mice compared to WT, highlighting the impact of PN-1 in inside-out signalling. Moreover, we observed that platelets from PN-$1^{-/-}$ mice exhibit a higher ADAM17 activity than WT platelets.
Summary/Conclusion: Despite its anti-thrombin effect, platelet PN-1 accounts for the formation of a tight structure of PRC. We thus identify a critical positive role of PN-1 on platelet-driven clot retraction. Moreover, our present data suggest that PN-1 may regulate platelet functions like adhesion and spreading via its ability to regulate ADAM17. We indeed previously demonstrated that PN-1 could be a regulator of ADAM17 activation on endothelial cells, a process likely involved in the shedding of numerous receptors and adhesion molecules. Future studies will be needed to determine which receptor is shed in platelets impaired for PN-1.

Platelets 2
PS 10.5
Board No. 59

Procoagulant activity of microparticles from stroke and mimics patients on endothelial cells

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Background: High levels of membrane-shed submicron microparticles (MPs) circulate in the peripheral blood of patients with thrombotic diseases including acute ischemic stroke (AIS). While circulating microparticles are useful biomarkers of cell activation/apoptosis, their role in the development of thrombosis remains unclear. Little is known about the effects of MPs from AIS patients on the anti-thrombotic phenotype of brain microvascular endothelial cells.
Aims: We have tested the hypothesis that MPs, isolated from blood of patients with AIS, could contribute to a procoagulant effect on brain microvascular endothelial cells, in the presence of platelets.
Methods: The studied population included patients suspected for AIS. Among the patients, we differentiated those eligible for reperfusion therapy from stroke mimics defined as a nonvascular disease presenting with stroke-like symptoms. Healthy volunteers were used as controls. MPs were purified from blood of healthy volunteers (controls), patients with AIS or mimics, and were quantified and characterized by flow cytometry.
We investigated the potential procoagulant effect of MPs from AIS patients on brain microvascular (hCMEC/D3) and macrovascular (HUVECs) endothelial cells by measuring thrombin generation at the cell surface by means of the Calibrated Automated Thrombogram in the presence of platelets.
Results: Total number of Annexin V-positive MPs was significantly higher in AIS patients (p=0.0001) and mimics (p=0.03) compared to controls No significant difference was observed between AIS and mimics patients. Platelet-derived MPs were the most abundant ones significantly increased in AIS compared to mimics (p=0.03) and controls (p=0.001). MPs from AIS and mimics significantly increased velocity index on the surface of both HUVECs (p=0.001 and p=0.006, respectively) and hCMEC/D3 (p=0.0055 and p=0.01, respectively). A significant reduction of LagTime was observed for HUVECs in the presence of MPs from AIS (p=0.009) and mimics (p=0.02).
Summary/Conclusion: Our results demonstrate that MPs from AIS patients and mimics are able to potentiate thrombin generation at the surface of brain endothelial cells in the presence of platelets suggesting a potential new procoagulant role, probably stress-mediated, for MPs in the pathophysiology of both AIS and mimics.

Platelets 2
PS 10.6
Board No. 60

Is laboratory testing for Heparin-Induced Thrombocytopenia (HIT) requested after the use of 4Ts score system? A review

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Background: The diagnosis of HIT envolves both clinical and laboratory assessment. Since laboratory testing results for HIT are not immediatly available, clinical assessment allows for faster decision-making. Clinical prediction is also important because isolated HIT antibodies are frequent and not all antibodies detected are capable of causing clinical HIT.
Scoring systems can be helpful in estimating the probability of HIT. The best studied of which is the 4Ts score system, which places patients at low, intermediate, or high pre-test risk for having HIT. The 4Ts system has a very high negative predictive value but a less reliable positive predictive value.
International guidelines recommend the use of a scoring system to determine the pre-test probability of HIT. It has been shown that if the 4Ts score is low, HIT can be excluded without the need for laboratory investigation. An intermediate or high score should be further evaluated by laboratory testing, and possible alternate anticoagulation should be given.
Aims: Our goal was to evaluate the adoption of this recommended strategy by medical practitioners in a tertiary referral hospital.
Methods: We performed a retrospective chart review of all patients that were tested for anti-PF4-heparin antibodies with enzyme immunoassays because of clinical suspicion of HIT during the year of 2016. We analyzed clinical and demographic data and calculated the 4Ts score in each patient at the time the immunoassay was requested. We proceeded to evaluate the adequacy for HIT laboratory testing based on the clinical probability of HIT.
Results: Fourty-eight patients were reviewed with median age of 66 years old. Thirty-five were men (72.9%) and thirteen were women (27.1%). Twenty-two (45,8%) were admitted for surgery (twenty-one of which for cardiac surgery) and twenty-six (54,2%) for other acute medical condition.
Thirty-two (66,7%) were being treated with unfractionated heparin (UFH) and fourteen (29,2%) with low-molecular-weight heparin (LMWH). Two patients were not treated with either UFH or LMWH the days prior to the test.
Thirty-two (66,7%) patients had a 4Ts score result between 0 and 3, indicative of a low probability of HIT. Eleven (22,9%) scored 4 or 5 (intermediate probability) and five (10,4%) scored 6 to 8 (high probability).
Six (12,5%) patients were confirmed to be positive for anti-PF4-heparin antibodies. Three of those had high clinical probability of HIT, two intermediate and one low. The patient with low probability score at the time of the immunoassay request did not develop clinical HIT.
Summary/Conclusion: Adoption of the 4Ts score strategy to calculate the pre-test probability of HIT was low in this institution. This may have led to redundant laboratory testing. Although the 4Ts score has a high negative predictive value, this tool shouldn't surpass a strong medical suspicion of HIT.

4 POSTER VIEW & DISCUSSION (INCL. BOARD NO)

Bleeding
P101
Board No. 61

A single centre experience on the safety of newly initiated oral anticoagulation therapy for non-valvular atrial fibrillation in adult patients

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Background: The introduction of the direct oral anticoagulants (DOACs) as an alternative therapy for stroke prevention in non-valvular AF (nvAF) has signaled a shift away from vitamin-K-antagonists (VKAs). Despite their growing popularity, little is known regarding their safety in "at risk" groups. There is limited research into how guidelines regarding safe initiation are implemented in clinical practice.
Aims: To provide real world safety data on DOACs by investigating appropriateness and safety of newly initiated anticoagulation therapy for nvAF in a London hospital from January-December 2015. Primary objectives are to characterise patterns of choice of anticoagulation agent and to determine whether patients were initiated on anticoagulation therapy appropriately and safely, according to local and national guidelines. Secondary objectives are to collect safety data on bleeding events assessing: nature, management and outcome of bleeding events as well as focusing on those secondary to falls and renal function status.
Methods: A retrospective, single-centre study on adult patients initiated on warfarin or DOACs for nvAF between January and December 2015 at a London hospital. Locally agreed measures were established to assess safe initiation of anticoagulation therapy and included: documentation of CHA_2DS_2-VASc and HAS-BLED scores (stroke and bleeding risk assessment), baseline renal function tests, appropriate dosing for renal function status, medicines reconciliation documentation and counselling on anticoagulation therapy. Compliance to measures to assess safe initiation of anticoagulation was reported by absolute numbers and percentages. Patterns of agent choice were summarised by descriptive statistics. Bleeding events, management and outcome were summarised descriptively.
Results: Compliance to safety measures varied, CHA_2DS_2-VASc and HAS-BLED scores were documented in 19% and 3% of cases, respectively. 96% of patients initiated on DOACs had baseline renal function tests, of these 82% were prescribed the appropriate renal dose. 85% of patients received counseling on the new anticoagulation agent. 142 patients were initiated on DOACs and 13 patients initiated on warfarin therapy. 19/155 patients experienced bleeding events (epistaxis, haematuria, per rectum bleeding commonly experienced); management varied on a case-by-case basis according to the clinical features of the event. Despite 27/155 of patients at risk of falls, only one patient experienced a minor bleeding event secondary to a fall (DOAC agent was restarted following investigations). 15/142 of patients on DOAC therapy had a creatinine clearance <30mL/min, with one patient experiencing a minor bleeding event (epistaxis).
Summary/Conclusion: Compliance to locally agreed measures to assess safety of newly initiated anticoagulation therapy for nvAF was encouraging, however further improvements are required. DOACs were the preferred agent of choice in practice, with patients at falls risk initiated on DOAC compared to warfarin therapy (favourable bleeding safety profile). 12% of patients in our study experienced at least one bleeding event and management varied on a case-by-case basis. Our study provided encouraging safety data for the use of DOACs in patients at falls risk and with severe renal impairment however sample sizes were small thus further research is required.

Bleeding
P102
Board No. 62

Anti-prothrombin antibody, case report

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Background: Anti-prothrombin antibody is a rare cause of hemorrhagic manifestations. It prevents the transformation of fibrinogen into fibrin. It can appear during an infection, an autoimmune disease, a medication or a myeloma. The treatment mostly found in the literature is corticosteroid therapy.
Aims: The aim was to describe the characteristics of patients with anti-prothrombin antibodies diagnosed in our hospital since 2010.
Methods: We contacted the hematology laboratory and the clinical hemostasis referent of our hospital to identify those cases. Then we collected the clinical and biological data of the patients, the treatment received and their future.
Results: Only one case has been diagnosed since 2010. It was a 92-year-old woman treated with Warfarin for an atrial fibrillation. Its recent history was marked by a recurrent erysipela treated with antibiotherapy in October 2017. In November 2017, the patient had ecchymosis with an INR (international normalised ratio) at 8 leading to the arrest of Warfarin and then to the administration of vitamin K. Despite this, the haemorrhagic manifestations worsened with epistaxis and melena, anemia. INR remained high. The biological assessment found prothrombin time at 10%, patient prolonged partial thromboplastin time>180 seconds, fibrinogen=3,69g/L, chronometric test II,V,X,VII,VIII,IX,XI,XII less than 10%, normal thrombin time, normal chromogenic factor VIII, normal von Willebrand factor antigen.
Initial management consisted of transfusion of packed red cells and prothrombin complex concentrates, administration of vitamin K without efficacy. Finally, because of the demonstration of the anti-prothrombin, corticotherapy was started but the patient died that evening.
Summary/Conclusion: Anti-prothrombin antibodies are a rare cause of hemorrhage. The cutaneous symptomatology was strongly suggestive of an acquired pathology of coagulation. The presence of measurable fibrinogen while the other factors were collapsed prompted us to search for an anti-prothrombin. The high INR in this context of treatment with vitamin K antagonist was a confounding factor.

Bleeding
P103
Board No. 63

Bleeding phenotype in cohort of patients with Von Willebrand disease in west Algeria.

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Background: The ISTH Bleeding Assessment Tools can be use to quantified the bleeding symptoms of Von Willebrand disease (VWD).
Aims: To evaluate bleeding symptoms in Algerian cohort of patients with VWD.
Methods: Bleeding severity was determined using Tossetto bleeding score (BS) in patients with VWF ≤ 30% (diagnosis criterion). The BS was compared to control groupe, VWD type, level of Von Willebrand factor, FVIII and blood group.
Results: Clinical data of 75 patients were analyzed, mediane age was 20 years (range 1-68) and 49% was female. The most frequent bleeding symptoms were ecchyoses (80%) and epistaxis (74.7%).
The patients with type 2 no difference was found in median BS (type 2A BS 6.71, type 2B BS 5.60, type 2M BS 5.89, type 2N BS 7.00, P = 0.686).
In type 1 patients blood groupe non O BS (8.33) was higher than type 1 patients blood groupe O BS (6.35), p = 0.04.
In type 2 patients no difference observed between blood groupe O and non O (BS 6.68 and 5.85 respectively, p = 0.141).
In type 1 patients BS was significativly associated with VWF:RCo and VWF:Ag.
In type 2 patients no association was found betwen BS and VWF level.
Summary/Conclusion: No difference in BS was found between VWD type and blood groupe O and non O, distinction between blood groupe O and non O is important in low VWD (VWF]30-50%]), the BS was dependent to age and severity of VWD.

Bleeding
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Board No. 64

Identification of Chinese Hospitalized Patients' Risk Profile for Venous Thromboembolism (DissolVE 2): Factors Affecting Venous Thromboembolism Risk and Prophylaxis in Hospital Care Setting in China

Chen Wang*

Background: Venous thromboembolism (VTE) is a common event in hospitalized patients. Studies in China have not assessed factors influencing VTE, risk of bleeding and prophylaxis in VTE. Therefore, we conducted DissolVE2 (Identification of Chinese Hospitalized Patients' Risk Profile for Venous Thromboembolism) study (ChiCTR-OOC-16010187) to address this knowledge gap.
Aims: To identify factors influencing VTE risk, risk of bleeding, and prophylaxis implementation in hospitalized Chinese patients.
Methods: Adult patients aged 18 years or over admitted to medical or surgical wards ≥72 hours were enrolled from 69 Chinese urban major tertiary hospitals. Factors affecting VTE risk and its prophylaxis were identified using Padua Prediction Scoring and Caprini risk assessment models in medical and surgical subjects, respectively. Logistic regression was used to analyze important patient characteristics associated with VTE prophylaxis. Rates of American College of Chest Physicians (ACCP) 9th edition-recommended prophylaxis administration were also assessed. Informed consent was obtained from patients before commencing the study.
Results: Medical (n = 6623; mean age 63.2 ± 15.6 years, 57.5% males) and surgical patients (n = 6986; mean age 50.4 ± 15.5 years, 37.8% males) were included from the 14000 eligible subjects, between March and September 2016. Acute infection (42.2%) and major open surgery (52.6%) were judged to be the major VTE risk factors (Table 1). Multivariate analysis showed high VTE risk (evaluated using Padua and Caprini risk models in medical and surgical patients) and age ≥60 years influenced prophylaxis in medical and surgery patients (Table 2). Other significant factors included congestive heart failure and cancer for medical; cardiac, vascular, orthopedic surgeries significantly affected prophylaxis in surgical patients. Acute infection and/or rheumatologic disorder and age did not influence prophylaxis in both groups. Among the included patients, 14.3%, 10.3% and 3.6% received any prophylaxis, appropriate prophylaxis and prophylaxis fully compliant with ACCP guidelines, respectively. Compared with medical patients, surgical patients received higher rates of any prophylaxis (19.0% vs. 9.3%), appropriate prophylaxis (11.8% vs. 6.0%) and prophylaxis fully compliant to ACCP 9th edition (4.0% vs. 2.2%).
Summary/Conclusion: With multiple risk factors found affecting VTE risk and prophylaxis in Chinese patients along with low rate of prophylaxis implementation, our study findings will be beneficial in improving perspective of physicians and VTE management.

Bleeding
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Board No. 65

IMPORTANCE OF THROMBINE GENERATION TESTS IN PATIENTS IN TREATMENT WITH DOACs

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Background: The thrombin generation test is a global test of hemostasis and high performance. For a great variability of thrombin generation capacity in patients with DOACS, TGT may provide a promising new approach to the clinical management of bleeding and thrombosis in these patients
Aims: Determine the usefulness of the thrombogram in patients of the Central University Hospital of Asturias, a treatment with direct acting anticoagulants (DOACS) of difficult control and to contrast with the usual results measured by the TTd or anti-Xa method
Methods: A retrospective study of 10 patients who received DOACS, was performed. 2 patients (20%) received Rivaroxaban and Dabigatran, and 6 patients with Apixaban (60%). The variables were measured at the peak levels (2-4 h after taking) of the blood anticoagulant and the thrombogram with the Calibrated Automated Thrombogram (CAT)
Results: we found a 2% or patients with underrated dose, of which 1% were with Dabigatran and another 1% Rivaroxaban. 2% were overdosed and corresponded to patients with Apixaban 2.5mg, 6% of patients were at normal levels, were with Apixaban (4), Dabigatran (1) and Rivaroxaban (1). When analyzing the thrombogram we observed that the direct inhibitors of thrombin (Dabigatran) reduced ETP in a greater percentage, Apixaban has longer latency times (Lag Time), patients with Rivaroxaban did not reduce the ETP according to their levels of DOACs, probably in one case he was breaking the treatment
Summary/Conclusion: TCG is a more representative test of the level of anticoagulation, so they could better identify the haemostatic phenotype in comparison with the quantitative measurement tests of plasma levels that only measure function and not quantity. The greater ability to inhibit the generation of thrombin of Dabigatran (ETP and Lag time), even when its plasma levels are infraterapeutics, stands out. Apixaban delay the onset of haemostasis but reducing much less the generation of thrombin despite supraterpetic levels. Rivaroxaban is the drug that least inhbides the generation of thrombin in our patients. More studies and more patients are needed, but it highlights the potential of TGT in the monitoring of DOACs and help us to prevent thrombotic-hemorrhagic complications

Bleeding
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Board No. 66

Hemorrhagic And Thrombotic Complications In Patients With Direct Action Anticoagulants According To The Type Of Drugs

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Background: Direct acting oral anticoagulant drugs (DOACs) are currently an attractive alternative to classical anticoagulation. There are different types of ACOD with pharmacodynamic and pharmacodynamic characteristics, and mechanism of action (anti-IIa or Anti-Xa).
Aims: It is vital to have studies in real life, in our environment, that endorse the efficacy and safety demonstrated in their pivotal studies. Also the knowledge of the basic times of haemostasis (APTT and PT), are fundamental for the correct treatment of complications
Methods: A retrospective study was conducted with 126 anticoagulated patients with DOACs of the Central University Hospital of Asturias. Patients were classified according to the prescribed drug and the appearance of thrombotic and / or hemorrhagic events (major bleeding complications, minor hemorrhagic complications and thrombotic complications). The drugs were prescribed by the following services: Hematology, Cardiology, Internal Medicine, Pulmonology and Vascular Surgery. The effect of each DOACs on prothrombin time (PT) elongation and activated partial thromboplastin time (APTT) in the basic coagulation study was analyzed.
Results: when evaluating hemorrhagic complications, we found that the 17.9 % of patients with Rivaroxaban presented hemorrhagic complication, 2.9% (2 patients) had severe hemorrhages and 15% minor hemorrhages. 20.6% of patients with Apixaban presented hemorrhagic complication, with minor hemorrhages in the majority. 36% of the patients with Dabigatran suffered hemorrhagic complication, in 8 cases had severe bleeding. When evaluating thrombotic, Apixaban is the drug with the highest frequency of this adverse effect, in 8.8% (3 cases) of the patients. 8% (2 patients) of patients treated with Dabigatran and 6% (4 patients) treated with Rivaroxaban suffered thrombotic complications. No spontaneous intracranial hemorrhage was detected with any of the drugs.
The drug that most frequently prolonged the activated partial thromboplastin time (APTT) is Dabigatran in 76% of the subjects who take it. Rivaroxaban has less influence on the prolongation of this time of coagulation, occurring in 25.4% of cases. Apixaban practically didn´t prolonged APTT (14%). If the effect of DOACs on prothrombin time (PT) is analyzed, Dabigatran is the drug that was most prolonged this time, in 40% of the patients studied. Apixaban prolonged it in 35% of the subjects of our population. The drug with less impact on the prothrombin coagulation time in Rivaroxaban, being elevated in 25% of cases.
Summary/Conclusion: With this results, it can be inferred that the three analyzed DOACs present a profile of efficacy and safety similar and concordant with their pivotal studies. Regarding the basic tests (TP and APTT) of Hemostasis included in the basic coagulation study, it can help to identify patients taking DOACs, and even to predict bioaccumulations of the drug if the typical prolonger profiles of each drug are known.

Bleeding
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Board No. 67

Extended half-life factor VIII : from biology to patients' management optimization

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Background: Management of patients with hemophilia A (PwHA) is currently moving ahead with extended half-life (EHL) Factor VIII (FVIII) products. Therapy adjustment is challenging due to discrepancies between FVIII dosages using one-stage-assay (OSA) and chromogenic-stage-assay (CSA) with some EHL FVIII. CSA is considered to be the most consistent method but is expensive and performed in few laboratories. OSA is cheaper and widely used, but more variable due to a diversity of reagents and devices.
Aims: Compare CSA and OSA methods for the dosage of FVIII in PwHA receiving a B-domain deleted recombinant FVIII linked to an IgG Fc domain (rFVIII-Fc, Elocta®, SOBI).
Methods: A monocentric study was conducted during 1 year (2017) in Bicêtre hemostasis laboratory, France. FVIII was measured in 195 samples from 68 PwHA receiving rFVIII-Fc using CSA (BIOPHEN™ FVIII:C, Hyphen Biomed) and OSA (STA-CKPrest®, STA-ImmunodefVIII®) on a STARMax® device (STAGO). Statistical analyses included a weighted Deming regression, mean absolute and relative errors and an intra-class correlation coefficient of type ICC(3,1) agreement.
Results: The two methods had an ICC equal to 0.970 (95% Confidence Interval (CI) : 0.960-0.980), but a slightly higher value of the CSA compared to the OSA measure with a Deming regression slope equal to 1.09 (95% CI : 1.04 to 1.13) and an intercept equal to −0.47 (95% CI : −1.17 to 0.18), meaning that OSA underestimated the FVIII by 8% compared to CSA. Absolute error was below 3.4% for FVIII levels ranging 1-30% while relative error decreased for FVIII ranging 1-30% and was stable, around 13%, for FVIII ranging 30-120%.
Summary/Conclusion: Our study shows that CSA and OSA FVIII dosages were in agreement in PwHA receiving rFVIII-Fc for the 1-120% range usually used to adjust treatment. The relative error did not exceed the 20% threshold commonly tolerated in the monitoring of anti-hemophilic factors. Thus, FVIII OSA, which is an inexpensive, easy and all-day long available method, can confidently be used in our laboratory hospital for PwHA receiving rFVIII-Fc. This study highlights the crucial role of biology to improve FVIII treatment in PwHA without increasing institutional costs and ultimately optimizing patients' management.

Bleeding
P108
Board No. 68

Development and characterization of neutralizing monoclonal antibodies targeting the direct oral anticoagulant edoxaban.

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Background: Direct oral anticoagulants (DOACs) are indicated for thromboembolism prevention and treatment. Dabigatran is a thrombin inhibitor while apixaban, rivaroxaban, edoxaban and betrixaban are inhibitors of factor Xa (FXa). Novel clinical research tools are required to better understand the mechanisms of action of this new therapeutical molecules.
Aims: The challenge was to generate high specificity and affinity monoclonal antibodies (MAbs) against chemical structures such as edoxaban.
Methods: Edoxaban molecules are conjugated to carrier proteins to obtained enhanced immune response to such reputed hapten. After immunizations, spleen cells from mice presenting with the highest titers are isolated for fusion. Specific binding of purified MAbs to DOAC of interest were analyzed in a competition ELISA where antibodies were pre-incubated with edoxaban before being added to hapten-carrier conjugate immobilized onto microtiter plates. FXa activity assay was performed on human citrated plasmas spiked with increasing edoxaban concentrations in the presence and in the absence of the selected MAbs. Isotypes and affinity were also determined by Surface Plasmonic Resonance (SPR) technology.
Results: We have generated 19 clones, all secreting IgG_1 isotype MAbs specific for edoxaban. MAbs presented with a total absence of reactivity against the other "xabans" as tested by ELISA. Moreover, our anti-edoxaban MAbs are able to neutralize the in vitro edoxaban and rivaroxaban anticoagulant effect on STA®-Liquid FXa assay (Stago), respectively at a MAb-DOAC molecular ratio of 2:1.
Summary/Conclusion: We therefore report the development of various MAbs specific to edoxaban and able to neutralize the anticoagulant effect of drugs. These MAbs could be very useful in diagnostic applications. Further steps are being undertaken to develop recombinant monoclonal antibodies for extended applications in clinical research.

Bleeding
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Board No. 69

Acquired von Willebrand syndrome associated with cerebral aneurysm

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Background: The acquired von Willebrand syndrome (AVWS) is a bleeding disorder associated with the same biological anomalies as those seen in hereditary von Willebrand disease (VWD), but which occurs in association with another underlying pathology, generally in elderly patients without any personal or family history of bleeding anomalies. Those underlying conditions are mainly represented by lymphoproliferative, myeloproliferative, autoimmune, malignant and cardiovascular disorders. The cardiovascular diseases with increased shear stress seem to be the most frequent of them.
Aims: We report the case of a 31 year-old women (blood type O) without any personal or family hemorrhagic history, presenting a non-ruptured aneurysm located in the M1 segment of the middle cerebral artery, already embolized in 2016. Recently (March 2018), increasing in the size of the aneurysm's neck has indicated a surgical clipping.
Methods: The preoperative hemostasis blood assessment showed a prolongation of both activated partial thromboplastin time and closure time (measured by a platelet function analyser, PFA-200). Dosage of factor VIII coagulant activity (FVIII:C), van Willebrand factor antigen (vWF:Ag) and ristocetin cofactor activity (vWF:Rco) were therefore made urgently, and results showed a decrease for all of them (respectively 47%, 30% and 25%). These results were confirmed on a second sample, knowing that the platelet aggregometry was strictly normal.
Results: The surgery was performed under a single infusion of vWF (Wilfactin®) and FVIII (Factane®) concentrates, without any hemorrhagic or thrombotic complications. The postoperative levels of FVIII:C, vWF:Ag and vWF:Rco have lastingly returned under normal values, this evocating an AVWS. We noted at the same time a persistent prolongation of the closure time, which was probably non-specific. Otherwise, the vWF multimers analysis and ADAMTS13 activity showed identical results in both pre- and postoperative situations, this excluding for the AVWS origin, an increase in vWF proteolysis secondary to the shear stress within the cerebral aneurysm.
The patient was seen in consultation 6 weeks after the surgery. Her clinical state was completely satisfactory, excepting the report of a post-craniotomy asthenia. Moreover, as there were no infectious or inflammatory syndrome, we could evaluate her basal levels of FVIII:C, vWF:Ag and vWF:Rco which were respectively 97%, 51% and 50%. The same results were observed on following samples, with a FVIII:C around 100%, and a vWF:Ag and vWF:Rco between 50% and 60%.
Summary/Conclusion: In conclusion, the diagnosis of AVWS have been retained, however, the pathogenic mechanism remains unknown to date.

Bleeding
P110
Board No. 70

Bariatric surgery in patients with inherited coagulation deficiencies: an experiment in Strasbourg University Hospital

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Background: Obesity, characterized by a BMI ≥ 30 kg/m², is an actual public health issue which concerns 9% of the French population, knowing that its prevalence is constantly increasing. The development and maintenance of obesity is multifactorial, involving actually several parameters such as genetics, diet, sedentary lifestyle, social determinants, metabolic disorders or medications. One of the consequences which are well-documented is apparition of arthritis, this last being induced by a mechanical stress, especially on bearing joints.
Regarding patients with haemophilia or other severe bleeding disorders, their BMI is quite similar to the one observed in general population. However, these patients having already a predisposition to joint damages, development of obesity can therefore worsen the joint prognosis.
Aims: Bariatric surgery, indicated for patients with a BMI > 40 or a BMI > 35 with complications, remains not much used on patients with coagulation or platelet disorders. We actually report the experiment of the Strasbourg University Hospital in these specific situations.
Methods: In fact since 2015, one severe haemophilia A patient, 3 patients with type 2 von Willebrand disease, and one patient with Glanzmann thrombasthenia have benefited from a gastric bypass surgery.
Results: The intervention was successful for all of them, thanks to an excellent interdisciplinary collaboration between respectively surgeon, biologist, hematologist, pharmacist, endocrinologist and psychiatrist. Surgeries were made by laparoscopy, with a replacement therapy for 5 days. Thromboprophylaxis with enoxaparin was decided for all the patients.
Summary/Conclusion: Finally, the clinical evolution was satisfactory, regarding both bleedings and joints.

Bleeding
P111
Board No. 71

A retrospective observational study on patients with moderate/severe (M/S) hemophilia A in France: HEMONIS study - Methodology and Results on the M/S population

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Background: In France, care procedures for patients (pts) with hemophilia A (HA) are well-organized. Nevertheless, data for French patients with HA were still limited in the literature in 2016. Therefore, knowledge of French epidemiology and treatment specificities of HA was not sufficient. In the context of clinical development of a new therapeutic approach, Roche/Chugai wanted to better describe the burden of the disease in French patients with HA in a real-world setting.
Aims: The primary objective of HEMONIS was to describe the treatment strategies currently used in a moderate or severe (M/S) population, including Immune Tolerance Induction (ITI)/on-demand/short-term or long-term prophylaxis. The main secondary objective was to describe the current treatment strategies in severe (SEV) HA pts. Other secondary objectives in both populations (M/S and SEV HA) were to describe pts and disease characteristics, joint condition and the current type of treatment. This abstract focuses on M/S pts; results in SEV HA pts are presented in a twin abstract.
Methods: HEMONIS is a national multicenter retrospective cohort study. In order to maximize the representativeness of the participating centers, the study was proposed to the 20 biggest centers (managing 90% of the HA patients). The selection process was carried out in 2 steps: first one, patients were randomly selected if they met the inclusion and exclusion criteria; second one, patients were selected randomly based on a birth period. Eligibility criteria were: constitutional M/S HA, pts ≥ 5 years-old at index date (the date of last visit within the last 2 years), having had a visit within the last 2 years prior to the index date. The study included 2 separate populations: M/S HA pts and SEV HA pts. SEV HA pts were then composed of 3 subpopulations according to their inhibitor status: current inhibitors, tolerized inhibitors and no history of inhibitors. Data were based on pts medical records and diaries that were collected during routine medical visits. For M/S pts, data was collected at index date only.
Results: A total of 230 patients from 9 participating hemophilia centers were included in the study, 221 were eligible for the study. 145 pts were included in M/S HA (39 being moderate and 106 being severe) and 132 pts in SEV HA. In M/S pts, all pts were males; median age was 33 years [5; 80] years. Most pts (n = 121, 83%) had no history of inhibitors and 24 pts had either current inhibitors (n=6, 4%) or tolerized inhibitors (n=18, 12%). Three pts with moderate HA had history of inhibitors. Among pts with moderate HA, 31 % had at least one disease-related musculoskeletal complication and 55% of pts with severe HA. Severe reduction in mobility was observed in 8% of pts, especially in the ankle (73% of these pts). In M/S pts, 58% [95% CI 50% - 66%] were treated prophylactically and 42% [34% - 50%] were treated on-demand. Pts with moderate HA were mostly treated on-demand with FVIII (90%) and pts with severe HA without current inhibitors were predominantly treated prophylactically with FVIII (76%). Pts with current inhibitor (n=6) were mostly treated on-demand (n=5, 83%).
Summary/Conclusion: Results of the retrospective HEMONIS cohort study provide valuable information on the profiles of M/S HA pts and the management of HA in France. In daily practice, most pts with moderate HA were treated on-demand and the majority of pts with severe HA were treated prophylactically.

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Study of Coagulation Factor Inhibitors in Patients with Hemophilia in Kermanshah

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Background: Hemophilia is the most frequent severe hereditary hemorrhagic disease due to de ciency of coagulation factors VIII (Hemophilia A) or IX (Hemophilia B) in plasma. We aimed to identify patients with hemophilia in Kermanshah, Iran and assess the incidence of inhibitors in this population and its associated factors.
Aims: Inhibitors assessment and ethnicity
Methods: is study was conducted on patients with hemophilia A and B admitted in hospitals of Kermanshah city, referred to coagulation laboratory of Kermanshah blood transfusion organization. Variables including age, sex, family history of the patients in terms of history of hemophilia and inhibitor formation, development of inhibitor in patients, age at starting the treatment, blood group, severity of hemophilia, average of factors received per month and liver disease were assessed in patients.
Results: Of 123 patients with hemophilia A, 119 (96.7%) were men. e mean±SD age of patients with hemophilia A was 25.9±15.74 years. Only ve men had developed factor VIII inhibitor. Of 25 patients with hemophilia B, 24 (96%) were men with a mean±SD age of 21.7±15.71 years. Factor IX inhibitor was not detected in any patient. ere was no association between incidence of inhibitors and age at the onset of the treatment, family history of hemophilia, blood group, severity of hemophilia, average of received factor per month and liver disease. However, a positive association between incidence of inhibitors and family history of inhibitors was found ($P<0.05$).
Summary/Conclusion: Association between family history of inhibitor and incidence of inhibitor formation in hemophilic patients was a new nding. erefore this outcome and genetic evaluation of these for nding relevant mutations should be considered.

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Surgical experiment on haemophilia B patients treated by eftrenonacog alpha (rFIXFc) in Strasbourg: about 3 case reports

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Background: The pivotal studies on eftrenonacog alpha (rFIXFc, Alprolix®) have particularly evaluated its haemostatic response on 19 severe haemophilia B patients who underwent a total of 29 major surgeries. The response, as well as the blood loss, was actually satisfactory, considering rFIXFc as safe and efficacious in these surgical situations. However, the pharmacokinetic properties of the molecule, especially with a tri-compartmental model, make its perioperative management quite specific.
Aims: Report the surgical experiment on haemophilia B patients treated by eftrenonacog alpha (rFIXFc) in Strasbourg
Methods: In Strasbourg Hospital, 5 patients with severe haemophilia B were switched toward rFIXFc since March 2018. These patients are in phrophylaxis, the regimen consisting on an infusion of 100 IU per kg every 10-12 days. Among them, 3 underwent a surgical intervention since the beginning of the switch, with 2 minor and one major surgeries.
Results: A first 55 year-old patient had a coloscopy with a polyp resection, which was performed with a single infusion of 100 IU/kg of rFIXFc. A second 32 year-old patient was surgically treated for a carpal tunnel syndrome. The intervention was performed with an infusion of 100 IU/kg, knowing that a second and third infusions of 50 IU/kg were needed respectively 24h and 72h after the first one. The haemostatic response was perfect for these 2 patients.
More interestingly, a third patient with a chronic kidney failure, underwent a total elbow arthroplasty. Given a limited incremental recovery (0.3 % per IU/kg) and an acquired renal thrombopathy, the patient received preoperatively an infusion of 180 IU/kg of rFIXFc, and one platelet concentrate. Then during the operation, another infusion of rFIXFc was needed because of a minor bleed. At the end of the intervention, a monitoring of FIX coagulant acitvity (FIX:C) was made every 4 hours with a chronometric method. As a result of a decrease in FIX:C, 2 additional infusions of 100 IU/kg were made, giving a total amount of 380 IU/kg for the surgery. From postoperative day 1 to day 3, the patient presented an important scar bleeding despite a FIX:C > 60%, leading to rFIXFc injections (60 IU/kg, twice per day), several red blood cells tranfusions and tranexamic acid administration, these associated with daily renal dialysis. The clinical outcome was progressively favorable from postoperative day 4 to day 7, with a transfer in a physiotherapy unit and a spacement in rFIXFc injections. Actually, a substantial global amount of rFIXFC has been consumed for the first 7 postoperative days (96 000 IU), knowing that the hemorrhagic complications were not accountable to a defect in rFIXFc infusion but to the precarious clinical state of the patient.
Summary/Conclusion: In conclusion, the 3 patients were reviewed a few weeks after their intervention, showing a satisfactory clinical state without FIX inhibitor development. The return to their original prophylaxis regimen was rapidly feasible, except the patient on dialysis who needed a FIX supplementation at each session.

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Estimating the long-term cost of bleeding events associated with the use of anticoagulant therapy in a European setting: a systematic literature review

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Background: Bleeding is the most significant complication of anticoagulants, and it affect patients' health and quality of life notably. Bleeding is also associated with a significant health care cost in relation to the immediate treatment of bleeding and the long-term management of the patient thereafter. Assessing the cost of anticoagulant-associated bleeding is relevant in order to better characterize the overall costs of associated with the anticoagulation treatment. Evidence relating to cost of bleeding has been dominated by studies from the United States, which is representative of a multi-payer healthcare system, and thus has proven challenging to transfer into a European setting in a single-payer healthcare system.
Aims: To identify evidence estimating the long-term healthcare resource use (HCRU), the overall costs and the cost patterns associated with patients on anticoagulation therapy in a European setting.
Methods: Database searches in Medline and Embase were conducted. Cohort studies estimating long-term (≥1 year) HCRU and costs of bleeding (defined by the International Society on Thrombosis and Haemostasis) in patients receiving anticoagulation therapy: Vitamin K antagonists (VKAs), low-molecular weight heparin (LMWH), irrespective of the indication for anticoagulation treatment. Only studies based on European data were eligible for inclusion.
Results: A total of 2,552 titles and abstracts were retrieved by the searches. One full text based on Danish data, and two abstracts based on data from the UK were eligible for inclusion; all 3 were retrospective cohort studies. Populations evaluated in the studies included patients with AF, and incident venous thromboembolism (VTE). Types of anticoagulant used were vitamin K antagonists (VKAs), and dabigatran. The studies evaluated different bleeding events. All studies reported increased HCRU and costs in the year/years following the event. An analysis of patients with AF who were on oral anticoagulation prior to the bleeding event was conducted as a subgroup analysis (authors provided the data on request). The analysis showed an average total costs over a three-year period varied from €23,142 (intracranial haemorrhage), €13,493 (gastrointestinal bleed) to €9,223 (other bleed). Healthcare costs accounted for 63% of total three-years societal costs attributable to intracranial haemorrhages while social care costs and production lost to society accounted for 25% and 12%, respectively. For gastrointestinal and other major bleeding events, healthcare costs accounted for 88% and 84%, respectively. The second and third year subsequent to a bleed accounted for approximately one-third of total 3 year costs. In contrast to the first year, the average social care costs exceeded average healthcare costs in the second and third year.
Summary/Conclusion: The current review highlights the limited robust evidence estimating the long-term costs of major bleeding related to anticoagulation therapies in Europe. The cost of a bleed indicated a significant economic burden both from a health care and a societal perspective. Evidence indicates significant short-term and long-term costs in the years following a major bleed.

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Betrixaban: Impact on routine and specific coagulation assays - A practical laboratory guide
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Background: Betrixaban is a novel oral direct factor Xa inhibitor approved by the Food and Drug Administration for prophylaxis of venous thromboembolism (VTE) in adult patients hospitalized for an acute illness at risk for thromboembolic complications. As for other DOACs, assessment of the anticoagulant effect of betrixaban may be useful in some situations. Also, clinicians need to know how routine coagulation assays are influenced.
Aims: To determine which coagulation assay(s) should be used to assess the impact of betrixaban on hemostasis and provide laboratory guidance for their interpretation.
Methods: Betrixaban was spiked at final concentrations ranging from 0 to 250 ng/mL in platelet-poor plasma. These concentrations cover the on-therapy range (from ± 9 ng/mL at C_{trough} to ± 122 ng/mL at C_{max} for 40 and 120 mg once daily dose according to the clinical pharmacology and biopharmaceutics reviews of the Center for Drug Evaluation and Research). We tested different reagents from several manufacturers (e.g. Stago, Werfen, and Siemens) and assessed betrixaban impact on Prothrombin Time (PT), activated-Partial Thromboplastin Time (aPTT), dilute Russel Viper Venom Time (dRVVT), chromogenic anti-Xa assays, Thrombin Generation Assay (TGA) and a large panel of hemostasis diagnostic tests.
Results: A concentration dependent prolongation of aPTT, PT and dRVVT is observed. The sensitivity mainly depends on the reagent. FXa chromogenic assays show high sensitivity and a linear correlation both depending on the reagent and/or the methodology. Several methodologies applicable for other direct factor Xa inhibitors have to be adapted. TGA may be efficient to assess the pharmacodynamics of betrixaban for low concentration but its turnaround time and the lack of standardization are limitations.
Summary/Conclusion: Chromogenic anti-Xa assays are the most appropriate assays to measure the pharmacodynamics of betrixaban. Betrixaban significantly affects several hemostasis diagnostic tests and this needs to be taken into consideration when requesting and interpreting such tests.

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Improvement of chromogenic anti-Xa assay to measure betrixaban concentration in plasma

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Background: Betrixaban, a novel direct oral factor-Xa inhibitor, has received its market authorization on the 23th of June 2017 in the United States (US) for the prophylaxis of venous thromboembolisms (VTE) in adult patients. Although direct oral anticoagulants (DOACs) do not require routine monitoring, the assessment of their effect on coagulation may be useful in some clinical situations (e.g. detecting drug accumulation in acute renal or hepatic failure; planning urgent invasive procedure; …). Our previous study mentioned that chromogenic anti-Xa assays seem to be useful to estimate the amount of betrixaban in plasma. Nevertheless, the sensitivity of available tests is limited and improvement is needed to encompass the on-therapy range which is from ± 9 ng/mL at Ctrough to ± 122ng/mL at Cmax for 40 and 120mg once daily dose which is quite lower, even transformed in molar, than other direct factor Xa inhibitors.
Aims: To improve the sensitivity of chromogenic anti-Xa assays to betrixaban by adjusting the sample dilution scheme of three frequently used commercial products.
Methods: Betrixaban was spiked at final concentrations ranging from 0 to 500ng/mL in normal pooled plasma. Three commercial tests were tested (Biophen® DiXaI® (Hyphen BioMed, France), STA® Liquid Anti-Xa (Diagnostica Stago, France), HemosIL® Liquid Anti-Xa (Instrumentation Laboratory, USA)) and adaptation of the sample dilution were performed. In parallel, our newly proposed dilution schemes were tested on plasma spiked with UNF (Heparin Leo®, Leo Pharma, Denmark), with LMWH (tinzaparin sodium, Innohep®, Leo Pharma, Denmark) or with fondaparinux (Arixtra®, GSK, UK) to evaluate the sensitivity of these adapted assays to heparins and derivatives.
Results: Results showed a concentration-dependent decrease in OD/min inversely proportional to the dilution of the samples. For the current (1/50) Biophen® DiXaI® showed a ½ OD/min of 447ng/mL whereas our adapted method of Biophen® DiXaI® with a sample diluted 10-fold showed a ½ OD/min of 81ng/mL. The CVs were always below 1.0%. Improvement of the LOD and LOQ were also noticed (from 10 to 31ng/mL for the current method and from 3 to 5ng/mL for the adapted method). Same results are observed with the other reagents. While modifications improve the sensitivity of this test, results also showed an increased sensitivity to heparins and fondaparinux for the Biophen® DiXaI® which is, with the normal dilution procedure (i.e. 1/50), insensitive to indirect FXa inhibitors.
Summary/Conclusion: Our results showed that the improvement of current methodologies makes the chromogenic anti-Xa assays more sensitive to betrixaban. However, this lower initial dilution of the samples makes the specific buffer of the Biophen® DiXaI® inefficient to prevent sensitivity towards heparins and derivatives. This may be a problem in case of bridging therapy due to an overestimation of betrixaban concentrations. To avoid the cross-interference between DOACs and heparin and/or LMWH, the conception and the optimization of a new buffer should be explored.

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Pharmacokinetic parameters, FVIII consumption and cost of 13 patients with severe haemophilia who have switched from conventional therapy to Efmoroctocog alfa:experience of a French haemophilia centre

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Background: Patients with severe Haemophilia A (HA) have the opportunity to get regular prophylaxis which improve life expectancy, prevent joint damage and improve their quality of life (QoL) Conventional FVIII replacement therapies have short half-lives in average 12h, with a high inter-individual variability (6-29h). Therefore the majority of HA patients inject themselves every day to 3/week. Efmoroctocog alfa (Elocta®) is a recombinant human FVIII bound to the Fc fragment of a human immunoglobulin (rFVIII-Fc) which enhances half-life *in-vivo* and may have the potential to reduce injection frequency.
Aims: the goal of this study was to evaluate pharmacokinetic (PK) parameters in patients treated with Elocta®, and to determine whether or not the switch from a conventional FVIII therapy to Elocta® reduced injection frequency, cost and improve their QoL.
Methods: Thirteen patients with severe HA previously treated with conventional FVIII concentrates (Advate®: n=7, Refacto®: n=2, Helixate®: n=2, Kogenate®: n=1, Novoeight®: n=1) were switched to Elocta®. All patients were older than 15 years, and treated either on demand (n=6) or on prophylaxis (n=7).
The WAPPS-Hemo database, which is coordinated by Mc Master University, is a web-accessible database for haemophilia patients and can provide individual PK estimates with a bayesian model requiring minimal factor VIII (FVIII) plasma levels. The PK were evaluated for each patient with Elocta® (3 to 5 FVIII plasma levels measured before injection and up to 48 hours after injection), tailored PK-driven prophylaxis was then assessed for each patient, in order to obtain a through level of FVIII >1%. FVIII half-lives calculated with Elocta® and the mean number of UI/kg per week injected with Elocta® were compared to those with regular products. In addition, after 1 to 14 months, patients were asked by telephone contacts or during scheduled visit to the Haemophilia center about their QoL with Elocta®.
Results: FVIII half-live with Elocta® increased for all patients with a mean half-life of 17.02 hours (h) [7.5-24.5] with Elocta® compared to 10.65h [6-14.75] with regular concentrates, with a mean gain of 62% [21%; 113%]. All the patients except one remained on prophylaxis with 1 to 2 injections per week. The patient «on demand» had significantly reduced his FVIII consumption.
The 13 patients were satisfied or very satisfied of the switch to Elocta®, either because of the lower frequency of injections or because their articular pain was reduced. These facts are highlighted by the decrease global FVIII consumption (75.4UI/kg/w |26-120] with conventional products to 62.9UI/kg/w [37-102]). No adverse effect was noticed.
Summary/Conclusion: PK studies are the only efficient way to determine the *in-vivo* elimination half-life of FVIII concentrates. All 13 HA adult patients, demonstrated that tailored PK-driven prophylaxis with Elocta® allows to reduce injections frequency in comparison to usual conventional FVIII therapy as previously described (Shapiro *et al*. 2014). Despite the lack of a standardized questionnaire of QoL, all patients have a positive feeling with Elocta®. Global FVIII consumption and cost evaluation are in favour of Elocta®. These preliminary results have to be confirmed on larger and prolonged study.

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Standardization and automation of thrombin generation assay: TG on its way to the clinical lab for haemophilia patients

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Background: Many research studies using thrombin generation (TG) in haemophilia settings have been published. Some show TG utility to predict the effects of hemostatic drugs in hemophilic patients (Dargaud et al, Blood 2010) or TG improved capacity to evaluate an individual's bleeding tendency with more accuracy compared with factor levels measured using routine assays has been published in research studies (Trossaert et al Haemophilia 2014). These results have not yet been translated into clinical utility through multicenter studies because of a lack of standardization of methods.
Aims: ST Genesia (Stago, Asnieres, France), a new analyzer intended to measure thrombin generation in a fully automated way, is evaluated in our laboratories for validation purposes with STG-BleedScreen reagent (Stago, Asnieres, France), a trigger containing low tissue factor (TF) concentration.
Aside from biological and clinical outcomes of our protocol in haemophilia patients populations, the study is also the opportunity to assess how precise and consistent can become TG in a bi-centric study.
Methods: Each of our 2 centers received the same lot of STG-BleedScreen reagent to assay patients plasmas. On each run, 3 freeze-dried samples were tested prior to testing fresh or frozen patient samples. 2 of these samples were internal quality control (IQC) samples (hypocoagulable and normocoagulable) and 1 is intended to be used as reference plasma for normalizing results (Perrin J et al, Thromb Res 2015).
Results: Due to difference of starting date on both sites, as of today one center has collected 27 results for each IQC while the other has completed 38 runs.

When comparing average results obtained in both centers, we observe the following relative deviations:
On the normocoagulable IQC level (before normalization / normalized):
Lag Time (LT): 1.6% / 0.3%
Peak Height (PH): 2.7% / 0.9%
Endogenous Thrombin Potential (ETP): 5.1% / 0.1%
On the hypocoagulable IQC level (before normalization / normalized):
LT: 7.5% / 8.5%
PH: 12.4% / 8.6%
ETP: 9.8% / 4.7%

Moreover, the coefficient of variation obtained over the total 65 measurements after normalization of results are: 5.9% and 6.9% (LT) , 6.8% and 8.1% (PH), and 4.1% and 4.3% (ETP) for normocoagulable and hypocoagulable IQC respectively.
Summary/Conclusion: Automation, enhanced control of temperature throughout the assay and normalization of results help to achieve good comparability and reproducibility of results when testing on two different sites and at low TF concentration. This enhanced standardization of TG is a pre-requisite to introduce the assay in the clinical lab.

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Establishment of a thrombin generation threshold in severe haemophiliac patients under prophylaxis to further determine an added value of this test in the personalized treatment: a pilot study

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Background: Treatment of severe haemophiliac patients is based on replacement of the deficient factor. Until recently, the theoretical through level admitted to avoid spontaneous bleeding in all patients was >1%. Current recommendation is to personalize the factor dosage to reach a through level that could be higher, depending of individual factors like age, life style, pharmacokinetics… On the other hand, it has been demonstrated that thrombin generation TG could better reflect the phenotype of the patient than the factor level.
Aims: We aimed to measure TG during pharmacokinetics undergone in every patient included in the study to assess the potential benefit of this test in the personalized follow-up/treatment. Chromogenic (CSA) and chronometric (OSA) FVIII levels were both measured.
Methods: 18 severe haemophilia A patients under prophylactic treatment were included after approval of the local Ethical committee. Annual bleeding rate ABR was collected for each patient. Citrated blood samples were obtained at 5 time points: before factor injection T0, 30 minutes, T1, T2 and T24 hours after injection. Poor platelets plasmas were obtained after a double centrifugation 15 minutes at 1900 g and stored at -80°C until processing. Factor half-life (T ½) was estimated thanks to WAPPS-Hemo. FVIII was measured on STA-Evo (Stago) using OSA (Cephascreen® aPTT reagent (Stago) and CRYOcheck® FVIII deficient plasma (Precision Biologic) and CSA (Hyphen Biomed®) assays.
TG using the Calibrated Automated Thrombogram® was triggered with low level of tissue factor (PPP LOW reagent). Endogenous Thrombin Potential (ETP) was monitored.
The ETP at T0 for each patient was expressed in function of the total dose of factor administrated in one year (RETP0). The performance of RETP at time 0 for ABR was determined using a ROC curve.
Results: The following patient's data are expressed in terms of median and range. Age: 19.5 y (2.9 – 60.8), weight 52.5 kg (15 - 97) follow-up 93.5 months (4 – 217), total dose of factors 2561.6 UI/kg/year (1171 – 4132), ABR 1.1 (0 – 3), T ½ 10.2 hours (4 -20).
FVIII levels measured by the two methods were significantly correlated at the 5 time points. Using the ROC curve, a RETP0 <0.115 reach a sensitivity of 84.6% and a specificity of 83.3% for ABR (Pvalue 0.01). Using this value, we determined an ETP threshold value of 190.9 nM below which the bleeding frequency would increase.
Summary/Conclusion: This pilot study encourages us to continue to use OSA to determine FVIII for classical factor concentrates, which is easier and less expensive cheaper than the CSA. We don't have yet enough data for long acting products.
Our study demonstrated that a relationship between ETP at time 0 and the patient phenotype could be established with the determination of an ETP threshold. This threshold could now be prospectively used to adapt prophylactic treatment to minimize bleeding risk, hoping to demonstrate an added value of this test in a larger cohort of patients.

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Primary hemostasis imbalance during ECMO therapy: preliminary study in Rennes hospital

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Background: Severe hemorrhages are the most frequent complications of extracorporeal membrane oxygenation (ECMO). They occur in about 50-60% of ECMO supports and are a major cause of mortality. They require frequent transfusions and may lead to surgical revision. Heparinization and anti-platelet therapy, surgery, inflammation and hemodilution: the causes of hemorrhages on ECMO are multifactorial but not yet clearly identified. Hemostasis balance seems to be disturbed and an Acquired von Willebrand Syndrome has been described. Yet characterization of Von Willebrand factor abnormalities and concomitant platelet functions, as a more global picture of primary hemostasis, is lacking.
Aims: The main objective of this study was to evaluate and characterize Von Willebrand factor abnormalities and possible platelet dysfunctions in patients on ECMO.
Methods: We performed a monocentric, observational study on 30 adult patients benefiting from ECMO between November 2016 and June 2018. Screening for acquired von Willebrand syndrome included the Willebrand antigen (VWF: Ag) and Willebrand activity (VWF: Act, with the ratio VWF: Act / VWF: Ag), Willebrand-collagen binding assay, and electrophoresis to assess the loss of Willebrand high molecular weight (HMW) multimers (reference value> 15%). Platelet functions were assessed by multiple electrode aggregometry following stimulation with different agonists as well as surface expression of platelet adhesive receptors (GPlb, GPIIb-IIIa) and activation markers with flow cytometry. Platelet counts were measured concomitantly.
Results: An acquired von Willebrand syndrome has been found in the first 10 patients analyzed: ratio VWF: Act/VWF: Ag <0.7, as well as a decrease or even a total loss of Willebrand HMW multimers. The proportion of Willebrand HPM multimers was <1.3% in the 5 patients with Medos systems (Xenios®), and between 2.5 and 6% in the 5 Rotaflow EMCO (Maquet®). Five patients had severe hemorrhages requiring surgery, 3 of 5 ECMO Medos and 2 of 5 ECMO Rotaflow. Platelet function assessments are currently under analysis.
Summary/Conclusion: This study shows the presence of an acquired von Willebrand syndrome in all analysed patients. However, the effect on primary hemostasis seems to depend on the type of device.

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A new rapid, specific and simple ELISA for VWF- Propeptide

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Background: Von Willebrand Factor propeptide (VWFPP), a large protein representing the D1 and D2 domain of VWF, is cleaved from VWF during intracellular processing. VWFPP is indispensable for storage and folding of VWF multimers in cell organells. VWFPP is secreted from endothelial cells and is found in plasma. For certain types of VWF deficiency, the assessment of VWFPP that has a different plasma half live than VWF is very helpful for characterizing the nature of the defect. VWFPP has also shown clinical value in acute vascular disorders such as HUS, TTP, in diabetes and in sepsis. ELISA based assays have been described for the measurement of VWFPP, but the methods are quite complicated and take more than a day.
Aims: Our aim was the development of a rapid, robust ready to use assay that could be implemented in each laboratory.
Methods: A monoclonal antibody against VWFPP was used for pre-coating of microtiter strips and a second one labeled with peroxidase served for detection. The assay is protected against interference by rheumatoid factor and heterophilic antibodies.
Results: The total assay time is 90 min. The assay has a measuring range from 0 -120 mIU/ml. The lowest detectable concentration with the standard calibration procedure is 1.7 mIU/ml. The intra assay precision is 9.8 % CV at a concentration of 3.8 mIU/ml and 0.6 % at 60.6 mIU/ml. After opening of the kit, all reagents can be stored in aliquots for several weeks. A study of 150 clinical samples from patients with different types of VWF deficiency and healthy donors revealed good correlation against a research assay from Sanquin (r= 0.947/ Passing Bablok) that was used as a reference.
Summary/Conclusion: The new rapid VWFPP ELISA showed at least equivalent performance as the reference. However, it is much simpler (ready to use microtiter strips), robust against common interfering factors, more rapid and can be used in all labs on standard equipment. Therefore it may become a useful tool for the workup of VWF deficiency, especially in the discrimination of type 1 and type 3 VWD, for calculating VWF/ VWFPP ratios, and as an innovative biomarker in certain vascular disorders.

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Diagnosis strategy of the Von Willebrand disease in the west Algeria: Single center experience.

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder, usually diagnosed on the basis of the presence of mucocutaneous bleeding symptoms and reduced circulating von Willebrand factor (VWF) levels. The genetic transmission is generally co-dominant or dominant. Although the first report of the disease dates back to 80 years ago.
Aims: Diagnose the Von Willebrand disease (VWD) in west Algeria.
Methods: Study conducted for 5 years in the west Algeria. We analyzed 701 samples of patients. For each patient was performed an activated partial thromboplastin time APTT, Prothrombin time, assay of Factor VIIIc, assays of von Willebrand factor antigen VWF: Ag (STAGO), ristocetine cofactor VWF: RCo by visual method (SIEMENS), collagene binding test VWF: CB, FVIII binding test VWF: VIIIB, blood typing ABO and blood count. All haemostasis tests were performed with normal, pathological and standard controls. The type of Von Willebrand disease is determined by calculating the reports (VWF: Rco/VWF: Ag, FVIII:C/ VWF: Ag, VWF: CB/VWF: Ag).
Results: The objective was to diagnose the Von Willebrand disease (VWD) in west Algeria.
We diagnose 96 patients with Von Willebrand diseases, 11% of the patients had VWD 3, 33% had VWD 1, 33% had VWD 2 and 22% low VWD.
The frequency of family history was 59%.
The frequency of consanguinity was 44% and 90% in VWD 3.
The epistaxis and ecchymose was the most common bleeding.
Summary/Conclusion: The Von Willebrand disease (VWD) was the most common congenital bleeding. the diagnosis of VWD3 are easy than VWD2 and VWD1.
The diagnosis has been difficult because of the extensive testing required for diagnosis compared to hemophilia or other rare deficiencies in coagulation factor.

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A retrospective observational study on patients with moderate/severe (M/S) hemophilia A in France: HEMONIS study - Results of the Severe population

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Background: The primary treatment of hemophilia A (HA) patients (pts) is infusion of FVIII concentrates; nearly 30% of pts with severe (SEV) HA (FVIII: <1%) develop antibodies (inhibitors) against this protein, thus neutralizing its effects. The management of pts with SEV HA is particularly complex especially for inhibitors pts and effective primary treatment options are still limited. Data on French pts with SEV HA are scarce, and further investigation is warranted to improve knowledge on disease characteristics, current primary treatment management and type, and frequency of significant events.
Aims: The methodology and main results of HEMONIS in moderate /severe HA population are presented in a twin abstract. This abstract focuses on SEV to describe the different primary treatment strategies (main secondary objective) and medical history with related conditions, patient's status, significant events (bleeding or physical limitations) and surgeries.
Methods: In this national multicenter retrospective cohort, SEV HA pts were included in 3 subpopulations according to their inhibitor status: current inhibitors (CI) pts with detectable inhibitors in the 2 years (yrs) before last visit (index date); tolerized inhibitors (Tol) pts with no inhibitors more than 2 years before index date and no history of inhibitors (NHI) pts who never had inhibitors. For patients in the SEV population, some clinical parameters were evaluated at different periods: medical history between diagnosis date and January 1, 2000, therapeutic scheme from January 1, 2000 to 2 years before index date, and treatment and significant events in the last 2 years preceding index date.
Results: SEV HA pts (n=132) included 36 NHI pts, 30 CI and 66 Tol. Median age were 32 [5; 80], 22 [5; 75]and 22 [6; 71] respectively.
At last visit, the most frequent primary treatment was FVIII prophylaxis in Tol (86%) and NHI pts (81%), and ITI in CI pts (40%). Infusion device was largely most used in CI (53%) and Tol (38%) than in NHI pts (6%).
Regarding ITI, 89% of Tol pts had a single ITI with a median duration of 10 months [1; 160], while 44% of CI pts were treated with ITI at least twice for a cumulative median of 47 months. Recombinant factor VIII concentrate was prescribed in 75% of ITI. Forty-nine surgeries were reported in the last 2 years preceding the index date: 21 in 13 operated CI pts (43%), 17 in 12 operated Tol pts (18%) and 11 in 9 operated NHI pts (25%).
Almost all SEV pts had mild or moderate bleeding episodes; only 3 pts (3%) experienced severe bleedings among NHI and Tol pts against 4 pts in CI pts (13%). Bleeding episodes occurred mainly in the joints. The percentage of target joints in NHI and Tol pts was 90% and 60% with on-demand against 19% & 24% in pts treated prophylactically, respectively. In CI pts, this percentage was 57%, 64% and 30% in pts treated prophylactically with by-passing agents, on-demand or with ITI, respectively.
Chronic hemophilic arthropathy occurred in 63% of pts with CI, 64% in NHI pts and 45% of pts with Tol; damaged joints were mostly knee and ankle.
Summary/Conclusion: The HEMONIS study analyzed the profile of pts with moderate and severe HA, with a specific focus on pts with SEV HA who develop anti-FVIII inhibitors. Their treatment is particularly complex as they cannot receive optimal treatment. Therefore, valuable support to understand the specificities and medical needs for these patients and new effective treatment options are needed.

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AN ALTERNATIVE APPROACH FOR IMPROVING FACTOR IX RESULTS IN HEMOPHILIA B PATIENTS TREATED WITH ALPROLIX®

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Background: The measurement of coagulation factors in plasma samples is mandatory in hemophilia patients. However there is significant inter-assay variability in factor IX (FIX) results observed in plasmas spiked with eftrenonacog alpha (ALPROLIX®). The Diagnostica Stago one-stage assay is particularly concerned with this variability. We have observed in the same plasma, sampled from a young patient treated with ALPROLIX, significant intra-assay variability between two laboratories (FIX results: 12 vs 19 IU/dl). This precludes the use of a correction factor when interpreting the test results but an improved standardization of the assays could be a solution.
Aims: Test if an improved assay standardization can correct the variability of the Stago one-stage assay in plasmas spiked with ALPROLIX and restore the expected increment in factor IX per IU/kg in treated patients.
Methods: Plasma samples with 3 different FIX potency levels (sample A: 80, sample B: 20, and sample C: 5 IU/dl) were provided by SOBI®. They were used to mathematically standardize the assay. Results before (method O) and after (method S) standardization are described. A pharmacokinetic study (t0, 30 min, 6h, 48h, 96h) at the introduction of ALPROLIX® was performed between March 26th and May 18th 2018 in 10 severe hemophilia B patients followed at the hemophilia center of the CHU de Toulouse. An informed consent was obtained for all patients. We used at the same time the methods O and S to measure FIX activity. All measurements were perfomed on STAR Expert Series coagulometers with CK Prest®, Immunodef FVIII® and Unicalibrator® (Diagnostica Stago). The concentration vs time relationship was studied with a nonlinear mixed effects model. All calculations were performed with the software R (2017).
Results: Before and after standardization the mean results of FIX were respectively, for plasma A: 49.3/80.1, for plasma B: 12.8/15.9 and for plasma C: 4.3/4.8 (IU/dl). The concentration vs time relationship was influenced by age, time since injection and the calibration method (original vs standardized). Method O shows a reduction of the - concentration vs time relationship - by more than 50% ($p<0.0001$). With method S the increment of factor IX per IU/kg administered for patients older than 6 years is normal (115% of the manufacturer's announced value). In the 2 younger patients, this increment is 48% of expected. For method O the increment is underestimated by 77% to 47%.
Summary/Conclusion: An improved standardization of the factor IX assay provided by Diagnostica Stago can correct the bias observed in the international study. This approach can be a practical and inexpensive solution for the heterogeneity of test results in patients treated with ALPROLIX®. With this approach, we observed normal increments of factor IX per IU/kg of concentrate administered in patients older than 6 years. In younger patients we found a reduced increment suggesting variability in the dose-response relationship. These results need to be confirmed in further studies.

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Effect of cell saver on haemostasis parameters during cardiopulmonary by-pass surgery

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Background: Viscoelastic testing is widely used as an aid to blood management strategies in bleeding patients after cardiopulmonary by-pass surgery. It has been shown that in these patients, intra-operative cell salvage (CS) reduces the risk of packed red blood cells transfusion but viscoelastic testing does not seem to be influenced by CS. In patients with high-risk of bleeding, CS impairs haemostasis either by fibrinolysis or excessive residual heparin. However, in our cohort, patients receive tranexamic acid systematically after general anaesthesia and never experience hyperfibrinolysis. Heparin and protamin dosing are adjusted according to HMS Plus (Medtronics) point of care results. In this context the effect of CS on haemostasis and more specifically thromboelastometry is unknown.
Aims: To study the effect of intraoperative cell salvage on thromboelastometry, residual anti-Xa activity and classic haemostasis parameters after protamin administration.
Methods: In this prospective observational study, fifty patients that underwent cardio-pulmonary bypass in January 2016 and January 2018 in the Rangueil hospital (CHU de Toulouse – France) were explored by thromboelastometry (ROTEM Delta – Werfen) immediately after protamin administration. Citrated blood was collected according to guidelines before and after administration of the CS. Patient management was performed according to local guidelines independently of tests results. Informed consent was provided by all patients. The relationship between the administration of the CS and the test results was studied with a generalized mixed effects model to account for longitudinal data and interaction between variables. All tests were performed with R (The R Foundation for Statistical Computing Platform©, version 3.4.3 – 30/11/2017).
Results: All patients had scheduled surgery for valve replacement (aortic 42%; mitral 4%), coronary by-pass (26%) and combined surgery (28%). Packed red blood cells were given during or after surgery to 12 patients (24%). The CS significantly and independently shortened the mean CT of EXTEM by 23% (85 to 65 secs; $p<0.001$). The maximum clot firmness was not influenced by CS nor any other ROTEM results. As previously described, mean anti-Xa activity increased by 20% after CS ($p=0.027$) but remained below 0.15 IU/ml in 95% of patients. Mean prothrombin time was reduced by 6,5% ($p<0.001$) but remained between 15-20 sec in all patients. Mean haemoglobin increased by 17% (9.9 to 11.6 g/dl; $p<0.001$).
Summary/Conclusion: CS administration during cardiac surgery allows for faster clot formation without reduction of clot strength. This may explain, at least in part, the observed reductions in blood transfusion. It is of interest to note that before CS, the mean CT of EXTEM was above 80 sec which is the threshold for prothrombin complex concentrate or fresh frozen plasma administration in algorithms for managing bleeding patients. This suggest that ROTEM should be performed after CS administration in bleeding patients to prevent inappropriate administration of coagulation factors.

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Which FVIII:C assay for the monitoring of patients with hemophilia undergoing surgery under the cover of FVIII-Fc fusion protein?

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Background: We analyzed 13 consecutive surgeries (8 major and 5 minor) that have been performed in Lyon comprehensive hemophilia care center between March 2017 and May 2018.
Aims: The aim of the study was to evaluate FVIII-Fc comsumption, infusions'frequency and FVIII assaies to monitor patients with hemophilia A undergoing surgeries.
Methods: These surgeries were performed in 13 patients with hemophilia A: 9 severe and 4 minor with a FVIII: C between 11 and 25 IU/dL.Factor VIII recovery was calculated before surgery in 11 of 13 patients. FVIII levels were measured with both one stage (Synthasil, Hemosil Factor VIII,Werfen) and chromogenic FVIII assays (Biophen FVIII, Hyphen).
For major surgeries such as joint replacement surgeries, patients received FVIII-Fc twice a day the day of the surgery (every 12 hours) and then once a day between postoperative days 1 and 7. After the first week, FVIII-Fc infusions were given every other day during two additional weeks until postoperative day 21.
For minor surgeries, daily infusions were prescribed during at least one week.
Target trough FVIII level was 80IU/dL during the first 7 postoperative days for major surgeries and 50IU/dL for minor surgeries.
For major surgeries, FVIII:C monitoring was performed using the one stage assay twice a day the day of the surgery and then, once a day until hospital discharge. One patient among 13 had FVIII: C monitoring with both chromogenic and one stage FVIII assays in parallel.
Results: Recoveries: we observed statistically significant discordance between FVIII levels measured with one stage and chromogenic assay (p=0.01; Mann Whitney test). We roughly observed 23% of difference (ranged from 6 to 39%) for normal FVIII levels i.e. 51 to 144IU/dL. The chromogenic assay systematically showed higher results than one stage FVIII: C assay.
The calculated recovery was therefore very different depending of the assay used i.e. average difference of 0.95%/UI/kg was observed (ranged from 0.4 to 2.08).
Among 11 patients who had FVIII recovery determination before surgery, dosages of FVIII-Fc to prescribe was decided on the basis of one-stage FVIII:C assay in 8 patients and on the basis of chromogenic FVIII:C assay in 3 patients.
Surgeries: Average total FVIII-Fc consumption on the surgery day was 84.5 UI/kg for major surgeries and 44.5 UI/kg for minor surgeries. Consumption results observed at post-operative week 1 were 44 UI/kg/j for major surgeries and 17 UI/kg/j for minor surgeries.
There was no excessive bleeding. Blood loss was in the expected average for these types of surgeries.
Two patients discharged on Day0 and 4 on Day1.
Summary/Conclusion: The use of a long-acting FVIII is effective, safe and more convenient compared to regular FVIII concentrates in surgical setting. Differently from the recommendations of the manufacturer, we observed a substantial difference between FVIII: C results measured with different methods; which may have a dramatic impact on the consumption of FVIII-Fc. In our series, an average saving of 2000 IU could be made in the first pre-operative infusion of FVIII-Fc only.

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Gastrointestinal Bleeding and Angiodysplasia in von Willebrand Disease: Results from the secondary interim analysis of the French HRQoL Study: WiSH-QoL.

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Background: Von Willebrand Disease (VWD) is primarily characterized by mucocutaneous bleedings as a result of primary haemostasis defect, excessive haemorrhage after invasive procedures, and, less commonly, by soft tissue haematomas and joint bleeds. Some patients with VWD may be subjected to recurrent overt or occult bleedings in the gastrointestinal (GI) tract related to angiodysplasia. The angiodysplasia occur most commonly in middle-age or elderly patients. These affected patients may need to be hospitalized for long periods of time and often require several weeks of different regimen of treatment. We report here the first results of GI bleedings impact on the Health-Related Quality of Life (HRQoL) of patients with VWD enrolled in the French WiSH-QoL study.
Aims: As both VWD and its treatment affect patients' and, possibly, their families' everyday life, the French non-interventional study aims to assess HRQoL in VWD patients of any age and to evaluate associated costs related to VWD management.
Methods: Clinical phenotype such as bleeding score (BS - Tosetto score), biological profile is documented. Patients are treated with the triple-secured plasma-derived VWF concentrate almost devoid of FVIII WILFACTIN. Clinical characteristics and therapeutic approach are recorded. HRQoL is assessed with the generic SF-36 (for adults) and the chronic-generic DISABKIDS Short Form (for children and adolescents) and the VWD-specific VWD-QoL (for adults, children and adolescents). In addition treatment satisfaction is evaluated. At least 350 patients will be followed during 2 years.
Results: Since October 2014, 357 patients have been included.
At time of the current analysis, out of 353 patients with evaluable data, special attention is given to the specific clinical subgroup of 40 patients with recurrent bleeding from the GI tract.
The patients' age ranged from 3.3 to 83.0 year-old with a 44.5 y.o median. Among them, 3 children were under 6 years old and 11 adults above 65 years. (Females: 24 (60%) and males: 16 (40%)).
Five patients had type 1 VWD (type 1Vincenza: 3; type 1: 2), 23 had type 2 (2A: 11; 2B: 7; 2M: 3 and 2 unspecified: 2), 10 had type 3 and 2 with a type undetermined.
The median Tosetto bleeding score (BS) reported for 40 patients with GI bleeds was higher +12 ranging from +4 to +28 compared to +5 (-1 to +31) in patients without history of GI bleeds. It was also able to distinguish disease severity by VWD type; Type 3 VWD had the highest bleeding scores (+26 (+7 to +28).
At time of inclusion, 28 patients were On-Demand treatment and 12 patients (4.7 – 70.4 years of age) were and are still requiring Long-Term secondary Prophylaxis (LTP) because of joint and/or GI bleeds. For 12 out of these 40 patients, the endoscopic procedure Argon plasma coagulation (APC) was used. For 5 of these patients, the procedure was associated with a LTP and they were on regular prophylaxis with VWF concentrate at study entry.
As frequently in VWD management, patients may need to stay on long-term iron therapy. This concomitant treatment has been given to 16 (40%) patients and 11 (27.5%) patients have also received anti-ulcer drugs.
Summary/Conclusion: Thus, this study will contribute to better assist VWD patients with GI episodes that are challenging due to their severity and recurrence. These findings may teach us what would be the best treatment such as LTP. With the HRQoL results a deeper insight into these patients' real daily life and their specific health care needs will be gathered as well.

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Massive Spontaneous Haemothorax in a Hemophilic Child: A rare case report

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Background: By far the most common clinical manifestations of specific Congenital Hemorrhagic disorders such as Hemophilia and type 3 of von Willebrand's disease are Hemathrosis and Hematoma.
Noteworthy to mention bleeding into pleural cavity is an extremely rare manifestation especially when it comes about spontaneously,
Spontaneous hemothorax is the commonest etiology of haemothorax. Which have been defined by bleeding without any evident trauma into the pleural cavity with wide spectrum of clinical manifestations, additionally the common etiology is the rupture of the adhesions between the parietal and the visceral pleura.
Aims: Here by we present a 12 year old boy with severe hemophilia A and factor 8 inhibitor (high responder) since he was 1 year old whom had spontaneous hemothorax without any history of trauma
Methods: Initially he was referred to our department due to chest pain, thorough examination and investigations such as Chest X-ray and cardiac echocardiogram revealed no specific finding, so patient have been discharged. but 2 day later patient were admitted with chest pain and respiratory distress with suspension to pleuropneumonia CT scan of thorax revealed mild pleural effusion which appropriated antibiotics and replacement therapy consisted of FEIBA or rFr VIIa were administered and patient were discharged in a good condition.
Results: He admitted again 3 days after discharge due to severe respiratory distress, initial evaluations consisted of CXR and CT scan revealed massive hemothorax in right lung replacement therapy with sequential method of administration of FEIBA or r Fr VII and packed red blood cell transfusion were initiated.
Summary/Conclusion: Immediate thoracocentesis with concomitant tube thoracostomy was carried out with no significant improvement hence Surgery was performed to remove extravasated blood and decorticate the right lung unfortunately all attempts were effortless and lead to death.
Despite it's rare incidence spontaneous haemothorax in hemophilia should be kept in mind even though the initial findings would be not specific. To the best of our knowledge this is the first case whereby reported in our country. Key words: Hemophilia, spontaneous Haemothorax

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Efficacy of escalating method of prophylactic low dose factor treatment in children with severe hemophilia A & B: Sonographic and clinical scoring outcomes

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Background: One of the long-term complications of bleeding in children with severe hemophilia is joint arthropathy. The prevention of bleeding is now accepted as the superior management of hemophilia to decrease joint damage instead of on-demand treatment; however both the cost and adherence issues –due to IV access problems in children-in the classic high dose and frequent prophylaxis regimens have been cruicial problem for its implementation in hemophilia care especially in developing countries.
Aims: This study was undertaken with the aim to evaluate efficacy of prophylactic low dose factor treatment in severe hemophilia A & B according to clinical and ultrasound imaging outcomes through one year follow up.
Methods: A total of 36 hemophilic boys (31 type A & 5 type B) aged 3-10 years who were initially treated with once weekly low dose clotting factor (25 IU/kg FVIII or 50 IU/kg FIX), were recruited in a cross-sectional study in one year period. Their prophylaxis regimen was changed to twice a week and then three times a week when they experienced three joint bleedings, four soft tissue bleedings or a one life-threatening bleed without a specific trauma history Joint status was assessed using the Hemophilia Joint Health Score (HJHS) and the Hemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) procedure of 6 joints in two subsequent yearly examination. The Two different scores were compared separately with paired t test.
Results: Median annual joint bleeding rate was 2.2. The mean pre-and post study HJHS scores of after one year were 2.47 & 1.27 respectively which showed statistically significant improvement (P value=……). Also, the mean HEAD-US scores were 1.69 & 1.44 which were not statistically significant. (P value = 0.0001) additionally 10 new target Joints were found during this period of care in 9 patients.Also 7 patients had to increase their frequency and doses of prophylaxis according to protocole
Summary/Conclusion: Our results showed that the escalating low dose factor prophylaxis can preserved joint health status according to both clinical and US assessments in pediatric patients with severe hemophilia A & B and can be advised in countries with limited resources.

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Management of prostate biopsy and prostate cancer therapy in patients with haemophilia: a retrospective French multicentre study

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Background: In a previous study in patients with haemophilia (PWH), excluding hepatocellular carcinoma, we found that urogenital tract tumours are the most frequent malignancies (1). In France, prostate cancer is the most commonly diagnosed cancer.
(1) Biron-Andreani C et al. Cancer detection and management in patients with haemophilia: a retrospective European multicentre study. Haemophilia 2014, 20, 78-82.
Aims: The aim of the present study was to collect data on cancer screening and diagnosis, clinical presentation, cancer type, treatment modalities, bleeding complications and outcome patient in a group of unselected PWH followed by haemophilia treatment centres over the last 15 years and evaluate the influence of haemophilia during the management.
Methods: The participating haemophilia treatment centres (Bordeaux, Caen, Chambery, Lille, Marseille, Montpellier, Nantes, Rennes, Rouen) reviewed their database of adult patients with mild, moderate or severe hereditary haemophilia followed. All cases of patients with suspect or confirmed prostate cancer were included in the analysis.
Results: In the interim analysis, 39 patients (37 haemophiliacs A and 2 B, 3 severe, 2 moderate, 34 mild, mean age 72 years, 60-87) were suspect of prostate cancer, 69% because of a systematic PSA screening by the general practitioner. Four patients had haematuria and one had haematospermia. Among comorbidities, the most frequent were hypertension in 43% (17/39) and cardiovascular diseases in 18% (7/39). Forty-six biopsies were performed mainly in university hospitals (80%). An adenocarcinoma was diagnosed in 28 patients (72%). Cancer was localized in 83% of cases. Among them, 42 (91%) were done on inpatients. Except one, factor concentrates or desmopressin was administrated. Biopsies were uncomplicated in 75% (35/46), 8 bleeding complications were reported with modified management in 5 cases. Half of the patients (15/28) underwent surgery including 9 robotic-assisted surgeries, 8 (28%) had radiotherapy, 6 (21%) had hormonal therapy and therapy abstention in 4 patients. Factor concentrates were administrated during all procedures, 5 received additional infusions for bleeding. At the time of writing, 3 patients had died, one being related to cancer. Haemophilia had influence the therapy choice in 13 out of 28 patients.
Summary/Conclusion: These findings underscore (i) the importance of an interdisciplinary management in order to critically weigh the risks and benefits of each therapy strategy and (ii) the improvement of specific guidelines for patients with haemophilia.

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Optimisation of the calibration curve for the accurate measurement of low FVIII levels with one-stage FVIII assays

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Background: The monitoring of factor VIII (FVIII) replacement therapy in haemophilia A patients relies on the accurate measurement of FVIII activity over a large concentration range. However, a significant overestimation of FVIII levels lower than 5 IU/dl with one-stage assays has been reported by recent studies with extended half-life recombinant FVIII (EHL rFVIII) (Bowyer et al., Haemophilia 2017; 23: e469-e470; Bulla et al. Haemophilia. 2017; 23: e335-e339). Previous studies have indicated that the reagents used to generate the calibration curves and to dilute the samples may determine the accuracy of the measurement of low FVIII levels (Cinotti et al. J Thromb Haemost 2006; 4: 828–33), although this method is not generally used on coagulation automates.
Aims: The objective of this study was to evaluate the impact of the reagents used to dilute the FVIII standard and the samples for the measurement of the levels of different types of FVIII using either a single dilution or multiple dilutions of the sample (parallelism method)**.**
Methods: We generated calibration curves by diluting a standard plasma pool with FVIII-deficient plasmas or with a commercial diluent buffer. Using these calibration curves, we measured the levels of FVIII in FVIII-deficient plasma spiked with plasma FVIII, a full length recombinant FVIII (FL rFVIII) and an EHL rFVIII. To evaluate the parallelism, the spiked samples were further prediluted two- and four-fold either in diluent buffer or in FVIII-deficient plasma. To limit the use of FVIII-deficient plasma in the parallelism experiments we also developed an automated method that dilutes the sample in 10% FVIII-deficient plasma on an ACL-TOP 500^{R}.
Results: The coagulation times of the FVIII calibration curves generated with diluent buffer were substantially longer than those with FVIII-deficient plasmas, which resulted in overestimation of FVIII levels lower than 50 IU/dl as well with plasma FVIII, FL rFVIII as with EHL rFVIII. The use of calibration curves generated with diluent buffer also resulted in the false detection of a significant FVIII activity (≥ 1 IU/dl) in FVIII-deficient plasma that had not been spiked with FVIII. Similarly, the parallelism tests carried out with samples prediluted in diluent buffer rather than in FVIII-deficient plasma led to unacceptable discordances between the FVIII levels measured at the different sample predilutions (CV of the FVIII levels measured with the different predilutions of the samples > 20%). By contrast, the automated method with predilution of the samples in diluent buffer supplemented with 10% FVIII-deficient plasma provided acceptable values (CV < 20%).
Summary/Conclusion: Our data confirm previous publications indicating that the generation of the calibration curve by dilution in FVIII-deficient plasma rather than in diluent buffer is crucial for the accurate measurement of low FVIII levels. For the determination of FVIII levels with the parallelism method, the predilution of the samples in FVIII-deficient plasma is also required. However, our automated assay using a dilution of the sample in diluent buffer supplemented with 10% FVIII-deficient plasma provides an accurate and cost-effective assay to measure FVIII levels with the parallelism method.

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Usefulness of anti-factor VIII IgG ELISA in acquired hemophilia A diagnosis and follow-up

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Background: Acquired hemophilia A (AHA) is a rare life-threatening bleeding disorder requiring a rapid diagnosis and management given the high risk of fatal bleeding event. Laboratory diagnosis is based on evidence of the association of FVIII deficiency and neutralizing anti-FVIII antibody usually detected by Bethesda assay (BA). However, BA suffers from high inter-laboratory variability, especially in low titer zone. An ELISA detecting anti-FVIII IgG has been recently commercialized and seems to present interesting performance in AHA diagnosis.
Aims: To assess the performance of a commercial ELISA detecting anti-factor VIII IgG in AHA patients at both diagnosis and during follow-up.
Methods: We retrospectively quantified anti-FVIII IgG antibodies in 8 patients with AHA followed in our center. ELISA (Zymutest®, Hyphen BioMed) was performed on citrate plasma samples frozen at the time of diagnosis and during follow-up (n=74). ELISA sensitivity was assessed on the samples from diagnosis and relapses (n=12). Its specificity was assessed on plasma samples from two populations: subjects with no coagulation disorder (n=18) and patients with an anti-phospholipid syndrome (n=18). All qualitative and quantitative results obtained with ELISA were compared with those obtained by BA. Anti-FVIII IgG ELISA is considered positive when OD>0.3. Anti-FVIII BA is positive over 0.6 BU.
Results: ELISA sensitivity was assessed at 91.7% (95%CI [76.0; 107]) because of 1 false negative result at relapse. Specificity was at 100% (95%CI [100; 100]) as no false positive was observed among the 36 AHA-free subjects. When comparing all the follow-up results obtained with both ELISA and BA, 12 results among 74 (17.6%) were discordant: 7 samples were negative with the BA while positive with the ELISA (ranging 0.306 to 0.654) and 6 samples were positive with the BA (ranging 0.7 to 1.2) but negative with the ELISA. Among these 12 discrepancies, only 3 (4.0%) ELISA results were inconsistent with patient AHA biological evolution. Anti-FVIII titers assessed by BA and ELISA were significantly correlated (p<0.0001, r=0.66).
Summary/Conclusion: The commercial anti-factor VIII IgG antibodies ELISA presents high sensitivity and specificity in AHA diagnosis and follow-up, and may be an alternative to BA for AHA biological management.

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Uncommon cause of the prolonged bleeding events – case report

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Background: Zymogen form of FXIII, that circulates in the plasma, is a pro-transglutaminase, giving a rise to cross-linked bonds of a previously formed fibrin clot after the activation by thrombin and ions of calcium. Therefore, it contributes to the stabilization of the clot and prevention of premature fibrinolysis. Acquired FXIII deficiency can be developed due to the formation of autoantibodies or its massive consumption. It is usually clinically manifested by bleeding of various intensity with the development of intramuscular, subcutaneous, intracerebral and other forms of life-threatening bleeding.
Aims: Description of an uncommon case of a patient with a rare acquired coagulation factor XIII (FXIII) deficiency in the context of the complex coagulopathy.
Methods: The authors present a case of a 86-year-old man, admitted firstly at the Department of Stomatology and Maxillofacial Surgery of the University Hospital in Martin for the prolonged bleeding after the extirpation of the cyst in the maxillar region. In laboratory parameters, there was the finding of deficiency of FXIII, mild plasminogen activator-inhibitor 1 (PAI-1) deficiency and coagulopathy with prolonged prothrombin time and hypofibrinogenaemia. During the last month, the patient reported the formation of the extensive haematoma spreading from the face to the thoracic region. The treatment with fresh frozen plasma, haemostyptic drugs and substitution of moderate anaemia and vitamin K deficiency enabled the stabilization of this clinical state.
Results: The treatment with the above-mentioned drugs was successful and the patient could be released home without the continuation of bleeding.
Summary/Conclusion: It is important to take FXIII deficiency into account when evaluating the differential diagnosis of the underlying cause of the bleeding episode. This case underlines the seriousness of the uncommon disorders of haemostasis.
Key words: factor XIII deficiency, bleeding, coagulopathy
Acknowledgement: We would like to thank the support of projects of the Agency for the Support of Research and Development (APVV) APVV-16-0020 and Scientific Grant Agency (Vega) 1/0168/16.
This study complies with the Declaration of Helsinki and informed consent ofthe patient was obtained.

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Successful management of delivery in rare bleeding disorder patients: An 11 year experience

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Background: Patients whom are influenced by rare bleeding disorders manifest a broad range of clinical symptoms which have tendency to become more complicated in affected women.
Two challenging issues in the management of women with rare bleeding disorders are Pregnancy and childbirth, which related information in the mentioned area is scarce hence we designed this long-term follow up study with pregnant women affected by factor deficiencies in southeast area of Iran.
Aims: The aim of this study is to determine the relationship of rare bleeding disorders and obstetric outcomes.
Methods: We carried out this prospective study on 75 women with rare bleeding disorders since 2006 to 2017.
The demographic features and clinical outcomes of affected cases were registered.
Results: A total of 75 pregnant patients with rare bleeding disorder who were followed at our clinic between 2006 and 2017 were retrospectively evaluated. Of the 75cases, 52 (69.3%) were Factor XIII deficiency, 3 (4%) were factor X deficiency, 3 (4%) were Glanzmann thrombasthenia 11 (14.6%) were Factor VII deficiency, 1 (1.3%) were Factor V and VIII combined deficiency, 4 (5.3%) were factor V deficiency, and 1 (1.3%) were afibrinogenemia.
All the 52 patients had successful delivery which none of them experienced antepartum or postpartum hemorrhage, all were on prophylaxis.
The antigenicity activity in all patients was less than 2 % and the detected mutation was Trp187Arg respectively.
The median of age at the last pregnancy was 23 years.
There were 3 births in patients with factor X deficiency all of them were prepared with the administration of Prothrombin complex concentrates prophylaxis additionally serum factor level in all were less than 1 %.
Three births in Glanzmann thrombasthenia patients were detected which all were prepared with the transfusion of single donor platelets.
All of our Factor VII deficiency patients were in mild category which median of serum level of factor VII 29 % , none of them received any treatment without any postpartum hemorrhage .
All of our Factor V and VIII combined deficiency, and factor V deficiency
Had successful delivery without PPH with appropriate prophylaxis
We had 1 afibrinogenemia patient 28 year old with history of multiple abrupt abortions which were treated with administration of fibrinogen concentrate which the last pregnant terminated successfully.
Summary/Conclusion: Antenatal and peripartum Care of obstetric patients with rare bleeding disorder should be given in the context of a multidisciplinary team setting with obstetricians and hematologists ideally in a tertiary hospital.
Noteworthy to mention, due to lack of reliable information on clinical management of rare bleeding disorders, designing large cohorts could provide evidence-based guideline about treatment.

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Emicizumab transforms lifestyle of patients with inhibitor: a qualitative research in haemophilia A

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Background: Emicizumab is a monoclonal antibody developed for the treatment of patients with haemophilia A (HA) which demonstrated significant efficacy in patients with inhibitor. Alongside the clinical development program, qualitative data have been collected from patients and healthcare professionals (HCPs).
Aims: The objective of this research was to assess the impact of emicizumab treatment in the daily lifestyle of patients with HA with inhibitor as reported by: the patients themselves in phase 1 study; and by HCPs involved in phase 3 studies (HAVEN 1 and 2).
Methods: Japanese patients from the emicizumab phase 1 study (n=11 patients with inhibitor) answered a series of open-ended questions about their daily life while treated with emicizumab. The English translation of their written answers was analysed. Six HCPs who had managed or been in contact with French patients with inhibitors treated with emicizumab as part of the HAVEN 1 and 2 phase 3 studies were interviewed by phone by a researcher experienced in patient-reported outcome sciences. Specific interview guides were used and aimed at collecting information about the impact of emicizumab treatment in the daily life of the patients. The interviews were exploratory and semi-structured, and lasted 45 minutes to 1 hour. They were recorded and transcribed word for word. A thematic qualitative analysis on the interview transcripts and written answers of the phase 1 study was conducted. Then, a conceptual model was developed to illustrate the experience of patients with HA treated with emicizumab as reported by both the patients themselves and the HCPs.
Results: Japanese patients (n=11) and French HCPs (n=3 physicians, 2 nurses and 1 CRA) reported "spectacular" changes in patients' daily lifestyle while they were switched to emicizumab. Patients reported changes that they qualified as significant in the following areas: decrease in bleeding frequency, severity and associated joint and muscle pain. This translated in various benefits from improved emotional state (e.g., less anxiety, more confidence) to increased daily activities (e.g., grocery shopping, sports, walking longer distances) resulting in better patients' autonomy and for some patients a major change in their daily lifestyle. Patients were able to travel further away from home thanks to portability of the treatment due to ease of use of subcutaneous injection combined with the low frequency of injections. HCPs reported that patients were feeling more confident in the future and were able to make plans again. They also reported that the treatment was so effective and easy to use that some patients were not feeling ill anymore.
Summary/Conclusion: Emicizumab significantly changed the way patients with Inhibitor handled HA, and resulted in a transformation to their lifestyle. This may also have an impact on organization in HCPs' health unit and medical practices. The level of detailed changes in patients' quality of life that we reported in this study may be difficult to capture with currently available generic and disease-specific instruments. Thus, new specific instruments could be developed to comprehensively capture in HA the transformation in patients' lives following newly developed treatments, including emicizumab.

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Palliative management of a giant iliopsoas haemophiliac pseudotumour under emicizumab prophylaxis

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Background: Iliopsoas haemophilic pseudotumour (HPT) is a rare but life-threatening complication unique to haemophilia. Curative management relies on complete surgical removal of HPT to prevent a fatal outcome secondary to fistulization, surinfection and/or uncontrolled bleeding. For patients ineligible to surgery, arterial embolization was reported to limit iliopsoas HPT evolution. We report the case of a severe haemophilia A patient with high responding inhibitor who underwent compassionate use of emicizumab prophylaxis for a giant iliopsoas HPT.
Aims: To assess the efficacy and safety of emicizumab prophylaxis in the management of a massive iliopsoas HPT inelegible to surgical removal and refractory to arterial embolization
Methods: A 59-year-old male with severe haemophilia A and high responding inhibitor (Historical titer = 1500 BU) was diagnosed with a massive iliopsoas HPT on a CT-Scan performed for inflammatory lombalgia. This patient was denied for surgery given a past medical history marked by several life-threatening bleeding episodes despite intensive clotting factor replacement with by-passing agents. An arterial embolization of HPT was performed but failed to control HPT growth. In this context of exhaustion of all treatment options in a patient with poor venous access, compassionate use of emicizumab prophylaxis was made available through request to Roche for compassionate use.
Results: Cutaneous fistulization of HPT occurred 6 days after the first loading dose (3 mg/kg weekly) of emicizumab. Under emicizumab maintenance therapy (1.5 mg/kg weekly), the clinical course was progressively hampered by chronic bleeding from cutaneous fistula requiring regular blood transfusions and confining patient to chair or bed most 50% of the day. No other bleeding events were observed under emicizumab prophylaxis. No thrombotic and microangiopathic events were observed in this patient including after a short course of recombinant factor VIIa (3 consecutive doses of 70 microgrammes every 3 hours). There was no indirect evidence for neutralizing antidrug antibodies. Six months after HPT fistulization, transfusion support and emicizumab therapy were withdrawn on patient's request. Death from severe anemia occurred two weeks later in the context of continuous bleeding from HPT cutaneous fistula.
Summary/Conclusion: A six-months survival was observed despite a late-onset of emicizumab therapy concomitantly of HPT cutaneous fistulization. Death was unrelated to emicizumab therapy which was withdrawn on patient's request two weeks before fatal outcome.

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Does a variation of +/- 2°C around 37°C in the incubation temperature influence the measurement of FVIII inhibitor titres?

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Background: Factor VIII (FVIII) inhibitors are antibodies directed against FVIII. They neutralize the coagulant activity according to a kinetic which is function of temperature and time. Validated FVIII inhibitor assays are based on a method measuring the decrease in FVIII activity after 2 h incubation at 37 °C in a mixture of normal pooled plasma (NPP) with the patient plasma suspected of containing inhibitor.
Aims: The aim of the study was to determine the impact of a variation of +/- 2°C of this incubation temperature to allow or not this variation in the validation of the water baths used in our laboratory. This work takes part of the metrological monitoring of the material according to ISO 15189 standards.
Methods: Thirteen known samples were selected based on titres, ranging from <0.4 UB to 80 UB / mL and studied. Patients were known with constitutive (n=11) or acquired (n=2) hemophilia A. Titration of FVIII antibodies was done according to the Bethesda method, by the same person, two series per temperature. Plasmas with FVIII level > 5% were preheated for 30 minutes at 58°C. The NPP used was homemade with healthy donors. The incubation was carried out in the same water bath, three temperatures were tested: 35, 37 and 39°C. All samples were tested at least for 2 dilutions (except a negative one). Residual factor VIII was determined on a STA R Max 2® (Diagnostica Stago, Asnières France; TriniCLOT™ Automated aPTT, T Coag®, Asnières, France; Coagulation FVIII Deficient Plasma, Siemens®, Marbourg, Germany). Titres were determined using the residual FVIII assay closest to 50%.
Results: All our controls mixtures showed a residual FVIII level between 46 and 51%. According to the measurement of uncertainty values and the reproducibility standard deviation these results are not significantly different.
Titres results ranged from <0.4 to 84.6 UB/mL. The Bland-Altman analysis of patients' inhibitor titres showed a good agreement between results at 37°C compared with respectively results at 35°C and at 39°C. No systematic bias was highlighted.
Whatever the temperature studied, the clinical care management based on the titres results would not have been different.
Summary/Conclusion: In conclusion, our study did not show any impact of a +/- 2 ° C variation around the recommended temperature of 37°C for the incubation step for FVIII inhibitors assay. These results allow us to accept such a variation.
However, further studies are required for validating these observations in a larger cohort of patients with FVIII inhibitors with variables titres.
In any case, for a good follow-up of patients, application of similar analytical conditions is recommended.

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Association of acquired hemophilia A and IgG4-related disease: case report and pathophysiological study

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Background: We report the observation of a 75 year-old patient who was referred to our tertiary care center for cervical lymphadenopathies. A pre-lymphadenectomy systematic blood work revealed an asymptomatic elevated aPTT with low factor VIII titers and high levels of anti-VIII antibodies, consistent with acquired hemophilia A (AHA). Histological work-up of a cervical lymphadenopathy revealed benign follicular hyperplasia with IgG4-positive plasmocyte infiltration; and serum IgG4 levels were markedly elevated, compatible with IgG4-related disease (IgG4-RD). He was thus diagnosed with IgG4-RD-associated AHA and successfully treated with a 9-month course of prednisone. Nine months after corticosteroid cessation, a relapse occurred, for which he was started on rituximab.
Aims: Building on this observation, we wanted to study whether there was a potential pathophysiological link between the two diseases by determining the isotype of the anti-VIII autoantibodies.
Methods: Using a modified ELISA assay approach, circulating levels of anti-factor VIII IgG4 and IgG1 antibodies (the most frequent isotypes of factor VIII inhibitors) were measured both in the serum of our patient and in a control group of 7 patients diagnosed with AHA but without known IgG4-RD.
Results: Factor VIII inhibitor was mostly of the IgG4 isotype, with an anti-VIII IgG4/IgG1 ratio of 42 at diagnosis and 268 at relapse in our patient with IgG4-RD-associated AHA. This ratio seemed lower in the control group (median [range]: 8.1[0.4;56]); however, controls #1 and #2 had elevated anti-VIII IgG4/IgG1 ratios in similar range than our patient at diagnosis (56 and 41, respectively). None of the controls had an anti-VIII IgG4/IgG1 ratio close to our patient's relapse value. Overall, these results suggest a preferential production of anti-VIII antibodies of the IgG4 subclass in our patient, although it may not be specific of IgG4-RD-associated AHA.
Anti-VIII IgG4 antibodies represented only a small proportion of our patient total serum IgG4 antibodies, with a ratio of $2.7x10^{-3}$ at diagnosis and $1.0\ x10^{-3}$ at relapse. These values seemed higher in the control group (median [range]: $4.6\ x10^{-2}$ [$4.4\ x10^{-3}$;$1.79\ x10^{-1}$]). This suggests that the production of anti-VIII IgG4 antibodies does not account for the increased serum IgG4 levels observed in our patient, comforting the polyspecific nature of the plasmocyte proliferation in IgG4-RD.
Summary/Conclusion: We report here a case of AHA associated with IgG4-RD, an unusual combination of two rare immunological diseases, and showed that anti-factor VIII antibodies were predominantly of the IgG4 isotype, especially at relapse, which may suggest a pathogenic link between the 2 conditions.

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Genetic analysis in Slovak family with inherited factor XIII deficiency

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Background: Inherited coagulation factor XIII deficiency is rare autosomal recessive bleeding disorder. Based on genotype, the most common is FXIII-A subunit deficiency, caused by mutations in the F13A1 gene. FXIII-B subunit deficiency is caused by mutations in the F13B gene. Homozygous or compound heterozygous patients experience severe lifelong bleeding, characteristically from the umbilical cord and intracranial bleeding. Also delayed wound healing have been reported.
Aims: Detection of genetic abnormalities in coagulation factor FXIII gene in patients with inherited factor FXIII deficiency.
Methods: Sequence analysis *F13A1* and *F13B* genes in patients with laboratory proven factor XIII deficiency using direct Sanger sequencing.
Results: We identified 2 mutations, where each parent is carrying one heterozygous mutation in coding part of F13A1 gene and affected children are compound heterozygous for these mutations. We also identified several single nucleotide polymorphisms.
Summary/Conclusion: The identified mutations are causally involved in mechanisms causing FXIII deficiency. Some of identified polymorphisms are associated with lower plasma levels of factor XIII.

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Can von Willebrand antigen level be able to predict the clinical outcome in patients with severe Hemophilia A at the initial presentation?

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Background: There is a large heterogeneity in bleeding events and arthropaty among patients with severe Hemophilia A which is multifactorial. Mostly FVIII gene mutations seems to influence phenotype however underlying prothrombotic risk factors and FVIII half life may both have an affect on the heterogeneity. Endogenous VWF is the specific carrier of factor VIII in plasma and protecs it from proteolytic degradation,prolonging its half life in circulation and efficiently localizing it at the site of vasculary injuiry.
Aims: Here, we want to evaluate von Willebrand antigen levels of the children with severe Hemophilia A at the initial diagnose and want to figure out whether low initial von Willebrand antigen (VWF:Ag) levels may asssociated with a frequent bleeding phenotype or not, retrospectively.
Methods: We retrospectively evaluate charts of 40 children with severe hemophilia A who was diagnosed in between 1997 and 2017 at the Hacettepe University, Department of Pediatric Hematology. All of these patients were receiving prophylaxis with recombinant products and among those 40 patients only 6 had a history of inhibitor in which 2 of them still persist
Results: Mean VWF:Ag levels were found to be 114±40(32-200)IU/dL. Ten out of 40 children VWF:Ag levels was found to be under 100IU/dL and from those 5 out of ten were found to be under 80IU/dL. Interestingly none of them were have the blood group 'O'. Initial bleeding age were found to be lower (median 6 months)in patients with low VWF:Ag(<100IU/dL) than that of the patients with high VWF:Ag (≥100IU/dL)(median 18 months)(p=0.05). 2/10 had a history of inhibitor (1 low titer 1 high titer) development and 7/10 had a target joint which requires radiosyneviectomy during the follow up period.On the other hand 4 out of 30 patients with VWF:Ag>100IU/dL had a history of inhibitor development (4 had high titre inhibitor : 2 was still found to be positive) and 5/30 had a target joint .
Summary/Conclusion: Despite we do not have the half lifes of FVIII, a significant shorter half life in patients with a low VWF:Ag level was previosly reported. Although there is no follow up of VWF:Ag levels in this small group of patients with a frequent bleeders initial VWF:Ag levels may give some clues about the clinical outcome.

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Acquired type 3 von Willebrand syndrome associated with splenic marginal zone lymphoma: a case report

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Background: Acquired von Willebrand syndrome (AVWS) is a rare bleeding disorder that occurs mainly in elderly patients with no history of bleeding. The diagnosis is often difficult given the variability of the underlying causes and the lack of a specific laboratory test. Lymphoproliferative disorders are the most common causes of AVWS, especially monoclonal gammopathy of undetermined significance (MGUS) accounting for almost 1/3 of all cases.
Aims: To demonstrate a rare case of AVWS associated with IgG kappa monoclonal gammopathy that turned out to be a rare presentation of splenic marginal zone lymphoma.
Methods: We carried out an in-depth investigation of the patient's medical records and conducted a thorough literature review.
Results: A 67-year-old female with a lack of personal or family history of bleeding was prescribed aspirin with no strong indication. Later on several large suffusions appeared on her body. The dose of aspirin was lowered then ceased but more suffusions and haemorrhoidal bleeding appeared. Prolonged activated partial thromboplastin time (APTT) was noted on the initial laboratory evaluation. Von Willebrand factor antigen and activity (VWF:Ag and VWF:GPIbm) were undetectable and acquired type 3 von Willebrand syndrome (AVWS) was diagnosed. PFA-100 was prolonged and factor VIII activity (FVIII:C) was 8%. Further investigation showed a normal VWF propeptid level but VWF-binding antibodies could not be detected. In search of the underlying disease serum protein electrophoresis and immunofixation showed IgG kappa monoclonal gammopathy. Additional extended laboratory findings were normal. Radiological investigation showed no signs of lytic bone lesions, enlarged spleen or lymph nodes, or any other alterations. Echocardiography revealed a mild mitral and aortic valve calcification without significant stenosis. Given the IgG kappa monoclonal gammopathy and the clinically significant symptoms, treatment with off-label intravenous immunoglobulin (IVIG) was decided in a dose of 1 g/kg body weight for two consecutive days. No adverse event occurred during IVIG treatment. One day later, the coagulation parameters were corrected completely and remained normal for 3 weeks. Bone marrow biopsy was carried out with normalized coagulation profile and it revealed the final diagnosis of splenic marginal zone lymphoma. It infiltrated only the bone marrow since PET/CT showed no pathological FDG uptake. The combination chemotherapy of bendamustine and rituximab was initiated that resulted in complete remission confirmed by bone marrow biopsy after 3 cycles.
Summary/Conclusion: Here we present an extremely rare case of AVWS associated with splenic marginal zone lymphoma. Clinical suspicion followed by thorough patient history and examination is crucial to distinguish AVWS from inherited von Willebrand disease since the autoantibody may not be detected. The variability of the underlying conditions makes the diagnosis more challanging while it is essential as the treatment should be aimed at the causative disease.

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Prenatal diagnosis of hemophilia a –five years experience

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Background: Identification of molecular basis in hemophilia A (HA) is very important for improving prenatal and postnatal care of patients and their families. Only after the detection of mutation in F8 gen (*F8*) in probants, it is possible to achieve adequate genetic counsel for family members, to determine eventual heterozygous carriers and perform prenatal diagnosis. Due to guidelines, analyses for heterozygous status are recommended only for females over 18 years old. Prenatal diagnosis is allowed just in mail fetuses.
Aims: Aim of this study was to analyze and present the molecular basis and genetic treatment in 12 families with HA.
Methods: After the detection of recurrent HA mutation in 12 severe HA patients, from 12 families, carrier status was defined in 16 female family members (mothers and sisters). According to that, from Jan 2013 to Dec 2017, after genetic counseling, prenatal diagnosis of HA was performed in 13 fetuses.
DNA samples were isolated from peripheral blood, chorionic villi tissue, amniotic fluid and cord blood. Analyses were obtained using inverse shifting PCR (IS-PCR) for presence of intron 22, *F8* inversions (inv22) and MLPA for detection of deletion and duplication of *F8* exones. The fetus's gender determination was performed using PCR method for sex detection.
Results: In our cohort of 12 HA patients from 12 families, inv22 was detected in 11/12 of them (inv22 type1 in 8/11, and inv22 type 2 in 3/11). After detection in probands, respective inv-22 was detected in all of their mothers and 4 sisters. That allowed the genetic counseling and performing 12 prenatal diagnoses. Primary analyses of fetal sex determination showed the female gender in 7/12 (59%) fetuses and male gender in 5/12 (41%) fetuses. Inv22 genotype was detected in 3/5 (60%) male fetuses; inv22 type 1 in 2/5 (40%) and inv22 type 2 in 1/5 (20%) cases. The genotype of 2/5 (40%) males were normal. In 1/12 HA patient and analyzed family, we found the large deletion of exones 1-22 of *F8* gene. Fetus of pregnant woman from this family, who carried the deletions, was female.
Summary/Conclusion: This study improve importance of molecular-genetic investigations in HA patients and converge IS-PCR and MLPA as method of choice at first line, for postnatal and prenatal detection of recurrent HA mutations.

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A case of severe acquired factor X deficiency: diagnosis approach and clinical management of a rare bleeding disorder

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Background: We report the case of a 76-year-old-man, with hypertension as unique medical history treated by calcic inhibitor. He had no surgical antecedent or no personal or familial bleeding history. He developed an epistaxis and went to emergency care unit because of persistence of the bleeding for hours and its resistance to haemostatic swab for nosebleeds.
Initial blood tests revealed a mild anaemia (11,6g/dL), normal platelet count and hyperleukocytosis (16,6G/L) related to a biological inflammatory syndrome. Renal function and hepatic enzymes were normal at diagnosis. Haemostasis tests revealed a significant reduction of prothrombin time (PT) (PT ratio 3.49; (Stago)) and a prolongation of activated partial prothrombin time (aPTT ratio 2.44; (Stago)). Further tests revealed an isolated factor X deficiency (3%) with normal or high levels of factors (F) II, V, VII, VIII, IX, XI and fibrinogen or von Willebrand factor. Disseminated intravascular coagulation markers and lupus anticoagulant were negative.
Aims: Congenital deficiency seems unlikely for this old man without bleeding history. The acquired FX deficiency is rare and mainly associated to amyloidosis since 8.7 to 14% of the patient with systemic AL amyloidosis can developed it (Thompson et al. 2010). Our patient had normal calcemia, protidemia, normal electrophoresis, absence of light chain and salivary gland biopsy was also normal. Finally, isolated FX deficiency can be associated to auto-antibody (Ab).
Methods: Bethesda assay was first used to identify anti-FX, but as it was negative, further experiments were made to try to identify antibodies and to explain physiopathology. Factor X antigen, anti-factor antibodies were measured with ELISA assay. To measure pentraxin-2 (PTX2) a capillary method was performed.
Results: The clinical patient's evolution was rapidly unfavorable with an aggravation of the bleeding syndrom leading to haemorrhagic shock with multiple organ failure and death, 3 days after admission. Bleeding was refractory to different procoagulant treatments. The patient received vitamin K, plasma transfusion, four-factor prothrombin complex concentrate and tranexamic acid. Factor X did not increase during this replacement therapy, and by analogy with acquired haemophilia and the hypothesis of an auto-Ab against FX, the patient received recombinant activated FVII (Novoseven® 90µg/kg every two hours) and then activated prothrombin complex concentrate (Feiba® 80UI/kg 3 times per day) without any clinical efficacy. Further experiments made *post-mortem* identified an Ab (IgG) directed against FX which cross-reacted with other vitamin K (VK) dependent coagulation proteins as FII, FVII, FIX, protein S and Z or non-coagulant protein as gas-6 but not with FVIII or vWF. Factor X antigen level was concordant with FX activity at diagnosis and increased during by-passing therapy with Feiba® whereas pentraxin-2 remains within normal range at diagnosis.
Summary/Conclusion: We report a case of a rare bleeding disorder with FX deficiency and dramatic evolution. Amyloidosis is unlikely, and clinical evolution associated with the detection of Ab against FX and others VK dependent proteins are consistent with the hypothesis of acquired FX Ab which is actually poorly reported in the literature (Lee et al. 2012). However, the specificity of the Ab remains unclear; it could be the GLA domain of vitamin K protein and it could impair FX/PTX2 interaction. Additional experiments are ongoing to clarify the mechanism of this acquired FX deficiency.

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Impact of extended half-life recombinant factor VIII Fc in consumption of FVIII in Haemophilia patients A with prophylaxis regimen

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Background: Prophylactic treatment of hemophilia A involves the regular intravenous infusions of clotting factor VIII (FVIII) concentrates to reduce the bleeding risk and to prevent joint damage. The recent development of the Extended Half-Life (EHL) recombinant FVIII (rFVIII) allows the reduction of injections frequency and improves the bleeding prevention. In October 2016, efmoroctocog alfa, a recombinant FVIII Fc fusion protein (rFVIIIFc), was the first EHL rFVIII commercialized in France. Therefore, we have been interested by consumption data in real-world use in our Haemophilia Care Center after 20 months of the rFVIIIFc's availability.
Aims: To evaluate the real-world changes in consumption data pre and post-initiation of rFVIIIFc in Patients With Haemophilia A (PWH A), treated on prophylaxis.
Methods: A retrospective analysis of factor consumption pre- and post-switch of FVIIIFc of PWH A was carried out from the Center database. Only patients with a prophylactic regimen are included. Additional doses dispensed in emergency haven't been taken in account. A collection of factor consumption data was realized for every patient. The impact of rFVIIIFc's cost was also evaluated. The cost has been calculated using the factor prices fixed by the Economic Committee on Health Care Products. It was approved at the same price as Standard Half-Life (SHL) FVIII (0,648€/International Units(IU)).
Results: In our Haemophilia Care Center, 91 PWH A on prophylaxis are followed. Among these 91 patients, 31 (34%), aged 5 to 67 years, were switched from Standard Half-Life (SHL) FVIII to EHL rFVIIIFc. Patients had received rFVIIIFc for a median of 12 months (range 1-18 months). Among the 31 patients who switched, 28 (90%) had severe haemophilia A and 3 (10%) had moderate haemophilia. Using rFVIIIFc allowed to decrease infusions number (10-4 injections/month) by 33%. And weekly factor consumption after switching to rFVIIIFc was reduced with 15 (48%) patients, increased with 12 (39%), and with 4 (13%) patients there were no difference. Overall weekly factor consumption decreased by 26 625 IU (16%). Considering average cost per IU of FVIII, weekly spending was reduced by 17 253€.
Due to good clinical outcomes and satisfactory rFVIIIFc PK assessments, intervals between injections were increased with 4 patients (13%).
Summary/Conclusion: This preliminary data, based exclusively on consumption, demonstrates that switching to rFVIIIFc has allowed to reduce infusion frequency and quantity injected, resulting a lower global consumption and lower expenses. Further data collection in Auvergne Rhône-Alpes region and other parameters analysis (patient satisfaction, adherence) could provide more information on the real impact of this long-acting rFVIII.

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Preliminary study aiming at collecting the opinion of patients and their caregivers about the development of an electronic diary for rare bleeding disorders in France

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Background: Communication between patients and haemophilia care centres is important in the management of the disease. Nevertheless, with the emphasis of home-based treatment, it has become difficult for physicians to monitor bleeding episodes and adherence to treatment. To help them, patients have to record, in a diary, all their bleedings and their injections of clotting factor concentrates. This registration is also essential to ensure patients' safety and maintain medicines' quality thanks to the batch traceability. Currently, the adherence to record keeping on paper diaries is problematic for the teenagers and the adults. The scientific literature describes some advantages for the patients using an electronic diary such as a superior adherence, a better data quality and a greater accuracy. Furthermore, several countries have already developed and implemented their own tool.
Aims: The aim of this study is to assess the interest of the development of an electronic diary to monitor patients with bleeding disorders in France.
Methods: This is a prospective study started in May 2018 and still ongoing. An anonymous self-administered questionnaire for the patients suffering from bleeding disorders and their family caregivers was established. The respondents used a Likert scale with five levels to give their opinion. We have questioned our cohort on different topics as the use of paper diary at home and during consultations in haemophilia care centres, as well as, their view and expectations for the development of an electronic diary. We're going to focus on this last part.
Results: 37 questionnaires have been distributed, 22 were picked up whose 20 included in the study according to the inclusion criteria. Out of twenty participants, 13 are patients, 5 are parents and 2 are spouses. Our cohort is comprised of various bleeding disorders whose Haemophilia A (75%), Haemophilia B (10%), Von Willebrand disease (10%) and Glanzmann's thrombasthenia (5%). 80% of the participants own a smartphone or a computer and 60% a tablet computer. Only 5% don't have any of these devices. 85% of participants define themselves as comfortable with new technology without any age distinction. Among them, 60% think they will use the electronic diary in preference to the paper version. Concerning the record keeping, 80% think the electronic diary could be improved the quality of data registered and 50% believe it could reduce the number of oversight. In addition, 85% think the data recording could be simplified by means of an electronic diary.
Summary/Conclusion: In our cohort, the mainly part of the participants have a positive opinion about electronic diary. They see the interest and the advantages of this tool comparatively to the paper version. It seems to haven't limit to access, indeed, only 5% don't own any connected devices. We can't note any distinction related to the age of participants. In a short-term, we would like to continue this study by expanding our cohort. In a second time, it would be interesting to interrogate the medical teams in haemophilia care centres. It would be the way to have their opinion about the use of electronic diary in the center and how they imagine the changes in the management of the diseases.

Clotting
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Thrombinography and fibrinography in geriatric patients over 80 years with atrial fibrillation receiving rivaroxaban, apixaban, or dabigatran

Geoffrey Foulon*

Background: Very elderly patients with atrial fibrillation (AF) are commonly frail: they have substantial comorbid conditions and are often polymedicated. The management of such patients regarding the use of anticoagulants is challenging. No study has specifically investigated direct oral anticoagulants (DOAC) pharmacokinetics (PK)/ pharmacodynamics (PD) including thrombin generation (TG) in elderly patients who are both at high hemorrhagic and thrombotic risk.
Aims: We sought to determine i/ the PK/PD profiles of DOACs in frail elderly patients assessed by thrombin generation (TG)/fibrinography as a function of DOAC concentration; ii/ the safety profile at six months.
Methods: ADAGE (NCT02464488) is an on going prospective, observational, academic, multicenter cohort study. We recruited patients aged 80 years or older, receiving DOACs for non-valvular AF since at least 4 days. Each patient had one to five samples collected at different time-points after DOAC intake at different days over a 20-day period. Plasma rivaroxaban / apixaban anti-Xa activity concentrations (ng/mL) were measured with a chromogenic assay (Liquid-anti-Xa®, Stago, France), and dabigatran concentration with a clotting assay (Hemoclot DTI®, Hyphen, France), using dedicated calibrators and controls. TG and fibrinography were measured using the innovative Thrombodynamics® system (HemaCore® - Russia). Non-anticoagulated young healthy volunteers and elderly patients were analyzed in parallel. Hemorrhagic and thrombotic events were collected at six months by phone.
Results: To date, 174 patients (mean age 87 ± 4 years, mean total CIRS-G comorbidity score 10.6 ± 4.7, mean creatinine clearance-Cockcroft 49.9 ± 16.4 mL/min) have been included in the cohort. A total of 335 plasma samples have been analyzed, 154 from 70 patients receiving rivaroxaban, 150 from 87 patients receiving apixaban mainly, 30 from 17 patients receiving dabigatran. Regarding anti-Xa activity in patients receiving rivaroxaban or apixaban, median peak (at 1-4h) and trough levels (percentiles p10-p90) were 237 ng/mL (110-400) and 43 ng/mL (<15-87) (n=62, rivaroxaban 15 mg/day); 152 (72-300) and 73 (39-148) (n=66, apixaban 2.5 mg x2/day), respectively. These values were comparable with PK data available in younger patients, except for peak level upper values which were higher. Regarding TG, all parameters were significantly associated with DOAC plasma concentrations: lag time, time to peak, peak height ($p< 10^{-4}$) and endogenous thrombin potential ($p< 10^{-3}$). Regarding fibrinography, the lag-time was the only parameter showing a significant association with DOAC concentration ($p< 10^{-4}$). Of the 116 patients for whom clinical follow-up had been completed at 6 months, 14 (12,1%) experienced bleeding whereas 3 (2.6%) experienced a thrombotic event.
Summary/Conclusion: This is the first study providing pharmacodynamic data associating DOAC plasma concentrations, thrombinography and fibrinography in polypathological and polymedicated elderly patients receiving DOACs. Overall, 13.7% of patients experienced haemorragic and/or thrombotic events, highlighting the frailty of such patients.

Clotting
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Clinical evaluation and implementation of a second generation assay in immuno-turbidimetric method for di-dimer testing in Cameroon

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Background: Improving the management of patients admitted for suspicion of venous thromboembolism remains a challenge in sub-Saharan Africa. In Cameroon, the prevalence of venous thromboembolism is reported to be around 1.6% in 2015**.** Their diagnostic aid goes partly through the D-dimer assay, although it is not purely specific to Deep Vein Thrombosis or Disseminated Intravascular Coagulation, D-Dimers belong to the ISTH defined score in 2001 for the diagnosis and prognosis of venous thromboembolism. The adoption of a D-dimer measurement method must be in phase with criteria of high sensitivity, reasonable specificity and especially of relatively short execution time adapted to the emergency.
Aims: In this work, we proposed to evaluate the performances of an immuno-turbidimetric method (Liatest D-DI Plus, STAGO) in relation to an Enzyme Linked Immunofluorescent Assay (ELFA) method (D-dimer Exclusion II, VIDAS) in assay D-dimer routine at the Pasteur Center of Cameroon.
Methods: A consecutive recruitment of 51 samples was performed by venipuncture on Vacutainer® citrate tube 3.2% or 0.109M. This cross-sectional prospective study was carried out on individuals coming to the Center Pasteur of Cameroon for D-dimer examinations. After centrifugation at 2500G for 10 minutes, the samples were simultaneously tested by ELFA method using monoclonal antibodies (P10B5E12C9; P2C5A10) with fluorescence detection on Vidas® Biomerieux instrument and by immuno-turbidimetric of latex microparticle suspension coated with monoclonal antibodies of human anti d-dimer mice (8D2;2.1.1.6) on STA satellite® Stago according to the manufacturer's recommendations. The sensitivity thresholds being respectively 0.045 and 0.27µg/ml FEU and a threshold value set on both sides at 0.50 µg/ml FEU, allowed us to discriminate the results in "negative" (<0.50µg/ml FEU) on the one hand and "positive" (>0.50µg/ml FEU) on the other hand. The samples showing different results from one method to another compared to the threshold of 0.50µg/ml FEU were further investigated.
Results: 32/56 (57.14%) positive samples and 17/56 (30.35%) negative were concordant between Vidas® and Liatest®; corresponding to a satisfactory KAPPA matching coefficient of 0.72. 4/51 (7.84%) samples were positive in Vidas and negative in Liatest against 3/51 (5.88%) negative samples in Vidas and positive in Liatest. A statistically significant linear correlation (R^2=0.9649, p<0.0001) is obtained between vidas and Liatest. This correlation is justified in particular by relatively close average values (Vidas: 1,779±2,244µg/ml) and (Liatest: 1,761±2,296µg/ml). The analysis of the Blant-Altman graph allowed us to obtain a mean difference of d=0.02≈0.00 (sdd=0.43) thus showing that all the values obtained in Liatest are replicable on those obtained in Vidas, which means that thus confirms the measurement concordance between the two methods. The partial analysis of the discordant results shows a value average around the cut-off (Vidas: 0.547, liatest: 0.737) with measurement ranges much higher in Liatest (0.310-1.930µg/ml) than in vidas (0.361-0.700µg/ml).
Summary/Conclusion: this study show a very satisfactory performance of the new Liatest® D-Di Plus immunoturbidimetric test as compared to the reference method. In addition, Liatest as the ability to perform each assay in duplicate and this, in a relatively shorter time (<10min) than that of Vidas, this caracteristic gives it a real advantage in its adaptation to the urgency of performing the D-dimer test in routine.

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Non-recommended dosing of direct oral anticoagulants in the treatment of acute pulmonary embolism is related to an increased rate of adverse events

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Background: Dose adjustment of direct oral anticoagulants (DOACs) is not required in the setting of acute PE treatment according to the manufacturer's labelling, beyond the contraindication in severe renal insufficiency.
Aims: We designed a prospective, multicenter cohort study to investigate the associated risk off of non-recommended DOAC doses prescription on 6-month adverse events.
Methods: The primary endpoint was a composite of all-cause death, recurrent VTE, major bleeding, and chronic thromboembolic pulmonary hypertension (CTEPH).
Methods: The primary endpoint was a composite of all-cause death, recurrent VTE, major bleeding, and chronic thromboembolic pulmonary hypertension (CTEPH).
Results: In total, among 656 patients discharge with DOACs between 09/2012 and 10/2016, 28 (4.3%) were not treated with a recommended DOAC dose. All the non-recommended DOAC dose prescriptions were under-dosed according to the drug labelling. After multivariate adjustment, age >70 years, a history of coronary artery disease, a CrCl <50mL/min and concomitant aspirin therapy were independent factors associated with non-recommended DOAC dose prescription (C-statistic: 0.82; Hosmer Lemeshow test: 0.50). The primary composite endpoint occurred in 7/28 patients (25.0%) in the non-recommended dose group and in 38/628 patients (6.1%) in the recommended dose group, yielding a relative risk of 3.19 in the non-recommended dose group (95%CI: 1.16-8.70; p<0.001). The higher primary endpoint rate observed in the non-recommended dose group was driven by a significantly higher rate of major bleeding (7.1% vs. 1.4%; p=0.008), with a non-significant trend toward a higher rate of death (7.1% vs. 2.2%; p=0.23), recurrent VTE (3.6% vs. 1.4%; p= 0.31), and CTEPH (7.1% vs. 1.6%; p=0.32).
Summary/Conclusion: Empiric dose reduction of DOACs was associated with 6-month adverse events in our real-life registry.

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Prescription Patterns of Direct Oral Anticoagulants in Pulmonary Embolism: A prospective multicenter multidisciplinary French registry

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Background: Data regarding the use of direct oral anticoagulants (DOACs) for the treatment of acute pulmonary embolism (PE) in clinical practice are sparse.
Aims: We conducted a prospective multicentre multidisciplinary registry study to describe patterns of DOAC prescription for the treatment of acute PE and the associated risk of 6-month adverse events in daily practice.
Methods: We included all patients discharged with an objectively confirmed diagnosis of acute PE in 11 centres. We recorded clinical data at discharge and 6-months outcomes.
Results: Between 09/2012 and 04/2017, 1,084 patients were included: 60.5% (n=656) were treated with DOACs and 39.5% (n=428) with another or no anticoagulant. The prescription rate of DOACs increased sharply just after their release in the market to reach a plateau over time, between 56% and 72% of the total prescription per year in PE patients (p for trend = 0.33). Active malignancy and renal function impairment were factors independently associated with non-prescription of DOACs. Overall, prescription of DOACs was appropriate in 95.3% of patients. The rate of use of non-recommended DOAC doses was 4.2% (n=28). The rate of death, recurrent venous thromboembolism, bleeding and chronic thromboembolic pulmonary hypertension were 2.4%, 1.2%, 7.2%, and 1.9%, respectively in the DOAC group.
Summary/Conclusion: DOACs have rapidly achieved a dominant market place. The choice to prescribe DOACs or not is related to patient characteristics. The overall appropriateness of prescription is high, while the rate of adverse events observed in patients treated with DOAC is low in our registry.

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Extended anticoagulation for the secondary prevention of venous thromboembolic events: an updated network meta-analysis

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Background: Extended treatment is preconized in a significant proportion of patients with unprovoked venous thromboembolism (VTE). However, limited direct and indirect comparisons are available to appropriately weight the risk/benefit ratio of the diverse treatments available for extended anticoagulation.
Aims: To compare the rate of symptomatic recurrent VTE (primary efficacy outcome), major bleeding (MB, primary safety outcome), the net clinical benefit (recurrent VTE+MB) and death on vitamin K antagonist (VKA), direct oral anticoagulants (DOAC) and antiplatelet drugs for the secondary prevention of VTE.
Methods: A systematic literature search (Pubmed, Embase, through May 31st 2017) was conducted to identify randomized controlled trials studying different pharmacologic therapies in the secondary prevention of VTE. Treatment effects were calculated using network meta-analysis with frequentist random-effects methods.
Results: 18 trials (18221 patients) were included in the analysis. Overall, all treatments reduced the risk of recurrence compared to placebo. Nonetheless, standard adjusted dose and DOAC were more effective than aspirin, whereas standard-dose VKA was more effective than low-dose VKA. The efficacy of DOACs was globally comparable to standard-adjusted dose VKA. Low- and standard-dose VKA also increased the risk of MB, which was no the case for any the DOAC. Low-dose VKA and DOAC had similar effects on MB compared to standard-doses. Although there was a trend for reduced MB and enhanced net clinical benefit for DOAC compared to VKA, this was not statistically significant. The specific anticoagulant therapies had no significant effects on deaths.
Summary/Conclusion: Standard-dose VKA and DOACs share similar effects on VTE recurrence and MB, whereas aspirin and low-dose VKA were associated with the worst risk/benefit ratio. Although appealing, low-dose DOAC was associated with a similar risk of MB compared to standard doses, as previously documented for low-dose AVK.

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Combined thrombofilia: experience in our centre.

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Background: Hereditary thrombophilias are a set of situations that predispose to develop secondary venous thrombosis due to certain mutations or DNA polymorphisms. They are classified into low-risk thrombophilias (V Leiden factor mutation and prothrombin gene mutation) and high-risk thrombophilia (antithrombin deficiency, protein C deficiency, protein S deficiency, and combined thrombophilias). The role of combined thrombophilia has been little studied, so little is known about its prevalence and the increased risk of venous thromboembolic disease (VTE).
Aims: Studying the prevalence of combined thrombophilia as well as its presentation.
Methods: A total of 319 thrombophilia studies that were performed in the coagulation service between January 2017 and May 2018 were included. Of the total, those with combined thrombophilia were selected and the manner of presentation of each case was studied.
Results: Of the total of 319 patients studied, 2.5% (8 patients) had a combined thrombophilia. Two was heterozygous for the prothrombin gene associated with a protein S deficiency, two was heterozygous for V Leiden factor associated with a protein S deficit, two patients were heterozygous for the V Leiden factor associated with a deficit of protein C, one had a deficit of protein C associated with a deficit of protein S and one patient was heterozygous for V Leiden factor associated with a deficit of protein C and a deficit of protein S. Age mean of presentation was 51.8 years.
Regarding the presentation form, two presented as deep vein thrombosis (DVT), two as superficial thrombophlebitis, two as repeat abortions, one study was carried out as part of a study prior to the feminization hormone therapy in the transsexual patient and one study was carried out because of a family history of V Leiden factor mutation.
As for the therapy, we opted for permanent anticoagulation in patients who had DVT. In those who debuted with superficial thrombophlebitis and in whom it was diagnosed due to a family history, we opted for prophylaxis in situations of risk. In patients with repeated miscarriages, prophylaxis with heparin would be chosen in future pregnancies. Finally, the patient who was going to receive feminization hormone therapy received anticoagulation during the hormonal treatment.
Summary/Conclusion: - The age of presentation of the thrombotic event in patients with combined thrombophilia does not differ from the age of presentation of the event in patients with simple thrombophilia. We have not observed a presentation at younger ages.

- The reasons for consulting the thrombophilia study have not been for severe thrombosis. There have not been reported cases of thrombosis in unusual territories or thrombosis disproportionate to the causal stimulus.
- The finding of a combined thrombophilia has changed the therapy in patients, in some cases receiving permanent anticoagulation and in others, receiving anticoagulation against hormonal treatment.

Clotting
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Role of the thrombophilia study in ischemic cryptogenic ictus in the young patient: experience in our centre.

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Background: The hypercoagulability states have been related to venous thrombosis, being its relationship with arterial thrombosis widely discussed. The patent foramen ovale (PFO) is defined as a communication between both atria due to a defect of the interatrial septum. This defect is prevalent in patients with young stroke, so it has been related as a pathogenic mechanism of paradoxical embolism. Therefore, the study of thrombophilia, which is not recommended systematically in patients with ischemic stroke, is recommended in patients who have a patent foramen ovale.
Aims: Studying the prevalence of hypercoagulable states in patients with ischemic stroke under 55 years with and without PFO.
Methods: We included patients younger than 55 years with acute ischemic stroke who were referred for study to the coagulation service between January 2017 and April 2018. These patients were asked to study complete thrombophilia performed in our center (mutation of the V Leiden factor, mutation of the prothrombin gene, protein C deficiency, protein S deficiency and antithrombin deficiency) and they were separated into two categories, according to whether they presented PFO or not.
Results: A total of 39 patients with a mean age of 43.6 years were included; 41% (16) were women. Of the total patients, 35.9% (14) had PFO.
Of the total number of patients with PFO, 57.14% (8) had a positive thrombophilia study (3 of them were diagnosed with high risk thrombophilia, 2 with protein C deficiency and 1 with protein S deficiency). Of the total number of patients who did not have PFO (25), 16% (4) had a positive thrombophilia study (one of them was high risk thrombophilia, protein C deficit).
In the statistical analysis, the presence of PFO in cryptogenic strokes in young patients was associated with the presence of a hypercoagulable state with an OR of 7 (95% CI: 1.5548-31.5154).
Regarding the presence of cardiovascular risk factors (CVRF), of the total of patients who had thrombophilia (12), 66.67% (8) had some CVRF. Of the total of patients who did not have thrombophilia (27), 55.56% (15) had some CVRF. No statistically significant differences were found between both groups, with an OR of 0.625 (95% CI: 0.1511-2.586).
Summary/Conclusion:
In young patients with cryptogenic stroke and the presence of PFO, the presence of thrombophilias was significantly more frequent than those without PFO.
The finding of high-risk thrombophilias was more frequent in the group of patients with PFO.
The inclusion of the study of thrombophilia in the study of acute ischemic stroke in the young patient should not be included systematically, but should be performed in the subgroup of patients with PFO.
Despite the finding of a hereditary thrombophilia, CVRF is an independent risk factor for acute ischemic stroke.

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Changes in renal function in patients with non valvular atrial fibrillation starting an oral anticoagulant

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Background: Direct oral anticoagulants (DOACs) are a mainstream therapy in non valvular atrial fibrillation (NVAF), but their plasma levels and dosage are dependent on the glomerular filtration rate (GFR). Therefore, low GFR is associated with an increased DOACs plasma concentration and bleeding in patients with NVAF. However, GFR changes over time, and data in real life patients with NVAF are scarce.
Aims: We aimed to investigate the changes in the prevalence of impaired renal function according to GFR variations in a cohort of NVAF patients receiving oral anticoagulants (OACs).
Methods: We collected a cohort of patients with NVAF consecutively referred between December 2013 and July 2015 to one of the participating hospital centers (17 cardiology units, 17 anticoagulation clinics and 10 internal medicine units) who started a new OAC therapy, either a vitamin k antagonist agent (VKA) or a DOAC. Clinical characteristics were recorded at enrolment and creatinine plasma levels were obtained at one, six and twelve months thereafter. GFR was estimated by Cockroft-Gault formula (eGFR). Subgroups analysis based on sex and age were performed.
Results: 416 out of 530 patients (51% male; mean age 75 ± 11) completed the follow-up at twelve months and were enrolled in the study. Of those, 280 (67%) started a DOACs, whether the others a VKA agent. Mean eGFR at baseline was 81 (±29) ml/min, and 24% of participants had impaired renal function (eGFR < 59 ml/min). The prevalence of impaired renal function increased only slightly during follow-up (22%, 25% and 25% at one, six and twelve months respectively). Men had a higher prevalence of impaired renal function than women (25% vs 22% respectively at enrolment) with similar percentages at the end of follow-up. Remarkably, up to 38% of patients older than 75 years had impaired renal function at enrolment, with a clinically relevant increased at both six (43%) and twelve months (43%), but not at one month (38%).
Summary/Conclusion: NVAF patients older than 75 years on treatment with DOACs require a renal function test at least at six months, regardless of their eGRF at the time of the first prescription, in order to determine whether a dosage titration is needed.

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Evaluation of the Cobas t711 coagulation analyzer: a single center experience

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Background: The Cobas t711 is a novel, fully automated coagulation analyzer from Roche Diagnostics. The first parameter release includes routine coagulation tests (APTT, PT, Fibrinogen and D-Dimer) and measurement of antithrombin (AT) activity.
Aims: We evaluated the performance characteristics of the routine coagulation tests (+AT) and conducted a method comparison study with the ACL TOP700/500 instruments (Werfen). We assessed FVIII- and lupus-sensitivity of the different APTT reagents ('APTT', 'APTT Screen' and 'APTT Lupus') and FVII-sensitivity of the PT reagent.
Methods: Between-run variation was assessed by running internal quality controls twice daily during 10 days. Within-run variation was determined on 10 repeat measurements of normal and abnormal pooled plasma. Method comparison with ACL TOP700/500 was performed on left-over samples from daily routine and, for AT, supplemented by samples from genetically confirmed AT deficient patients. Reagents used on ACL TOP700/500 were APTT-SP, PT Recombiplastin 2G, Q.F.A. Thrombin and D-Dimer HS500 (Werfen) and Innovance Antithrombin (Siemens). Factor sensitivity was assessed with dilution series of normal pooled plasma in factor deficient plasma of the factor of interest. For FVIII-sensitivity, APTT was measured with the different APTT reagents on Cobas t711 and with APTT-SP on ACL TOP700. For FVII-sensitivity, we measured the PT on both Cobas t711 and ACL TOP700 with their respective PT reagent. Lupus sensitivity was assessed by measuring APTT on samples from 10 patients positive for lupus anticoagulant (LA).
Results: Between-run variations fell within the specifications provided by the manufacturer, except for fibrinogen and AT (normal control). However, all were <7%.
Within-run precision was excellent for all parameters (<2%), except for D-Dimer. This higher CV (6.1%) can be explained by the high concentration of D-Dimer in the plasma pool (i.e. ~4200 ng/mL FEU).
The method comparison showed good agreement between 'APTT Screen' on Cobas t711 with APTT-SP, while 'APTT' and 'APTT Lupus' did not. A possible explanation could be the use of silica as activator in both 'APTT Screen' and APTT-SP. PT % and INR on Cobas t711 showed good correlation with PT Recombiplastin 2G. Fibrinogen values correlated well in the normal range but tended to be lower in the higher range when compared to fibrinogen values on TOP700/500. For D-Dimer, 8 samples with positive result on ACL TOP700/500 had a normal result (<500 ng/mL FEU) on Cobas t711. None of these patients suffered from venous thromboembolism. There was an overall good agreement between AT activity on both systems, except for AT deficient samples. Some samples from type II AT deficient patients yielded normal results, a known shortcoming of anti-IIa based assays, as the one on Cobas t711.
Off the three different APTT reagents, 'APTT Screen' was the most sensitive to FVIII while only 'APTT Lupus' was sensitive to LA. Factor FVII-sensitivity of PT on Cobas t711 was identical to PT Recombiplastin 2G.
Summary/Conclusion: All evaluated assays on the Cobas t711 showed good analytical and clinical performance. The differences in APTT and AT observed in the method comparison, can be attributed to reagent-specific characteristics like activator (for APTT) or analysis method (for AT).

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Lack of standardization of the anti-factor (F)Xa activity and the anti-FXa-correlated aPTT therapeutic ranges used in monitoring unfractionated heparin treatments

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Background: Due to the risk of thrombosis in case of under-dosing and the risk of hemorrhage in case of over-dosing, monitoring UFH treatments is mandatory. The activated partial thromboplastin time (aPTT) is the most widely used method as it is simple, cheap, and widely available. However, it is also poorly standardized and affected by many parameters, both analytic and pre-analytic, that are unrelated to the heparin effect. Particularly, the UFH-induced prolongation of aPTT is highly dependent on the reagent and analyzer used. In an effort to improve inter-laboratory agreement in the monitoring of UFH, the 9thACCP Consensus guideline recommends monitoring UFH treatments by using either the anti-FXa activity, with the therapeutic range between 0.30 and 0.70 IU/mL, or the anti-FXa-correlated aPTT.
Aims: To compare the inter-laboratory agreement of the anti-FXa activity and the anti-FXa-correlated aPTT for monitoring therapeutic UFH treatment, we conducted a cross-validation study among 3 accredited coagulation laboratories that used different technical conditions.
Methods: 125 inpatients on UFH were sampled in one of the centers. Blood was collected into evacuated polymer tubes containing 3.2% tri-Na citrate. Tubes were centrifuged twice within 2h after collection, and plasma was stored frozen in aliquots at -70°C that were shipped in dry ice to the other centers to be locally evaluated. Anti-FXa activity (in IU/mL) was evaluated using 4 chromogenic assays i.e. Biophen Heparin LRT (Hyphen BioMed), HemosIL Liquid Anti-Xa (Instrumentation Laboratory, IL), Innovance Heparin (Siemens), and STA-Liquid Anti-Xa (Stago). APTT was evaluated using 4 reagents i.e. HemosIL SynthASil and HemosIL APTT SP (IL), Pathromtin SL (Siemens), and STA-PTT Automate 5 (Stago). Combinations of reagents and analyzers i.e. ACL TOP 700 (IL), CS-5100 (Siemens) and STAR (Stago) from the same manufacturer were used at the participating centers.
Results: Anti-FXa test results were found to be highly significantly different depending of the assay used, with the median activity ranging from 0.57 IU/mL with one reagent to 0.37 IU/mL with another, intermediate results around 0.44 IU/mL being obtained using the 2 other reagents. Test results obtained using the different reagents were well correlated ($r>0.91$ in all cases) even though some comparisons performed according to Bland-Altman demonstrated unacceptable bias. If 0.30 to 0.70 IU/mL was used as the therapeutic range of anti-FXa activity, such a discrepancy in test results led to a lack of agreement as to whether a sample was subtherapeutic, therapeutic or supratherapeutic in more than 25% of the patients.
The median aPTT test result (ratio) ranged from 2.22 with the less sensitive to 2.93 with the most sensitive reagent). Using the anti-FXa-correlation method, aPTT therapeutic ranges were found to be highly different from one combination of reagent/analyzer to another, and the same applied when correlations were made between one single aPTT reagent and different anti-FXa assays performed on the same analyzer. Consequently, agreement among all aPTT reagents was found in in less than 40% of the patient samples
Summary/Conclusion: The reported discrepancy between test results evaluated using commercially available anti-FXa assays that are consistent with the results of various national and international EQA programs, clearly suggests a lack of standardization of that assay. As the consequence, the anti-FXa-correlated aPTT therapeutic range could be significantly impacted.

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Monitoring treatments by unfractionated heparin (UFH). Stability of plasma anti-Xa activity for up to 4 hours in citrated tubes

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Background: Current guidelines recommend a maximum delay of 2h between blood sampling and testing for anti-Xa activity and aPTT prescribed for monitoring treatments by UFH, when citrated tubes are used.
Aims: As such a short delay could be an issue, particularly at multisite centers, we evaluated the potential impact of longer delays.
Methods: For that purpose, 2 citrated tubes (0.109 M) were obtained from 73 patients on UFH: one centrifuged and tested within 1h after sampling and one stored for 4h at room temperature before being centrifuged.
Anti-Xa activity was evaluated using 2 reagents [Biophen Heparin LRT (Hyphen Biomed) and HemosIL Liquid Heparin (Werfen)], and aPTT using HemosIL SynthASil (Werfen) automated on an ACL TOP 700 CTS (Werfen).
Results: Anti-Xa activity was significantly ($p<0.0001$) lower after a 4h- than after a < 1h-delay [0.38 (0.16 1.18) vs. 0.45 (0.19 1.20) with the Hyphen reagent, and 0.34 (0.11 1.21) vs. 0.38 (0.11 1.22) with the IL reagent]. However, the mean bias calculated according to Bland-Altman was below 0.05 IU/mL for both reagents, a value lower than the technique imprecision. The mean bias was similar for anti-Xa activity below (n=45 with Hyphen reagent and n=54 with IL reagent) or above 0.50 IU/mL (n=28 and n=19 respectively). Considering 0.30 to 0.70 IU/mL as the therapeutic range, there were 5 cases of discrepancy (6.8%) with one reagent, and 12 with the other (16.4%). Most of them were around the lower limit of the therapeutic range and would have had no impact on anticoagulation management of the patients.
aPTT was significantly shortened ($p< 0.0001$) after a 4 h-delay [1.63 (0.96 >5.0) vs. 1.91 (1.02 >5.0), $p<0.0001$] with a mean bias of -0.26 decrease in ratio, corresponding to a shortening of -8.1 sec. If aPTT therapeutic range was calculated to correspond to anti-Xa activities between 0.30 and 0.70 IU/mL, 9 cases of discrepancy were found, some of them could have induce UFH dosage change.
Summary/Conclusion: These results suggest that it is safe to extend to 4h the delay between blood sampling and measurement of anti-Xa activity for monitoring UFH treatments, with the tested reagents that contain dextran sulphate, a compound able to bind PF4 with a high affinity and so to displace heparin from its complex with PF4. Changes in aPTT were higher, suggesting a shorter acceptable delay. This point is currently investigated.

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The use of the age-adjusted D-dimer threshold to exclude pulmonary embolism

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Background: The diagnostic management of pulmonary embolism (PE) is based on a combination of clinical variables, D-dimer testing and diagnostic imaging (computerized tomography pulmonary angiography – CTPA). D-dimer measurement is a sensitive test to rule out PE, and a normal result presents a high negative predictive value. In the elderly, the specificity of the test decreases as D-dimer levels normally increase with advancing age. Recently, a new age-adjusted D-dimer threshold was derived for screening strategy in patients over 50 years old.
Aims: According to the diagnostic approach of the clinicians, all patients with non-high clinical probability for PE and D-dimer value >500 μg/L FEU (Fibrinogen Equivalent Units) are referred for CTPA. The aim of our study was to retrospectively validate the new age-adjusted D-dimer threshold in patients with non-high clinical probability for PE, aged over 50 years.
Methods: We retrospectively recorded the data of outpatients presented with suspected PE during one year period. 48 consecutive patients (29 males/19 females), aged over 50 years (median age 72.5 years), with non-high clinical probability for PE, D-dimer value >500 μg/L FEU (conventional threshold) (INNOVANCE® D-dimer, Sysmex® CS-2100*i* System) and CTPA (CTP Siemens® 2013 16 Slices) interpretation "negative for PE", were enrolled. We calculated the age-adjusted D-dimer threshold as followed: patient's age x 10 μg/L.
Results: Based on the age-adjusted D-dimer threshold, 10 out of 48 patients presented a normal D-dimer value for their age. According to the diagnostic approach (non-high probability and normal age-adjusted D-dimer value), the CTPA testing could be avoided. Finally, PE could have been excluded without CT scanning in 21% of patients.
Summary/Conclusion: Applying the age-adjusted D-dimer threshold reduces the need for CT scanning in patients with suspected PE. Our results are in accordance to those of other studies. Further studies will be needed to establish the age-adjusted D-dimer threshold in everyday clinical practice.

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Fibrinolytic portrait for different thrombotic conditions in women

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Background: We are considering various thrombotic conditions, such as (1) thrombotic readiness when a thrombosis can be occurred any time due to the combination of thrombogenic conditions, (2) permanent (micro)thrombosis as the cycle in which the (micro)thrombi are formed and impact the clinical picture, however the most of clots dissolve fast, and (3) occurred thrombosis as acute thrombosis requiring long time for natural lysis or post-thrombotic condition within 12 months after the event.
Aims: To study the fibrinolytic patterns specific to thrombotic condition in women with some disorders.
Methods: The study included women with some myeloproliferative neoplasms (MPNs n=57), with chronic cerebrovascular diseases (CCVD; n=88) and with CCVD comorbided with Ph-negative myeloproliferative neoplasms (CCVD+MPNs, n=76), with acute ischemic stroke (AIS; n=62) and within 12 months after AIS (Post-AIS, n=44), and pregnant women with preeclampsia (PE, n=23). Fibrinolisys was tested with fibrinogen, plasminogen (PLG), tPA, PAI-1, TAFI, Alfa-2-Antiplasmin (a2-AP), D-Dimer. Thrombin generation rate was characterized via TEG-Angle, and modulus G was used to estimate a clot.
In addition 98 markers reflecting of blood clotting, anticoagulants, platelets and vascular wall, angiogenesis and cytokines were analyzed in each patient as well as calcium, creatinine and patient age.
Results: Thrombotic readiness was presented patients with MPNs, CVS and CVD+Zns. Compared with thrombotic patients, they showed less thrombin generation rate and plasminogen activity but higher tPA, PAI-1 and TAFI were lower, and a2-AP had a strong variation, D-dimer was slightly less, and the clot had the same properties.
Among all thrombotic patients Post-AIS had the lowest profibrinolytic activity, the highest antifibrinolytic activity and most high firmness of clot.
High thrombin generation rate and plasminogen were combined with extremely low tPA and extremely high PAI-1 and TAFI in women with preeclampsia. However, the clot firmness was not high in them.
Multivariate analysis revealed that each fibrinolytic parameter is under fine regulation by means of microenvironment. A set of factors making an assembly is formed due to the dominant pathology. Values of the powers for each affecting assembly were calculated by means of model analysis of data obtained.
Summary/Conclusion: Studied disorders have specific fibrinolysis pattern. Profibrinolytic balance prevails in thrombotic readiness compared to the permanent and/or occurred thrombosis. Microenvironment factors form ensembles which become drivers in fine regulation of fibrinolysis.

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SIGNIFICANCE OF THE APPLICATION THE THERAPEUTIC PLASMA EXCHANGE (TPE) IN PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare systemic disease that belongs to the group thrombotic microangiopathy. Therapeutic plasma exchange (TIP) is the basic measure of therapeutic treatment of TTP.
Aims: Analyze laboratory parameters before and after plasma exchange in patients with code TTP and examine the effect derived TIP on the clinical condition of the patient.
Methods: We studied a retrospective analysis of the five-year period at the Blood Transfusion Institute of Serbia conducted 300 therapeutic plasma exchange in 45 patients (31 women-71.77% and 14 men - 28.23%, aged 19 to 66 years of life). We used two types of apparatus therapeutic plasma exchange with centrifugacionom technique and filtration technique. In all patients who has performed therapeutic plasma rye are laboratory parameters before and after plasma exchange (platelet count, serum LDH and haptoglobin). Volume changes of plasma ranged from 1.1 to 2.0 liters, per one cycle. It is also accompanied by the clinical picture of the patient whether the disease first appear or in case of relapse and relapse, which was after the order and whether to emerge early relapse of disease and the number of days required for the recovery of patients. The incidence of complications in patients with TTP were: first attack of the disease occurred in 19 (42, 22%) patients; first recurrence in 8 (17.78%) patients; Third disease recurrence occured in 4 (4.89%) patients; fifth relapse in 2 (4.44%) patients and in 2 (4.44%) patients early ralaps i.e. exacerbation of disease after 7 days.
Results: Laboratory findings suggestive of TTP are signs of hemolytic anemia (fragments of platelet reticulocytosis, erythroblasts in smear-elevated LDH, elevated indirect bilirubin, decreased haptoglobin, and megakaryocytic hyperplasia), thrombocytopenia, fibrinogen and factor VIII high, signs of disseminated intravascular coagulation are rare, while other tests are within normal limits. Average values of the main laboratory parameters that we followed before the start of TIP were: hemoglobin: 81±12 g / L; hematocrit: 0,2±0,03 L / L; platelet count: 16±8 $x10^9$ / L; LDH: 3432 ±1136 U / L; haptoglobin: 10±10 mg / dL. Plasma volume removed in one cycle changes therapeutic plasma ranged from 1.1 L to 2.0 L. Therapeutic plasma exchange are made daily until normalization of platelet count, normalization of LDH levels and repairing neurological status. Thrombocytopenia and high LDH levels were normalized within one to two weeks, and neurological abnormalities earlier. Efficiency TIP levels were observed using LDH. The increase in LDH activity in the course of a TIP is pointing to the maintenance of the disease and require more aggressive treatment, such that the TIP performed twice a day with the corticosteroid therapy. After a favorable response TIP was done on the second or third day, and with the achievement and maintenance of continuous remission is interrupted treatment with TIP.
Summary/Conclusion: TIP has proved to be an excellent treatment modality TTP, a severe and deadly diseases. During our study, which lasted 5 years, the outcome therapeutic TIP in 80% of patients had complete remission, while in 3 (6.66%) patients occurred exacerbation and repeated TIP.

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Bone Marrow Necrosis in Patient with Antiphospholipid Syndrome: Case Report and Review of Literature

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Background: Bone marrow necrosis (BMN) considered a rare complication of malignant or non-malignant conditions. Cytopenia with bone pain is the hallmark of the BMN. Since the BMN is considered rare, the combined presentation of BMN and Antiphospholipid syndrome (APS) is, even more, more unusual and very few cases reported in the literature.
Aims: We are reporting a case of APS with BMN confirmed by trephine biopsy and searched the literature looking for cases with the same presentation. In those cases, we will find the similarity and the differences between them and review their presentation criterion.
Methods: We used the online resources to search extensively in the literature. We looked for the relevant articles in the PubMed and Google Scholar with the keywords: Bone marrow necrosis, Antiphospholipid syndrome and Bone marrow necrosis and Antiphospholipid syndrome. We excluded the cases that not published in English and the cases that diagnosed without bone marrow trephine biopsy.
Results: We founded only 3 cases in the literature. All 4 cases (3 reported in the literature and our case) diagnosed previously with APS and all of them stated a history of multiple miscarriages. Bone pain is one of the important presenting features, however, 2 of the reported cases display no bone pain. Bone marrow trephine biopsy examination shows extensive necrosis (Grade III) in all 4 cases.
Summary/Conclusion: BMN is not rare anymore but BMN and APS category is unusual and reporting of these cases are important. The few cases reported where already diagnosed with APS and bone marrow trephine was done to assess the cause of the peripheral cytopenia. The evidence of the presence of microthrombi cannot be demonstrated for all the cases using the bone marrow biopsy but those patients were not diagnosed with any primary disease but the APS and presence of miscarriages history support this hypothesis. These cases highlight that, the primary causes of the triad of APS, cytopenia and bone pain is BMN. Bone marrow trephine biopsy could be not important to reach such diagnosis unless the primary physician suspecting other cause for cytopenia.

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Direct Oral Anticoagulants For Treatment Of Cerebral Venous Sinus Thrombosis

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Background: Cerebral venous sinus thrombosis (CVST) typically affects young adults and requires anticoagulant treatment for a period that varies from 3 months to lifelong. Direct oral anticoagulants (DOAC), because of their safety profile and the lack of laboratory monitoring, may represent a valid alternative to vitamin K antagonists (VKA) for the treatment of CVST. To date, no phase III trials on DOAC included patients with CVST and only three case series of respectively 2, 6 and 7 patients with CVST treated with rivaroxaban have been published, supporting their safety in this particular setting.
Aims: To investigate the safety and efficacy of DOAC in a cohort of patients with CVST.
Methods: All consecutive patients with CVST treated with DOAC from September 2013 to May 2018 were included in the study. Recurrence, recanalization and bleeding complications were the study outcomes, expressed in terms of counts (minimum-maximum range for continuous variables) and percentages.
Results: Eighteen patients with CVST at a median age of 35 years were the study population. Two patients (11%) had thrombosis limited to one sinus, while the remaining (89%) had at least two sinuses involved. Eight patients (44%) had also thrombosis of the cerebral veins and other 8 (44%) had thrombosis extended to the internal jugular vein. After treatment with low molecular weight heparin in the acute phase, 10 (56%) received DOAC, while 8 (44%) received VKA for a median time of 227 days (range 41-521 days) and then was switched to DOAC. The median time in DOACs was 302 days (range 44-688 days). Fourteen patients (78%) received rivaroxaban (20 mg od), 3 (17%) edoxaban (two 60 mg od and one 30 mg od) and one (5%) apixaban (5 mg bid). At 12 months from the index event, 5 patients (28%) obtained a complete recanalization, 7 (39%) a partial recanalization and the remaining 10 (56%) did not complete the follow-up period yet. Two patients (11%) had recurrent CVST during treatment with DOAC (one rivaroxaban and one apixaban) and were switched to VKAs. Bleeding occurred in 3 patients (17%) on rivaroxaban: two (11%) had menorrhagia (in one case associated with anemia requesting oral iron supplementation) and one had asymptomatic subdural hematoma.
Summary/Conclusion: Considering the limitation of the small sample size, our results raise some concerns on efficacy and safety of anticoagulant treatment with DOAC in patients with CVST. Further data from randomized controlled trial are needed.

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Predictors Of Venous Thromboembolism In Patients Undergoing Neurosurgery For Glioma

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Background: Patients undergoing surgery fro glioma have a high post-operative risk of pulmonary embolism (PE). A stratification of the risk of PE is warranted to optimize post-operative antithrombotic prophylaxis, that at our Hospital is usually started the second day after surgery.
Aims: Aim of the study is to identify individual variables predicting the risk of PE after surgery for glioma.
Methods: Consecutive patients undergoing glioma resection at our Neurosurgery Unit between 2012 and 2016 underwent a Q-scan at baseline (day 0, before tumor resection) and the second day after surgery (day 2). Results of Q-scans were used to estimate the risk of PE expressed as incidence risk with its 95% confidence interval (CI). Blood samples for D-dimer were collected at baseline and immediately after surgery. A multivariate logistic regression model was fitted with sex, age, BMI, tumor volume, duration of intervention, D-dimer (categorized at the 50th percentile [i.e., 627 ng/mL]) and histologic grade (high vs low) as predictors of PE. The predictive capability of the model was measured as the area under the ROC curve (AUC) with its 95%CI.
Results: 59 patients were included in the study, 33 (56%) men and 26 (44%) women, with a median age at surgery of 57.5±13.7 years. All patients had a negative Q-scan at day 0. 19 patients (32%) developed PE at day 2, for an incidence risk of 32.2% (95%CI 21.7-44.9%). All PE were asymptomatic. The variables associated with the risk of PE were D-dimer, (adj. odds ratio 3.0, 95%CI 0.7-12.7), histologic "high malignancy grade" (2.9, 0.3-28.4) and BMI (2.0, 0.7-5.2 for every 5 kg/m^2 increase). The AUC of the ROC curve of the whole predictive model was 0.7 (95%CI 0.6-0.9) (Figure).
Summary/Conclusion: We found a 32% incidence of early PE after glioma resection. A predictive model including D-dimer, histologic grade and BMI showed a promising capability to identify patients at risk of PE who may deserve an early start of antithrombotic prophylaxis.

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High dietary intake of marine n-3 fatty acids is associated with reduced risk of recurrent VTE

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Background: Venous thromboembolism (VTE) is associated with a significant risk of recurrence, particularly following an unprovoked or a cancer-related index event. Experimental studies have demonstrated that polyunsaturated fatty acids of marine origin (n-3 PUFAs) exert antithrombotic effects, and it has been suggested that dietary intake of n-3 PUFAs may lower the risk of incident VTE. However, the impact of n-3 PUFAs-intake on the risk of recurrent VTE remains virtually unexplored.
Aims: To investigate the association between dietary intake of n-3 PUFAs and the risk of recurrence in VTE-patients derived from the general population.
Methods: A total of 567 patients with incident VTE and data on dietary intake of n-3 PUFAs were derived from the Tromsø Study survey 4 (1994-95) and 6 (2007-08). A comprehensive n-3 PUFA- variable was constructed based on self-reported intake of fat and lean fish, fish as spread and fish oil supplements, and participants were categorized according to quartiles of weekly intake in grams (Q1: <4.7, Q2: 4.7-13.4, Q3: 13.4-29.1, Q4: >29.1). The assessment recorded in closest proximity to the index event was used for analysis. Participants entered the study on the date of the incident event and were followed until recurrence, death, migration or the end of the study period (31.01.2016). Hazard ratios (HRs) for VTE recurrence adjusted for age (as time scale), sex and body mass index were calculated using Cox regression models with Q1 as the reference. Analyses restricted to unprovoked index events and to cancer-free patients were also performed. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: There were 92 recurrences during a median follow-up of 3.2 years. Overall, there was no significant association between weekly intake of n-3 PUFAs and the risk of recurrent VTE. However, there was a trend towards a lower risk in the highest quartile of n-3 PUFAs-intake (HR Q4 0.77, 95% 0.41-1.48), which appeared to be driven by an effect on recurrence following an unprovoked index event (HR Q4 0.31, 95% CI 0.11-0.93). The effect of n-PUFAs on recurrence risk following VTE was also amplified (HR Q4 0.47, 95% CI 0.22-1.02) when patients with cancer-related VTE were excluded from the analysis.
Summary/Conclusion: We found that a high dietary intake of n-3 PUFAs was associated with a tendency of a lower risk of recurrent VTE. Stratified analyses revealed that the effect was mainly confined to recurrence following an unprovoked index event and to cancer-free VTE-patients.

Clotting
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Board No. 124

Prognostic factors of catheter-related thrombosis (CRT) are different than prognostic factors of VTE in patients with cancer, Data from the ONCOCIP study.

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Background: Systematic thromboprophylaxis is not recommened in cancer outpatients, including those with central venous catheters. There is a need to better characterize patients at high risk for venous thromboembolism (VTE). Several risk assessment models have been developed to identify patients at high risk for VTE. But, data regarding risk factors for catheter-related thrombosis (CRT) are scarce.
Aims: To identify prognostic factors of 1) CRT; 2) VTE unrelated to catheter on the other hand.
Methods: In a prospective, multicenter cohort study, consecutive adult patients with solid tumors and requiring central venous catheter (whether at inclusion or within one month prior to inclusion) were followed for 12 months. CRT was defined as ipsilateral symptomatic upper-limb deep-vein thrombosis with or without pulmonary embolism. VTE unrelated to the catheter was defined as any symptomatic deep-vein thrombosis or pulmonary embolism, and incidental pulmonary embolism. All events were objectively confirmed and centrally adjudicated. Rate assessment integrated the competing risk of death. Potential prognostic factors for CRT and VTE unrelated to the catheter were determined by two multivariate models.
Results: Overall, 3032 patients were included in 29 centers (median age: 63 years; women: 58%; ECOG performance status of 0: 58%). The most frequent cancer locations were breast (33.7%), lung (18.5%) and colorectal (15.6%); cancer was metastatic in 43.2% of patients. Most patients (97.1%) received chemotherapy. By 12 months, 48 patients (1.6%) had been lost to follow-up and 656 (24.6%) had died; 111 patients (3.8%, 95% confidence interval, 3.2 to 4.5) presented at least one CRT event. Use of the cephalic vein for catheter insertion was associated with an increased risk of CRT, whereas ongoing antiplatelet therapy was protective (Table 1). VTE unrelated to the catheter occurred in 276 patients (9.6%, 95% confidence interval, 8.7 to 10.7). Prognostic factors were advanced age, previous venous thromboembolism, cancer site, and low hemoglobin level or increased leukocyte count before chemotherapy (Table 1).
Summary/Conclusion: This large prospective cohort study shows that the frequency of CRT is smaller than the frequency of VTE unrelated to the catheter (3.8% versus 9.6%). This risk is not negligible, this especially as it can make the continuation of chemotherapy harder. The identification of different prognostic factors for CRT and VTE unrelated to the catheter argues for the evaluation of distinct anticoagulant prophylaxis for these two profiles of patients at risk.
NCT02025894

Clotting
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Joint effects of atrial fibrillation and prothrombotic genotypes on the risk of ischemic stroke. The Tromsø Study

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Background: Atrial fibrillation (AF) is the most common arrhythmia of clinical significance, and the main cause of cardioembolic stroke. It is not known to what extent prothrombotic genotypes increases the risk of atrial thrombus formation and subsequent stroke in AF patients.
Aims: To investigate the joint effects of prothrombotic genotypes and AF on the risk of ischemic stroke in a population-based cohort study.
Methods: A randomly selected sub-cohort (n=3663) was sampled from the fourth survey of the Tromsø study (1994/1995) and incident cases of ischemic stroke (n=314) were identified through December 31, 2012. DNA isolated from blood of all participants was genotyped for rs8176719 (non-O blood type) in ABO, rs6025 (factor V Leiden) in F5, rs1799963 (prothrombin G20210A) in F2, rs2066865 in FGG, and rs2036914 in F11. Cox proportional hazards regression models were used to calculate hazard ratios (HR) with 95% confidence intervals (CI) for incident ischemic stroke by individual SNPs and categories of risk alleles (de Haan 5-SNP score; 0-1, 2 and ≥ 3) in subjects with and without AF. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: In total, 482 participants were diagnosed with AF, of whom 67 developed subsequent ischemic stroke during the study period. The presence of prothrombotic risk alleles in factor V Leiden, prothrombin, F11, and FGG did not increase the risk of ischemic stroke in subjects without and with AF. However, a joint effect was observed for ABO and AF on the risk of ischemic stroke. In the absence of AF, ≥ 1 risk allele(s) in ABO were not associated with risk of ischemic stroke (HR 1.17, 95% CI 0.81-1.70), whereas AF alone yielded a 3-fold increased risk (HR 3.26, 95% CI 1.74-6.13). Participants with both the ABO risk allele(s) and AF had a 5-fold increased risk of ischemic stroke compared to subjects without AF and risk alleles (HR 4.90, 95% CI 2.95-8.14). There was no linear increase in risk of ischemic stroke across increasing risk alleles in the de Haan 5-SNP score in participants either with or without AF.
Summary/Conclusion: We found a joint effect between AF and risk alleles in ABO (non-O) on the risk of ischemic stroke. Our findings may suggest that risk alleles in ABO should be considered when assessing the thromboembolic risk in patients with AF. Our findings merit further investigation in larger prospective studies.

Clotting
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Board No. 126

Joint effects of atrial fibrillation and prothrombotic genotypes on the risk of venous thromboembolism. The Tromsø Study

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Background: Recent studies have demonstrated that individuals with atrial fibrillation (AF) have increased risk of venous thromboembolism (VTE). While prothrombotic genotypes increase the risk of VTE in the general population, it is not known to what extent the combined effects of AF and prothrombotic genotypes contribute to the VTE risk.
Aims: To investigate the joint effects of prothrombotic genotypes and AF on the risk of VTE in a population-based case-cohort study.
Methods: Cases with incident VTE (n=664) and a randomly selected age-weighted sub-cohort (n=3746) were recruited from the fourth survey of the Tromsø study (1994/1995) and followed until December 31st 2013. DNA isolated from blood was genotyped for rs8176719 (ABO), rs6025 (Factor V Leiden), rs1799963 (Prothrombin G20210A), rs2066865 (FGG) and rs2036914 (F11). Cox proportional hazards regression models were used to calculate hazard ratios (HR) with 95% confidence intervals (CI) for incident VTE by individual SNPs and categories of risk alleles (de Haan 5-SNP score; 0-1, 2 and ≥ 3) in subjects with and without atrial fibrillation. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: There were 665 participants diagnosed with AF, of which 75 developed subsequent VTE during the study period. Subjects with AF had higher risk of VTE than those without AF (HR 1.85, 95% CI 1.44-2.38). Among the individual SNPs, a joint effect on VTE risk was found for ABO and Factor V Leiden. Subjects with ≥ 1 risk allele in ABO (non-O) without AF had a 1.4-fold higher VTE risk (HR 1.35, 95% CI 1.13-1.60), whereas those with ≥ 1 risk allele in ABO and AF had a 2.8-fold higher VTE risk (HR 2.84, 95% CI 2.07-3.91). Similarly, subjects with ≥ 1 risk allele in Factor V Leiden without AF had a 2.3-fold higher VTE risk (HR 2.33, 95% CI 1.85-2.92), whereas those with ≥ 1 risk allele in Factor V Leiden and AF had a 4-fold higher VTE risk (HR 3.96, 95% CI 2.17-7.24). (HR 1.35, 95% CI 1.01-1.80). The risk of VTE increased linearly ($p<0.001$) across categories of risk alleles in the 5-SNP score. In contrast, there was no linear increase in risk across categories of risk alleles in AF patients.
Summary/Conclusion: We showed joint effects between AF and risk alleles in ABO and Factor V Leiden on the VTE risk. Our findings may suggest that risk alleles in ABO and Factor V Leiden should be considered when assessing thrombosis risk in patients with AF.

Clotting
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Slow infusion of reduced dose of alteplase decreases 30-day mortality in patients with intermediate-risk pulmonary embolism

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Background: According to current guidelines, routine thrombolytic therapy is not recommended for patients with intermediate-risk pulmonary embolism because of excessive risk for major bleeding and insufficient decrease of the composite of need for the escalation to reperfusion therapy and mortality.
Aims: Aim of this study was to investigate whether slow infusion (1-2 mg/hour) of reduced does of alteplase (maximum dose per patient was 50 mg) either systematically or by catheter will reduce mortality in patients with intermediate risk PE. The secondary end-point was the incidence of major bleeding (International Society of Thrombosis and Hemostasis criteria) associated to thrombolytic therapy.
Methods: Among 604 patients with acute PE from the multicenter data-base of acute PE patients in Serbia (founded during the 2016), 355 patients fulfilled criteria of intermediate-risk PE. Out of this cohort of patients, 208 (58.6%) did not receive any thrombolytic protocol, 90 (25.4%) treated with full dose of recommended (by European guidelines) thrombolytic protocols for PE, and 57 (16.1%) were treated with slow infusion (1-2 mg/hour) of reduced dose of alteplase (range 25-50 mg) given either through the systemic venous infusion in 37 (10.4%) or by catheter ultrasound assisted thrombolysis in 20 (5.6%) patients.
Results: Three cohorts of patients were not different regarding to gender distribution, number of patients older than 75 years, frequency of PESI higher than 0, the presence of malignant disease, right ventricle systolic pressure and BNP level. All cause of death and PE cause of death in 30 days follow-up were 11.1% vs 16.7% and 1.8%, p=0.019; and 2.9% vs 10.0% vs 1.8%, p=0.021 in patients without thrombolytic therapy compared to recommended thrombolytic protocols and slow infusion of reduced dose alteplase protocol, respectively. Major bleeding associated to thrombolysis was more often presented in patients with slow infusion of reduced alteplase protocol, 17.5% vs 8.9% (p=0.130) compared to currently recommended protocols. However, the half of this bleeding events in slow infusion group was related to local-skin hematoma and there was no fatal bleeding in either groups.
Summary/Conclusion: Slow infusion of reduced dose alteplase reduces 30-day mortality rate in patients with intermediate-risk PE with increase of non-fatal major bleeding compare to no reperfusion strategy and therapy with recommended thrombolytic protocols.

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Evaluation of human-sourced lipid interferences on three different D-dimer enhanced latex immunoassays

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Background: The Clinical and Laboratory Standard Institute, in its guideline EP07, recommends that each potential interfering substance should be tested at two different measurand levels. High lipid concentrations usually but not systematically associated with a cloudy aspect of the plasma sample are recognized as interferents encountered in immunoturbidimetric assays. Nevertheless, there is a great variability in the manufacturers' methods for testing these lipid or turbidity interferences.
Aims: Evaluate the interference of human-sourced triglyceride-rich lipoproteins on three different D-dimer enhanced latex immunoassays.
Methods: To obtain eight levels of lipid concentrations, two native plasma samples with different D-dimer concentrations (about 0.65 and 3.0 micrograms per ml FEU respectively) were spiked with increasing quantities of concentrated triglyceride-rich lipoproteins extracted from human-sourced materials. The D-dimer and triglyceride concentrations of these different spiked samples were measured respectively with three D-dimer latex immunoassays and with one biochemical colorimetric assay.
Results: For the plasma with D-dimer concentration at about 0.65 micrograms per ml FEU, lipid addition only slightly affected the D-dimer measurement with the three D-dimer enhanced latex immunoassays tested. On the opposite, in the plasma with a high D-dimer concentration at about 3.0 micrograms per ml FEU, lipid addition had an impact on the D-dimer results that depended on the reagent/instrument system used. This difference can be explained by different human-sourced lipid interference sensitivities of the three systems tested.
Summary/Conclusion: Regardless of analytical considerations, the use of human-sourced triglyceride-rich lipoproteins is an interesting approach to evaluate lipid interference on enhanced latex immunoassays. Moreover, this study performed on three D-dimer immunoassays shows different lipid interference sensitivities when the D-dimer concentration is near 3.0 micrograms per ml FEU, and no difference at a D-dimer concentration near 0.65 micrograms per ml FEU.

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Health education and clinical-biological monitoring in patients with DOACs and their impact on thrombotic and hemorrhagic complications

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Background: Thrombotic disease, arterial and venous side, is the first cause of mortality in the western world. The pharmacological treatment with direct acting oral anticoagulants (DOACs) supposed a revolution in the therapeutic approach of this pathology, but recent studies have shown a lower therapeutic adherence in patients anticoagulated with these drugs. Is logical to think that in those patients who receive health education about these anti-thrombotic and have to a regular clinical-biological monitoring, the lack of adherence to the drug will be detected more effectively. This translates into a decrease in the incidence of hemostatic complications
Aims: Evaluate the influence of health education and clinical-biologic monitoring, on the incidence of thrombotic and hemorrhagic complications. Also, the frequency of these complications in both groups is compared
Methods: A retrospective study was conducted with 126 anticoagulated patients with DOACs of Area IV of Health of Asturias, that divided into two groups depending on the Clinical Service that makes the indication of the drug, the Thrombosis and Haemostasis Unit of the Hematology Service of the Central University Hospital of Asturias (group 1) vs other medical services (group 2)
Results: The characteristics of the studied population are detailed below: Group 1: 76 patients: 37 males and 39 females. Age: 52 - 97 years. 46 patients (60.5%) treated with Rivaroxaban, 20 (26.3%) with Apixaban, 10 (13.2%) with Dabigatran. Group 2: 50 patients; 32 males and 18 females. Age: 33 - 92 years. 22 patients (44%) treated with Rivaroxaban, 12 (24%) with Apixaban and 16 (32%) with Dabigatran.
Regarding adherence to treatment, it was observed that in group 1 the percentage of patients who did not take the drug correctly or assumed an erroneous dosage was correct. This fact was verified by determining the concentrations of the drug in blood, seeing which were above and below the therapeutic levels. The frequency of hemorrhagic and thrombotic complications was lower in group 1. A significant difference ($p = 0.008$) was observed between hemorrhagic and thrombotic complications in favor of patients who had received health education and clinical-biological monitoring.
Summary/Conclusion: Patients treated with DOACs who receive prior health education and clinical-biological follow-up by personnel specialized in haemostasis present fewer hemorrhagic and thrombotic complications than those who do not follow this protocol of action. Severe hemorrhagic events and thrombotic events occur more frequently in patients who do not receive health education or clinical-biological monitoring

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Plasma Fibrinogen Level in Sudanese Patients with Type2 Diabetes Mellitus

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Background: High plasma fibrinogen level has been described as independent risk factor for cardiovascular diseases in the general population. Increased fibrinogen plasma concentrations have been reported in type 2 diabetic patients and it has been suggested to be involved in the excess rate of cardiovascular diseases in patients with type 2 DM.The mechanisms leading to hyperfibrinogenemia in type 2 diabetes are not known but several studies have shown that underlying hyperglycemia responsible for increased glycation of fibrinogen leading to shorter fibrinogen circulating half-life. This higher clearance rate means that synthesis is actually even more increased than suggested by the plasma level elevation. Also, the glycation of fibrinogen results in the formation of a denser fibrin clot with finer fibers that is resistant to fibrinolysis. Another finding is the consequent oxidative stress of diabetes, may give rise to increased thrombin formation. This process causes increased Production of prothrombin fragments (F1 + 2) and increased turnover of fibrinogen, with increased production of fibrin and consequently increased release of fragment D. F1 + 2 and fragment D regulate the production of fibrinogen in the liver; increased release of them into the circulation may produce an increase in circulating fibrinogen.
Aims: This study aimed to assess plasma fibrinogen level in Sudanese patients with type 2 DM
Methods: Non interventional analytical case control study was conducted at Soba University Hospital from March to May 2011. A total of 40 Sudanese patients with type 2 diabetes mellitus and 10 non diabetic individuals as controls were enrolled in this study, including both genders with age group 20-85 years old. The patients were 12 males and 28 females, most of them have been diabetic for more than 2 years. The blood samples were collected from cases and controls, in 3.2% tri sodium citrate for estimation of fibrinogen level using a full automated coagulometer sysmex CA – 500.
Results: Mean plasma fibrinogen level in Sudanese patients with type2 diabetes mellitus (mean =210.75mg/dl) was significantly increased (P=.013) than in non diabetic individuals (mean=185 mg/dl) and there was no statistically differences between age and gender groups.
Summary/Conclusion: Increased plasma fibrinogen level in Sudanese patients with type2 diabetes mellitus is reported and it is helpful to bear this in mind, and to include fibrinogen assay as routine investigations for better cardiovascular risk management of these patients.

Clotting
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Board No. 131

Prognostic Impact of Non-Compliance with Guidelines-Recommended Treatment of Acute Pulmonary Embolism: Results from a Prospective Multicenter Registry

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Background: Initial management of pulmonary embolism is the subject of international evidence-based guidelines.
Aims: To describe patterns of non-compliance with guidelines and to evaluate the impact of non-compliance on the occurrence of clinical events.
Methods: This is an observational, multicenter, multidisciplinary registry of acute PE. Inclusion criteria were all pulmonary embolism patients admitted in the participating centers between 01/11 and 04/17. The measure of 100% compliance was used to allocate patients in the compliant or non-compliant groups. The primary outcome was all-cause death at 6 months. Secondary outcomes included recurrent venous thromboembolism, and major bleeding.
Results: In total, 1,285 patients were included. Treatment was not in compliance with the guidelines in 172 patients (13.4%). Four factors were found to be independently related to non-compliance with PE guidelines: Shock or hypotension, renal insufficiency, active cancer, and right ventricular dysfunction at admission. The primary endpoint of all-cause death at 6 months occurred in 62 of 172 patients (36.1%) in the non-compliant group and in 131 of 1113 patients (11.8%) in the compliant group, for a relative risk of 2.02 in the non-compliant group (95%CI: 1.45-2.81; $p < 0.001$). At follow-up, the rates of recurrent venous thromboembolism (7.0% vs. 1.3%, $p < 0.001$) and major bleeding (13.4% vs. 4.8%, $p = 0.04$) were higher in the non-compliant group as compared to the compliant group.
Summary/Conclusion: Non-compliance with guidelines was associated with worse outcomes including death, recurrent venous thromboembolism, and bleeding.

Clotting
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Thrombotic risk evaluation of factor V Leiden mutation with thrombin generation test: a retrospective study in Rouen University hospital

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Background: Factor V Leiden (FVL) mutation is the most common genetic risk factor of venous thromboembolism (VTE). In families with history of thrombosis, FVL can be present in 20 %. Thrombin generation test (TGT) evaluates the activation and inhibition of clotting system. This assay is commonly used as an evaluation tool of thrombotic risk.
Aims: The objective of this study was to evaluate thrombogenic potential of FVL in different patients: asymptomatic carriers, familial or personal history of thrombosis.
Methods: 136 patients were retrospectively included between 2015 january the 1st and 2018 june the first. All patients have had a FV Leiden mutation study in Hemostasis unit of Rouen University hospital. Among them, 40 had personal history of thrombosis: idiopathic (n=20) or provoked (n=20); 116 had only familial history of thrombosis: either in first degree relatives (n=83) or in second degree relatives (n=33). Other biological thrombophilia were excluded. Thrombin generation (TG) was realized in frozen platelet poor plasma with 1 pM of tissue factor and 4 µM of phospholipid. Pooled platelet poor plasma from healthy volunteers was used as control group (n=25).
Results: FVL mutation was associated with global increase of TG compared to controls for endogenous thrombin potential (ETP) as forand thrombin peak. No difference was observed between patients with second degree history of thrombosis and controls in ETP (1255.2±259.0 nM.min versus 1194.9±152.7 nM.min respectively). No difference was observed between patients with provoked thrombosis and patients with familial first degree history of thrombosis (ETP: 1501.0±316.4 nM.min and peak: 253.4±71.5 nM versus 1520.4±283.8 nM.min and 268.6±68.0 nM respectively). An increase of TG has been observed between idiopathic (n=20) and provoked thrombosis (n=20) (ETP: 1819.5±319.8 nM.min and peak: 332.3±55.8 nM).
Summary/Conclusion: Our results suppose an increase thrombin generation is associated with the risk of unprovoked VTE in FVL carriers. Large population prospective studies are required to validate our results and confirmed a thrombin generation cut off to predict unprovoked VTE risk in FVL.

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After One Month Of Treatment With Apixaban Pulmonary Embolism Patients Have Higher Activity Of Coagulation Factor II, As Compared With Patients Treated With Rivaroxaban And Dabigatran

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Background: Direct oral anticoagulants (DOACs) have been frequently in use for the treatment of pulmonary embolism (PE). There are still no sufficient data for direct comparison of those drugs and which would depict the differences in their influence on coagulation cascade system.
Aims: To compare the difference in thrombin (coagulation factor II) activity in PE patients after one month of treatment with either rivaroxaban, apixaban or dabigatran.
Methods: This is single-center cross-sectional study of consecutive PE patients for the period of 2014 till 2017. After initial treatment patients were randomly assigned to receive oral anticoagulation with one of the three DOACs: rivaroxaban, apixaban or dabigatran. After one month of stable therapy blood was taken at trough drug concentration for the measurement of the activity of coagulation factor II.
Results: Overall 100 consecutive patients were enrolled in the study. Among them, 36 (36%) were on rivaroxaban, 32 (32%) on apixaban and 32 (32%) on dabigatran treatment. Patients on apixaban had significantly higher factor II activity, as compared with patients on rivaroxaban and dabigatran (1.45±1.12 vs.1.13±0.92, $p<0.001$ and 1.45±1.12 vs. 1.20±0.96, $p=0.003$, respectively). The difference in factor II activity was not significant between patients on rivaroxaban and dabigatran ($p=0.869$).
Summary/Conclusion: Pulmonary embolism patients treated with apixaban, after one month of treatment, have significantly higher activity of coagulation factor II, as compared with patients on rivaroxaban and dabigatran. Higher factor II activity might be responsible for better hemostasis by thrombin activation of platelets.

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Regular physical activity and risk of recurrent venous thromboembolism (VTE) and VTE-related mortality

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Background: Venous thromboembolism (VTE) is a common disease that represents a growing public health concern. The disease is associated with several complications, such as high recurrence rates and increased risk of premature mortality. Current evidence suggests that physical activity (PA) may lower the risk of incident VTE, but the effect on recurrent disease and VTE-related mortality remains unknown.
Aims: To investigate the impact of habitual PA, assessed prior to the incident VTE event, on the risk of recurrence and all-cause mortality in a cohort of VTE-patients recruited from the general population.
Methods: A total of 786 patients with incident VTE and data on PA derived from the Tromsø Study surveys 4-6 (1994-95, 2001-02 and 2007-08) were included. Information on habitual physical activity was obtained from self-administered questionnaires, and the participants were categorized according to the PA level reported in the survey preceding the incident VTE event (inactive: <1h per week or active: ≥1h per week). Recurrent VTE and all-cause mortality were registered up to December 31, 2015. Hazard ratios (HRs) for VTE recurrence and all-cause mortality adjusted for age (as time scale), sex and body mass index were calculated using Cox regression models with the inactive group as reference. Analyses were also performed in subgroups stratified by characteristics of the index event (i.e. provoked or unprovoked VTE, and deep vein thrombosis (DVT) or pulmonary embolism (PE)). The Regional Committee for Medical and Health Research Ethics approved the study, and written informed consent was obtained from all study participants.
Results: There were 139 recurrences and 395 deaths during a median follow-up of 2.9 years. Habitual PA was not associated with the risk of recurrent VTE (HR 1.04; 95% CI 0.70-1.56). However, active individuals had 36% lower risk of mortality during follow-up (HR 0.64; 95% CI 0.51-0.79). Sub-analyses revealed that the effect was essentially similar for unprovoked (HR 0.60; 0.41-0.88) and provoked (HR 0.67; 95% CI 0.52-0.87) index events, but appeared to be largely driven by an effect on mortality following DVT (HR 0.53; 95% CI 0.41-0.70) and not PE (HR 0.89; 95% CI 0.62-1.27).
Summary/Conclusion: Our results suggest that habitual PA prior to incident VTE does not influence the risk of recurrence. However, active individuals were at a lower risk of VTE-related mortality, particularly following a DVT.

Clotting
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Determination of reference values of factor II and factor X activity levels in our laboratory for the monitoring of vitamin K antagonist therapy

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Background: Lots of diseases required oral anticoagulation by Vitamin K Antagonist (VKA) therapy. VKA therapy is closely monitored using prothrombin time (PT) expressed as international normalized ratio (INR), in order to ensure safe and effective levels of anticoagulation and to make dosage adjustment. However, PT can be influenced by clotting factor deficiency independent of VKA therapy and haematocrit > 55%, thus over- or underestimating the INR. In these cases, factor II and factor X clotting activity (FII:C and FX:C) measurements may be indicated as alternative VKA anticoagulant effect markers.
Aims: The objective of this study was to provide FX:C and FII:C reference values for VKA therapy monitoring, using our own analytical system.
Methods: 106 patients having well-balanced VKA therapy for at least 3 months and normal FV:C were included in this study. Depending of their INR value, three groups of patients were defined: INR value between 2 and 3 (n=36), INR value between 3 and 4.5 (n=35) and INR value over 4.5 (n=35).
FII:C, FV:C, and FX:C activities were determined simultaneously using Instrument laboratory reagents (HemosIL® RecombiPlasTin2G and HemosIL Factor II/V/X deficient plasma) on ACL TOP® 750 (Werfen).
Results: The 3 groups were comparable in terms of sex, age and VKA molecule. 67% of patients included received fluindione. The FII:C and FX:C values were significantly different in each group (Kruskal-Wallis test, $p<0.0001$). Mean ± standard deviation of FII:C values were 25 % ± 7 for the group with INR 2-3, 19 % ± 4 for the group with INR >3-4.5, and 14 % ± 5 for the group with INR > 4.5. FX:C values were 12 ± 2%, 10 ± 3% and 7 ± 2% in the group with INR values of 2-3, >3-4.5 and > 4.5, respectively. A non-linear correlation was observed between INR and both FII:C and FX:C (Spearman coefficient r=-0.74, p<0.0001; and r=-0.76, p<0.0001, respectively). In our laboratory, the therapeutic window for VKA therapy corresponds to INR 2-4.5. Within this therapeutic range, mean [IC95%] of FII:C were 22% [14-33%] and FX:C were 11% [7-17%]. FII:C < 14% and FX:C < 7% were associated with an overdosage of VKA.
Summary/Conclusion: Our results showed that FII:C and FX:C could be used as an alternative to INR for the measurements of VKA anticoagulation efficacy and safety. Assuming the tighter dispersion of observed values, FX:C seems more accurate than FII:C for monitoring VKA therapy. Considering the numerous different thromboplastin commercially available that have various sensibility to clotting factor deficiency and the different values proposed in the published literature, it is recommended that each laboratory determine their own FII:C and FX:C reference values used to monitor VKA therapy.

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Direct comparisons of brain natriuretic peptide and cardiac troponin I for the prediction of thirty day all-cause death and pulmonary embolism-cause death in patients with acute pulmonary embolism

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Background: Cardiac troponins (cTn) and brain natriuretic peptide (BNP) are used for the risk stratification in pulmonary embolism (PE) patients, though direct comparison of their value for the prediction of all-cause death and PE-cause death from the registries are lacking. Since patients with PE very often have a huge burden of comorbidities the prediction for all-cause death and PE-cause death might be quite different.
Aims: The aim of this study was to compare value of plasma BNP and serum cTnI levels at admission for the prognosis of all-cause and PE-cause 30-day mortality.
Methods: Among 507 patients with PE form the Serbian University Pulmonary Embolism Registry (SUPER 2015-2017) 334 and 287 patients had measured BNP and cTnI blood levels at admission. Both markers are measured in 211 patients. Brain natriuretic peptide and cTnI was measured on Siemens ADVIA Centaur system.
Results: Using the cTnI cut-off value of 0.04 ng/L, 30-day all-cause mortality rate was 19.2% vs 7.6% (p=0.006) and for PE-cause mortality 11.5% vs 2.3% (p=0.003) for comparisons of values above and under the cut-off level, respectively. Similarly, using cut-off for BNP of 100 pg/ml, 30-day all-cause mortality was 15.1% vs 2.5% (p<0.001) and PE-cause mortality 9.9% vs 0.0% (p<0.001) comparing values above and under the cut-off values, respectively. Areas under the receiver operating characteristic (ROC) curves for all-cause mortality were 0.700 (95%CI 0.614-0.786) and 0.650 (95%CI 0.560-0.740) and for PE-cause mortality 0.762 (95%CI 0.691-0.832, p<0.001) and 0.714 (95%CI 0.609-0.820) for BNP and cTnI separately, respectively. The areas under ROC curves for 211 patients who had both markers measured, were as follows: for all-cause mortality 0.787 (95%CI 0.688-0.887, p<0.001) and 0.608 (95%CI 0.488-0.729, p=0.084) and for PE-cause mortality 0.837 (95%CI 0.760-0.913, p<0.001) and 0.727 (95%CI 0.576-0.878, p=0.008) for BNP and cTnI, respectively.
Summary/Conclusion: Brain natriuretic peptide is better predictor than cTnI for all-cause death and both BNP and cTnI are respectable predictors for PE-cause death at one month in patients with acute pulmonary embolism.

Clotting
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Identification of Chinese Hospitalized Patients' Risk Profile for Venous Thromboembolism (DissolVE 1): a Nationwide, Multi-center, Observational study

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Background: Appropriate venous thromboembolism (VTE) prophylaxis for at-risk patients is not aggressively followed in China despite recommendations by the American College of Chest Physicians (ACCP) guidelines, 9th edition. Moreover, Chinese data regarding treatment pattern and outcomes is limited.
Aims: To identify the proportion of patients who received appropriate VTE prophylaxis (fully compliant with ACCP guidelines, 9th edition) among those who were discharged within 6 weeks prior to diagnosis of symptomatic VTE and were at risk of VTE, and to investigate the VTE treatment pattern 3 months after symptomatic VTE diagnosis.
Methods: Adult (≥18 years) patients discharged within 6 weeks prior to diagnosis of symptomatic VTE and patients admitted due to acute medical conditions or surgical operations and diagnosed with VTE were enrolled after obtaining consent from 40 hospitals. VTE risk was evaluated using Padua Prediction Scoring (PPS) in medical inpatients while Caprini Risk Assessment model (CRA) was used for both medical and surgical inpatients. Data regarding VTE risk, prophylaxis prescribed in the 6 weeks before VTE diagnosis, and treatment prescribed in the 3 months after VTE diagnosis were collected and analyzed based on Full Analysis Set (FAS) using SAS version 9.2 or later (SAS Institute, Inc., Cary, NC, USA).
Results: From 1203 eligible patients, 577 medical and 560 surgical patients were added into the FAS (n=1141); with 67.7%, 18.8%, and 13.5% suffering from deep vein thrombosis (DVT), pulmonary emobolism (PE), and both, respectively. CRA showed a greater number of medical inpatients to be at high risk of VTE (n=352/577; 61.0%, 95% CI 56.9–65.0) than PPS (n=267/577; 46.3%, 95% CI 42.1–50.4). However, high risk was larger in surgical (n=431/560; 77%, 95% CI 73.3–80.4) than in medical inpatients. Despite high risk of VTE as predicted by CRA and PPS, very few patients (n=60/827; 7.3%, 95% CI 5.6–9.2) received appropriate VTE prophylaxis. Among these 60, 14/267 were high risk medical (5.2%, 95% CI 2.9–8.6) and the remaining 46/560 (8.2%, 95% CI 6.1–10.9) were surgical patients. Among these 46 surgical patients, 8/114 (7.0%, 95% CI 3.1–13.4) were at medium and 38/431 (8.8%, 95% CI 6.3–11.9) were at high VTE risk. Post-diagnosis treatment was prescribed to 96.4% (n=1100/1141, 95% CI 99.7–100.0) of the patients with anticoagulation (n=1065/1141; 93.3%, 95% CI 91.6–94.6) being highly preferred. Low molecular weight heparin (LMWH) was the highest prescribed anticoagulant (n=998/1065; 93.7%). Anticoagulant therapy (mean treatment duration = 66.4±47.8 days) was completed by 456 patients. Monotherapy (n=752; 65.9%, 95% CI 63.1–68.7) with anticoagulants (n=716; 95.2%, 95% CI 93.4–96.6) was prescribed more than combination treatment (n=348; 30.5%, 95% CI 27.8–33.3), the majority of which was anticoagulants plus fibrinolytic therapy (n=115; 33.0%, 95% CI 28.1–38.3).
Summary/Conclusion: Despite the overall VTE risk being high in China, the rate of implementation of ACCP-recommended prophylaxis is low. Post-diagnosis treatment mostly consisted of monotherapy with an anticoagulant such as LMWH. Our findings emphasize the need for improving awareness among physicians for effectively managing VTE prophylaxis in China.

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Real Life Spanish Cohort Of Direct Oral Anticoagulants In Patients With Atrial Fibrillation: Refase Registry

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Background: Currently in Spain there are few cohorts with a sufficient number of patients to study the clinical profile, effectiveness and safety of direct action anticoagulants (DOACs) in real clinical practice globally and in different subpopulations.
Aims: Due to the lack of information in this area we consider the creation of the REFASE registry.
Methods: Retrospective study of patients with atrial fibrillation (AF), who initiated anticoagulant treatment with DOACs consecutively for the prevention of stroke between December 2012 and December 2016 in the south-east of Spain. The patients were followed until February 2018, registering clinical events in 99.7% of them. We collected more than 60 variables related to atrial fibrillation and anticoagulant treatment of the patient as well as comorbidities and concomitant medication. The registration obtained the approval of the CEIC.
Results: A total of 2505 patients were included. Follow-up (months) 22,7±7,4. The mean age was 76.0 ± 9.4 years (63.5%> 75 years), 52.9% were women, and 51.1% had permanent AF. With respect to thromboembolic and hemorrhagic risk, the mean CHA2DS2-VASc was 4.0 ± 0.9 (95.5% ≥2) and the average HAS-BLED 2.4 ± 0.9 (38.3% ≥3) , respectively. The presence of cardiovascular risk factors was frequent (arterial hypertension 86.8%, diabetes 31.5%), as well as vascular disease (stroke / transient ischemic attack 20.6%, heart failure 18.6%, ischemic heart disease 15,3%, peripheral arterial disease 3.1%). In a high percentage of the patients, 40,4, we found a creatinine clearance (Cockroft-Gault) <50 ml/min and a history of major bleeding in 8.8% of them. Regarding anticoagulant treatment, 43.2% of the patients had been previously anticoagulated with vitamin K antagonists, 3.9% with another direct oral anticoagulant, and the rest (56.6%), started directly. The doses of ACOD prescribed were Apixaban 2.5-5 mg (13% and 24%) Rivaroxaban 15-20 mg (17 and 23%) dabigatran 110-150mg (7 and 10%) edoxaban 30-60 (1.5% and 1, 4% mg. If we analyze these data according to renal clearance, in patients with creatinine clearance ≥50 ml / min / 1.73m2 the most used ACOD was rivaroxaban 603 (40.4%) this is maintained in patients with a clearance <50 ml / min / 1.73m2 419 (41.4%). Results were studied in the most relevant subpopulations, renal insufficiency, advanced age, valvular heart disease, heart failure, use of antiplatelet drugs. The most relevant thromboembolic and hemorrhagic events are stroke, % (events per 100 pacients-year) 3,0 (1,7) and mayor bleeding, % (events per 100 pacients-year) 5,0 (2.9). In the major bleedings, 56.2% were of gastrointestinal origin, 10.3% hematomas / ecchymoses.
Summary/Conclusion: The REFASE registry offers a very relevant information since it is a study with a large volume of patients with AF treated with ACODs. The data from this registry indicate that patients treated with ACODs have an advanced age and a high thromboembolic and hemorrhagic risk greater than that offered in the pivotals. Despite this, the rates of thromboembolic and hemorrhagic complications offer data which are similar to the international registries.

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DIRECT ORAL ANTICOAGULANTS IN REAL LIFE. Experience of a center during the year 2017. MGJ-API-2018-01.

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Background: The oral anticoagulants have recently extended their options with the introduction of the Direct Oral Anticoagulants (DOACs). The initial trials have shown that DOACs are comparable in anti-thrombotic effectiveness with warfarin and have less intracranial hemorrhage risk. The scientific evidence and the accumulated experience in clinic since the inclusion of DOACs, would justify the need of a monitored follow-up of these patients in real life.
Aims: Evaluate the incidence of complications that happen in patients being treated with DOACs because of a non-valvular atrial fibrillation, depending on the drug and its dose; as well as the adherence and its correct dosage.
Methods: Observational and retrospective study, approved by the Clinical Research Ethics Committee of Álava (Spain), and classified and codified by the AEMPS as MGJ-API-2018-01.
141 patients have been analysed since January 2017 until January 2018. The patients were interviewed for one year (initial visit, at 3, 6 and 12 months after their inclusion) and the data were collected in a digital application form, specifically prepared for this study, and included in their computerized medical history.
Results: 77 patients out of 141 patients analysed were men (54,6%). The mean age was 77,28 years, with 61% of the sample older than 75 years. Thrombotic risk (CHA_2DS_2-VAS_C) had a median of 4 points, which is much higher than median in pivotal trials. 39,7% of the patients had have a stroke before and 38% of them suffered from renal failure. 21,3% of all the patients received an inadequate dose, attending to DOACs Thecnical Sheet criteria. 36 complications occurred: 19 cases were mild bleedings while 5 were major bleedings. 3 patients had a stroke through the year. There were 4 events of oral intolerance, all of them in dabigatran group. The statistical analysis showed no significant differences between the DOACs and the complications occurred (χ^2=5,096; p=0,165), neither between dose and complications (*rho*=0,109). Throughout the year 19 interventions were performed, 11 procedures (57.9%) had low risk of hemorrhage and 8 (42.1%) high risk. All patients underwent ACOD suspension according to the perioperative protocol established at the center, and only one case of hemorrhage, requiring transfusional support, was documented. The rest did not suffer complications and the post-intervention ACOD could be reintroduced as appropriate.
Summary/Conclusion: The population studied in real life was older and had more thrombosis risk than the patients included in the clinical trials, with an expected rate of complications according to their clinical profile. Of the 36 complications registered, 24 were hemorrhagic (66.7%) and 3 cerebrovascular accidents (8.3%), without significant differences between the different DOACs. The therapeutic adherence was very high, but up to 16.3% of the patients were under-treated, similar to the evidence in the literature; These facts demonstrate that the medical follow-up of the anticoagulated patient with DOACs could optimize its management and offer a quality medical assistance.

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Myocardial infarction as a trigger of incident venous thromboembolism: Results from a case-crossover study

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Background: A bidirectional relationship exists between arterial cardiovascular disease (CVD) and venous thromboembolism (VTE). We have previously shown that patients with a myocardial infarction (MI) are at increased risk of VTE, particularly during the first months after the MI. The mechanisms behind this association are yet unclear. Moreover, the role of MI as a trigger of VTE is scarcely investigated.
Aims: To investigate the impact of acute MI as a trigger of incident VTE in a case-crossover study of VTE patients recruited from a general population.
Methods: We conducted a case-crossover study of VTE patients (n=707) recruited from the fourth survey of the Tromsø study. VTE triggers, risk factors and hospitalizations were registered during the 90-day period before the VTE (hazard period) and in four 90-day control periods. Conditional logistic regression was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE according to acute MI with further adjustment for other triggers. In Model 1, we calculated the crude OR for the association between MI and VTE. In model 2, we adjusted for immobilization and infection since these variables are potential triggers for VTE that often co-exist with MI. In model 3, we additionally adjusted for other VTE triggers including major surgery, trauma, red blood cell transfusion and central venous catheterization by entering them simultaneously and one-by-one in the regression model. We applied the KHB-method for mediation analysis in order to quantify how much the known triggers could account for the effect of MI on VTE risk. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: MI was recorded in 13 (1.8%) of the hazard periods and in 6 (0.2%) of the control periods, which yielded a crude OR of 11.9 (95% CI: 3.9-36.7). After adjustment for immobilization and infection the OR was 2.7 (95% CI: 0.6-11.2). When these variables were entered into the model one by one, the OR for MI adjusted for immobilization was 8.1 (95% CI: 2.2-30.2) whereas adjustment for infection yielded an OR of 3.9 (95% CI: 1.0-14.7). The OR was further attenuated to 2.6 (95% CI: 0.6-11.9) after addition of major surgery, trauma, red blood cell transfusion and central venous catheterization into the model. Approximately 60% of the total effect between MI and VTE was potentially mediated by concomitant infection and immobilization.
Summary/Conclusion: Our results showed that the increased VTE risk after MI to a large extent could be explained by concomitant conditions related to the MI, particularly infections and immobilization. Our findings suggest that both concomitant infection and immobilization should be considered in clinical decision making on thromboprophylaxis after MI. Further studies are required to investigate the potential discriminatory power of infection and immobilization on VTE risk in MI patients.

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Joint effects of myocardial infarction and prothrombotic genotypes on the risk of venous thromboembolism: The Tromsø Study

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Background: The risk of venous thromboembolism (VTE) is increased after a myocardial infarction (MI), particularly during the initial months after the MI. Some prothrombotic genotypes associated with VTE risk have also been associated with increased risk of MI. Whether the presence of prothrombotic genotypes further increases the risk of VTE in MI patients is scarcely investigated.
Aims: To study the joint effect of MI and prothrombotic genotypes on the risk of VTE in a case-cohort with participants recruited from a general population.
Methods: Cases with incident VTE (n=641) and a randomly selected subcohort weighted for age (n=1761) were sampled from 3 surveys of the Tromsø Study (1994-95, 2001-02 and 2007-08). DNA was isolated from blood, and participants were genotyped for rs8176719 (*ABO blood type*), rs6025 (*FVL*), rs1799963 (*Prothrombin G20210A*), rs2066865 (*FGG*) and rs2036914 (*F11*) single nucleotide polymorphisms (SNPs). Cox regression models were used to calculate hazard ratios (HR) with 95% confidence intervals (CI) for incident VTE by individual SNPs and categories of risk alleles (de Haan 5-SNP score; 0-1, 2, 3 and ≥4 risk alleles) in subjects with and without MI. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: There were 274 subjects with MI, of which 47 had a VTE. In subjects without MI, an increased risk of VTE was observed for each of the individual SNP in those with ≥1 risk alleles compared to no risk alleles. However, in patients with MI, only F11 yielded an effect on VTE risk that exceeded the sum of the individual risk components (HR 1.58 95% CI: 1.10-2.30, RERI: 0.33 95% CI: -0.66-1.31). In subjects without MI, the risk of VTE increased linearly across increasing risk alleles in the 5-SNP score (P<0.001), and subjects with ≥4 risk alleles had a 1.78-fold higher risk of VTE (95% CI: 1.37-2.31) than those with 0-1 risk alleles. There was no linear increase in VTE risk across increasing number of risk alleles among MI patients. The results of subgroup analysis with deep vein thrombosis and pulmonary embolism as outcomes were similar to the results for overall VTE.
Summary/Conclusion: Except for F11, the combination of MI and prothrombotic genotypes did not result in an excess risk of VTE, suggesting that there is no biological interaction between MI and genetic predisposition for VTE.

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Determinants and prognostic impact of diagnosis delay in pulmonary embolism

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Background: Signs and symptoms of pulmonary embolism (PE) are not specific and this can lead to a diagnostic delay. Little is known about the determinants of this delay and its prognostic implication.
Aims: The aims of the study were to identify the determinants of the diagnostic delay and to evaluate its prognostic impact in patients with a first episode of PE.
Methods: We conducted a retrospective analysis of a prospective cohort involving patients with a first episode of PE. The diagnostic delay was defined as a time from first symptom onset to diagnosis of> 3 days, corresponding of the median time in the population. Multivariable logistic regression analysis was performed to identify determinants of diagnostic delay. Prognostic implication was measured as the occurrence of 30-day all-cause mortality, haemodynamic collapse or recurrent PE.
Results: A total of 240 (47%) among 514 patients had a time from first symptom to diagnosis > 3 days. Previous deep vein thrombosis (OR 0.55, 95% Confidence Interval (CI), 0.32-0.93), immobilization (OR 0.52, 95% CI, 0.28-0.96), surgery (OR 0.31, 95% CI, 0.16-0.62), chest pain (OR 0.58, 95% CI, 0.39-0.86), syncope (OR 0.48, 95% CI, 0.23-1.01), dyspnea (OR 2.48, 95% CI, 1.57-3.91) and hemoptysis (OR 3.57, 95% CI, 1.40-9.07) were associated with diagnostic delay. Twenty-two patients (4.3%, 95%CI, 2.8–6.5) experienced an outcome event within 30 days. Among them, 15 patients (6.2% 95%CI, 3.7- 0.3) had a diagnostic delay and 7 (2.6%, 95% CI 1.1-5.4) did not (p=0.039).
Summary/Conclusion: Diagnostic delay is associated with the absence of major risk factors for PE or clinical features such as chest pain or syncope and the presence of dyspnea or hemoptysis. Diagnostic delay is associated with a worse 30-day prognosis.

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Evaluation of haemostasis perturbations in two mice models of Chronic Kidney Disease

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Background: Bleeding and thrombosis are frequent in patients with chronic kidney disease (CKD). The coexistence of these conflicting haemostatic disorders is poorly understood. We and other have previously demonstrated that indoxyl sulfate (IS), an indolic uremic toxin, induced tissue factor (TF) in both endothelial and vascular smooth muscle cells. Animal models are therefore important tools to understand haemostatic disturbances occurring during kidney diseases.
Aims: To compare the haemostasis in two mouse models of CKD: a new one, adenine diet and a well-established model, nephrectomy of 5/6th.
Methods: C57BL/6 mice were fed with 0,25% dietary adenine for 2 weeks, then with normal diet for 1 week or underwent a 5/6th nephrectomy (Nx). Serum creatinine and blood urea nitrogen (BUN) were monitored to assess renal function. IS was assayed by High Pressure Liquid chromatography. Renal inflammation and fibrosis were evaluated by hematoxylin/eosin and Sirius red staining on kidney sections, and by measuring the expression of target genes (*TNF-a, IL6, PAI-1, CCL2; TFG-b1, Col 1A1, Col 3A1, Acta-2*) by RT-qPCR. TF expression in organs was analyzed by RT-qPCR and immunohistochemistry. Plasma thrombin/antithrombin (TAT) complexes were assayed by ELISA. Tail bleeding time was measured and platelet count in plasma was performed by flow cytometry. intravital microscopy was used to determine the kinetics of platelets thrombi formation and fibrin generation at the site of injury performed by a laser on the cremaster arteries.
Results: Mice (n=8) from the adenine group showed higher levels of BUN, serum creatinine and IS compared to Nx mice (n=10) and controls (n=10). Morphological analysis and gene expression revealed more severe renal inflammation and fibrosis in the adenine group.
TF mRNA expression was up-regulated in both models in kidney tissue but not in the other organs tissues tested. Histological sections of kidney showed that TF is localized in the peritubular capillaries. TAT concentration was significantly increased (2 fold) in similar levels in plasma from adenine diet and Nx mice.
No significant difference in platelet count was observed but bleeding time increased significantly only in adenine fed mice compared to normal diet fed mice (respectively 254 ± 30s and 108 ± 19s).
Results obtained by intravital microscopy after laser–induced endothelial injury showed impaired platelet function in mice from the adenine group and an increase of fibrin generation in mice from Nx group compared to their respective control mice.
Summary/Conclusion: Adenine diet is a more severe model of CKD compared to the 5/6th nephrectomy model. TF expression and TAT concentration were increased similarly in both CKD models reflecting activation of coagulation. Bleeding was observed only in the more severe model of CKD. Adenine diet in mice recapitulate the whole spectrum of hemostasis abnormalities observed in humans.

Clotting
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Attenuation of monocytes induced procoagulant activity on CoCr surfaces with thin films of degradable polar/hydrophobic/ionic polyurethanes

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Background: Cobalt chromium (CoCr) alloy is commonly used in cardiovascular medical devices such as stents. However, it has been shown to be pro-thrombotic and pro-inflammatory with activation of platelets and coagulation and induction of leukocyte adhesion and activation. On the other hand, polyurethanes (PUs) have attracted great interest in the biomedical field because of their broad range of chemistry, tailored mechanical properties and generally favorable blood compatibility.
Aims: The aim of this work was to develop controlled thin films of novel polyurethane coatings on CoCr and to evaluate monocyte and macrophage reactivity on these surfaces.
Methods: PUs synthesis was carried out by free radical polymerization of divinyl oligomers (DVO), methacrylic acid (MAA) and methyl methacrylate (MMA) in the presence of tetrahydrofuran at 66°C for 1h and then at 110°C for 24h under nitrogen. Two PUs with different molar ratios of the above monomer were synthesized, D-PHI (degradable polar/hydrophobic/ionic) and HHHI (high hydrophobic and high ionic). Immunoglobulin G (IgG) and fibronectin (FN) binding characterization on the surfaces were carried out as these proteins are substrates for monocytes/macrophages. Monocytes procoagulant activity (PCA) was studied by a global coagulation assay, the Calibrated Automated Thrombogram (CAT) and by measurement of tissue factor (TF) activity through the ability of TF to promote factor X activation in the presence of activated factor VII using a chromogenic activity assay. TNF-α (a pro-inflammatory cytokine) and IL-10 (an anti-inflammatory cytokine) levels were quantified by specific ELISA. Both monocytes and macrophages were imaged by Scanning Electron Microscopy (SEM).
Results: Controlled and tailored thin films of PUs with a 5 µm thickness were produced. When PUs were coated on CoCr, they showed a significant reduction in the Fab binding site exposure from human IgG as well as in the cell binding and the N-terminal domains of human FN, reflecting a potentially less reactive surface towards monocytes and macrophages. This was confirmed by SEM as the surface area related to bound monocytes was significantly ($p<0.001$) reduced on PUs (55.5 ± 37.3 and 40.8 ± 22.6 µm^2 for D-PHI and HHHI, respectively, mean ± SD) compared to CoCr (232.4 ± 123.4 µm^2). Moreover, the PU coatings also prevented CoCr-induced thrombin generation by purified monocytes with a two fold decrease of the CAT velocity index ($p<0.05$). CoCr-induced PCA of monocytes was identified as TF activity. TF activity was 2 fold lower at the surface of PU-coated CoCr ($p<0.001$). CoCr-induced secretion of TNF-α and IL-10 by monocytes was also significantly reduced on samples with PU coatings relative to CoCr ($p<0.01$). Finally, macrophages activation was largely decreased by PU coatings of CoCr surfaces as demonstrated by SEM and by the reduction of TNF-α secretion compared to CoCr whereas Il-10 secretion was equal for all surfaces.
Summary/Conclusion: Biocompatibility of metallic materials can be improved by specific PU coatings that lead to a reduced global activation of monocytes and macrophages and more specifically to a reduced PCA of monocytes due to a decrease in TF activity.

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A new assay to evaluate microvesicle plasmin generation capacity: validation in disease with fibrinolysis imbalance.

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Background: Among extracellular vesicles, leukocyte-derived microvesicles (LMVs) have emerged as complex vesicular structures. Primarily identified as procoagulant entities, they were more recently ascribed to plasmin generation capacity (MV-PGC).
Aims: The objectives of this work were 1) to develop a new hybrid bio-assay combining the specific isolation of LMVs and measurement of their PGC, and compare its performance to the original method based on centrifugation 2) to validate MV-PGC in septic shock, combining increased of LMVs and fibrinolytic imbalance.
Methods: Control samples were generated from plasma spiked with LMVs featuring different levels of PGC. The MV dependency of the test was demonstrated using electron microscopy, high speed centrifugation, nanofiltration and detergent-mediated solubilization and the MV-PGC specificity using plasmin-specific inhibitors, or antibodies blocking elastase or uPA. The validation of the new immunomagnetic bio-assay was further performed in patients with septic shock.
Results: Using control samples, we demonstrated that CD15-beads specifically extracted LMVs. Thanks to a reaction booster (e-ACA), we showed that the assay was more sensitive and reproducible than the original method and exhibit a good repeatability, inter-operator and inter-experiment reproducibility. In patients with septic shock, we showed that MV-PGC values were significantly lower in septic shock patients who died compared to patients who survived, both at inclusion and 24h later (1.4 [0.8-3.0] *vs* 3.1 [1.7-18] $A_{405}\times10^{-3}$/min, p=0.02; 1.4 [1-1.6] *vs* 5.2 [2.2-16] $A_{405}\times10^{-3}$/min, p=0.004). Interestingly, combining both MV-PGC and PAI-1 in a ratio significantly improved the predictive value of PAI-1.
Summary/Conclusion: This strategy, a hybrid capture bioassay to specifically measure LMV-PGC using for the first time, opens new perspectives for measuring subcellular fibrinolytic potential in clinical settings with fibrinolytic imbalance.

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A simple, inexpensive and robust assay to measure levels of negatively charged phospholipids – a modified PPL assay

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Background: Growing evidence support a role of extracellular vesicles (EVs) in haemostasis and thrombosis partly due to exposure of negatively charged procoagulant phospholipids (PPL) which facilitates coagulation activation. Current clotting assays uses resource-demanding chemically phospholipid-depleted plasma to measure PPL activity in FXa-dependent clotting assays.
Aims: We aimed to modify the FXa-dependent clotting assay to measure PPL activity by substituting phospholipid-depleted plasma by EV-depleted plasma (EVDP) obtained by stepwise centrifugations with final ultracentrifugation (100 000 x g for 1 hr), and to introduce a standardized PPL reagent to allow for comparisons of results between laboratories.
Methods: Blood was collected from healthy volunteers. Clotting tests were carried out in duplicate on a StarT4 instrument (Diagnostica Stago) by pre-warming a mixture of 25 µl test sample and 25 µl EVDP for 2 min at 37ºC, and the reaction was initiation by an assay buffer containing bovine FXa. A commercially available standardized phospholipid source was used (UPTT, BioData Corporation, Horsham, PA, USA). Assay properties of our modified assay were compared to a commercial PPL assay (STA®-Procoag-PPL assay, Diagnostica Stago, Asnières sur Seine Cedex, France).
Results: The two PPL assays displayed similar sensitivity to exogenously added standardized phospholipids (UPTT) and the PPL activity measured by the two assays in plasma samples from healthy volunteers (n=10) were highly correlated ($r=0.97$ $p<0.0001$). The intraday- and between-days coefficients of variation (CV) varied between 2-4% depending on the PPL activity. The modified PPL activity was insensitive to increased postprandial lipoproteins in plasma after a standard fat tolerance test and to tissue factor-positive EVs isolated from whole blood exposed to LPS-stimulation (5 ng/ml) and PMA (30 ng/ml) for 4 hrs. The PPL activity in the modified assay was inversely associated with Annexin V^+ EVs determined by flow cytometry ($r=-0.55$, $p<0.001$).
Summary/Conclusion: The modified PPL assay performed equally to the comparator and was insensitive to postprandial lipoproteins and TF^+ EVs. The modified clot-based PPL assay had low assay costs, which make it suitable for large-scale applications.

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Coagulation factors FVIII, FXI, and FXII and outcomes after first ischemic stroke in PROSCIS-B

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Background: Coagulation factors VIII and XI have been implicated in risk of first ischemic stroke and myocardial infarction. The relationship between factor XII and thrombosis is less clear, as study results are heterogeneous. Though recurrence risk is high following ischemic stroke, little is known about risk factors for secondary events.
Aims: Our aim was to elucidate the role of coagulation factors VIII, XI, and XII in cardiovascular outcomes and mortality after first stroke using data from the PROSpective Cohort with Incident Stroke Berlin (PROSCIS-B).
Methods: The PROSCIS-B followed participants aged 18 and older after first mild to moderate ischemic stroke event for a median of three years until occurrence of recurrent stroke, myocardial infarction or all-cause mortality (combined endpoint). High coagulation factor activity levels (p75) were compared to low/normal levels, and quartiles were used to assess dose response. We used Cox proportional hazards models adjusted for age, sex, and cardiovascular risk factors to estimate hazard ratios (HRs). We additionally calculated the relative excess risk due to interaction (RERI) for the FXI and FXII exposure categories.
Results: In total, 92 events occurred in 570 included participants, resulting in an absolute risk of 6.5/100 person-years. High FVIII showed the strongest relationship with the combined endpoint (HR=2.05, 95%CI 1.28-3.29). High FXI levels were also associated with an increase in relative risk (HR=1.80, 95%CI 1.09-2.98) and quartile analyses indicated this relationship is dose-responsive. Contrarily, high FXII levels were not significantly associated with the combined endpoint (HR=0.86, 95%CI 0.49-1.51). We observed no clear evidence of biological interaction between levels of FXI and FXII on the outcome.
Summary/Conclusion: High levels of coagulation proteins FVIII and FXI are associated with an increased risk of combined cardiovascular/all-cause mortality endpoint in the three-year period after first ischemic stroke. High FXII levels did not increase risk for a secondary event.

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Frequency of FV Leiden (R506Q), prothrombin 20210G>A and MTHFR 677C>T variants in a group of pediatric patients with stroke from Serbia

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Background: Stroke is heterogeneous life treating manifestation that can cause long term disability in children, with incidence 2-13/100000 live births per year. There are three primary types of pediatric stroke: arterial ischemic stroke (AIS), cerebral sinovenous thrombosis (CSVT) and hemorrhagic stroke (HS). Theoretically, children with hereditary thrombophilia have increased risk of stroke as their body has the increased tendency to form intravascular blood clots. Most commonly described genetic variants of hereditary thrombophilia with potential role in this condition are factor V (FV) Leiden (R506Q), prothrombin 20210G>A and MTHFR 677C>T.
Aims: Thrombophilia testing is recommended by Perinatal/Paediatric Scientific Sub-Committee of the International Society of Thrombosis and Haemostasis. Purpose of this study was to investigate the incidence of FV Leiden (R506Q), prothrombin 20210G>A and MTHFR 677C>T variants in a group of pediatric patients with AIS, CVST and HS.
Methods: DNA samples were extracted from peripheral blood lymphocytes of 51 children (30 with AIS, 11 with CSVT and 10 with HS). Simultaneous qualitative in vitro detection of FV Leiden (R506Q), prothrombin 20210G>A and MTHFR 677C>T variants was performed by PCR-ARMS method using ELUCIGENE™ TRP commercial kit (Elucigene Diagnostic, Manchester, UK).
Results: Final allelic frequencies in analyzed group for FV Leiden (R506Q), prothrombin 20210G>A and MTHFR 677C>T were 5.89% (6/102), 0.98% (1/102) and 35.29% (36/102), respectively. No homozygous variant was found, except for MTHFR 677C>T in 7.84% (4/51) patients. In heterozygote carriers incidence was: 11.76% (6/51) for FV Leiden (R506Q), 1.96% (1/51) for prothrombin 20210G>A and 54.90% (28/51) for MTHFR 677C>T. In patients with AIS, these frequencies were: 1.66% (1/60) for FV Leiden presented in 3.33% (1/30) heterozygous carrier and 33.33% (20/60) for MTHFR 677C>T variant presented in 10% (3/30) homozygous and 46.67% (14/30) heterozygous carriers. In HS patients frequency of FV Leiden (R506Q) variant was 15% (3/20) presented in 30% (3/10) heterozygous carriers; prothrombin 20210G>A variant was detected in only one heterozygous carrier, with prevalence of 5% and frequency of MTHFR 677C>T variant was 45% (9/20) presented in 10% (1/10) homozygous and 70% (7/10) heterozygous carriers. In cohort with CSVT frequency of FV Leiden (R506Q) variant was 9.09% (2/22) presented in 18.18% (2/11) heterozygous carriers and frequency of MTHFR 677C>T was 31.82% (7/22) presented in 63.64% (7/11) heterozygous carriers.
Summary/Conclusion: Results of this study showed that only FV Leiden (R506Q) variant had higher prevalence of in a group of patients with stroke, compared to healthy control group. In addition, higher frequencies of FV Leiden (R506Q) and prothrombin 20210G>A was detected in patients with HS. Children with CSVT had higher frequency of FV Leiden (R506Q) variant. In group with AIS there were no difference found compared to healthy controls. Despite the fact that these results must be statistically proven on bigger sample, our study showed the importance of using these genetic tests.

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Management of high-risk pregnant patients on anticoagulant treatment

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Background: Thrombophilia increases the risk of thrombotic and pregnancy complications. The increased thrombin formation may cause the development of microthrombosis at the site of implantation of the blastocyst or later the impairment of the blood flow. Prophylaxis with low molecular weight heparin (LMWH) has anticoagulant, immunomodulatory and also anti-inflammatory effect. According to the current guidelines, in such high-risk pregnant women, LMWH is preferred over other anticoagulant drugs.
Aims: To assess the results of the monitoring of selected changes of haemostasis and its use in the management of high-risk pregnant patients on anticoagulant treatment.
Methods: Women with high-risk pregnancy (past history of pregnancy loss, preeclampsia or other pregnancy complication, thrombosis, thrombophilia) were included in this study. Selected parameters of haemostasis were measured in 5 intervals of blood sampling during pregnancy and postpartum period.
Results: Dose of LMWH was modified according to the results of the tests including standard coagulation tests, quantitative analysis of D-dimer levels, anti-Xa activity, rotational thromboelastometry and other tests of special haemostasis, and with regards to the clinical state of the patient.
Summary/Conclusion: Pregnancies were successful without any life-threatening thrombotic and pregnancy complications.
Key words: thrombophilic state, anticoagulant thromboprophylaxis, haemostasis, high-risk pregnancy
Acknowledgement: Authors thank the support of the projects of the Scientific Grant Agency (Vega) 1/0168/16 and Agency for the Support of Research and Development APVV-16-0020. This study complies with the Declaration of Helsinki and informed consent of the patient was obtained.

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Board No. 150

Inherited and acquired risk factors of thrombosis – a single-centre experience

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Background: The development of venous thromboembolism (VTE) and arterial thrombosis is associated with the presence of inherited and acquired risk factors. However, there are many unresolved questions. In some of the individuals, despite the coincidence of several prothrombotic risk factors, the thrombotic event does not occur. On the contrary, in some patients, the thromboembolic episode can be diagnosed without known provoking factor.
Aims: The analysis of the impact of inherited and acquired risk factors on the development of arterial and venous thrombosis.
Methods: The authors analyzed risk factors and circumstances of the development of thromboembolic events in subjects enrolled in the National Centre of Haemostasis and Thrombosis in Slovak Republic.
Results: In this complex study, the authors confirmed the influence of the acquired risk factors on the development of arterial and venous thrombosis. In the selected groups of patients, they also assessed the impact of the acquired thrombophilic changes of haemostasis, such as the increased activity of coagulation factor VIII, protein S deficiency and antithrombin deficiency on the development of thrombotic complications.
Summary/Conclusion: The results of these laboratory tests can be used in the targeted management of the primary and secondary thromboprophylaxis.
Key words: inherited and acquired thrombophilia, thromboembolic event, thromboprophylaxis
Acknowledgement: We would like to thank the support of projects of the Agency for the Support of Research and Development (APVV) APVV-16-0020 and Scientific Grant Agency (Vega) 1/0168/16.
This study complies with the Declaration of Helsinki and informed consent of the patient was obtained.

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Micro-ribonucleic acid in high-risk pregnancy

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Background: The presence of inherited or acquired thrombophilia increases the risk of thrombotic and pregnancy complications. In such high-risk pregnant patients, according to the current 9th Guidelines of the American College of Chest Physicians, the administration of low molecular weight heparin is preferred over other types of anticoagulation. Quantitative detection of the expression of selected molecules of microRNA regulating the development of placenta during the periimplantation period and later during pregnancy could play a role in the management of anticoagulant thromboprophylaxis.
Aims: To evaluate the results of the analysis of micro-ribouncleic acid (microRNA) and the use of such detection in the management of high-risk pregnant patients.
Methods: Authors analyzed the samples of high-risk pregnant patients with the past history of thrombotic and/or pregnancy complications and presence of thrombophilia and assessed the dynamics of the quantitative expression of the selected molecules of microRNA during pregnancy and postpartum period.
Results: Using this abstract, we report on the preliminary results of our analysis of microRNA that were compared to changes in haemostasis of the tested patients.
Summary/Conclusion: Changes in microRNA expression in the correlation with clinical state and changes in the parameters of haemostasis may be important in the management of anticoagulant thromboprophylaxis.
Key words: microRNA, management, high-risk pregnancy
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High-risk pregnant women and genetic changes in the expression of deoxyribonucleic acid

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Background: The inherited or acquired thrombophilic state aggravates the risk of development of thrombotic and pregnancy complications. Except changes in the genetic material responsible for the development of thrombophilia, there are several further polymorphisms predisposing to the development of preeclampsia, repeated pregnancy loss, placental abruption or intrauterine growth restriction. According to the results of one of the recent meta-analyses, the administration of low molecular weight heparin increased the success rate of high-risk pregnancies and is recommended mode of anticoagulation during pregnancy.
Aims: To evaluate the results of deoxyribonucleic acid (DNA) analysis and their use in the management of high-risk pregnant patients.
Methods: Authors analyzed the samples of the patients with the past history of the thrombotic and/or pregnancy complications and thrombophilic state and detected the presence of the selected genetic polymorphisms of DNA.
Results: In some of the patients, we confirmed the presence of selected high-risk polymorphisms of DNA.
Summary/Conclusion: The presence of genetic changes in the correlation with the actual clinical state and changes of haemostasis could contribute to the effective management of anticoagulant thromboprophylaxis.
Key words: genetic polymorphism, anticoagulant thromboprophylaxis, high-risk pregnancy
Acknowledgement: We would like to thank the support of projects of the Agency for the Support of Research and Development (APVV) APVV-16-0020 and Scientific Grant Agency (Vega) 1/0168/16.
This study complies with the Declaration of Helsinki and informed consent of the patient was obtained.

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Rationale and update of Hokusai post-VTE study: a follow-up study on long-term outcomes of venous thromboembolism in patients treated with heparin/edoxaban vs heparin/warfarin

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Background: Post-thrombotic syndrome (PTS) and post-pulmonary embolism syndrome are long-term complications of deep vein thrombosis (DVT) and pulmonary embolism (PE), respectively. Both syndromes are associated with decreased quality of life and curable therapeutic options are limited. Hence, prevention of PTS and post-PE syndrome is of great importance. For development of PTS, it is known that poor quality treatment with vitamin K antagonists (VKA) - subtherapeutic INR - is a risk factor. Direct oral anticoagulants have a more stable pharmacologic profile than VKA. We hypothesize that treatment of venous thromboembolism (VTE) with the direct oral anticoagulant edoxaban leads to improved thrombus resolution and thereby less long-term sequelae, translating into a lower incidence of PTS and better PE- and DVT-related quality of life compared to treatment with heparin followed by VKA.
Aims: To assess the cumulative incidence of PTS after acute DVT and to assess the long-term quality of life following DVT and/or PE, in patients treated with heparin plus edoxaban in comparison to patients treated with heparin plus warfarin.
Methods: A subset of centers that participated in the original Hokusai VTE trial (2010-2012) were invited to participate in this follow-up study. In the current study we collect data of previously enrolled patients at least 5 years after the index VTE. Patients with an index DVT will be asked to complete the validated generic (SF-36) and VEINES-QOL questionnaires to assess the (venous disease-specific) quality of life. The Villalta score will be used to assess PTS. The objectively and subjectively obtained Villalta score – known as the Patient Reported Villalta - will be compared. Among patients with an index PE, the qualiy of life will be assessed by the SF-36 and pulmonary embolism specific (PEmbQoL) questionnaires. Patients diagnosed with both PE and DVT at index will be examined for PTS and will be asked the complete all questionnaires.
Results: The study protocol was approved in Amsterdam in October 2016. 77 centers were invited for this follow-up study. Centers were selected based on feasibility and number of recruited patients and quality of data in the original Hokusai VTE trial. Of these 77 invited centers, 38 agreed to participate in the current study. The first patient was included in April 2017 in the Academic Medical Center in Amsterdam. In all centers, ethical approval is required in order to conduct the follow-up study, even though follow-up can be as non-invasive for patients as completing questionnaires. In some centers, the protocol was declined by the ethical committee since it was considered an interventional trial. Up to date, 164 patients are included in 10 centers the Netherlands, Belgium, Germany, Canada, Italy and Norway. We hope to start recruitment in France, Australia and New Zealand soon.
Summary/Conclusion: The Hokusai post-VTE study is an important study that investigates the effect of treatment of acute VTE with heparin plus edoxaban in comparison to heparin plus warfarin, on long-term outcomes such as PTS, post-PE syndrome and quality of life. So far, 164 patients have been included in 10 centers and more centers are in the process of ethical approval.

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Board No. 154

Influence of arterial hypertension on different venous thrombosis types

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Background: Venous thrombosis (VT), including deep vein thrombosis (DVT) and pulmonary embolism (PE) is one of the leading causes of morbidity and mortality worldwide. Although it is classified into provoked and unprovoked type one of the questions arising from knowledge about ethiopathogenesis of this disease is can we consider all venous thrombosis as provoked ones, and that is why recognition of all potential risk factors is of great interest. There are conflicted data regarding influence of arterial hypertension on VT risk and studies, taking into account different types of venous thrombosis, could help to lighten the role of this link in VT chain.
Aims: To investigate the influence of the arterial hypertension on the risk of various venous thrombosis type development.
Methods: The research involved 986 participants, classified in two groups: a group of patients that previously had deep vein thrombosis or pulmonary embolism and healthy control group. Participants were further classified to the participants with or without hypertension. The group of patients was also classified to the ones with provoked and spontaneous (unprovoked) venous thrombosis. In order to estimate the VT risk we used unconditional logistic regression analysis to calculate odds ratios (ORs) with 95% confidence intervals (CIs). The analysis was adjusted to "confounding" factors in order to avoid the bias within the obtained results and to assure the interpretation of the results is valid. An informed consent was obtained from all participants and approved by the Ethical Committee of Clinical Center of Vojvodina.
Results: We had 486 participants in patient group, among which 272 (56%) had unprovoked VT and 214 (44%) had provoked VT, and 500 healthy controls. The presence of hypertension was higher in the group of patients compared to the control group (41% vs. 29%). Significantly higher percentage of people with hypertension were present in the group of patients with unprovoked VT compared to a group of patients with provoked VT (61% versus 16%; p = 0,000). Observing the effect of arterial hypertension on the risk of VT among all patients, fully adjusted OR was 1.64 (CI 0.73-3.67). Within the group with provoked VT fully adjusted OR was 0.45 (0.13-1.54), but within the group with unprovoked venous thrombosis, fully adjusted OR was 2.93 (CI 1.13-7.60).
Summary/Conclusion: Arterial hypertension triples the risk of spontaneous venous thrombosis occurrence.

Clotting
P202
Board No. 155

Thrombosis of v.portae and v.lienalis in pregnanat woman with thromboophilia – Case study

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Background: Pregnant woman 28 years old, in gestational week of 34 with diagnosed thrombosis of v.portae has a congenital thrombophilia following the type of heterozygous mutation of the FV Leiden gene and the burden of family anamnesis in the sense of thrombosis.
Aims: The patient was monitored in the sense of diagnosis and an adequate therapy.
Methods: Doppler echosonography of the portal system, blood vessels of the lower limbs and the heart Color doppler.
Results: Due to an evidently diagnosed thrombus and newly developed thrombosis at the root of v.lienalis, and the compressive uterine syndrome, the increase in D-dimer of 9.23 and the prophylactic value of Anti Xa (on two occasions 0.41 IU/ml and 0,25 IU/ml) the patient had to receive Fragmin 7500IU twice daily .Control Anti Xa 0,84 , AT III 78%. Doppler of v.portae : no signs of thrombosis in the system of v.portae.
Summary/Conclusion: The pregnant woman had a successful vaginal delivery in a week of 40, on which occasion she gave a birth to a live female child of weight of 3300 g. There were no postpartal complications. Hospital release was followed with a therapy of Fragmin 5000 IU twice daily and with an indication for haematological control after a month of delivery.

Clotting
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International Pediatric Thrombosis Network: advancing the field of pediatric thrombosis

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Background: Thromboembolic events (TEs) are increasingly recognized in children resulting from increased number of children with underlying diseases, medical and technologic developments and increased awareness. However, pediatric TEs remain relatively rare and prospective management studies are lacking. Current guidelines for clinical care are based on low evidence and mainly extrapolated from adult studies. Larger, international, higher quality clinical trials studying the efficacy and safety of direct oral anticoagulants are under way. However, many important questions will not be answered by industry trials such as the natural history of pediatric arterial and venous TE, optimal diagnostic modalities, and effective and safe treatment of pediatric TE in special populations, such as neonatal renal vein thrombosis (RVT). Much further research is needed to improve evidence in order to promote the best care for children with TE. As the number of patients per center is limited, international collaboration is essential to reach that goal. To empower international research collaboration, the International Pediatric Thrombosis Network (IPTN) has been initiated at the International Society of Thrombosis and Haemostasis (ISTH) congress in Berlin in 2017.
Aims: The aims of the IPTN include 1) development of an international trial network to conduct epidemiological research, to link academic and industry partners to initiate and conduct clinical trials, and to incorporate translational research into clinical trials, 2) harmonize clinical care via educational tools, lectures and publications.
Methods: An IPTN steering committee has been established and a core group of participating centres identified. Furthermore, a prospective pediatric thrombosis registry has been developed for a survey on the epidemiology of pediatric thrombosis worldwide, using the REDcap database, provided by the ISTH. In addition, this registry will be supplemented by additional data collection for future research projects. Every centre taking care of neonates and/or children with TE is invited to participate in the IPTN and can include patients in the general thrombosis registry and in the various research projects.
Results: The survey collects data about thrombotic events in children (0-18 years old), including age at diagnosis, gender, type of thrombosis, location, risk factors and treatment of thrombosis. The first research project is about neonatal RVT and will study the epidemiology and management of neonatal RVT worldwide as well as the risk factors for recurrent thrombosis and long-term renal sequelae to define patients at risk, which may benefit from antithrombotic therapy.
Summary/Conclusion: IPTN is a worldwide group of pediatric thrombosis experts whose ultimate goal is to bring the best treatment to children with thrombosis. Therefore, we have developed a prospective pediatric thrombosis registry and will perform collaborative research of all types to address unmet needs and further improve diagnostic and treatment strategies in children with thrombosis.

Clotting
P204
Board No. 157

Coagulopathy and Pregnancy - Overview of Case

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Background: There is not a state of the human body that is more interesting than pregnancy. This is due to the fact that there is a battle for two life simultaneously. In order to take a proper stance on the subject of the treatment of coagulopathies, we have to recognise the physiological changes that happen in the body during pregnancy, like: hypercoagulability, the decrease in the number of platelets (hemodilution, placenta previa). The incidence for thrombosis during pregnancy increases.
Aims: Display a timely-discovered case of Papain positive pregnancy with an AT III deficiency, presence of Fibrinogenolysis inhibitors and hyperlipidemia.
Methods:
LISS – COOMBs cards for an indirect Coombs test and Papain test by DIAMED,
Screening tests for hemostasis (PT, TT, APTT) with a coagulometer by BENC ELECTRONIC 4,
Number of platelets,
Immunochemical determination of ATIII,
AFA (IgG and IgM),
Echo Doppler,
Level of lipids
Since the beginning of the fifth month, the patient received low molecular weight Heparin (Clexan 40mg) until the end of the pregnancy.
Results:
The patient is diagnosed with a hyperthrombotic state with:

- ATIII deficiency (0,12 g/L),
- Platelets 128x10/L,
- Fibrinogenolysis inhibitors (18%)
- AFA (elevated levels of IgG and IgM),
- Echo Doppler (decreased flow),
- Cholesterol 8,5 mmol/l,
- Coombs test, negative for the entire duration.

Since the beginning of the fifth month, the patient received low molecular weight Heparin (Clexan 40mg) until the end of the pregnancy.
After delivery, the results normalized:

- ATIII (0,3 g/L),
- Platelets 172x10/L,
- Cholesterol 5,6 mmol/l.

Summary/Conclusion: The pregnancy ended successfully with the close monitoring of all the parameters and a treatment with Clexan. These same parameters were most probably the reason for the first pregnancy resulting in a miscarriage.

Clotting
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Board No. 158

Thrombotic events associated with central venous catheter in patients admitted at a Pediatric Intensive Care Unit of Sao Joao University Hospital

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Background: Although central venous catheters (CVC) facilitate care for patients in intensive care units, catheter occlusions and catheter-related thrombosis (CRT) are common complications with a potential for significant morbidity. Widely divergent incidence rates of CRT in pediatric patients have been reported, from 1.7% to as high as 81.0%. The presence of CVC itself is the single most important risk factor for deep venous thrombosis (DVT) in children, accounting for 85% of DVT in this population. Despite that, there is a lack of consensus about the need for thromboprophylaxis in children with CVC.
Aims: To determine the incidence of CRT in children admitted in a Paediatric Intensive Care Unit (PICU) during 2017.
Methods: We performed a retrospective analysis of children with CVC that were admitted in the PICU of Sao Joao University Hospital, from January to December 2017. Age, sex, position of CVC, incidence of thrombotic events during the admission period and its location were evaluated.
Results: In PICU, 169 children with CVC were admitted. The median age was 14 years old, and 67% were male. Only 21 (12,4%) patients were under anticoagulation: 9 patients had been submitted to cardiac surgery and the other 12 presented identifiable hypercoagulable states. Only 3 thrombotic events were registered (1.8% incidence rate): 3 DVTs were confirmed with Duplex ultrasound, in patients under 2 months of age, with no previous anticoagulation. CVCs were inserted in the right femoral vein and the DVTs were also located in the right femoral/iliac vein.
Summary/Conclusion: Pediatric critical care practitioners should recognize the importance of venous thromboembolism in critically ill children to allow early identification and treatment. Thrombosis in children with CVC is an important complication that can lead to increased morbidity. We observed a CRT incidence rate of 1,8% in accordance with previous international studies. It seems to exist an association between the location of insertion of the CVC and the occurrence of the thrombotic event. In a recent review of infants younger than 1 year with CVC, it was reported that DVT were more commonly associated with CVC in the femoral veins than in the jugular or subclavian veins. These results show the necessity of additional investigation of measures to prevent CRT.

Clotting
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Board No. 159

Acquired Factor XI Inhibitor interfering with the essays of intrinsic pathway factors: a case report.

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Background: Factor XI is a dimeric glycoprotein of coagulation formed from two identical subunits united by a disulfide bridge. Factor XI deficits, inherited or acquired, exist but are rare. The acquired deficit can be observed in the presence of a specific inhibitor directed against factor XI, but inhibitors of contact factors are particularly rare.
Aims: exploration of prolonged APTT (activated partial thromboplastin time) on the sample without hemorrhagic symptomatology
Methods: We report the case of child G.A aged 12 known for nephrotic cortcicorésistant syndrome in terminal renal failure sent to the hemostasis laboratory of our department for exploring of prolonged APTT. There was no history of personal and familial bleeding or altered coagulation tests. The APTT was tested with two different reagents, containing kaolin and ellagic acid as an activator. A mixing study was performed. In the absence of hemorrhagic symptomatology, we have performed lupus anticoagulant. An assay of the factors of the intrinsic pathway represented by factor VIII, XI, XI, XII has been done.
Results: The laboratory tests showed a PT to 95%, prolonged APTT to 140 Sec (reference range 26- 35 Sec) with reagent containing kaolin and to 90 Sec (reference range 24- 32 Sec) with reagent containing acid- ellagic which could not be corrected by mixing with normal plasma. The presence of lupus anticoagulant was suspected, but the results were not in favor, intrinsic pathway factors essay revealed a factor VIII: C, IX: C and XI: C less than 1% (reference range 60- 150 %). Increasing dilutions of patient plasma gave a total correction of factor VIII: C, and factor IX: C at the 1/80 and 1/320 dilutions respectively, except for the factor XI: C. the rate of factor XI has not been corrected even at the highest dilutions (1/2560) evoking an acquired deficit in factor XI. The patient was remotely controlled, the APTT remained prolonged at 76 Sec (reference range 26- 35 Sec) with factor VIII: C 100%, IX: C 91%; XII: C 73% and factor XI: C lower than 1%. negative lupus anticoagulant and the index of circulating anticoagulant (ICA) at 31% have been found . The search of circulating antibodies, anti-Factor XI was positive at 16 Bethesda units. The most likely diagnosis was an antibody anti-factor XI interfering with the assays of intrinsic pathway factors
Summary/Conclusion: Inhibitors anti-factor XI are very rare, sometimes, their discovery is fortuitously on the occasion of a hemostasis assessment.

Clotting
P207
Board No. 160

Protein C deficiency - a case report

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Background: The inherited thrombophilias are a heterogeneous group of genetic disorders associated with an elevated risk of venous thromboembolism(VTE). Causes of inherited thrombophilia include the factor V Leiden mutation, the protrombin gene mutation, dysfibrinogenemia, and deficiencies of protein C, protein S, and antithrombin III. The function of protein C is to inactivate factor Va and factor VIIIa.
Aims: Protein C deficiency by plasma level alone is found in 1 in 200 to 1 in 500 persons in the general population. However, many affected individuals remain asymptomatic throughout life. Protein C deficiency is present in approximately 2-5% of patients presenting with VTE. This studies have demonstrated an increased risk of recurrent venous thromboembolic disease in patients with protein C deficiency.
Methods: A 55-year-old woman got pain in the left hypohondrium accompanied by fever. She was hospitalized and during her stay in the hospital she started complaining about pain in her left lower leg. The following methods were used:CT of the abdomen and thorax, Colour doppler sonographic screeninig of the circulation of the lower extremities, Ehocardiogpraphy of the heart, Laboratory tests (coagulatory and biochemical).
Results: A CT of the abdomen and thorax was performed and it showed the following findings: a) The scanned part of the chest shows a small pleural effusion on the left side, with small plate-like atelectasis; b) On the level of mediastinum trunkus pulmonalis with defects in filling the lobar and segmental parts which points to pulmonary thromboemboly; c) Abdominal aorta with defects in filling (possible thrombosis) in the length of 5,4sm – 1,8sm proximally from trunkus celiacus; d) Liver, gallbladder and pancreas with normal findings; e) Spleen with diffuse hypodense zones with different sizes which do not show colouring after application of i.v. contrast and point to infarct zones; f) The other organs in the small pelvis are KT regular. A colour Doppler sonographic screening of the circulation of the lower extremities showed DVT of the left lower leg. EKG and Ehocardiogpraphy of the heart with normal findings. Laboratory tests (coagulatory and biochemical) with the following findings: lightly increased cholesterol 5,45 (3,50 – 5,20), LDH 660 (208 – 378), D-Dimeri positive (3200 ng/ml), PT, aPTT and TT point to hypercoagulation condition. Diagnosis: DVT of the lower leg, Pulmonary thremboemboly, Thrombosis of the abdominal aorta, Infarct of the spleen. The therapy was as follows: LMWH (Low Molecular Weight Heparin) in therapeutic doses together with oral anticoagulant therapy for 10 days until achieving a therapeutic INR (target 2,5; range 2,0-3,0) as well as acetylsalicylic acid of 100 mg per day. The control tests performed after one month show slight regression in relation to the previous CT findings. Further tests for exclusion of neoplastic processes were carried out as well as genetic tests for hereditary thrombophilia and tests for acquired thrombophilia. A heterozygous deficit of Protein C was determined.
Summary/Conclusion: The risk of VTE increases with age and among heterozygotes thrombosis is unusual before age 20 years. Treatment of a patient with protein C deficiency depends upon the individual patient's risk of thromboembolic disease. Patients that have had multiple thromboembolic episodes or are at high risk of further episodes may be considered for long-term oral anticoagulation therapy.

Clotting
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Evaluation of thrombotic risk in antithrombin deficiency variant by thrombin generation assay

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Background: Antithrombin (AT) deficiency is the first inherited thrombophilia describe by Egerberg in 1965. This coagulation abnormality is due to various gene mutations in SERPINC 1 gene. AT deficiencies are divided into type 1 (quantitative decrease) and type 2 (functional activity decrease) deficiencies. Among type 2 deficiencies, there is 2 subtypes: “reactive site” (RS) or “heparin binding site” (HBS). In this last variant, thrombotic risk is lower than in RS variant or quantitative deficiencies. However, one particular mutation (AT Budapest) generates a HBS variant of AT deficiency but is associated with a high risk of thrombosis
Aims: The objective of this study is to evaluate the thrombotic risk of HBS variant AT deficiency by thrombin generation assay.
Methods: Patients were recruited in Hemostasis unit of Rouen University hospital. They have had a biological thrombophilia study because a personal or familial history of thrombosis. 31 patients with AT deficiency were enrolled: 13 with type 1 AT deficiency, 13 with type 2 AT HBS, 4 with AT Budapest and 1 Type 2 reactive site. Other causes of congenital thrombophilia were excluded. Thrombin generation (TG) was realized in frozen platelet poor plasma with 1 pM of tissue factor and 4 µM of phospholipid. Pooled platelet poor plasma from healthy volunteers was used as control group (n=25).
Results: AT deficiency was associated with global increase of TG compared to control for endogenous thrombin potential (ETP) and thrombin peak. We observed a significant increase of ETP in type 1 AT compared to Type 2 HBS (ETP: 2152.7±531.5 nM.min versus 1620.2. ± 442.6 nM.min) but no significant difference for thrombin peak (275.9±65.7 nM versus 245.1±76.5 nM). An important increase of thrombin generation was observed in AT Budapest (ETP: 2580.6±319.3 nM.min and peak: 334.1±39.4 nM) compared to others AT HBS deficiencies. For type 2 RS dficiency, thrombin generation was 2204.6 nM.min and 312.0 nM for ETP and peak respectively.
Summary/Conclusion: Our results suggest that thrombin generation increase is more important in AT Budapest compared to others AT HBS deficiencies. This mutant appears to have the same thrombin generation curve than type 1 AT deficiency illustrating the thrombotic risk of AT HBS Budapest. A larger population study could confirm if thrombin generation differentiate AT HBS and AT Budapest.

Clotting
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Board No. 162

Interest of IgG and IgM anti-prothrombin autoantibodies in the exploration of anti-phospholipid syndrome: a 5 years retrospective study

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Background: Non-conventional anti-phospholipid autoantibodies have been described in patients presenting clinical manifestations highly suggestive of anti-phospholipid syndrome (APS) but persistently negative for conventional markers. Among them, the detection of autoantibodies against prothrombin, which is involved in the coagulation cascade, has been proposed to improve the diagnosis and thus the management of these patients
Aims: The aim of the study was to retrospectively evaluate the interest of IgG and IgM anti-prothrombin antibodies (aPT) in the exploration of APS in a large cohort of patients consecutively referred to the Immunology laboratory over a period of 5 years.
Methods: 441 patients (63.5% women; 45 ± 18 years) presenting a medical prescription for exploration of APS, were enrolled from September 2011 to September 2016. Clinical and biological data including conventional APS serological markers results such as anti-cardiolipin, anti β2 glycoprotein I autoantibodies and lupus anticoagulant were collected. Detection of aPT was performed by using an inhouse ELISA.
Results: A total prevalence of 17% of aPT positive patients (75/441) was established. Regarding clinical data, a significant association was found between aPT and thrombosis (p=0.035), aPT positivity was significantly more frequent in venous than in arterial thrombosis (p=0.031). Among aPT positive patients with thrombosis, 30% of patients (11/37) presented thrombotic events in an APS context, whereas the majority of them (70%, 26/37) had unexplained thrombosis showing that aPT are mostly detected in the absence of any conventional markers of APS.
Regarding aPT isotype, no difference was observed between IgG and IgM aPT. However, interestingly, aPT IgG ratio were higher in recurrent thrombosis than isolated thrombosis (p=0.013).
Summary/Conclusion: Our results underline the interest of assessing both IgG and IgM aPT in thrombosis, in particular when conventional markers are negative. We suggest that quantification of these markers could predict the recurrence of thrombosis but prospective clinical studies are now required to comfort these findings.

Clotting
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Board No. 163

Potential value of magnetic resonance direct thrombus imaging in preventing overdiagnosis by compression ultrasound in patients with suspected ipsilateral recurrent deep venous thrombosis

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Background: Compression ultrasonography (CUS) is the imaging method of choice in the diagnostic management of a first episode of deep venous thrombosis (DVT) of the leg. In the setting of suspected ipsilateral recurrent DVT (sirDVT) the specificity of CUS is inadequate due to persistent intravascular abnormalities after a first DVT in 30-50% of patients. Magnetic resonance direct thrombus imaging (MRDTI) can reliably distinguish acute DVT from chronic thrombotic changes. The value of MRDTI in clinical practice is still unknown.
Aims: To evaluate the proportion of patients with sirDVT where MRDTI may prevent a false positive DVT diagnosis by CUS.
Methods: This is a subanalysis in one study site of the ongoing Theia study (NCT02262052), a prospective international outcome study in which patients with sirDVT are managed according to the result of MRDTI. Informed consent is obtained prior to study inclusion as consecutive patients are recruited prospectively. In case of an abnormal MRDTI patients are treated with anticoagulants, in case of a normal MRDTI patients are left untreated and are followed for 3 months. All patients with normal MRDTI are subjected to a reference CUS as well, but management decisions are based entirely on the MRDTI results.
Results: Currently, 71 consecutive patients have been included from the Danderyd Hospital. Their mean age was 62 years (SD 16) and 55% were male. MRDTI was indicative of DVT in 16 patients (DVT prevalence 23%). Of the 55 patients with normal MRDTI, 24 had an abnormal CUS, indicating that 24 of a total of 40 (i.e. 16 plus 24; 60%, 95%CI 45-74) abnormal CUS examinations may be false positive.
Summary/Conclusion: MRDTI may prevent overdiagnosis and subsequent overtreatment in 45-74% of patients with abnormal CUS in the setting of sirDVT. The safety of withholding anticoagulant therapy in these patients is still to be determined in the Theia study, the results of which are expected in 2019.

Clotting
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Board No. 166

Monitoring argatroban with rotational thromboelastometry: an emergency alternative to activated partial thromboplastin time?

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Background: Heparin-induced thrombocytopenia treatment by argatroban, a specific direct thrombin inhibitor, is monitored by activated partial thromboplastin time (aPTT). Rotational thromboelastometry is another method enabling the evaluation of the coagulation intrinsic pathway, and could be an interesting alternative considering its availability bedside in intensive care units.
Aims: The aim of this study was to assess if the aPTT is a trustworthy test to monitor argatroban levels using our lab configuration (STA R Max 2/STA-PTT Automate, Stago®), and to evaluate the clotting time under argatroban with the use of rotational thromboelastometry (ROTEM® delta/INTEM analysis).
Methods: Using three healthy volunteers, argatroban dilutions were added to whole-blood samples to obtain plasmatic concentrations (plasmatic volume was calculated with hematocrit) of 0, 0.25, 0.5, 1, 1.5, 2 µg/ml (therapeutic range), and 5, 10 µg/ml (supra-therapeutic range). The aPTT and ROTEM® analysis with INTEM were then performed, respectively on plasmatic samples and whole-blood samples. Correlation analyses were performed using the Spearman and the Pearson correlation analyses.
Results: The aPTT values corresponding to argatroban therapeutic range were between 1.5 to 3 time aPTT initial baseline value, as expected according to official recommandations. There was a linear correlation between aPTT and clotting time (CT) in INTEM analysis ($p<0.0001$ and $r=0.99$). We found a non-linear correlation between argatroban concentrations and aPTT ($p<0.0001$ and $r=1$), as well as between argatroban concentrations and CT in INTEM analysis ($p<0.0001$ and $r=1$).
Summary/Conclusion: Our lab aPTT configuration proved to be excellent to monitor argatroban.
Monitoring argatroban using ROTEM® analysis with INTEM seems to be as reliable as aPTT, with a significant correlation between concentrations and results. ROTEM® analysis with INTEM could be an alternative to monitor argatroban, especially in emergency situations.

Clotting
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Board No. 167

Joint effects of D-dimer and prothrombotic genotypes on the future risk of incident venous thromboembolism

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Background: D-dimer, a fibrin degradation product, is a diagnostic marker of acute venous thromboembolism (VTE) and a predictor of VTE-recurrence. However, limited data exist regarding the association between plasma levels of D-dimer and risk of incident VTE in the general population. Moreover, a high basal D-dimer may reflect not only genetic but also acquired risk factors for VTE, and whether its combination with established prothrombotic single nucleotide polymorphisms (SNPs) has synergistic effects on VTE risk remains uncertain.
Aims: To investigate the association between plasma levels of D-dimer and future risk of VTE in a nested case-control study, and to further assess joint effects of D-dimer and prothrombotic genotypes on VTE risk.
Methods: Participants comprised 414 cases with incident VTE, and 843 sex- and age-matched controls who were randomly selected from the fourth survey of the Tromsø Study (1994-1995), a population-based cohort study. Unconditional logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE across quartiles of plasma D-dimer levels set in controls. A randomly selected subset of 389 cases and 403 controls were genotyped for the five SNPs included in the de Haan score: rs6025 (factor V Leiden [FVL]), rs1799963 (FII), rs8176719 (ABO), rs2066865 (fibrinogen-g), and rs2036914 (FXI). ORs for VTE were estimated by combined categories of risk alleles (de Haan 5-SNP score; 0-1, 2-3 and ≥4) and D-dimer levels (<75th and ≥75th percentiles). All regression analyses were adjusted for age, sex and body mass index. The regional committee for research ethics approved the study and informed written consent was obtained from all study participants.
Results: The risk of VTE increased linearly across quartiles of D-dimer levels (p for trend 0.018). Subjects in the highest quartile of D-dimer (≥183 ng/mL) had an OR for VTE of 1.49 (95% CI 1.02-2.16) compared to those in the lowest quartile (<102 ng/mL). Stratification by provoked and unprovoked events and location (deep vein thrombosis and pulmonary embolism) yielded similar results. For the joint analysis, subjects with D-dimer <183 ng/mL and 0-1 risk alleles were set as the reference in the 5-SNP model. Participants with D-dimer <183 ng/mL and ≥4 risk alleles had an OR for VTE of 2.19 (95 % CI 1.20-3.67), and those with D-dimer ≥183 ng/mL and 0-1 risk alleles had an OR of 1.39 (95% CI: 0.70-2.76). Having both high D-dimer (≥183 ng/mL) and ≥4 risk alleles yielded an OR of 3.93 (95% CI 1.77-8.68), which was greater than the sum of the separate effects of the two exposures. In analyses of the individual SNPs, a supra-additive effect on VTE risk was observed for the combination of high D-dimer and FVL.
Summary/Conclusion: D-dimer levels were associated with future risk of VTE in a dose-response manner. Our results further suggest a joint effect of high D-dimer (≥183 ng/mL) and either FVL or having ≥4 prothrombotic risk alleles on VTE risk. Taken together, our findings suggest that the number of risk alleles in the de Haan 5-SNP score were particularly predictive of venous thrombosis risk in subjects with high D-dimer.

Clotting
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Does in-vitro addition of activated charcoal allow lupus anticoagulant testing with dRVVT in plasma of patients treated with DOAC?: the CAVIAR study

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Background: In patients receiving Direct Oral AntiCoagulants (DOAC), Lupus Anticoagulant (LA) testing is challenging since these drugs interfere with dilute Russell Viper Venom Time (dRVVT) test. The addition of agents able to *in vitro* neutralize DOAC could potentially avoid such interference. DOAC-remove® (5-Diagnostics®) tablets containing activated charcoal are intended to be used *in vitro* for removal of DOAC compounds from human citrated plasma samples.
Aims: In the CAVIAR (CArbon in VItro Anticoagulant Removing) study, the main objective was to assess the performances of DOAC-remove® tablets to neutralize DOAC, and to allow performing dRVVT test without any interference, in plasma from patients receiving DOAC and requiring LA testing. We also aimed to check that DOAC-remove® tablets did not interfere with dRVVT test.
Methods: In this on-going two-center study, we intended to test 100 plasmas from patients receiving DOAC and referred to us for thrombophilia testing. The effect of the activated charcoal was also tested on known LA-positive and negative plasmas from patients without anticoagulant. Tablets were added to ~1 mL citrated plasma and handled according to slightly modified manufacturer requirements. DOAC plasma concentrations were measured using STA-Liquid-anti-Xa® (Stago) for apixaban and rivaroxaban and Hemoclot® (Hyphen) for dabigatran, with dedicated calibrators and controls, on STA-R Evolution® analyser (Stago). The lower limit of quantification (LLOQ) was 20 ng/mL for all drugs. LA testing was performed using either Staclot dRVVT Screen/Confirm® (Stago) and LAC Screening/Confirm® (Siemens) depending on the center. Tests were performed before and after addition of the tablet.
Results: To date, 24 plasmas from patients on DOAC at various concentrations have been tested: 11 with rivaroxaban (min–max: 46-468 ng/mL), 8 with apixaban (min–max: 25-514 ng/mL) and 5 with dabigatran (min–max: 20-221 ng/mL), The residual DOAC concentration after addition of the tablet was <LLOQ in all but one plasma samples; in 1 sample containing 400 ng/mL rivaroxaban, the residual concentration was of 31 ng/mL.
Before charcoal addition, all samples with rivaroxaban showed LA-positive whereas, with apixaban or dabigatran all samples showed LA-negative.
After charcoal addition, with rivaroxaban all samples but one were LA-negative; it is likely that the remaining positive elderly patient had a weak LA (normalized ratio: 1.26) related to sepsis. With apixaban and dabigatran, all samples remained LA-negative.
In addition, activated charcoal did not affect dRVVT testing on plasmas of patients without anticoagulant treatment with low positive-LA (n=4, normalized ratio 1.28 to 1.31) or with high positive-LA (n=5, normalized ratio 1.72 to 2.55), and on LA-negative plasmas (n=2).
Summary/Conclusion: These preliminary results show that in patients treated with DOAC, *in-vitro* addition of activated charcoal to plasma samples seems to be able to neutralize DOAC up to 500 ng/mL, and allow performing dRVVT test. Further data are required to ascertain that high concentrations of DOAC can be neutralized.

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Interactions between argatroban and vitamin K antagonists on clotting tests (APT and aPTT) and interest of argatroban measuring during perioperative management: case report.

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Background: In patients with heparin-induced thrombocytopenia (HIT), a cross-allergy to danaparoid sodium occurs in 5 to 10%, requiring the use of other molecules. Argatroban, a direct thrombin inhibitor, is approved for the treatment of HIT and its prevention in patients with a documented history of HIT. Several laboratory tests are available to monitor the anticoagulant effect of argatroban. The activated partial thromboplastin time (aPTT) and the activated clotting time (ACT) are the most commonly used, but the interactions between argatroban and the traditional clotting tests complicate the monitoring. Global clotting times or plasma concentrations could provide an alternative for perioperative management of argatroban.
Aims: To report our experience of argatroban's perioperative management by using traditional clotting tests and plasma concentrations.
Methods: We report the case of a patient with documented history of HIT and cross-allergy to danaporoid sodium diagnosed after an aortic valve replacement by a mechanical valve. Recently, a lymph node biopsy had been planned due to a suspected lymphoma. Fluindione was then discontinued and switched for argatroban at a dosage of 2 mcg/kg/min, according to guidelines. Argatroban had to be started after that INR decreased under 2.5. We also had to monitor daily aPTT ratio (Triniclot Stago) with a therapeutic range between 1.5 and 3 and argatroban plasma concentration (Hyphen BioMed) with a range between 0.25 and 1.5µg/mL. Argatroban had to be stopped 12 hours before the biopsy and restarted 12 hours after the procedure. Fluindione would be restarted the day after in order to overlap with argatroban for five days. Argatroban would be stopped when the INR became higher than 4.
Results: Fluindione was stopped on March 14th. Argatroban was started on March 15th at a dosage of 2 mcg/kg/min with baseline INR at 1.8 and aPTT ratio at 1.04. Two hours after initiation, aPTT ratio was measured at 2.1. Daily monitoring of aPTT ratio was in therapeutic range between 1.5 and 3. Plasma concentrations of argatroban were between 0.85 and 1.2. Argatroban was discontinued for 24 hours for the biopsy between March 20th and 21st. INR and aPTT ratio were measured at 1.2 and 1.1 respectively 6 hours after stopping argatroban, allowing biopsy. Fluindione was started on March 21st and INR was higher than 4 on March 26th. A first attempt to stop argatroban failed: INR after six hours was under therapeutic range (4.1 to 2). A second attempt failed despite INR at 4.5 decreasing to 1.9 after six hours. A third attempt failed despite INR at 5.9 decreasing to 2.4. Finally, argatroban was stopped on March 29th with INR at 7.7 and control at 3.3. Next day, INR remained stable at 3.4. Neither thrombotic nor bleeding complication was observed.
Summary/Conclusion: There was a good correlation between aPTT ratio and plasma concentrations during preoperative period. INR reached a normal level six hours after interruption of argatroban. After biopsy, aPTT ratio and plasma concentrations remained correlated, while INR was inconclusive under treatment with argatroban. As recommended, INR monitoring is crucial after argatroban interruption. In some cases, INR threshold should be set higher than usually recommended before stopping argatroban.

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Comparision between the standard thrombin generation method and the automated thrombin generation technique

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Background: Thrombin generation assay (TGA) is global test for monitoring of the overall potential of a sample to generate thrombin.
One of the main drawbacks is the lack of standardization with the Calibrated Automated Thrombogram (CAT), the semi-automatized technique used until now. Recently, an automated method, ST-Genesia® for the determination of thrombin generation (TG) in plasma, has been developed.
Aims: The objective of this study is to compare the determination of TG by CAT versus ST-Genesia®.
Methods: We processed 79 healthy controls samples of platelet poor plasma. The TG analysis was performed out with both devices: the CAT (ThrombinoscopeTM) and the ST-Genesia® (Stago®). In both cases (PPP-Reagent for CAT® and Thromboscreen® for ST-Genesia®) the tissue factor and phospholipids concentration were comparable, according to the manufacturer.
The comparison analysis of both methods was performed by Passing & Bablok regression. The parameters analysed were: The Lag Time (latency time), the time to reach the maximum peak (Time to peak, TTP), the maximum peak of thrombin and the endogenous thrombin potential (ETP). Student's t test for paired data was used to assess the differences between the two methods.
Results: No significant differences were observed for the maximum peak and ETP, but we found significant differences between the Lag Time 0,22 min (95% CI 0.14-0.30 p = 0.0001) and TTP 0, 58 min (95% CI: 0.38-0.78 p = 0.0001). The correlation between the different parameters were 0.78, 0.75, 0.79 and 0.57 for lag time, maximum peak, TTP and ETP respectively and significant in all cases ($p<0.0001$). The regression of Passing & Bablok showed proportional differences between Lag Time **(0.75, IC95%:0,60-0,92)**, TTP **(0,76, IC95%:0,65-0,87)** and ETP **(0,71, IC95%: 0,53-0,91)** . No systematic differences were observed in any of the parameters studied.
Summary/Conclusion: Despite finding significant differences between Lag time and TTP these differences are not clinically relevant. Moreover, ST-Genesia® and CAT are globally well correlated.
In the case of ETP, not all the variability of the results obtained on ST-Genesia® device is explained by the CAT. This difference can be partly explained by a change in temperature management between those devices, with a rigorous temperature control on ST-Genesia®. These characteristics confer more precision during the evaluation of TG in haemorrhagic and thrombotic settings.
Standardization, automation and precise management of the temperature make ST-Genesia® compatible with its use in daily practice.

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ABO blood group and factor VIII levels in thrombophilic families

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Background: Venous thromboembolism (VTE) is a multicausal disorder involving acquired and genetic risk factors. The ABO locus has functional effects on von Willebrand factor (vWF) and factor VIII (FVIII) levels. Specifically, non-O individuals have higher vWF and FVIII levels than group O individuals. The ABO blood group system is controlled by a single gene with three main alleles (two codominant and B and one recessive O). FVIII has a high heritability, meaning that genes explain 40% of the variance of FVIII levels. Non O-blood group and high FVIII levels are independent risk factors for VTE. In fact, those individuals with A1 variant are overrepresented in the population with VTE

Aims: We aimed to study the influence of ABO blood group and factor VIII levels in thrombophilic families in which the clustering of thrombotic events cannot be explained by other known genetic risk factors
Methods: We describe three families, each one with several first-degree relatives affected by objectively confirmed VTE. Clinical data including age at first or subsequent episodes, the location of the thrombosis (DVT- deep vein thrombosis, PE- pulmonary embolism), and recognized predisposing factors were recorded. Thrombophilia testing was performed including laboratory determinations of antithrombin (AT), protein C (P.C), protein S (P.S), homocysteine and FVIII clotting activity. Genetic analysis was done for factor V Leiden (FVL) and the 20210A prothrombin (PT20210A) mutations. ABO phenotype and A1 subtype was determined by Blood Bank at our hospital. Informed consent was obtained from all individuals
Results: In these thrombophilic families most of the episodes were idiopathic venous thrombosis and some of them debuted at young age. Three of them presented recurrent VTE. The clustering of thrombotic events could not be explained by deficiencies of anticoagulant proteins or prevalent genetic mutations. Instead significantly high FVIII levels (>230%) and non-O blood group were found as risk factors. All individuals were non-O except one whose blood group is not known and A1 allele was demonstrated in six of nine individuals
Summary/Conclusion: ABO blood group and factor VIII levels may explain familiar thrombophilia in some cases and should be considered part of the thrombophilia study

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Macrophages stimulated in vitro with LPS and ATP produce large IL-1ß-positive microparticles which can be detected in patients with systemic-onset juvenile idiopathic arthritis

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Background: IL-1ß is barely detectable in the serum of patients, including those with a well-known IL-1ß-mediated disease such as systemic-onset juvenile idiopathic arthritis (SoJIA), possibly due to the fact that IL-1ß is a leaderless cytokine with poorly characterized secretory mechanisms. Leukocyte microparticles (MP) may be a mechanism of IL-1ß secretion, despite the fact that nothing is known of their detection in human diseases and of their biological functions *in vivo*.
Aims: MPs issued from activated monocytes/macrophages have been shown to contain bioactive IL-1ß *in vitro*, however their pro-inflammatory functions *in vivo* as well as their detection in human diseases have not been reported to date. In this study, we characterized IL-1ß-positive MPs for their pro-inflammatory functions both *in vitro* and *in vivo* and asked whether it was possible to detect these MPs in patients with SoJIA.
Methods: PBMCs or THP-1 cells were stimulated with LPS (10 micrograms/ml) for 16 hours then with ATP (1 mM) for 1 hour. Microparticles were then harvested by centrifugation of cell-culture supernatants at 70000g and characterized for the presence of IL-1ß, P2X7R, NLRP3, caspase-1 and tissue factor (TF) using ELISA, western-blotting or flow cytometry. MP biological properties were further investigated *in vitro* by assessing their tissue factor activity using a factor-Xa generation assay and their capacity to trigger HUVEC activation through IL-1ß. IL-1ß-dependent MP inflammatory properties were also studied *in vivo* in a murine model of neutrophilic inflammation. Finally, we searched for the presence of IL-1ß-positive MPs in the serum of active SoJIA patients using flow cytometry.
Results: Macrophages stimulated with LPS and ATP released MPs, which conveyed the 33 kDa precursor and 17 kDa mature forms of IL-1ß, inflammasome components NLRP3 and caspase-1 and also bioactive TF. These MPs expressed the P2X7 purinergic receptor and released soluble IL-1ß upon stimulation with ATP. They induced HUVEC activation *in vitro* and neutrophil peritoneal infiltration in mice, which were both reduced by IL-1 receptor antagonist (IL-1ra). IL-1ß was preferentially detected in a subpopulation of annexin V-positive MPs larger than 500 nm, which could also be evidenced in the plasma of 9 patients with active SoJIA.
Summary/Conclusion: Large MP issued from activated macrophages contain IL-1ß and NLRP3 inflammasome components. They constitute complete pro-inflammatory entities, which can be detected in active SoJIA patients.

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Removal of DOACs from plasma: performance comparison and pre-analytical considerations of three different devices

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Background: Given the plethora of coagulation tests influenced by Direct Oral Anti-Coagulants (DOACs), it would be of particular interest to remove DOACs from a sample. This could enable a better investigation of an underlying plasma defect potentially hidden by a DOAC interference. In this context, several DOACs-removal devices have been developed for a potential use in clinical routine. The transfer of plasma through a filter or the addition of an adsorbing agent is currently under investigation.
Aims: The aim of this study was to evaluate the performances of three devices enabling the removal of DOACs from spiked plasma samples. Their efficiency to eliminate DOACs from plasma, the impact of the transfer through the filters on the coagulation and their ergonomics were investigated.
Methods: Fresh normal pooled plasma from 6 healthy volunteers were mixed with either dabigatran, rivaroxaban or apixaban at 0-125-250-500 ng/mL theoretical final concentration. Six hundred µL of plasma were tested before and after filtration on DOAC filter (Stago, France), DOAC Stop (Haematex Research, Australia) or on Hemofilter (Hemosafe, Belgium) on a STAR Max2 analyzer using calibrated STA-Liquid anti-Xa or STA-ECAII for dosage of anti-Xa and anti-IIa drugs, respectively (all products from Stago, France). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were also analyzed on the STA-R Max2. Several usage data regarding these devices were collected throughout the study.
Results: Some discrepancies can be observed between theoretical and actual dosages of dabigatran, rivaroxaban and apixaban in spiked samples. Results also show that all devices enable a sufficient removal of rivaroxaban and dabigatran to lower measurements under the limits of detection of the instrument. For apixaban, only the DOAC Stop and the Hemofilter enable this sufficient elimination while residual amounts of apixaban were measured after the use of the DOAC filter on samples containing the highest concentrations tested (250 and 500 ng/mL theoretical). All the DOACs-removal devices were able to restore normal PT and aPTT, but Hemofilter introduced a shortening of PT under every condition. Regarding their ergonomics and usage data, the DOAC Stop procedure is the quickest (7 min). However, the complete elimination of the adsorbing agent is difficult and black residues in the sample were still visible after the procedure. The use of DOAC filter and Hemofilter include less steps in their protocol. The Hemofilter induces the lower loss of sample volume.
Summary/Conclusion: In conclusion, the DOAC Filter and Hemofilter are the easiest to use and no visible residues potentially interfering with measurement are observed with these devices as opposed to DOAC Stop. However, the DOAC Filter is not able to eliminate apixaban at concentration higher than 250 ng/mL.

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The presence of anti-ADAMTS13 autoantibodies does not change ADAMTS13 antigen levels measured by an in house developed ADAMTS13 antigen ELISA

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Background: Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is characterized by the presence of autoantibodies (autoAbs) against ADAMTS13. ADAMTS13 is a metalloprotease which consists of multiple domains: metalloprotease (M), disintegrin-like (D), cysteine-rich (C), spacer (S), 8 thrombospondin type 1 repeats (T1-8) and 2 CUB domains. ADAMTS13 antigen (Ag) levels are moderately to severely reduced in most iTTP patients, indicating that antibody-mediated clearance is the major pathogenic mechanism. In addition, a severe reduction in ADAMTS13 Ag has recently been shown to be associated with a higher mortality rate.
Aims: We aimed at validating our in-house ADAMTS13 Ag ELISA in terms of sensitivity, reproducibility and background and investigated whether ADAMTS13 Ag determination is influenced by the presence of anti-ADAMTS13 autoAbs.
Methods: In our in-house ADAMTS13 Ag ELISA, ADAMTS13 is captured by the anti-ADAMTS13 monoclonal antibody (mAb) 3H9 (anti-M) and detected using a mixture of biotinylated anti-ADAMTS13 mAbs 17G2 (anti-CUB1) and 19H4 (anti-T8) and HRP-labeled streptavidin. Detection limit (DL, 3xSD above mean of blank) and quantification limit (QL, minimum measurable concentration with coefficient of variation (CV) < 20%) were determined by performing six replicates of serial dilutions of normal human plasma (NHP), in six assays. The influence of iTTP autoAbs on ADAMTS13 Ag determination was tested using 20 purified iTTP IgG samples. First, NHP was pre-incubated with the purified iTTP IgGs and ADAMTS13 Ag levels in this mixture were determined. Next, the presence of overlapping epitopes between the assay mAbs (3H9, 17G2 or 19H4) and iTTP IgGs were studied in a competition ELISA on coated recombinant human (rh)ADAMTS13.
Results: The DL of our in-house ADAMTS13 Ag ELISA is 2.52 ng/mL (1/396.9 NHP dilution) and the QL is 7.00 ng/mL (1/142.9 NHP dilution). The CV for each NHP dilution (1/6.7; 1/11.1; 1/18.5; 1/31.0; 1/51.4; 1/85.7; 1/142.9) was 3.0%, 3.5%, 3.1%, 3.0%, 2.6%, 2.3% and 3.6%, respectively. Additionally, the ADAMTS13 Ag measurements in NHP were similar with or without pre-incubation of purified iTTP IgGs, indicating that iTTP autoAbs do not interfere with the ADAMTS13 Ag determination. Moreover, a competition ELISA in which the binding of the assay mAbs (3H9, 17G2 and 19H4) to rhADAMTS13 in the presence of purified iTTP IgGs was tested, showed that none of the purified iTTP IgGs competed with 3H9 or 17G2 for rhADAMTS13 binding, and only one iTTP IgG sample competed with 19H4 for rhADAMTS13 binding.
Summary/Conclusion: Our in-house ADAMTS13 Ag ELISA has been validated in terms of sensitivity, reproducibility and background. Additionally, we showed that the presence of anti-ADAMTS13 autoantibodies does not change ADAMTS13 antigen levels. The high affinity of our murine anti-ADAMTS13 mAbs for plasma ADAMTS13 possibly explains why the presence of low(er) affinity patient anti-ADAMTS13 autoAbs do not interfere in the ELISA.

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An optimized method to measure tissue factor activity on microvesicles.

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Background: Tissue factor-dependent procoagulant activity of microvesicles (MV-TF) is associated with an increased risk of thrombosis in different clinical settings. Several methods have been described to measure MV-TF but their sensitivity and TF specificity remain major issues.
Aims: Therefore, the aim of this work was to optimize and validate a new method to measure the MV-TF-dependent procoagulant activity.
Methods: The assay is based on measuring factor Xa generation from MVs purified from platelet-free plasma (PFP) using a fluorogenic substrate. This study evaluates the assay's sensitivity, specificity and reproductibility. The assay was then tested on normal and LPS-treated plasma and in plasma from different pathological settings associated with increased TF activity (sepsis, burn patients and acute myeloïd leukemia (AML)).
Results: A high sensitivity was obtained by using i) 10 nM of factor VII and 760nM of factor X, ii) high initial volume of PFP: 500 µL, iii) low plasma viscosity, iv) a 24,000g during 1h centrifugation. Provided that the first milliters of blood were removed after venous puncture, high specificity of the method was warranted using an anti-TF antibody (SBTF1) which shows a more potent inhibition than HTF1 (94±2% versus 70±6% at 2.5µg/mL). Specificity was confirmed by the absence of activity on MVs derived from knock out-TF cell line. A good reproducibility around 4% was found using purified MV and 20% including the centrifugation step, provided however, that the same rotor type was used. Taken together, these optimizations allow us to detect a measurable level of MV-TF activity in normal PFP samples (26±15 fM, n=9) and a significant increase of MV-TF activity after LPS activation of these blood samples (4 to 28 fold, n=9, p=0.008). Comparison of this assay with others methods used in the litterature shows a higher sensitivity of our in-house test with the others tests on lowers values (normal samples) and a high correlation on higher values (LPS-treated samples). Finally, compared to normal samples (28 [13-37] fM, n=9) MV-TF activity was very heterogeneous with an increase tendancy in sepsis patients (37 [26-62] fM, n=11) and significantly increased in burn patients (45 [33-80] fM, p=0,01, n=11] and AML patients (634[93-2200] fM, p=0,0008, n=18).
Summary/Conclusion: The new method shows a good sensitivity without loosing specificity but would benefit from future development allowing to avoid centrifugation step. Such methodological improvements are mandatory to better evaluate the clinical interest of TF-dependent procoagulant activity of MVs as a potential biomarker of thrombotic risk.

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The effect of lower-leg injury and knee arthroscopy on coagulation factor levels

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Background: Patients with lower-leg injury treated with cast immobilization have an eightfold increased risk of venous thrombosis (VT) as compared to the general population.(Van Adrichem RA, JTH 2014) Patients with minor leg injuries that do not require cast immobilization or surgery have a fivefold increased risk of VT as opposed to the general population.(Van Stralen KJ, Archives of internal medicine 2008) A possible mechanism of the increased risk could be a procoagulant state as a reaction to the injury. Patients undergoing knee arthroscopic surgery have a six fold increased risk of VT as compared to the general population. (Van Adrichem RA, JTH 2015) As knee arthroscopy is supposed to be accompanied by a low degree of tissue damage, it is interesting to find out whether the increased risk of VT is due to a different mechanism than that in patients with lower-leg injury.
Aims: To describe the coagulation profile of patients with lower-leg injury and of patients undergoing knee arthroscopy and compare to a reference population (controls).
Methods: Patients who participated in the POT-CAST trial (Prevention Of Thrombosis following CAST immobilization) had lower-leg injuries and were included for analyses. They provided a blood sample directly after trauma, before receiving a plaster cast or surgery. In patients undergoing knee arthroscopy (the POT-KAST trial), both pre- as postoperative blood samples were taken. Both patient groups were compared to subjects derived from the control group of the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study, a population-based case-control study. Factors (F)VIII and XI activity, fibrinogen (FG) and von Willebrand Factor antigen (vWF) and D-dimer were measured. Information on risk factors of VT was derived from questionnaires. Linear regression models were used to estimate the mean difference of coagulation factor levels of patients compared to controls with 95% confidence intervals (95%CI), adjusting for sex, age, body mass index, comorbidity, malignancy, and use of oral contraceptives.
Results: A total of 4209 persons (1204 plaster cast patients, 2921 controls) were eligible for analyses. Injuries involved lower-leg fractures (89.4%), Achilles' tendon rupture (6.9%), ankle distortion (2.4%), and contusion (1.1%). Patients with lower-leg injury had higher levels than controls regarding FVIII (mean difference: 35.5%, 95%CI 32.6-38.4), FXI (mean difference: 16.8%, 95%CI 15.3-18.3), and vWF (mean difference: 45.1%, 95%CI 41.6-48.6). Patients had 2.67 (95%CI 2.54-2.81) times higher levels of D-dimer than controls (logarithmically transformed due to skewed distributions). After adjusting for other factors we also found an association for lower-leg injury and FG (mean difference: 11.6 mg/dL, 95%CI 6.7-16.6), in which age had the strongest effect on adjustment. Of all injuries, tibia and/or fibula fractures, and ankle fractures had the highest mean FVIII and vWF levels. Phalanx fractures had the lowest mean FVIII and vWF. The results of the analyses of knee arthroscopy patients are still ongoing and will be presented at the ECTH 2018 congress.
Summary/Conclusion: Trauma in lower-leg injuries leads to elevated levels of FVIII, FXI, FG, vWF, and D-dimer. For FVIII and vWF in particular, this was correlated to the severity of the injury. Comparison with the situation in knee arthroscopy patients will be made at the time of the ECTH 2018 conference.

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Genomic Variations in the Fibrinolytic cascade and their role in Venous Thrombosis

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Background: Hypofibrinolysis due to abnormalities in factors in the fibrinolytic pathway is a known cause of venous thrombosis (VT). Reports of association of polymorphisms of fibrinolytic proteins with VT have been contradictory. Renin-angiotensin system (RAS) partially regulates fibrinolytic pathway. Variations in RAS genes may affect fibrinolysis.
Aims: To evaluate the role of single nucleotide polymorphisms of fibrinolytic and RAS genes in patients with venous thrombosis and investigate their association with fibrinolytic proteins.
Methods: 339 VT patients [144 cerebral vein thrombosis (CVT), 157 deep vein thrombosis (DVT), 28 abdominal thrombosis (AVT), 10 retinal vein occlusion (RVO)] were compared to 170 healthy individuals. SNPs were genotyped by restriction fragment length polymorphism or allele-specific PCR for PAI-1 (-675 4G/5G and -844 G>A), tPA (-7351 C>T and Alu I/D), TAFI (505 G>A and 1040 C>T), THBD 1418 C>T, angiotensin type 1 receptor AGT1R 1166 A>C, angiotensinogen AGT (T174M and M235T), ACE Alu I/D, renin (T9435C and G6567T) and bradykinin receptor 2 BDKRB2 (C181T and C58T). Fibrinolytic proteins PAI-1, tPA, TAFI and THBD were measured by ELISA.
Results: 4G/4G PAI-1 genotype imparted a 1.85 fold overall VT risk (95%CI= 1.05-3.28, p= 0.027), 1.94 fold DVT risk (95%CI= 1.01-3.72, p= 0.044) and a 2.87 fold AVT risk (95%CI= 1.05-7.86, p=0.0041). Patients with PAI-1 4G/4G genotype had significantly high PAI-1 level (mean: 100.86 ± 68.67 ng/ml) (p <0.0001). A/A genotype of -844 PAI-1 associated with a 1.65 fold overall VT risk (95%CI= 0.91-2.98, p= 0.01), 1.68 fold DVT risk (95%CI= 0.86-3.28, p= 0.019) and a 3.27 fold AVT risk (95%CI= 1.09-9.75, p=0.016). Patients with A/A -844 PAI-1 genotype also had significantly high PAI-1 level (mean: 96.79 ± 71.39 ng/ml) (p <0.0001). -7351 T/T tPA genotypic frequency was higher in VT cases against controls (p=0.032, OR= 3.60, 95%CI= 0.79-16.34). No correlation was found between -7351 tPA genotypes and level. Genotypes of tPA Alu I/D and TAFI 505 G> A polymorphisms were not significantly different in cases and controls and did not affect levels. RVO patients had higher T/T genotypic frequency of TAFI 1040 C>T. T/T genotype associated with lowest TAFI mean levels. THBD 1418 C>T polymorphism associated with overall VT and DVT risk (p<0.05, OR >1.5). A strong correlation was seen between C allele of AGT1R 1166 A>C and VT risk (p<0.0002, OR>2.9) alongwith DVT, CVT and RVO risk (p<0.005). Significant correlation was observed for AGT T174M in dominant model (C/C vs C/T+T/T) in overall VT (p=0.045, OR=1.63, 95%CI=1.0-2.65) and separately in CVT (p=0.032, OR=1.85) and PVT (p=0.016, OR=3.08). Genotypes of AGT M235T, ACE Alu I/D, Renin (T9435C and G6567T) and BDKRB2 (C181T and C58T) polymorphisms were not significantly different in cases and controls. Although no statistically significant association was seen, numerous variants of RAS did show to have variable mean levels of PAI-1 and tPA thereby establishing the fact that RAS polymorphisms may indeed have a small contributing effect on fibrinolysis.
Summary/Conclusion: Fibrinolytic polymorphisms of PAI-1, TAFI and THBD and RAS polymorphisms AGT1R 1166 A>C and AGT T174M imparted an increased VT risk by affecting fibrinolytic protein levels.

Clotting
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Clot waveform analysis: Determination of optimal wavelength to assess the fibrin coagulation process

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Background: The fibrin generation test (FGT) monitors the coagulation process using a TECAN analyzer and provides an analysis of the optical waveform generated during clotting test. This allows the exploration of the coagulation process with more information than global coagulation tests (i.e. prothrombin time, thrombin time or activated partial thromboplastin time). The principle behind this test is also called clot waveform analysis (CWA), which is used to measure and record the time required for a plasma to clot. This technique assesses coagulation endpoint by measuring change in optical density. In the literature, routine tests generally measure fibrin clot formation at different wavelengths (340 nm, 635 nm or 671 nm). The choice of wavelength for detection is crucial to the sensitivity of the assay, but the question is not dealt in the previous SSC report from subcommittee of the working group of the FVIII and IX on CWA.
Aims: This study tries to define which wavelength provide the best signal-to-noise to monitor fibrin clot formation.
Methods: Clot formation was monitored at wavelength from 280 to 700 nm (by step of 1 nm on a TECAN analyzer) to provide absorbance spectrum of both non-clotted and clotted plasma. Normal pool plasma (NPP) incubated with an inducer of the intrinsic pathway of the coagulation (Actin FS®; Siemens) or inducers of the extrinsic pathway (PPP reagent® or PPP reagent Low®; Thrombinoscope BV). Briefly, 20µL of reagent were mixed with 80µL of NPP in a 96-well microtiter plate and incubated for 3minutes at 37°C.For clotted plasma, the coagulation process was triggered by the addition of 20 µL of $CaCl_2$ while 20 µL of physiological saline was added in non-clotted plasma to harmonize the total volume of the reaction. The signal-to-noise between the 2 conditions was then calculated.
Results: At baseline (i.e. before the sample starts to clot for the experiment with the addition of $CaCl_2$), no difference in the absorption was observed between the 2 conditions, whatever the wavelength used, showing that the addition of $CaCl_2$ (before the coagulation process has started) has no impact on the absorbance of the sample. However, as expected, once the coagulation process has begun, clotted samples showed higher absorbance values than non-clotted samples, whatever the wavelength used. Interestingly, the absorbance of clotted and non-clotted samples was higher at 340 nm than at 635 and 671 nm ($p<0.05$). However, the signal-to-noise ratio was significantly higher at 635 or 671 nm than at 340 nm (e.g. 3.8 at 340 nm; 6.8 at 635 nm and 6.7 at 671 nm with the PPP Reagent Low®) due to very low baseline absorbance at 635 and 671 nm. Of note that the signal-to-noise ratio is higher with inducers of the extrinsic pathway of the coagulation (i.e. PPP-Reagent® and PPP-Reagent Low®).
Summary/Conclusion: This study confirms that the choice of the wavelength is crucial for CWA and the subsequent FGT. Wavelengths above 550 nm seems preferable to assess fibrin clot formation. At 340 nm, the signal-to-noise is lower and the analysis could be affected by individuals' characteristics (e.g. bilirubin) and should therefore be avoided.

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Clot waveform analysis for measuring and quantifying fibrin clot formation

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Background: The most widely known global coagulation assays are the thrombin generation test (TGT) and the viscoelastometric assays (ROTEM or TEG). They both analyze an endpoint of the coagulation cascade (i.e. thrombin or fibrin clot for TGT or ROTEM/TEG, respectively) and allow the detection of several coagulation abnormalities. The clot waveform (CW) defines changes in light absorbance that occur during the process of clot formation. The changes in light absorbance are determined by continuous measuring during aPTT/PT/TT or any specific inducer of the coagulation cascade and represented by a clot waveform. The computation of the first derivative allows to compare these methods.
Aims: This study aims at investigating which test between TGT and fibrin generation test (FGT), defined as the first derivative of the clot waveform analysis, is the more sensitive in regards to the coagulation process.
Methods: Thrombin generation in plasma was monitored during 45 minutes using a calibrated automated thrombogram (CAT®). The fibrin clot generation was monitored during 15 minutes at 671 nm on a TECAN® analyzer. Normal pooled plasma (NPP) was incubated at 37°C with an inducer of the intrinsic pathway of the coagulation (Actin FS®; Siemens) or inducers of the extrinsic pathway (PPP reagent® or PPP reagent Low®; Thrombinoscope BV). Briefly, 20 µL of reagent were mixed with 80 µL of NPP in a 96-well microplate and incubated for 3 minutes at 37°C. The coagulation process was triggered by the addition of 20 µL of FluCa® solution (Thrombinoscope BV®) for TGT or 20 µL of 100mM $CaCl_2$ for FGT, respectively.
The first derivative curves of fibrin generation were analyzed and compared to thrombin generationcurves.
Results: As expected, clot waveforms were best fitted by a sigmoid equation. Depending on the activation pathway, the clot waveforms are different in the fibrin generation experiment. The amplitude of the clot waveform is more important with inducers of the extrinsic pathway than with inducers of the intrinsic pathway ($p<0.05$). Based on these results, the first derivative curves were calculated to obtain Gaussian curves.
Actin FS® induces a more important peak (0.004836) than reagent PPP Reagent Low® (0.002390). These results demonstrate that height of the peak is due to both steepness of slope (sigmoid curve) and amplitude of clot waveform.
Comparison between the fibrin generation test (FGT) and the thrombin generation test (TGT) demonstrated that the curves of the first derivative of the fibrin generation process start earlier for all reagents tested. This suggests that the well-established TGT provide information on the coagulation process after the clot has been completely formed and reflects that fibrin generation occurs before the CAT® analyzer is able to detect the thrombin generation. This supposes FGT has higher sensitivity than TGT in analyzing the coagulation process.
Summary/Conclusion: This study unlocks new perspectives about analysis of the coagulation process. Further experiments have to be done to analyze the impact of impairments and/or interference on the coagulation process (i.e. hemophilia, sepsis, anticoagulant treatment, thrombophilia …) on FGT. In perspectives, the implementation of this technique and the subsequent computerized analyzes on automated analyzers is also planned.

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The relationship between absorbance and fibrinogen in the clot waveform analysis

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Background: The clot waveform analysis (CWA) can be considered as a global coagulation test despite the lack of information. The principle of CWA is to measure and to record the time required for a plasma to clot. This technique assesses coagulation endpoint by measuring changes in optical density. The CWA could make the link between absorbance intensity and concentration of fibrinogen in plasma and provide similar parameters to thrombin generation assay such as time to peak, peak, endogenous thrombin potential (ETP) and additional parameters with first and second derivative curves.
Aims: The aim of this study is to highlight parameters that we could use to determine the relationship between absorbance intensity and fibrinogen concentration.
Methods: We used frozen plasma with fixed fibrinogen concentration (Cryopep, France) covering a range of 0 to 800 mg/dL. Depending on the plasma fibrinogen concentration, the process of fibrin clot formation was monitored from 4 hours to 30 minutes at 671 nm. Twenty µL of reagent of intrinsic (Actin FS®, Siemens, Marburg) or extrinsic coagulation pathway (PPP Reagent Low® and PPP Reagent®, Thrombinoscope BV, France) were mixed with 80 µL of plasma in a 96-well microplate and incubated for 3 minutes at 37°C. The coagulation process was triggered by the addition 20 µL of 100mM $CaCl_2$. The first derivative curves of fibrin generation were calculated from sigmoid curve. We determined 5 parameters such as $logIC_{50}$ (time to reach peak of generated fibrin), slope (velocity of fibrin generation), delta (difference between maximum and minimum absorbance), peak and area under the curve (AUC) obtained from first derivative curve.
Results: Based on results, we can observe that absorbance intensity is correlated with fibrinogen concentration (i.e. plasma clotted absorbance increases with fibrinogen concentration). A difference is observed in the maximum absorbance of the clot between the both coagulation pathways.
Concerning slope parameter, the steepness of the curve is stronger with reagent of the intrinsic pathway.
We can observe that $logIC_{50}$, on sigmoid curve, is equivalent to time to peak in the first derivative. For this parameter, we observed a diminution for the intrinsic pathway which means that the time to reach the peak of generated fibrin is shorter with this pathway.
The delta increases dose-dependently with fibrinogen concentration due to higher absorbance after clot formation. This parameter is higher for the extrinsic pathway.
Derivation of the sigmoid function allows the analysis of the peak and AUC. An increase of peak with fibrinogen concentration is observed. The peak, due to both steepness of slope and delta of clot waveform, is higher with the intrinsic pathway.
As regards AUC, we observed that AUC value (first derivative curve) correspond to absorbance value of the delta (sigmoid curve).
According to the studied parameters, our results showed a high variability between both coagulation pathways. The clot waveform seems to be different depending on the activation pathway.
Summary/Conclusion: As the absorbance of plasma increases with fibrinogen concentration, these results showed that the analysis of the clot waveform by optical density provides information on fibrin generation. Many parameters to analyze the clot formation are available and provide fingerprint of the clot process. The important parameters to analyze a CWA should be delta, AUC and peak. A correlation between delta and peak parameters and fibrinogen concentration is also observed.

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Clot waveform analysis: Do the reagents of intrinsic, extrinsic and common coagulation pathway interfere with baseline absorbance

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Background: The principle of clot waveform analysis (CWA) is used to measure and to record the time required for a plasma to clot. This technique assesses coagulation endpoint by measuring change in optical density. In the literature, routine tests generally measure fibrin clot formation at different wavelengths (340 nm, 635 nm or 671 nm). The choice of wavelength for optical detection is crucial to the sensitivity of the assay and the use of colored activating reagents of coagulation could interfere with initial absorbance plasma in this optical measurement method.
Aims: This study aims to determine if reagents from coagulation pathway can interfere with absorbance plasma in clot waveform analysis.
Methods: The fibrin clot formation is initially measured from 80 µL of plasma mixed with 20 µL of inducer of the intrinsic or extrinsic pathway. The coagulation process is then triggered by the addition of 20 µL of 100mM $CaCl_2$. To investigate effect of reagents from coagulation, the 20 µL of $CaCl_2$ are replaced by 20 µL of phosphate buffer saline (PBS) to prevent plasma clotting. Normal pooled plasma (NPP) was obtained from 58 healthy patients. The mean absorbance and standard deviation (SD) values were calculated at 340, 635 and 671 nm. The choice of these wavelengths is based on our previous experiments.
Baseline absorbance was obtained from 80 µL of individual plasma (IP) mixed with 40 µL of PBS to respect the volume of the fibrin clot formation test. Then, to evaluate effect of aPTT and thrombin generation (TG) reagents on plasma samples, the 40 µl of PBS were replaced by 20 µL of reagent and 20 µL of PBS and mixed with 80 µL of NPP.
As reagents used for PT testing and for detection of lupus anticoagulant (DRVVT) contain calcium ions, plasma samples were replaced by serum samples to avoid activation of the coagulation process. Normal pooled serum (NPS) was obtained from 6 healthy patients. The mean absorbance and standard deviation (SD) values were also calculated at 340, 635 and 671 nm. As before, with the IP samples, 80 µL of individual serum (IS) was mixed with 40 µL of PBS to obtain baseline absorbance. To measure effect of reagents from extrinsic and common pathway, 20 µL of reagents were mixed with 20 µL of PBS and 80 µL of NPS.
For tested reagents (aPPT, TG, PT and DRVVT), mean, standard deviation and coefficient variation (CV) were calculated at 340, 635 and 671 nm.
Results: Whatever the wavelengths assessed, reagents of the intrinsic, extrinsic and common pathway had very slight effect on the baseline absorbance except for CK Prest® which contain kaolin.
For aPTT and TG reagents, except CK Prest®, the absorbance was always within range of the individual plasma +/- 1*SD (0.2977 ± 0.07928 at 340; 0.06201 ± 0.01616 at 635 and 0.05694 ± 0.01403 at 671 nm).
For PT and DRRVT reagents, the absorbance was always within range of the individual serum +/- 1*SD (0.3885 ± 0.1485 at 340; 0.07902 ± 0.03166 at 635 and 0.0692 ± 0.02772 at 671 nm).
Summary/Conclusion: Despite the colorful appearance of some reagents, these do not seem to interference with absorbance of plasma and serum, except CK Prest® (CV>10%) which contains kaolin. The volume of reagent (20µL) is insignificant in relation to total volume (120 µL). In conclusion, most of reagents currently on the market can be used for clot waveform analysis, except CK Prest®.

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Towards point-of-care diagnosis of TTP: Detection of anti-ADAMTS13 autoantibodies using FO-SPR technology

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Background: Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a life-threatening disorder, characterized by the presence of anti-ADAMTS13 autoantibodies (autoAbs). Current diagnostic assays for iTTP are expensive, time-consuming and labor-intensive, however, rapid diagnosis is vital for adequate treatment. Biosensors, such as the fiber optic-surface plasmon resonance (FO-SPR), have emerged and showed to be sensitive, inexpensive and easy-to-use. In addition, FO-SPR platform offers real-time monitoring and fast response time, being well-suited for point of care (POC) purposes.
Aims: The aim of this study is to develop an easy to use, fast and sensitive FO-SPR-based bioassay for the detection and quantification of anti-ADAMTS13 autoantibodies in iTTP plasma samples.
Methods: In our FO-SPR bioassay (on white fox 1.0 device), gold-coated optical fibers were functionalized with a nitrilotriacetate self-assembled monolayer (NTA-SAM) and chelated with cobalt. Next, recombinant human ADAMTS13 (rhADAMTS13) was immobilized on the optical fiber via its His-tag and the optical fiber was exposed to sample with autoAbs. Signal amplification was achieved using gold nanoparticles (AuNP) functionalized with anti-human IgG antibodies. The cloned patient anti-ADAMTS13 antibody (Ab) II-1 or iTTP patient plasma, containing high anti-ADAMTS13 autoAb titers, were used as calibrator. Normal human plasma or pooled human IgGs (both negative for anti-ADAMTS13 autoAbs) were used as negative control. Validation was performed by testing six iTTP plasma samples in both the FO-SPR-based bioassay and ELISA, using a dilution series of iTTP patient plasma as the calibration curve. The read-out, when using optical fibers precoated with rhADAMTS13, was obtained within 10 minutes.
Results: Effective immobilization of rhADAMTS13 on the FO probe and subsequent signal amplification, after adding a source of anti-ADAMTS13 autoAbs, was observed, whereas negative controls showed no significant signal. The calibration curve using the cloned patient Ab II-I demonstrated a dynamic range from 6.25 to 200 ng/mL (inter-assay CV = 10.1%, n=3), and the calibration curve using the iTTP sample (high anti-ADAMTS13 autoAb titer) ranged from 10 to 1600 times diluted plasma (inter-assay CV = 12.7%, n=3). Next, anti-ADAMTS13 autoAb titers were determined in six iTTP plasma samples and the values were compared with the titers obtained in ELISA. Antibody titers obtained via the FO-SPR-based bioassay and ELISA demonstrated a significant correlation ($p < 0.05$, ICC=0.8562, n=3).
Summary/Conclusion: An in-house FO-SPR-based bioassay for the detection of anti-ADAMTS13 autoAbs has been established. Anti-ADAMTS13 autoAb titers were measured in six iTTP plasma samples and showed a good correlation with ELISA. Due to the speed, simplicity and high degree of automation, this new FO-SPR bioassay could have great potential to become a POC diagnostic tool for iTTP.

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Immature neutrophils circulate in peripheral blood of acute coronary syndrome patients

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Background: Acute coronary syndrome is a leading cause of death worldwide. Though, the mechanisms of disease onset, i.e. plaque rupture and atherothrombosis, are not fully elucidated. There is evidence that neutrophils could play a role, as their count in circulation, especially neutrophil-to-lymphocyte ratio (NLR), is among the most robust predictors of acute coronary events, and is associated with disease severity. Neutrophils can notably contribute to thrombus formation by releasing neutrophil extracellular traps (NETs) with potent pro-inflammatory and prothrombotic ability. Furthermore, NET markers are independently associated with the severity, extent and occurrence of adverse cardiac events.
Neutrophil heterogeneity has been described in several acute and chronic inflammatory conditions, and in cancer. In these diseases, neutrophils consist of distinct subsets showing either immunosuppressive or pro-inflammatory functions. Some of them sediment within the peripheral blood mononuclear cells (PBMCs) fraction after density gradient centrifugation and are generally named "low density neutrophils" (LDNs).
However, neutrophil subsets in ACS patients and their role in disease onset remain unexplored.
Aims: The goal of our study was to determine if ASC patients display heterogeneous peripheral blood neutrophils, and to study the association between the presence of neutrophil subpopulations, NET markers, and severity of acute coronary events.
Methods: We performed a pilot prospective study on 36 patients with stable coronary artery disease (CAD), 16 with Non-ST-Elevation Myocardial Infarction (NSTEMI) and 16 with ST-Elevation Myocardial Infarction (STEMI). After density gradient centrifugation, phenotype of LDNs and neutrophils from "normal density" fraction (NDNs) were analyzed by flow cytometry using different markers of neutrophil activation and maturation (CD66b, CD11b, CD16 and CD10). The proportions of CD66b-positive cells were determined in PBMC fraction (LDNs). The ability of NDNs to release NETs was evaluated in vitro upon activation with phorbol ester. NET markers (nucleosomes) were measured in patient plasma.
Results: We detected LDNs in the peripheral blood of ACS patients (stable CAD: 1.7±1.9; NSTEMI: 2.2±2.6; STEMI: 6.0±5.4 % in PBMCs, P=0.0025 STEMI *vs* stable CAD). LDN percentage was positively correlated with neutrophil count and NLR (Spearman's r= 0.4 and r=0.5, respectively, P <0.0001). NDNs from every patient were all positive for CD10 and CD16, but NDNs from ACS patients expressed lower levels of CD16 (MFI: ACS: 86282±23576, stable CAD: 110162±49821, P=0.0131). NDNs from ACS patients also expressed higher levels of CD11b (MFI: ACS: 14242±2568, stable CAD: 11664±3095, P=0.0004). LDNs from ACS patients were a mix of cells at different stages of maturation, as some cells did not express CD10 or CD16. On average, LDNs express lower levels of CD10, CD16, and CD11b on their surface as compared to NDNs, depicting a more immature phenotype. NDNs isolated from STEMI patients were more prone to release NETs. Interestingly, nucleosome levels were higher in plasma of ACS patients (P=0.028), and were inversely correlated with the expression of NDN CD10 and CD16 (Spearman's r=-0.2 and P=0.0361 and 0.0470 respectively), suggesting a relationship between the presence of immature neutrophils in blood and NET release.
Summary/Conclusion: ACS patients exhibit circulating immature neutrophils that could be implicated in the onset of acute MI, possibly via increased NET release.

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Caveats in detection of tissue factor

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Background: Throughout the years reports of tissue factor (TF) expression in blood cells have been a controversial issue. It all started by the publication of blood borne TF (Giesen et al. 1999). The basic for this assumption was the observation that when whole blood was allowed to pass ex vivo along a collagen-coated glass slide or over porcine arterial tunica media, a TF-dependent clotting occurred. In a follow-up study, it was shown that monocytes and possibly polymorphonuclear leukocytes might be involved in the transfer of TF onto platelets. Thus, the origin of the blood born TF appeared to be monocytes expressing TF whereas others claimed the presence of TF in circulating platelets. Later, several studies were published on the TF synthesis and expression of TF in platelets as well as neutrophils.
Aims: In our opinion, the conflicting results on the TF detection in platelets can be explained by: 1) contamination of platelet preparations with monocytes, 2) non-specific TF antibodies, and 3) TF activity assays lacking both sensitivity and specificity.
Methods: Platelet preparations, based on merely density centrifugation and not immune-affinity removal of monocytes, are likely to be contaminated by the latter that even at rest possess palpable amounts of TF antigen and mRNA.
Results: Controversial reports showing TF on platelets could also stem from the choice of anti-TF antibody, as well as their higher-than-optimal concentrations. Some antibodies, even at low concentrations, lead to false positive TF detection due to non-specific binding to various cell antigens. Thus, the anti-TF antibodies from the clones CLB/TF-5 and VIC7 used in several studies, appeared to detect large amounts of TF in resting and activated platelets, as well as in resting monocytes and extracellular vesicles isolated from platelet-free human plasma. A third anti-TF antibody, clone VD8, showed no binding to resting platelets but to Ca-ionophore activated platelets or other cells expressing high amount of phosphatidylserine (PS), suggesting non-specific binding as pertinent controls have been lacking. Furthermore, ELISA assays have been most often regarded as a specific assay for the TF protein detection, however numerous studies have failed to show any correlation of plasma TF levels in patients with ongoing thrombosis or predict the likelihood of having thrombosis. The TF activity measurements have mainly been based on the activation of FX by TF in the presence of FVII/FVIIa, a system dependent on 1-2 h incubation followed by measurement of formed FXa. Although traces of TF may be required, as perceived by the neutralization of TF by antibodies, FVIIa alone is capable of activating FX in the presence of PS and measured FXa could merely represent an auto-activated FX (Jesty et al. 1974). Thus, the FXa-based assays are neither sensitive nor specific for detection of TF activity in cells/EVs due to the inevitable presence of membranes rich in PS. Thrombin generation assays (CAT) succumb the same problem since traces of TF trigger PS-dependent thrombin generation.
Summary/Conclusion: Further refinement of TF measuring is needed to further research in this field.

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Microfluidic modeling of thrombolysis: effect of antiplatelet and anticoagulant agents on tissue plasminogen activator-induced fibrinolysis.

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Background: Despite the high clinical relevance of thrombolysis, representative models for the study of human blood under flow conditions are lacking.
Aims: Our objective was to develop a microfluidic model for comparative evaluation of thrombolytic therapeutic strategies
Methods: Citrated human blood was supplemented with $DIOC_6$ and Alexa Fluor 647 fibrinogen conjugate, recalcified, and perfused through microfluidic flow chambers coated with collagen and tissue factor under an adjusted shear stress for 3 or 4 min in order to obtain non-occlusive thrombi. A second perfusion was performed for 10 min with rhodamine-6G-labeled citrated whole blood, supplemented or not with recombinant tissue-type plasminogen activator (rtPA), FITC-conjugated rtPA, and Alexa Fluor 568 plasminogen conjugate. rt-PA was associated or not with anticoagulants [heparin 0.5 U/ml or hirudin (20 U/mL), thrombomodulin (5 nM)], or the antiplatelets [Abciximab (3 µg/mL), ticagrelor or a blocking anti-GPVI Fab (50 µg/mL)] on selected preformed fibrin rich thrombi. Real-time fluorescence microscopy permitted the quantification of fibrinolysis and platelet accumulation. Fibrinolysis was also assessed by measuring D-Dimers in the output flow.
Results: Plasminogen and rtPA bound to pre-formed thrombi. rt-PA (15 µg/mL) induced progressive and significant lysis of the fibrin network compared to the control in absence of rt-PA ($p= 0.0026$). The level of fibrinolysis was dependent on shear stress and measured 50% and 70 % in arterial and venous conditions, respectively. Fibrinolysis increased in a rt-PA dependent concentration manner. The extent of fibrinolysis measured by fluorescence was well correlated to the increased levels of D-Dimers ($p<0.005$). Remarkably, despite ongoing fibrinolysis, new platelets continued to be recruited to the thrombus under lysis. Combining rtPA with hirudin enhanced fibrinolysis, but did not prevent the recruitment of new platelets, which was, in contrast to antiplatelet agents (ticagrelor or the GPVI-blocking Fab 9O12). Conversely thrombomodulin strongly delayed rt-PA induced fibrinolysis.
Summary/Conclusion: This microfluidic model is suitable for studying thrombolysis in venous or arterial flow conditions and for testing the efficacy of drugs used in combination with r-tPA. Real time analysis of fibrin and platelets during rtPA-mediated fibrinolysis under arterial flow conditions showed that platelets continue to accumulate during fibrinolysis. Such platelet accumulation may impair rtPA-mediated recanalization.

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GWAS identifies new risk variants in ADAMTS13/STKLD1 locus for deep venous thrombosis

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Background: Deep Venous Thrombosis (DVT) is a common multifactorial disease that is influenced by environmental and genetic factors. Previous genome-wide association studies (GWAS) identified several thrombophilic mutations in coagulation factors, as well as in the fibrinogen alpha and gamma chains (*FGA/FGG*) and the *ABO* locus. Further susceptibility loci (e.g. *ZFPM2*, *TSPAN15*, *SLC44A2*, *GP6*, *KNG1*, *STXBP5*, *HIVEP1* and *NME7*) have been suggested. However, a large proportion of the heritability of DVT remains unexplained.
Aims: In this study, we aim to identify new DVT susceptibility genes using a GWAS approach. Identified variants were further tested for association with ADAMTS13 activity and vWF levels to gain further insights in disease aetiology.
Methods: A GWAS was performed on 962 adult DVT cases and 902 healthy controls of European descent. Genotypes were generated using the Illumina Infinium Global Screening Arrays MD24 v1.0. Logistic regression assuming an additive genetic model, adjusted for sex, age and the first five principal components was used for association analysis. The genomic inflation factor was estimated as 1.049 indicating no relevant population stratification. Additionally, two linear regression models were performed on active ADAMTS13 levels and von Willebrand-Factor (vWF) levels.
Results: Previously described loci FV-Leiden ($p = 9.35 \times 10^{-29}$), *ABO* ($p = 6.14 \times 10^{-16}$), *FGA/FGG* ($p = 3.48 \times 10^{-09}$) and *NME7* ($p = 3.66 \times 10^{-19}$) exceeded genome-wide significance (5×10^{-08}) in our study. Additionally, we were able to find a variant in the von Willebrand cleavage protease *ADAMTS13* on chromosome 9 (rs4962153, $p = 6.36 \times 10^{-08}$) nearing genome-wide significance, as well as one in the neighbouring serine/threonine kinase like domain containing 1 gene (*STKLD1*, rs17474001, $p = 1.78 \times 10^{-08}$). These newly identified SNPs also showed a significant association with vWF levels in linear regression analysis (rs4962153: $p = 4.12 \times 10^{-09}$; rs17474001, $p = 2.17 \times 10^{-10}$). However, these results need to be viewed with caution, since the distribution of vWF levels was skewed and failed statistical criteria for normality.
Summary/Conclusion: We confirm susceptibility loci from earlier GWA studies, providing further support for their role in DVT aetiology. Here we provide novel evidence of *ADAMTS13* having an impact on DVT in addition to previous reports on its role in ischemic stroke and arterial thrombosis. *STKLD1* and *ADAMTS13* have been associated with vWF levels in stroke in earlier studies[1-3] and a variant located in *STKLD1* was shown to decrease plasma ADAMTS13 levels[4]. Our findings support an impact of *STKLD1* on vWF levels within the context of DVT potentially mediated via ADAMTS13 levels. However, this finding requires further validation in functional studies. Taken together, our results implicate the *ADAMTS13/STKLD1* locus as a general susceptibility locus for thrombotic diseases.

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Bring of the high multiplex Real-time chain polymerase reaction (PCR)

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Background: Thrombosis is the formation of a blood clot inside a blood vessel, obstructing the blood flow of the cardiovascular system. Several thrombosis associated single nucleotide polymorphisms (SNPs) have been identified and reported to significantly increase the risk of venous thrombosis.
The high multiplex Real-time PCR allows to detect and discriminate several SNPs in a single tube.
Aims: The goal of our work is to compare multiplex real-time PCR with simplex real-time PCR.
Methods: A genetic study looking for the most frequent mutations, responsible for thrombosis was performed in twelve patients referred to the hemostasis laboratory of our department for exploration of a thrombotic syndrome, and whose thrombosis assessment showed resistance to activated protein C. For this study, we used real-time PCR in simplex (R506Q mutation of Factor V Leiden, G20210A mutation of Factor II Laiden) and in multiplex (mutations R506Q, H1299R and Y1702C of the factor V gene, the factor G20210A mutation. II Leiden, C677T and A1298C) in the MTHFR gene.
Results: The results obtained by the two real-time PCR methods (simplex and multiplex) are concordant with identification of other mutations by multiplex PCR, which explains the intensity of the thrombotic syndrome.
Summary/Conclusion: the high multiplex Real-time PCR detects multiple genetic variations in a single trial, dramatically accelerating diagnosis and optimizing patient treatment.

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Immunoglobulins of healthy donors – a source of anti-idiotypic antibodies against anti-ADAMTS13 autoantibodies in immune-mediated thrombotic thrombocytopenic purpura (iTTP) patients?

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Background: Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is caused by autoantibodies against ADAMTS13. Specific targeting of those anti-ADAMTS13 autoantibodies to restore ADAMTS13 activity remains challenging. Indeed, nowadays Rituximab is frequently used to remove anti-ADAMTS13 autoantibodies, however by depletion of all B cells. Intravenous immunoglobulins (IVIG) have sporadically been used as a treatment for iTTP patients who are refractory to the regular treatment of plasma exchange, and have contraindications to Rituximab. The immunosuppressive mechanism of action of IVIG (an IgG pool from healthy donors) is likely multifactorial, with one of the possibilities, the presence of anti-idiotypic antibodies that might neutralize anti-ADAMTS13 autoantibodies.
Aims: To investigate whether healthy donor plasma contains anti-idiotypic antibodies that can inhibit the binding of the iTTP patient's autoantibodies to ADAMTS13.
Methods: An ELISA assay was set up to test whether 4 purified IgG pools from healthy donors could inhibit the binding of iTTP patient anti-ADAMTS13 autoantibodies to coated recombinant human (rh)ADAMTS13. The human anti-ADAMTS13 monoclonal antibody (TTP73-1) cloned from an iTTP patient, and a pool of purified IgG from an iTTP patient were biotinylated and incubated with the purified IgGs of a healthy donor. Next, the solution was transferred to a 96-well plate which was pre-coated with rhADAMTS13. The bound anti-ADAMTS13 autoantibodies (TTP73-1 or purified iTTP IgGs) were detected with HRP-labelled streptavidin.
Results: The four purified IgG pools from healthy donors (range 0.5-0.9 mg/ml) were tested for inhibition of the binding of the anti-ADAMTS13 autoantibodies from iTTP patients (TTP73-1 or purified iTTP IgGs) to coated rhADAMTS13. None of the healthy donor IgG pools individually or combined inhibited the binding of the patient autoantibodies.
Summary/Conclusion: The four IgG pools tested did not contain anti-idiotypic antibodies that interfere with the binding of autoantibodies to ADAMTS13. Hence, this suggests that the limited successful treatment of refractory iTTP patients with IVIG is probably not due to the presence of anti-idiotypic antibodies. To confirm these results, we are currently investigating whether IVIG inhibits the binding of anti-ADAMTS13 autoantibodies to rhADAMTS13.

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From autoantibodies to therapeutics: en route to novel treatment for immune TTP

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Background: A cryptic epitope in the ADAMTS13 spacer domain is targeted by autoantibodies in most of the immune TTP patients. Mutations on the epitope residues of spacer domain can protect ADAMTS13 against the neutralizing activity of most of the anti-ADAMTS13 autoantibodies (Pos et al, 2010).
Aims: Our aim is to employ *in silico* approaches, cryo-EM and functional characterisations to map the interactions between the spacer domain and autoantibodies, such as to provide structure-function data that can be translated into therapeutic use.
Methods: Patient-derived monoclonal anti-ADAMTS13 antibodies I-9 and II-1 were modelled by Bioluminate module in Schrodinger suite and HADDOCK protein-protein docking was employed to study the interactions between these autoantibodies and spacer domain of ADAMTS13. Proposed complexes between ADAMTS13 and autoantibody complexes were subjected to binding free energy calculation with AMBER16 over a 100ns molecular dynamics simulation. Most likely complexes were selected through agreement with functional data, with residue substitutions on the epitope (Pos et al., 2010, 2011) being accompanied by a predicted less-favoured binding free energy throughout the simulations.
Results: Binding affinities were calculated for the interaction between the ADAMTS13 spacer domain and I-9 and II-1, to give binding energies of -66±6 kcal/mol and -59±5 kcal/mol, respectively. These values are in accordance with experimentally determined binding affinities as measured by surface plasmon resonance (Pos et al., 2009). Selected poses were subsequently exploited to investigate essential residues of the ADAMTS13 spacer domain for binding with autoantibodies. Apart from known major epitope residues (Pos et al., 2010, 2011), several other residues within the spacer domain were found to contribute to autoantibody binding. The detailed structural knowledge of the predicted models was utilized to design peptides that bind to epitopes of the spacer domain to prevent autoantibody binding. These peptides were produced and functionally tested for their ability to compete with autoantibody binding. These peptides are now being optimized *in silico* to obtain stronger binding affinities against spacer domain.
Summary/Conclusion: In summary we have used the available structural bioinformatics tools to predict the nature of autoimmune antibody binding to the human ADAMTS13 protein. The autoantibody bound ADAMTS13 models will be further verified by the use of cryo-EM. The derived knowledge will be employed to facilitate the design of novel therapeutic approaches for treatment of immune TTP.

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The balance between fibrin clot formation and fibrinolysis may predict cardiovascular events in patients with stable coronary artery disease

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Background: Despite antiplatelet treatment with aspirin, adverse cardiovascular events, like acute myocardial infarction and cardiovascular death, are common complications in patients with coronary artery disease. We have previously demonstrated an unfavorable fibrin clot structure with reduced fibrinolysis in patients with coronary artery disease and type 2 diabetes mellitus and/or prior myocardial infarction treated with aspirin. It remains to be investigated whether a dense fibrin clot structure resistant to fibrinolysis may predict adverse cardiovascular events in stable coronary artery disease patients.
Aims: We aimed to determine whether increased fibrin clot formation and reduced fibrinolysis are associated with cardiovascular events in patients with stable coronary artery disease treated with aspirin monotherapy.
Methods: We included 786 patients with angiographically documented coronary artery disease and either prior myocardial infarction, type 2 diabetes mellitus or both. Fibrin clot formation and fibrinolysis was evaluated using an in-house turbidimetric assay employing thrombin (0.03 U/ml in clot) and calcium (7.5 mmol/L) as coagulation triggers and tissue plasminogen activator (83 ng/mL) as lysis agent. We evaluated a) fibrin clot maximum turbidity, which reflects fibrin clot formation, b) clot lysis time reflecting fibrinolysis and c) area under the curve, which integrates the balance between fibrin clot formation and fibrinolysis. The primary endpoint was the composite of nonfatal myocardial infarction, ischaemic stroke, and cardiovascular death. Hazard ratios were estimated using multivariable Cox proportional hazards survival regression. The study was conducted in agreement with the Declaration of Helsinki and all participants gave written informed consent.
Results: At 3-year follow-up, a total of 70 primary end points had occurred. The primary endpoint occurred more frequently in CAD patients with increased fibrin clot formation and fibrinolysis time evaluated by the integrated clot area under the curve (crude hazard ratio for first versus fourth quartile: 2.4 [95% confidence interval (CI) 1.2-4.6], p=0.01). This finding remained significant after adjusting for sex, age, prior myocardial infarction, diabetes mellitus, active smoking, renal function and fibrinogen level (adjusted hazard ratio: 2.4 [95% confidence interval (CI) 1.2-4.8], p=0.01), and also when adjusting for fibrinogen level alone (adjusted hazard ratio: 2.4 [95% confidence interval (CI) 1.2-4.7], p=0.01). However, this finding was not confirmed in CAD patients with high levels of clot maximum turbidity (crude hazard ratio: 1.9 [95% CI, 1.0-3.7], p=0.05), and prolonged lysis time (crude hazard ratio: 1.5 [95% CI, 0.8-2.7], p=0.25). High levels of fibrinogen did not predict the primary endpoint of cardiovascular events (crude hazard ratio: 1.5 [95% CI, 0.8-3.0], p=0.22).
Summary/Conclusion: We demonstrated for the first time that increased fibrin clot formation and prolonged lysis time, reflected by high fibrin clot area under the curve, predicts cardiovascular events in stable CAD patients treated with aspirin. However, our findings were not confirmed when evaluating fibrin clot formation and fibrinolysis time individually. Increased fibrinogen levels did not explain the association between increased area under the curve and cardiovascular events alone.

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ANNUAL COST OF CLOTTING FACTORS IN PATIENTS WITH SEVERE (SEV) HEMOPHILIA A (HA) IN FRANCE - AN ANCILLARY ANALYSIS OF HEMONIS STUDY

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Background: The management of patients with Sev HA is particularly complex and costly especially for patients with inhibitor. Data on the real consumption of clotting factors and associated costs are scarce and further investigation is needed to improve knowledge on the economic burden of this disease.
Aims: The objective of this analysis was to estimate the annual cost per patient of clotting factors in Sev HA patients according to their therapeutic strategy and inhibitor status.
Methods: HEMONIS is a national multicenter retrospective cohort study describing the treatment patterns of French patients with HA. The core methodology and main results are presented in two other abstracts. Sev HA patients were included in two subgroups according to their inhibitor status: current inhibitor (CI) and no history of inhibitor (NHI). Clotting factors consumptions to prevent or manage bleeds were used to define the resources used according to treatment regimen (prophylaxis, on-demand (OD) and ITI)). Unit cost of clotting factors were based on the French tariffs excluding VAT (Feiba®: €0.903/U; Novoseven®: €0.608/µg; FVIII: €0.648/UI). Total cost was then estimated at individual patient level and annualized to improve comparability of results.
Results: A representative sample of 66 Sev HA patients was analyzed: 36 NHI patients (median age 32; median weight 75.3 kg) and 30 CI patients (median age 22; median weight 66.8 kg). At last visit, the most frequent treatment was FVIII prophylaxis in NHI pts (81%), and ITI in CI pts (40%). In NHI patients treated with FVIII, the annual mean (median) cost per patient was €216,118 (€204,928) for patients under prophylaxis (n=25) and €78,685 (€55,690) for patients under OD treatment (n=9). In CI patients under ITI (FVIII only), mean annual cost per patient was €1,215,799 (€641,520) for background infusions (n=13) and €76,592 (€4,209) for the management of bleeds (n=11). When treated with prophylaxis (n=6) or OD (n=9) (BPA only), the annual mean cost per patient was €811,268 (€795,294) and €184,196 (€140,659) respectively.
Summary/Conclusion: This cost-of-illness study shows the very high economic burden of Sev HA patients in France especially among patients with inhibitor, for which the cost of prophylaxis is estimated above €800,000 per patient per year. However, this strategy is still associated with limited benefit on bleed reduction and high treatment burden for patients. This study highlights the potential need for more cost-effective treatments in Sev HA. The HEMONIS study was sponsored and conducted by Roche SAS.

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A RARE CASE OF ANTIPHOSPHOLIPID SYNDROME-ASSOCIATED MIDDLE CEREBRAL ARTERY STENOSIS IN A YOUNG PATIENT PRESENTING WITH MOYA-MOYA PATTERN: A THERAPEUTIC CHALLENGE FOR THE INTERNIST

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Background: Moyamoya disease (MMD) is a rare entity of the cerebrovascular system usually involving stenosis of the proximal middle cerebral (MCA) or the internal carotid artery (ICA). The affected vessels trigger the growth of a network of collateral vessels, visible on angiography as a puff of smoke, leading to the disease's interesting name. Common presentation symptoms include headaches, ischemic stroke or intracerebral hemorrhage and- less frequently- seizures or cognitive changes. Moyamoya syndrome (MMS) refers to typical moyamoya vasculopathy with well-recognized associated conditions such as sickle cell disease, type 1 neurofibromatosis as well as systemic lupus erythematosus. However, there have been no systematic studies on MMS with antiphospholipid antibodies (aPL).
Aims: Herein, we present an interesting case of a 38-year-old Caucasian male patient, who presented with multiple primary exercise headaches and was finally diagnosed with aPL-associated Moyamoya syndrome.
Methods: A 38-year-old Caucasian male patient, with no previous medical history and no use of alcohol or illicit drugs, was referred to our outpatient clinic for multiple primary exercise headaches during the last three months lasting, at least 10 minutes until a day the worse. No history of other neurologic complaint was reported apart from several headache episodes after smelling spicy perfumes lasting for several hours. His physical examination was unremarkable apart from a mitral valve murmur of 1-2/6. His neurological examination was excellent too. Routine laboratory blood tests were normal. The patient underwent brain MRI tomography and angiography, which both revealed an occlusion of the right middle cerebral artery with rich basal collateral vessels. Brain angiography confirmed the presence of Moyamoya- like vasculopathy. Coagulation studies were normal, lupus anticoagulant (LA) was borderline positive, while immunoglobulin IgG and IgM anticardiolipid antibodies (aCL) levels were 9 U GPL/ml and 38 U MPL/ml (normal for both <15 U MPL/ml), respectively, and IgG/IgM anti-beta2-glycoprotein I antibodies (ab2GPI) were normal.
Results: Based on the typical radiological findings and laboratory results, a diagnosis of aPL-associated MMS was made. The patient was started on aspirin (100 mg/day) with clinical remission. A year after initial diagnosis, he remains asymptomatic and his brain MRA findings remain unchanged. aCL were twice measured out of the normal range establishing antiphospholipid syndrome (APS) diagnosis.
Summary/Conclusion: The relationship between MMS and APS remains unclear, aPL though may play a role in promoting further thrombosis and recurrent ischemic events through damaged vascular structures or with thrombosis arising from the abnormal vasculature or with an unidentified systemic disease underlying MMS. Such patients are always a therapeutic dilemma involving the risk of thrombosis in definite APS and the tendency toward fatal hemorrhage in MMS. Although MMS is a rare entity, medical community should be alerted for its diagnosis, which should be made as early as possible, due to the associated high disability.

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State of the knowledge on the set up of medical compression in patients at high risk of thrombosis

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Background: In France, the Institut de Veille Sanitaire (InVS) reports in 2010, 5,137 deaths for which ThromboEmbolic Venous (VTE) Disease was the initial cause. Different treatments for primary prevention and / or recurrence are recommended, including medical compression, an effective method accessible to all. During consultations with patients at risk of venous thrombosis, it was found that few patients knew the value and the good use of venous compression. This observation led us to set up pharmaceutical interviews for therapeutic information following the medical consultation.
Aims: The aim of this study is to assess the level of knowledge of patients on the set up of venous compression during pharmaceutical interviews.
Methods: This is a prospective study started in March 2018, still ongoing. A questionnaire was developed based on the recommendations of the French Society of Phlebology (SFP). Various items were selected such as the method of placement used, the presence of difficulty during donning, compliance, as well as the existence of associated advice during an earlier wear of venous compression. The survey was conducted in a university hospital center by a pharmacist, based on this questionnaire during a therapeutic information interview, following a medical consultation. Only patients who had a thrombotic event or several risk factors requiring medical compression were included in the study.
Results: Out of 46 interviews, 20 patients were included in the study according to the predefined criteria. All patients are women with deep veinous thrombosis, complicated or not with pulmonary embolism, and / or genetic risk factors. 80% of patients in our cohort have an ongoing pregnancy, representing an additional thrombotic risk factor. The average age is 32.5 years (25 – 50 years).
At the time of dispensation of venous compression, 40% (n = 8) of patients had received advice on compression placement from a community pharmacist (75%, n = 6) or nurse (25%, n=2).
During the pharmaceutical interview, 55% (n = 11) know the recommended method by GEMMAT as a reference, and 45% (n = 9) use it.
Regarding compliance, 30% (n = 6) of patients wear compression daily. Other patients report different reasons for their bad compliance, such as discomfort, aesthetic, negligence, or difficulty in donning. Indeed, 15% (n = 3) of patients report difficulties in set up of their compression.
Summary/Conclusion: Although the set up of this medical device is simple, an insufficient number of patients know the reference method of donning. One reason may be the lack of information during the dispensation. As when setting up an anticoagulant treatment, it would be relevant to carry out systematic therapeutic information at each dispensation. In a second time, it would be interesting to be able to assess the state of knowledge of these patients after our interviews.

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Mice Lacking the Novel Thrombosis Susceptibility Gene Slc44a2 Have Normal Hemostasis but an Aberrant Response to Vascular Injury

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Background: Recent genome wide association studies (GWAS) identified a novel susceptibility locus for venous thromboembolism (VTE), harbouring the *SLC44A2* gene, which encodes Solute Carrier Family 44 Member 2 protein (SLC44A2). Thus far, SLC44A2 has not been linked to hemostasis pathways and may therefore be a unique contributor to VTE.
Aims: To uncover a possible role of SLC44A2 in normal hemostasis by evaluating several parameters of hemostasis in mice lacking *Slc44a2* (*Slc44a2*$^{-/-}$).
Methods: Wild type and *Slc44a2*$^{-/-}$ littermates were evaluated in key aspects of normal hemostasis i.e. thrombin generation, transcription of coagulation related genes and platelet aggregation. Von Willebrand factor (VWF) plasma levels, multimerization and localization were studied as SLC44A2 was previously described to be a potential binding partner of VWF *in vitro*. Additionally, the response of *Slc44a2* deficient mice upon vascular injury was assessed using the cremaster muscle arterioles laser injury model on *Slc44a2*$^{-/-}$ mice with an FVB and B6 background.
Results: *Slc44a2*$^{-/-}$ mice displayed comparable levels of thrombin generation and gene expression of coagulation related genes in the lung and liver, as compared to littermate wild type controls. No detectable levels of fibrin deposition in lung and liver were observed, indicating no pro-thrombotic state in these mice. Platelets counts were unaffected by *Slc44a2* status with similar collagen and thrombin induced aggregation kinetics. Interestingly, a 20% drop in VWF plasma levels was observed for *Slc44a2*$^{-/-}$ ($p<0.05$), while no differences in VWF multimerization and vascular localization were detected. Upon *in vivo* laser injury of the cremaster arterioles, we observed an altered response for *Slc44a2* deficient mice on both a B6 and FVB background. The FVB mice displayed similar dynamics of platelet accumulation, however fibrin accumulation was significantly impaired in *Slc44a2*$^{-/-}$ mice ($p<0.0001$), which may suggest clot instability. Interestingly, the response upon laser injury in *Slc44a2*$^{-/-}$ mice on B6 background was also impaired, which was due to a significantly decreased platelet accumulation as compared to control mice ($p<0.05$).
Summary/Conclusion: *Slc44a2*$^{-/-}$ mice are normal for the several hemostasis parameters that we evaluated; apart from a reduction of circulating VWF levels. This observation, in addition to the recorded impact of *Slc44a2* deficiency upon vascular challenge, suggests that SLC44A2 contributes to hemostasis. Thereby these findings substantiate the reported GWAS data and encourage further investigation of the role of SLC44A2 under pathological conditions i.e. thrombosis and if any, the underlying mechanism.

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Mean platelet volume predicts the complications of arteriovenous fistula in hemodialyzed patients

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Background: Arteriovenous fistula (AVF) is the life line of the hemodialyzed (HD) patients. Complications of the AVF are responsible of 30% of hospitalizations in HD patients. The two main complications are dysfunction related to stenosis or thrombosis. The high incidence of stenosis is related to surgery and uremia. Thrombosis is secondary to anatomical anomalies and the prothrombotic state associated with uremia. The best treatment of these complications is prophylaxis. To identify it the best approach is ultrasound examination. Unfortunately, this exam is time consuming to be perform repeatedly to all patients. So, we are searching an easily biomarker to pre-screen the HD patients. Increased mean platelet volume (MPV) is associated with increased cardiovascular complications in HD patients. We hypothesized that increased MVP could be a predict the risk of complications of AVF.
Aims: The main objective was MPV is a predictor of AVF events (AVF dysfunction requiring endovascular treatment and thrombosis).
The secondary objective was MPV variation at 6 months intervals.
Methods: All the patients on chronic hemodialysis at the Conception Hospital (Marseille) on June 2014 were included. Exclusion criteria were: missing laboratory data (platelet count, mean platelet volume), presence of platelet clusters, thrombocytopenia <50 G / L, hemodialysis duration <3 months, refusal to participate in study, inability to give informed consent. All AVF events were prospectively recorded. The analysis of the VPM was carried out on Sysmex automate by impedancemetry and dynamic focusing method. The MPV is calculated according to the plateletocrit formula (%) / total number of platelets (G / L) x 105. Statistical analyzes were performed with the GraphPadPrism software and R software. A value of $p < 0.05$ is statistically significant.
Results: We included 153 patients. At the end of the follow-up, 81 patients were still on dialysis in the center, 11 had changed dialysis centers but their medical data was available, 20 patients had renal transplantation, 40 patients died, and one was lost to follow-up. The median follow-up was 644. The mean MPV was 10.8 fl [7.8-13.5] and the quartiles were: group 1: MPV ≤10fl; group 2: 10fl ≤ MPV <10.7fl; group 3: 10.7 fl ≤ VPM <11.5 fl; group 4: MPV ≥ 11.5fl. Four groups were comparable excepted for creatinine ($p = 0.05$) and albumin ($p = 0.06$) lower in group 1. 43 AVF dysfunctions and 29 AVF thromboses (72 events) occurred in 54 patients (35%) with a median time of 259 days. The Kaplan-Meier analysis shows a significant difference ($p = 0.001$) in the rate of AVF events with a higher incidence for each MPV quartile increase: group 4 (59%), group 3 (34%), group 2 (27%), group 1 (18%). Multivariate analysis by Cox's method shows an independent association between the MPV and the risk of occurrence of an AVF event, the Odd Ratio (OR) is 1.66 [1.24-2.23] for each fl ($p = 0.0007$). Having a native AVF and not taking platelet antiaggregant are protective factors with respectively an OR 0.46 [0.19-0.95] $p = 0.04$ and 0.50 [0.26-0.98] $p = 0.04$. MPV values at 6 months of intervalle are stable.
Summary/Conclusion: In conclusion, the value of MPV is predictive of AVF events in hemodialyzed patients. Our results suggest the use of this easily available biomarkers to identify a group of high risk patients. This group could benefit of a more intense follow-up by echography allowing a best allocation of a limited resource.

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Role of factor VIII in clot growth and sensitivity to reversible factor Xa inhibitors

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Background: Plasma clot growth is a new phenotype, recorded by video and characterized by clot sizes achieved in defined time periods and clot growth rate at each time point.
Aims: We evaluated the role of factor VIII and the effects of reversible factor Xa inhibitors, rivaroxaban, apixaban and edoxaban.
Methods: Clot growth from a surface with immobilized tissue factor in citrated plasma, with added corn trypsin inhibitor was recorded with the thrombodynamics analyzer. Factor VIII and IX depleted plasma were obtained commercially. Clot size was recorded in µm after 5000 secs; clot growth rate in µm/min at 5000 sec.
Results: The clot size and clot growth rate after 5000s were about 60% and 40% in factor VIII or IX depleted plasma compared to normal pooled plasma. We discern a factor VIII/IX portion and a TF/FX portion. In the factor VIII/IX portion the growth is proportional to the factor VIII concentration from 0-200% FVIII and varies with a factor of 3.
The TF/FX portion is insensitive to inhibition by rivaroxaban, apixaban and edoxaban. The Factor VIII/IX portion is dose dependently inhibited by the factor Xa inhibitors, attributed to inhibition of factor VIII activation by factor Xa.
The inhibition by factor Xa inhibitors is stronger when factor VIII is low (50%) compared to higher levels (200%). The apixaban to achieve full inhibition of FVIII/IX related growth rate is from 0,1- 0,5 µM for 50% FVIII to 200% FVIII. The inhibition for 50% FVIII concerns a smaller effect (50% relative to normal plasma)) compared to that of higher FVIII, example 300% for FVIII 200%.
Summary/Conclusion: It is concluded that the size of effect and the effective concentration for effect of reversible factor Xa inhibitors is related to the factor VIII concentration. This is attributed to competitive inhibition of the inhibitors for factor VIII activation.
It suggests a difference in risk of bleeding and efficacy for a fixed dose of inhibitor due to factor VIII concentration. It suggests adjusting dosing to the factor VIII in a patient.

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Rheumatoid arthritis closes the gap in hemostatic balance between pre- and postmenopausal women

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Background: Together with the progressive disability secondary to the joint impairment, cardiovascular (CV) risk is a major issue in patients with rheumatoid arthritis (RA). The association with traditional CV-risk factors does not entirely explain the extent of premature atherosclerosis in this group of patients. Chronic systemic inflammation in RA shifts the hemostatic mechanisms in favor of thrombosis by activation of the coagulation system, inhibition of fibrinolysis and decreases in natural anticoagulant pathways. Thus, the presence of prothrombotic condition acts synergistically with traditional CV-risk factors and contributes to the increased CV-risk. This is of particular importance knowing that RA occurs more commonly in women and frequently onsets in women of childbearing age.
Aims: To assess hemostatic disturbances in female patients with established RA in relation to menopausal status and disease activity.
Methods: Ninety women were included in the study, 42 patients with RA and 48 age-matched healthy controls (mean age 54.8±9.1 and 54.1±6.2, respectively). The mean disease duration in patients was 12.8±8.0 years and the mean value of DAS28 was 3.8±1.1 at the moment of blood sampling. All patients were treated with the standard treatment protocol (methotrexate and Prednisolone), however only 9 patients (20%) were in remission measured by DAS28. There were no differences between the investigated groups regarding the presence of traditional CV-risk factors: smoking status, hypertension, diabetes mellitus, body-mass index (BMI), hyperlipidemia and the presence of previous CV event was the exclusion criteria. Patients with RA had higher CRP, SR and fibrinogen levels ($p<0.01$, $p<0.05$). Two global hemostatic assays were employed i.e. endogenous thrombin potential (ETP) and overall hemostasis potential (OHP). The parameters of ETP assay (area under the curve reflecting ETP, C-max, t-lag, t-max) and OHP assay (overall coagulation potential – OCP and overall fibrinolytic potential –OFP) were assessed. Moreover, parameters of fibrin structure (lag time, Max Abs, Slope, Max Abs time, Slope time) were measured by clot turbidity.
Results: All participants were divided in two subgroups according to menopause: (1) premenopausal controls (n=14); (2) postmenopausal controls (n=34); (3) premenopausal patients (n=11); (4) postmenopausal patients (n=31). Women in subgroups 1 and 3 were younger ($p<0.01$), had lower BMI ($p<0.01$), cholesterol- and LDL levels ($p<0.01$, respectively) compared to the respective subgroups. Premenopausal controls in the subgroup 1 differed significantly from all other subgroups by the diminished levels of OCP, OHP, ETP, C-max, Max Abs and Slope, while OFP was increased. This tendency ceased to exist in the premenopausal RA patients (subgroup 3). The disease activity measured by DAS28 correlated with disease duration, OCP and OHP indicating persistent hypercoagulable condition in the whole group of RA patients.
Summary/Conclusion: This extensive assessment points out to the persistent coagulation activation in premenopausal women with established RA, despite the low number of investigated subjects. Still, the patients were well characterized for the presence of traditional CV-risk factors, ongoing medications and had moderate- to high disease activity, which enabled assessment in the real life setting. Larger studies are mandatory to confirm these preliminary findings.

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Thrombin generation is increased in patients with chronic urticaria that associate angioedema

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Background: Chronic urticaria (CU) is a disease characterized by the appearance of wheals or/and presence of angioedema (AE). These symptoms occur when immunologic, physical or infectious stimuli trigger the release of inflammatory mediators. Patients with CU show increased activation of coagulation and fibrinolysis. The thrombin generation test (TGT) is a global coagulation assessment which quantifies *in vitro* the ability of plasma to generate thrombin.
Aims: To determine if TGT could be related with the presence of AE in CU patients, evaluating the association between the coagulation pathway and the course of the disease.
Methods: We have recruited 68 patients with active CU, 40 of them with a history of AE. Informed consent was obtained from all subjects. Plasma from patients was incubated with a mixture of tissue factor (4 pM), fluorogenic substrate and CaCl2. The thrombin generation curve was estimated using 5 parameters: lag time (LT, min), endogenous thrombin potential (ETP, nM·min), peak height (PK, nM), time to peak (ttPK, min) and the maximum slope of thrombin formation (Vo, nM/min). The severity of the CU was assessed using the Urticaria Severity Score (USS) questionnaire. TGT parameters were related to the CU severity, the presence of AE and other clinical parameters.
Results: CU patients with AE showed an increase in the PK and Vo (187.6±53.2 nM and 46.5±20.5 nM/min) compared with CU patients without AE (164±59 nM and 36.4±21.3 nM/min) (P=0.0625 and P=0.0355, respectively). There were no differences between CU patients with or without AE in the LT (5.37±1.07 and 5.56±1.38 min, respectively) or in the ttPK (10.33±1.99 and 10.86±2.49 min, respectively). We observed no relationship between the different TGT parameters according to the severity index. We also found no correlation between the different TGT parameters and clinical parameters associated with CU.
Summary/Conclusion: The generation of thrombin is increased in patients with CU who associated AE, indicating the presence of a hypercoagulable state. However, the clinical relevance of these findings is still under investigation. These results further suggest that the TGT can be useful to evaluate the role of the coagulation cascade in diseases with inflammatory component.

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Utility of the Thrombin Generation Test in various clinical phenotypes to predict thrombotic recurrences in cancer patients without anticoagulant treatment

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Background: Venous thromboembolism (VTE) is a frequent complication in cancer patients, which causes greater morbidity and mortality in these patients. In addition, patients with cancer and VTE have a high rate of thrombotic recurrences.
Aims: To explore the ability of the thrombin generation test (TGT) in various clinical phenotypes to predict thrombotic recurrence in cancer patients who had already suffered a VTE event, but in whom the anticoagulant treatment was withdrawn.
Methods: We prospectively recruited 167 cancer patients who suffered VTE, all participants gave written informed consent. We obtained a sample of citrated blood at anticoagulation withdrawal (recruitment), as well as 21 days and 3 months after. Of these, 22 patients (13.2%) suffered a recurrence (median: 3 months, 1-40 months). At each visit we measured FVIII, tissue factor, soluble P-selectin, D-dimer, C-reactive protein, procoagulant phospholipid activity, and the different TGT parameters by automated test (CAT, Thrombinoscope). Differences between the variables studied during the patients' follow-up were analyzed with the Wilcoxon test using R (v3.5.0).
Results: The variables that allowed us to differentiate cancer patients with thrombotic recurrences were: P-selectin at 21-day follow up (P=0.006), D-dimer at recruitment (P=0.034), D-dimer at 21 days (P=0.0003), C-reactive protein at 21 days (P=0.0098) and lag time at 3-months (P=0.039).
Summary/Conclusion: The concentration of P-selectin, D-dimer, C-reactive protein and the lag time of TGT are parameters that seem to have a prognostic utility during the follow-up of oncological patients who have suffered VTE, in order to predict a thrombotic recurrence. This information could lead to personalized thromboprophylaxis in these patients to avoid recurrences. ISCIII-FEDER (PI11/02308, PI14/00079, PI14/00512, FI14/00269, PI15/01085, CPII15/00002, PI17/00495), Generalitat Valenciana (PrometeoII/2015/017, ACIF/2017/138), Sociedad Española de Trombosis y Hemostasia and LEO-Pharma.

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Assessment of hypercoagulable state in patients with preeclampsia using global haemostatic assays

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Background: Haemostatic balance shifted toward hypercoagulability in normal pregnancy is even more pronounced in preeclampsia (PE). While routine coagulation assays provide static data on single coagulation factors and thus reflect acute situation, a new generation of global haemostatic assays gain an overall assessment of haemostasis. The endogenous thrombin potential (ETP) measures the total amount and kinetics of thrombin generated over time, and therefore enables the monitoring thrombin-forming capacity of plasma beyond the initiation of clot formation. On the other hand, assay of Overall Haemostatic Potential (OHP) gives additional insight in whole dynamics of the haemostatic process providing additional information concerning the rates of fibrin formation and fibrin degradation.
Aims: To analyze hemostatic disturbances in PE employing two global haemostatic assays and to investigate their correlation with maternal and fetal outcomes.
Methods: Forty-seven pregnant women diagnosed with PE were enrolled in the study. Blood sampling was done next morning after admission to hospital with second blood sample obtained from 2 - 10 days after delivery. Control group included 80 healthy pregnant women matched by age and gestational stage.
Results: Three thrombin generation parameters (ETP, lag time and peak height) and OHP were significantly increased in preeclamtic pregnant women compared to pregnant controls ($p<0.001$, $p<0.05$, $p<0.001$ and $p<0.05$, respectively), whereas Overall Fibrinolytic Potential (OFP - determined as a parameter of the OHP assay) had significantly lower values ($p<0.001$). Additionally, significantly prolonged clot lysis time (CLT) was found in patients with PE ($p<0.001$).
In PE group after delivery we observed significant elevation of the peak height and reduction in time to peak and OFP ($p<0.01$, respectively) compared to values before delivery.
To assess the contribution of hemostatic imbalance to maternal and fetal outcomes in the PE group, we examined the relationship between investigated parameters and reported complications in mothers, as well in a newborns. Among all investigated maternal complications (HELLP; renal complications – oliguria, nephrotic proteinuria, nephrotic syndrome; thrombocytopenia; placental abruption and neurological disorders) only patients with renal complications had significantly higher values for ETP, peak height and D-dimer compared to the patients without these complications ($p<0.05$, $p<0.01$ and $p<0.05$, respectively). Regarding fetal complications, moderate correlation was observed between ETP and 5 minute APGAR score.
Summary/Conclusion: Evaluation of global haemostatic parameters in this study confirmed the presence of enhanced coagulation and impaired fibrinolysis in patients with preeclampsia before, but even after the delivery. The presence of renal complications in preeclampsia should alert the clinicians to make a screening for the haemostatic disturbances in these patients and consider thromboprophylaxis in patients with confirmed hypercoagulable disorder.

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Point-of-care-INR testing: Challenges

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Background: We educate each year about sixty to seventy new cardiac patients in our anticoagulation school (AK-school) to use point-of-care (POC) devices for self INR monitoring. Literature data show that conditions with high and law hematocrit values (anemia and polycythemia vera) can affect INR measurements performed by POC devices.
Aims: To investigate how often this problem occurs practically.
Methods: We reviewed medical records in our AK-school from January 2017 until May 2018 to find out the amount of patients, who were advised to use conventional laboratory INR measurements due to medical conditions, influencing INR monitoring by POC devices.
Results: We found two cases.
Case 1.
A 56-year old man was prescribed Warfarin therapy due to chronic atrial fluctuation and was learned to use a POC device for self-INR monitoring. Later he reported measurement errors, not allowing performing INR measurements. A new device was delivered, but the problem was not resolved. The patient was invited to AK-school and INR was measured by laboratory technician on four devices, which reported the same measurement errors as mentioned. Blood samples were drawn to measure B-Hemoglobin, B-Hematocrit and B-Erythrocytes. The results showed high values of all three parameters (B-Hemoglobin 12,9 mmol/l (reference range 8,3-10,5 mmol/l); B-Hematocrit 0,60 (reference range 0,40-0,50); B-Erythrocytes 6,5 x 10^{12} l (reference 4,3-5,7 x 10^{12} l). The patient was diagnosed with secondary JAK-negative polycythemia vera due to chronic heart and lung diseases and was advised to use conventional laboratory method for INR control.
Case 2.
A 65-year old man was prescribed Warfarin therapy due to paroxysmal atrial fluctuation. He got a kidney transplantation 22 years ago and took immunosuppressive medication. The patient was routinized to perform POC INR measurements.
Medical personally observed divergent INR values measured by a POC device and conventional laboratory method. POC INR measurement showed INR values 6,1 and 4,1 compared to 4,2 and 2,8 measured by laboratory method. B-Hemoglobin showed lowered values between 6,9 and 7,3 mmol/l (reference range 8,3-10,5 mmol/l). The patient was advised to use conventional laboratory method for INR control.
Summary/Conclusion: Our observations confirm literature data regarding challenges in INR measurements by POC devices in some medical conditions.

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The significance of the fibrinolysis state in cancer patients receiving chemotherapy

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Background: The frequency of venous thromboembolic complications (VTE) in cancer patients (OP) increases by 10% during chemotherapy (CT).
Aims: To evaluate the role of fibrinolysis (F) during CT in OP.
Methods: We studied 86 cancer pts treated with chemotherapy in different regimens. Pts were observed for 7-14 days during CT. Test Thromboelastography (TEG): fibrinolysis was initiated in vitro by adding plasmin to kaolin-activated blood sample. Recorded parameter was TEG reaction time (r). The difference between r kaolin TEG (ABC) and plasmin+kaolin TEG (ABC+pl) (rF) was calculated as percentage of rF prolongation time (normal range rF=70-130%).

Results: There was the only one significant difference between the fibrinolysis state before CT (F=97±21) and after it (F=43±12, p<0, 01).
Summary/Conclusion: The hypofibrnolysis may by the main reason of hypercoagulation and thrombosis risk in cancer Pts receiving chemotherapy.

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Congenital dysfibrinogenemia – a single centre experience

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Background: Congenital dysfibrinogenemia is a very rare condition with variable clinical features. It leads to a mild bleeding disorder in about 30% of patients, whereas in another 20-25% of patients, the specific molecular defects are associated with thrombotic complications.
Aims: To present experience in management of patients with congenital dysfibrinogenemia who have been diagnosed and followed up in our centre.
Methods: Eighteen patients from twelve families were diagnosed with congenital dysfibrinogenemia in our centre between 2006-2017. Mutations in the fibrinogen molecule were found in all cases and two of them were published as novel mutations. Seven patients presented with bleeding diathesis that was mild in most of the cases. Two patients had thrombotic complications which were severe in one of them (repeated pulmonary embolism). One patients suffered from repeated spontaneous abortions. One half of the patients were asymptomatic.
Results: Fibrinogen replacement therapy was used to cover surgical procedures in four cases, including one total knee replacement. A total of four deliveries in three patients were covered with fibrinogen replacement therapy. Hemostasis was succesfully achieved in all the cases. We did not observe any thrombotic complications associated with fibrinogen replacement therapy.
Summary/Conclusion: Congenital dysfibrinogenemia, although it is a very rare condition, is a challenging diagnosis for clinician due to the variety of clinical features. Data regarding the phenotype are not often sufficient in the international databases. Thus, evaluating patient‘s history regarding the bleeding and thrombotic events is always extremely important for subsequent management of the patients even in cases of known causal mutation.

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The shortened activation peptide of Pseudonaja textilis venom FX abrogates activation by the intrinsic tenase complex

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Background: The Elapidae are a family of snakes characterized by venom prothrombin activators consisting of factor X (FX)- and factor V-like proteins that structurally and functionally resemble the human prothrombinase complex. One of these complexes, Pseutarin C, is found in the venom of the eastern common brown snake (*Pseudonaja textilis*). Its catalytic subunit (v-ptFX) comprises ~80% sequence identity to its liver counterpart and ~50% to human FX. Factor X is maintained in a zymogen state by an activation peptide that is proteolytically removed upon activation into factor Xa (FXa). Interestingly, while all vertebrate liver-expressed coagulation FX homologs, including that of *P. textilis*, comprise an activation peptide of at least 40 residues, the activation peptide of v-ptFX is significantly shortened to 27 residues.
Aims: Here we aim to assess the functional role of the shortened activation peptide in *P. textilis* venom FX and its effect on the activation by the intrinsic and extrinsic tenase complexes.
Methods: The non-conserved, shortened activation peptide of v-ptFX was exchanged for the human FX activation peptide, thereby generating ptFX-hAP and hFX-ptAP. All variants were stably expressed in HEK293 cells and purified to homogeneity employing ion-exchange and benzamidine-affinity chromatography.
Results: Assessing FX activation by the extrinsic (factor VIIa-tissue factor) tenase complex revealed comparable kinetic parameters for hFX-ptAP relative to wild-type (wt)-FX (K_m: 0.25 vs. 0.29 µM; k_{cat} 36 vs. 34 min^{-1}, respectively). In contrast, while the ptFX variants were similarly activated by RVV-X, FXa formation by the extrinsic tenase complex appeared to be strikingly inefficient. We therefore evaluated ptFX activation via real-time measurements, which suggested a 1.5-fold increased catalytic conversion rate of ptFX-hAP compared to v-ptFX. We next determined the activation kinetics by the intrinsic tenase complex (factors VIIIa-IXa). Surprisingly, activation of hFX-ptAP was virtually undetected in conditions up to 2.5 µM FX (0.078 vs 24.1 mOD/min/nM enzyme). Similar results were observed for ptFX variants in real-time assays, revealing little or no FXa formed by the human intrinsic tenase complex. Finally, as zymogen activity has been reported for v-ptFX, we determined whether the shortened activation peptide is at the basis of this remarkable characteristic. Preliminary results employing a purified prothrombinase assay revealed that, hFX-ptAP comprised a ~4000-fold increased factor Va-dependent zymogen activity compared to wt-FX, which was similar to that of v-ptFX.
Summary/Conclusion: Here we demonstrate a crucial role for the human FX activation peptide in the activation by the intrinsic tenase complex. Exchanging the activation peptide for the snake venom ortholog may eliminate an important binding site or may disturb recognition of the cleavage site by the FVIIIa-FIXa complex. Furthermore, we revealed that v-ptFX is sparsely activated by the human tenase complexes, despite the presence of the human FX activation peptide in our chimeric variant ptFX-hAP. At last, our preliminary results suggest that the shortened activation peptide confers FX zymogen activity. As such, the shortened activation peptide characteristic for snake venom FX could be one of the examples of by which *P. textilis* venom FX was modified into a toxin during evolutionary selective pressure.

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Measuring Anti-Factor Xa Peak Levels in Pregnant Women Receiving Therapeutic Enoxaparin Dose: A Prospective Observational Study

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Background: Venous thromboembolism is the leading pathological risk of morbidity and mortality in pregnant women. Enoxaparin is a low molecular weight heparin used during pregnancy.
Aims: To compare the anti-factor Xa peak levels (therapeutic, subtherapeutic, or supratherapeutic) between pregnant and non-pregnant women who are receiving therapeutic doses of enoxaparin and to evaluate whether anti-factor Xa peak levels correlated with Age, maternal weight, body mass index, weight-based enoxaparin dose or pregnancy.
Methods: Prospective, observational cohort study conducted between July 1st, 2017 and January 30th, 2018 at King abdulaziz Medical City, Riyadh, Saudi Arabia. Sixty-eight patients (36 pregnant and 32 non-pregnant women) who received a therapeutic doses of enoxaparin every 12 hours and had a three periodic steady-state anti-factor Xa peak levels collected during the study period
Results: Sixty-eight patients (36 pregnant and 32 non-pregnant women) were available for three periodic measurement of anti-factor Xa peak levels and included in the analysis. The anti-factor Xa peak levels within therapeutic range were achieved in the first measurement by only 14 (38.9%) pregnant women compared to 21 (65.6%) in the non-pregnant group ($p=0.028$). Similarly, in the second anti-factor Xa measurement, there was a significant lower pregnant group with therapeutic anti-factor Xa peak levels 20 (55.6%) compared to 25 (78.1%) in the non-pregnant women ($p=0.008$). Likewise, in the third anti-factor Xa measurement, there was a significant higher non-pregnant women achieved therapeutic anti-factor Xa peak levels 26 (81.3%) compared to 20 (55.6%) in the pregnant group ($p=0.003$). Pregnancy was the only variable has a positive correlation with the fluctuating anti-factor Xa peak level ($p<0.0001$).
Summary/Conclusion: Periodic anti-factor Xa peak levels measurement for dose adjustment should be considered for all pregnant women prescribed weight-based therapeutic enoxaparin.

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Two mutations in the FGB gene in patients with a quantitative fibrinogen disorder with bleeding and thrombotic phenotype

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Background: Congenital fibrinogen disorders are caused by mutations in the homozygous or compound heterozygous state in one of the three fibrinogen genes that affect the synthesis, assembly, intracellular processing, stability or secretion of fibrinogen. Patients can have bleeding manifestations or thrombotic complications and they can be also asymptomatic. Homozygous or heterozygous mutations in the three fibrinogen genes (Aα, Bβ, and γ) can lead to congenital quantitative fibrinogen disorders. Functional studies of mutant Bβ-chains revealed the importance of individual residues as well as three-dimensional structures for fibrinogen assembly and secretion.
Aims: This study describes two homozygous fibrinogen Bβ chain mutations in two Slovak families with afibrinogenemia and hypofibrinogenemia.
Methods: To identify the causative mutation peripheral blood samples were collected from all subjects. The coagulation-related tests and rotational thromboelastometry were performed. All exons and exon-intron boundaries of the fibrinogen genes (*FGA, FGB* and *FGG*) were amplified by PCR followed by direct sequencing.
Results: A Bβ chain truncation (BβGln180Stop) was detected in a 28-year-old afibrinogenemic man with bleeding episodes including repeated haemorrhaging into muscles, joints, and soft tissues, and mucocutaneous bleeding and a Bβ missense mutation (BβTyr368His) was found in a 62-year-old hypofibrinogenemic man with recurrent deep and superficial venous thromboses of the lower extremities. The missense mutation was confirmed by molecular modelling.
Summary/Conclusion: Studying the molecular anomalies and the modeling of fibrinogen mutants helps to understand the extremely complex machinery of fibrinogen biosynthesis and finally to better assess the correlation with the patients' clinical course.

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Risk factors, thromboprophylaxis and occurrence of thrombotic events in multiple myeloma patients attending a university hospital.

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Background: Patients with multiple myeloma (MM) are at increased risk of venous and arterial thrombosis, which leads to increased patient morbidity and mortality. Several factors contribute to this risk, like patient comorbilities or treatment with thalidomide or lenalidomide in combination with dexamethasone or multi-agent chemotherapy. As such, thromboprophylaxis must be considered in these patients.
Aims: In a retrospective single center study, risk factors, use of thromboprophylaxis, and the occurrence of venous and arterial thrombosis in patients with MM admitted in a university hospital were evaluated.
Methods: Patients attending the hematology department in a university hospital diagnosed with MM during the year 2015 and followed-up until the end of 2017 were selected. Several risk factors for thrombosis were analyzed, namely diabetes, cardiac disease, acute and chronic renal failure, obesity, transplant, prior thromboembolic disease, chemotherapy scheme, immobilization and use of central venous catheter (CVC). The use of thromboprophylaxis and thrombotic events were also evaluated. The statistical analysis was performed using IBM SPSS® Statistics. A p-value of <0.05 was considered statistically significant.
Results: There were a total of 36 patients with a median age of 66.0 years [IQR 58.5; 69.0] and 66% were male. A total of 5 patients had a thromboembolic event (3 deep vein thrombosis, 1 pulmonary thromboembolism and 1 acute ischemic stroke). Only one patient was not under thromboprophylaxis due to high hemorrhagic risk. The rate of thrombotic events was 13.8% which is in accordance with other published studies. According to our study, independently of age and gender, patients with venous and arterial thrombosis, had a statistically significant higher frequency of acute renal failure ($p=0.017$) and use of CVC ($p=0.028$). There was no association between the other variables and outcome.
Summary/Conclusion: There are a number of factors that contribute to the thromboembolic risk in patients with MM. For a better understanding of the association of these risk factors, studies with larger samples are needed. Therefore, care must be taken in optimizing thromboprophylaxis in these patients, with careful individual risk stratification when defining the best prophylaxis strategy.

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Does addition of three SNPs to the genetic investigation of thrombophilia give better risk prediction in a cohort that was clinically tested after VTE or family history of VTE?

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Background: Venous thromboembolism (VTE) is a common but underdiagnosed condition with serious morbidity and mortality, where prophylaxis generally is effective and well tolerated, although increased bleeding risk is an ever present concern. Acute symptoms aside, 25% of VTE patients will also develop chronic symptoms (pulmonary hypertension and post thrombotic syndrome). The heritability of VTE is around 50% and genetic risk factors can be suspected particularly in younger patients with unprovoked VTE. Patients with identified prothrombotic Single Nucleotide Polymorphisms (SNPs) as part of a significantly increased risk for VTE will benefit from anticoagulative prophylaxis. The only SNPs used in clinical practice are Factor V Leiden (FVL) and Prothrombin (G20210A) (FII). Addition of three additional prothrombotic SNPs (Fibrinogen γ-chain (FGG), Factor XI (FXI) and the ABO blood group gene (ABO)), has been shown to improve the risk prediction for first time VTE and for recurrent VTE, with the risk increasing in proportion to the number of risk alleles. Risk prediction with 5 SNPs is also superior to assessment based on only clinical factors.
Aims: Evaluating if a larger proportion of patients that were tested for thrombophilia could be explained by an expansion of the genetic panel. We will also investigate how many of the prothrombotic risk alleles that are present in Swedish patients with a clinically suspected thrombophilia compared to controls.
Methods: All patients that were subject to thrombophilia investigation at the special coagulation lab between 2014-2016 were invited to participate. After written informed consent was obtained, the 250 patients who fulfilled the inclusion criteria were included. Study participants were comprised of 100 with thrombosis < 50 years of age, as well as 100 with at least one first grade relative with thrombosis < 50 years of age, and 50 with both these inclusion criteria. An age- and sex-matched control group was collected from healthy blood donors without any of the aforementioned inclusion criteria or any anti-coagulation drugs.
DNA was extracted from EDTA whole blood and stored in -20° C. TaqMan SNP Genotyping Assays was used for genetic testing of FVL (rs6025), FII (rs1799963), ABO (rs8176719), FGG (rs2066865) and FXI (rs2036914).
Results: In a pilot study of 76 patients, analysis with 2 SNPs (FVL + FII) showed that 33% had no risk alleles and 67% had one risk allele. For 5 SNPs (FVL + FII + FGG + FXI + ABO) 3% had no risk alleles, 16% had one, 38% had two, 37% had three and 6% had four risk alleles. The proportion of patients with at least one prothrombotic allele increased from 67% to 97%.
Summary/Conclusion: The 5 SNP genetic testing increased the proportion of patients where a genetic risk factor could be identified as a contributing risk factor for venous thrombosis. Greater knowledge of the individual patients' genetic predisposition for thrombosis and number of risk alleles can be used to tailor individual treatment, in order to treat those patients that would benefit most from anticoagulant prophylaxis. The clinical value is considerable, because prophylaxis to the right patient at the right time could prevent major morbidity and mortality.

Ref:

De Haan et al., Blood, 2012
Van Hycklama et al., Circ Cardiovasc Genet, 2014

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Clinical factors associated with PE in patients initially subjected to diagnostic cardiac work up: A Swedish cross-sectional study from over ninety thousand emergency department visits

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Background: Pulmonary embolism (PE) often presents with dyspnea, chest pain and/or coughing making it one of the most common differential diagnoses at the emergency department (ED). Other diseases, e.g. myocardial infarction (MI) and angina pectoris (AP) present with similar chief complaints, consequently many PE patients are initially subjected to diagnostic cardiac workup. In this setting, factors associated with PE are poorly studied.
Aims: To identify factors associated with PE in patients subjected to diagnostic cardiac work up at the ED.
Methods: This was a Swedish cross-sectional study based on register data from four tertiary hospitals in Stockholm county between 2013 and 2016. All consecutive patients admitted to the ED and subjected to diagnostic cardiac workup were included. Comparisons were made between patients with PE and those without, patients with acute coronary syndrome (ACS) being excluded and instead used for a secondary comparison. PE patients were identified through discharge diagnosis. ACS patients were identified through the discharge diagnosis of MI or unstable AP that included a revascularization procedure and/or 30-day MACE. Clinical factors were identified by cross-referencing a combination of national registers with electronic medical records. Categorical variables were compared using Chi-square test and included sex, chief complaint, heredity for ischemic heart disease (IHD) and comorbidities. Numerical variables, i.e. vital signs, laboratory findings and age, were compared using Mann-Whitney U Test and presented as median values. P-values of
Results: A total of 90249 patients met the inclusion criteria out of which 780 patients had PE and 3136 ACS. PE patients were older than those without (71 vs 59 years) and had a more even sex distribution compared to ACS patients (50 vs 30% female). PE patients more often presented with dyspnea and less with chest pain compared to both non-PE and ACS patients (50 vs 14 and 10% respectively; 26 vs 50 and 76% respectively). Comorbidities were similar in PE and non-PE patients although a higher prevalence of hypertension (51 vs 41%) but a lower of atrial fibrillation (8 vs 12%) was observed in patients with PE. ACS patients had an overall higher prevalence comorbidities including heredity for IHD. Both CRP and Troponin T was higher in PE patients compared to those without (24 vs 2 mg/L and 20 vs 6 ng/L respectively). Compared to ACS patients the troponin T level was lower in PE patients (20 vs 44 ng/L). Vital signs differed on a general basis, with PE patients having both a higher heart and respiratory rate compared to non-PE patients (92 vs 79 bpm and 20 vs 16 breaths respectively). The differences were similar compared to ACS patients but in addition PE patients had a lower systolic blood pressure (140 vs 149 mmHg).
Summary/Conclusion: In patients subjected to diagnostic cardiac work up the ED, PE was quite rare and found only in one out of hundred patients with ACS being four times more common. A clinical profile for PE patients was recognized and included dyspnea and more impaired vital signs. Conversely, ACS patients had a higher prevalence of comorbidities.

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Association of an Afibrinogenemia and a FV Leiden in two patients within the same family.

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Background: Afibrinogenemia is a rare dyscrasia characterized by a constitutional deficit in fibrinogen. About 150 families have been reported around the world since the first German observation published by Rabe in 1920. Hemorrhagic manifestations vary, ranging from a minimal or moderate bleeding to cataclysmic hemorrhage. Afibrinogenemia may be paradoxically associated with venous and arterial thrombotic complications. These complications could occur spontaneously or after a massive substitution in fibrinogen concentrate or in very rare cases associated with a biological risk factor for thrombosis.
Aims: interest to complete the hemostasis assessment to identify the associated deficits
Methods: We report here the case of two brothers known for severe fibrinogen deficiency since childhood. In adulthood, one of the two brothers had severe deep vein thrombosis complicated by pulmonary embolism. A complete thrombosis checkup was undertaken in the patient and other members of his family in search of a biological risk factor for thrombosis.
Results: In addition to severe fibrinogen deficiency with a fibrinogen level <0.1 g / l in activity and antigen, the thrombosis checkup revealed a corresponding activated protein C resistance in both the afibrinogenemic brothers and other family members. This resistance to activated protein C corresponds to a mutation of FV Leiden in the heterozygous state.
Summary/Conclusion: Congenital afibrinogenemia is a rare, autosomal recessive disorder that results in multiple life-threatening haemorrhagic manifestations. The occurrence of thrombosis remains exceptional.
The achievement of a complete hemostasis assessment is required for the diagnosis of rare associated deficits of hemostasis responsible for hemorrhagic and thrombotic syndrome.

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The incidence of coagulopathy after adverse reactions to platelet transfusions in pediatric hematooncology patients

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Background: Transfusion of platelet concentrates are frequently complicated with occurrence of allergic and febrile nonhemolitic transfusion reactions.
Aims: The aim of this study was to investigate whether adverse transfusion reactions on platelets in pediatric patients could cause hemostatic disturbances, assess whether disturbances impact on the further use of platelet transfusion.
Methods: In the 3-year study period, 239 pediatric hematooncological patients received platelet products. In a total of 52 patients (35 boys-67% and 17 girls-32%, average age 10.29 ± 5.6 years) 70 transfusion reactions were recorded. The control group consisted of 21 patients who received platelets but without adverse transfusion reactions. Evaluation of hemostatic disturbances after adverse reaction to platelet transfusion included: platelet counts, screening hemostatic tests, fibrinogen concentration (Clauss assay), D-dimer test and von Willebrand factor antigen.
Results: Allergic reactions to platelet transfusions were the most frequent transfusion related event in our group of patients (72.8%), followed by FNHTR (17.1%), other (10.1%). The results of the coagulation tests in patients with reactions were as follows: prothrombin time 69.3±26.3%, partial thromboplastin time 38.9±45.3s, thrombin time 24.6±44.2s, fibrinogen 3.75±1.7g/l, vWF antigen 188.8±95.5%, D-dimer 1146.6±2351.0 ng/l. Platelet count of patients after reaction was 38.73±20.12 $x10^9$/l, in control group 23,92±11.67 $x10^9$/l ($p<0.001$). Interval between administration of the platelet doses in patients with reaction was 6.06±13.7, compared to the group without reaction 2.14±1.52 days ($p=0.045$). Platelet count of patients with allergic reaction was highest 41.03±20.02 $x10^9$/l, ($p<0.001$), they also had the fewest number of days with a platelet count of less than 10 $x10^9$/l, 2.93±6.2 compared to the group of patients with no reaction 9.92±9.05 days ($p= 0.019$). D-dimer in control group was 630.62±950 ng/ml. D-Dimer concentration were increased in 56.2% of patients with allergic reactions, and only 18.8% with FNHTR ($p = 0.017$).
Summary/Conclusion: There was no difference between the results of coagulation tests regarding occurrence of transfusion reactions to platelets. D-dimers concentration was higher in patients with transfusion reactions but without clinical significance. Allergic reactions resulted in the degradation of fibrinogen in more patients but also a higher increase in their platelet count. The explanations for these phenomena are probably mediators released from activated mastocytes.

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Overall hemostatic potential and thrombin generation in patients with atrial fibrilation receiving rivaroxaban
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Background: Direct oral anticoagulants (DOAC) are used for prevention of stroke in patients with non valvular atrial fibrillation (AF) without need for routine laboratory monitoring. However there are obvious situations in which measurement of drug concentration and/or intensity of anticoagulation are desirable, e.g. in preparation for surgery, patients with major bleeds, with suspected interactions with other drugs. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered to be gold standard for following the effect of drugs which is available only in specialized centers. Furthermore, there is little real-world clinical experience about the safety and efficacy of DOAC in everyday clinical practice settings. At the same time, there is a shortage of data about their relative effect on hemostatic parameters based on a direct comparison. In that respect, involvement of new methods for measuring anticoagulant effect of hemostatic potential in AF patients is very important.
Aims: To evaluate the effect of rivaroxaban on global hemostatic methods-overall hemostatic potential (OHP) and thrombin generation (TG) test: endogenous thrombin potential (ETP) and to compare these methods to LC-MS/MS.
Methods: We studied 50 patients with AF treated with rivaroxaban 15 or 20 mg OD and 20 controls (healthy individuals). Plasma concentrations were measured using LC-MS/MS. Two global hemostatic assays were employed, ETP and OHP. The parameters of ETP assay (area under the curve reflecting ETP, C-max, t-lag, t-max) and OHP assay (overall coagulation potential – OCP and overall fibrinolytic potential –OFP) were assessed.
Results: A wide range of rivaroxaban concentrations was determined by LC-MS/MS (<2–464 ng/mL). There was significant difference between rivaroxaban and control group for all of the parameters ($p<0.01$). OHP and OCP were significantly increased while OFP was decreased in rivaroxaban group (75.02-298.38; 188.50-455.75; 20.34-74.59 respectively) compared to control group (46.31-172.78; 190.46-390.21; 34.86-76.291 respectively). Regarding TG test, we noticed significantly lower values of ETP and C-max while values of t-lag and t-max were longer in the rivaroxaban group (58.77-93.72; 35-104.9; 22.52-61.19; 59.19-236.19 respectively) compared to control group (72.65-109.08; 78.25-105.5; 16.02-27.72; 49.39-83.34 respectively). Plasma concentration of rivaroxaban correlated with ETP, C-max, t-lag and t-max while OHP, OCP and OFP were not in correlation with rivaroxaban plasma concentration.
Summary/Conclusion: This data shows that treatment with rivaroxaban lowers TG in dose dependent manner but has no effect on OHP which is paradoxally high. Possible explanation for those results can be insensitivity of OHP for rivaroxaban or that rivaroxaban influence only TG but no later phases of hemostasis such as clot binding and stability. LC-MS/MS is the gold standard for measurements of rivaroxaban in plasma but the question is if the concentrations always correspond with total anticoagulant activity. Therefore, TG as global hemostatic assay presenting as ETP may be used for monitoring levels of rivaroxaban. Interestingly it seems that OHP method is relatively insensitive to the presence of rivaroxaban.

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Advanced proteomic approach to identify a specific protein plasma profile associated to venous thromboembolism

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Background: Venous thromboembolism (VTE) is a multifactorial disease which comprises several known environmental and genetic risk factors. Nevertheless, approximately 40% of VTE patients have no known risk factors, and in 20-30% of cases it is recurrent. Current laboratory techniques do not identify all individuals at high risk of VTE. Therefore, it is necessary to identify new specific biomarkers of VTE, which could interact with those already known, identifying new routes associated with this pathology.
Aims: We performed an advanced proteomic method in order to establish a characteristic profile of plasma proteins in patients with VTE.
Methods: We selected citrated plasma from 18 patients with a history of VTE and 18 healthy volunteers. Informed consent was obtained from all subjects. First, depletion of the 14 major proteins in human plasma samples was performed using an HPLC system with a specific column (*MARS, Agilent Technologies*). Next, we generated a library of MS/MS spectra (*Protein-Pilot Progroup Algorithm*), identifying a total of 522 proteins with a threshold of 95%. The study of differential non-targeted proteomics was carried out using SWATH LC-MS/MS. The results were analyzed by logistic regression analysis with ELASTIC NET penalty (*R v3.2.3*), identifying the variables that have the discriminating information.
Results: We obtained a list of 28 proteins able of distinguish between patients and controls, with a fold-change that ranged between -2.9 and 2.6. The predictive model containing those proteins showed an area under the ROC curve of 0.997 (95% CI: 0.988-1). Next, we identified the biological pathways in which they participate (*UniProt, ProteinDataBase, String, SwissProt*). We found 12 proteins involved in coagulation, platelet activation, lipid metabolism, complement activation, glycosylation or response of the immune system. In addition, we identified a set of 16 proteins whose function within the thrombotic pathology remains still unknown.
Summary/Conclusion: Our results demonstrate the usefulness of non-targeted proteomics, and may play an important role in future strategies for the diagnosis, treatment and follow-up of patients with VTE. Our next aim will be to validate these findings in a larger number of samples to corroborate our results, as well as ascertain the implications of these proteins in the coagulation system, in order to describe new cofactors to be taken into account in VTE.
ISCIII-FEDER (PI14/00079, PI14/00512, FI14/00269, CPII15/00002, PI17/00495), Generalitat Valenciana (Prometeo II/2015/017, ACIF/2017/138), IIS La Fe (VLC_BIOMED 2014/0718, 2016/0820, IIS La Fe_UPV 2014/0421, 2017/0408).

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Haemostatic disturbances in children with acute leukemia at presentation and during induction therapy-single center experience

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Background: The occurrence of various coagulation abnormalities in children with acute leukemia is well established. The major haemostatic problem is usually haemorrhage secondary to thrombocytopenia, whereas thrombosis is relatively rare complication and is usually consequence of chemo therapy. Disseminated intravascular coagulation (DIC) is responsible for most cases of clinical significant bleeding or thrombosis and is very important contributing factor of early death. Although more commonly recognized in association with acute promyelocytic leukemia, all types of leukemia can trigger DIC.
Aims: The aim of the investigation is to evaluate haemostatic disturbances in children with acute leukemia at presentation and during the induction treatment.
Methods: This study represents retrospective analysis of 79 newly diagnosed patients with acute leukemia treated on hematology ward of Mother and Child Health Care Institute of Serbia. Patients were diagnosed in the period from January 2013 up to June 2018.
Results: Acute lymphoblastic leukemia was diagnosed in 68 children (86.1%) and acute myeloid leukemia (AML) in 11 patients (13.9%). 57% of patients were boys and 43% girls, age between 3 months up to 17 years and 7 months (median age was 53 months). Children with ALL were treated according to ALLIC BFM 2009 and children diagnosed with AML with AML BFM 98 protocol. Twenty seven children (34%) had various haemostatic complications: 6 patients (7.6%) had signs of DIC, 19 patients (24.1%) had hemorrhages and in two patients (2.5%) had peripheral venous thrombosis. Disseminated intravascular coagulation was defined by International Society of Thrombosis and Hemostasis criteria. In the cohort of patients with DIC there were 3 children with AML (1 patient with APL and two patients with M5 FAB subtype) and 3 patients with T-ALL, age range between 6 months and 17 years and 7 months (median range 15 years). Two of them died in first 7 days of treatment due to intracranial hemorrhage (15 year old boy with APL and 17 year old girl with hypercellular T-cell ALL). Hemorrhage was more frequent in children with AML: 36.4% patients with AML and 22% patients with ALL had signs of hemorrhage at the diagnosis, although the hemorrhagic complications not associated with DIC were categorized as mild. Thrombotic complications occurred in two patients, 14 year old boy with T-cell ALL as one of the first signs of illness and in two year old girl with B-II common ALL on the 26th day of treatment as a complication of therapy. In our cohort of patients with acute leukemia, early death was observed in 3 patients: in two children with catastrophic DIC and in one girl due to infective complications.
Summary/Conclusion: Coagulation disturbances, especially due to hemorrhage and DIC, are common at presentation in children with acute leukemia. Older age, T cell morphology in patients with ALL, or M3 and M5 subtypes in patients with AML, are associated more frequently with DIC. Thrombosis is typical complication in ALL patients. Early recognition of haemostatic disturbances and adequate treatment approach can diminish morbidity and early mortality in newly diagnosed pediatric patients with ALL.

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Management of intraoperative anticoagulation for cardiopulmonary bypass heart transplantation in a patient with acute heparin-induced thrombocytopenia undergoing heart transplantation.

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Background: We reported here the management of anticoagulation of a young patient with subacute heparin-induced thrombocytopenia who urgently required heart transplantation.
Aims: A 17 years old young man was addressed in Nancy Hospital for severe dilated cardiomyopathy discovered in a context of ischemia of left lower limb occurred after intracardiac thrombus embolization. Six days after treatment initiation, platelet number dropped and heparin-thrombocytopenia was diagnosed. Anticoagulation was then performed using argatroban and the patient registered on a list for extremely urgent heart transplantation. Heart transplantation was performed with cardiopulmonary bypass, under bivalirudin according to the following protocol (adatpted from Koster and Selleng, 2013):
Methods: argatroban perfusion was stopped 4h before operating room entry in order to obtain normal coagulation basal test. At this time, TP, aPTT, fibrinogen and FV were measured before the intervention. Two hours after argatroban infusion arrest, the concentration measured by ecarin clotting time (ECT) was 8 µg/mL and then controlled at 13 µg/mL i.e. overdosed at 10 times the target. This level jeopardized the heart transplantation because CPB under argatroban is highly not recommanded. This plasma level was due to an error of the nurse who pushed the residual content of the infusion circuit. Finally at the time of the beginning of the intervention, the residual concentration of argatroban was decreased to 0,72 µg/mL allowing the intervention under bivalirudin. Bivalirudin was initiated with a priming bolus of 50 mg in the CPB circuit and an initial bolus iv infusion of 1mg/kg followed by continuous infusion of 2,5mg/kg/h . The bivalirudin treatment was monitored every 15 minutes using ACT in the operating room (target > 400s) but always in parallel with laboratory assay of bivalirudin concentration using a specific ECT calibrated with plasma overloaded with bivalirudin at concentrations between 1 and 4 µg/mL (Target 12 to 16 µg/mL). In order to give results quickly available (15 minutes maxi) and then useful to dose adjustment, plasma was centrifuged at 4000g for 2min. If target is not reached, supplementary bolus of bivalirudin (0.1 to 0.5 mg/kg) was administered. If concentration is too high, dose could adjusted by 0.25 mg/kg/h. Cell saver had to be prefered at cardiotomy succion because of the accelerated degradation of bivalirudin during blood stasis.
Results: This protocol was followed and concentrations of 7 and 7,2 µg/mL were reached. Then a supplementary bolus of 30 mg was administered allowing to reach 9,6 and 11,4 µg/mL. The concentration measured in the cell saver was < 1,5µg/mL. Bivalirudin was stopped after 5 hours and according to its plamsa half-life of 25 minutes, was eliminated in 4 hours. Post CPB bleeding complications occured due to this prolonged anticoagulation as compared to heparin neutralization by protamin but were overcomed using CGR, platelets and FFP transfusion, PPSB and finally recombinant FVIIa administration.
Summary/Conclusion: Finally heart transplantation was succeed. The thrombosis aetiology was attributable to congenital hyperhomocysteinemia. Anticoagulation was restarted with argatroban few hours after heart transplantation and VKA were hen intoduced for long term anticoagulation.

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Anticoagulant Protein Level Differences Between Young and Elderly Patients With Myocardial Infarction

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Background: The role of natural anticoagulants is much better known in patients with venous thromboses than in the pathogenesis of arterial thrombosis while the role of anticoagulant proteins in the pathogenesis of arterial thromboembolism in elderly and young patients with myocardial infarction is still insufficiently clarified.
Aims: To detect the difference in the anticoagulant proteins (antithrombin and protein C) in patients with myocardial infarction younger than 45 years and the group of patients with myocardial infarction older than 65 years.
Methods: Our prospective study encompassed 143 patients, i.e. 80 patients with myocardial infarction younger than 45 years and 63 patients with myocardial infarction older than 65 years. The values of antithrombin and protein C were respectively analysed using standard coagulometer assays. In the statistical analysis The Student T - test, $\chi 2$ test, Mann - Whitney U test were used.
Results: In the group of patients older than 65 years there was the significantly lower ($p = 0.000$) activity of antithrombin (87,2±12,6 vs 105,5±12,4%) and protein C (104,8±17,2 vs 117,9±18%) than in the group of patients with myocardial infarction younger than 45 years. The percentage of patients with decreased values of protein C (<70%) was statistically significantly higher in the elderly group of both sexes compared to younger patients of both sexes (6.3% in the elderly vs 0.0% in younger patients, $p = 0.028$). The percentage of patients of both sexes with antithrombin values decreased below the reference range was higher in the group of elderly patients with myocardial infarction compared to young patients with myocardial infarction (6.7% in the elderly versus 1.2% in the younger), though no statistically significant difference ($p = 0.074$) was determined.
Summary/Conclusion: The results from this study suggest the presence of different pathogenetic mechanisms regarding anticoagulants proteins, i.e. antithrombin and protein C in young and elderly patients with myocardial infarction, which would require further research for more reliable results.

Platelets
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Platelet aggregation induced by serine proteinase isolated from Vipera lebetina snake venom: involvement of purinergic signalling pathway

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Background: Snake venom components can target various steps of the blood coagulation and platelet aggregation. These molecules affect the hemostasis system and induce pro or anticoagulant effect. They also act as pro- or anti-platelet aggregants.
Aims: This study is undertaken to investigate the role of isolated serine proteinase from *Vipera lebetina* venom in the interaction between platelet-and platelet receptor. The initiation of purinergic signalling pathway and coagulation cascade by the serine proteinase was also investigated.
Methods: The effects of this serine proteinase on human platelet aggregation in presence of clopidogrel, ticlopidine and aspirine (acetylsalicylic acid) was evaluated. The platelet receptor antagonists were incubated with Platelet-Rich Plasma (PRP) and the isolated serine proteinase. The aggregation was monitored for 5 to 10 min at 37ºC using an aggregometer. Platelet stimulated by ADP (0.2 mM), Arachidonic Acid (AA) (5 mg/mL), or collagen (100 µg/mL) was added to PRP as a positive control. Furthermore, the coagulation activity of the serine proteinase on deficient human plasmas was also tested. Deficient plasmas in factor II, V, VII, IX and X were incubated with the purified serine proteinase at 37°C and the clot formation was observed.
Results: The serine proteinase was able to induce platelet aggregation without lag time. The aggregating effect was reduced markedly by the P2Y12 ADP receptor inhibitors, clopidogrel and ticlopidine, than the cyclooxygenase (COX-1) inhibitor, aspirin. Platelet aggregation induced by this serine proteinase was reduced by 99% in presence of clopidogrel compared to that aspirin. These results suggest the involvement of ADP signaling pathway. This serine proteinase may activate platelet aggregation by a mechanism similar to that of thrombin which is able to hydrolyze thrombin receptor (PARs). The interaction between P2Y12 and PAR1/PAR4 signalling pathways occurs on the level of cAMP-calcium crosstalk. PAR4 acts with the P2Y12/PI3K pathway to stabilize platelet aggregates. The purified serine proteinase presents high coagulant activity against human plasma and is able to replace missing factors V and VII in deficient plasmas leading to activation of factor X. This molecule seems to induce coagulation by the activation of factors of the extrinsic pathway of coagulation cascade.
Summary/Conclusion: The proaggregating activity of this molecule involved the purinergic signalling pathway. The isolated molecule from snake venom can be useful for the development of new diagnostic tools or therapeutic drugs for the treatment of haemostatic disorders.

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Does The Response To Antiplatelet Therapy Influence The Appearance Of Thromboembolic Events In Patients With Carotid Stenosis And Eversion Endarterectomy-Pilot Study

Tina P. Novakovic*

Background: Antiplatelet therapy (AT) with acetylsalicylic acid has a key role in preventing thromboembolic events in patients with carotid stenosis submitted to eversion endarterectomy (EA). Aspirin might decrease the perioperative stroke rate and long term risk of thromboembolic events after surgery. It is well established that patient response to AT is variable depending on measuring method. It is not known if patient response to AT has impact on thrombotic event in carotid surgery.
Aims: AIM of this study was to observe correlation between low responding to AT with neurological complication of carotid surgery.
Methods: This was retrospective review of consecutive patients undergoing EA at university clinic for vascular surgery. Data including patient demographic, operative details, preoperative use of Aspirin, response on AT therapy and outcome were collected. Endpoints included thromboembolic events in hospital period. Platelet function was assessed by impedance aggregometry (Multiplate®, Roche Diagnostics). Anti-platelet therapy response was defined by the following AUC results: ASPI>600 as no responders, ASPI< 600 as responders, ASPI<300 as high responders. All patients were on chronic AT.
Results: During 4 months, 180 patients (125 men and 55 women, median age (min-max) 69 (50-85) years) undergoing EA were included. Good response to AT was recorded in 89 patients (49%), while 91(51%) had shown no response to AT. Frequency of thromboembolic event was 5%. All of 9 patients with thromboembolic event had ASPI>300 (p=0.401), 5 (56%) of them were resistant to AT, ASPI>600 (p=0.514).
Summary/Conclusion: Based on our results non responders to AT are not at higher risk from neurological events after carotid surgery. These results should be tested in a prospective study on a higher number of patients.

Platelets
P266
Board No. 218

Flavonoid metabolites with antiplatelet effects

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Background: Flavonoids are a group of naturally occurring second plant metabolites, whose intake in human diet can be very high. Therefore, these compounds have been attracting large interest for their possible effects on human health, such as the antiplatelet activity. However the bioavailability of parent flavonoids is considered to be very low. Most of them are metabolized by human colon bacteria to smaller phenolic compounds, which are subsequently absorbed into systemic circulation in higher concentrations than parent flavonoids.
Aims: To analyse the antiplatelet potential of available flavonoid metabolites on platelet aggregation triggered by different inducers and to determine their mechanism of action.
Methods: Screening of the antiplatelet activity (AA /arachidonic acid/, collagen) and ADP, TRAP (thrombin receptor activating peptide) were investigated by impedance aggregometry. Future mechanistic experiment were targeted toward formation of prostaglandin H_2 by cyclooxygenase-1, production of thromboxane A_2 via platelet thromboxane A_2 synthase and analysis of antagonism at the thromboxane A_2 receptors.
Results: The initial screening of 30 metabolites has shown no inhibition effect of the most tested compounds on platelet aggregation induced by AA. However, some metabolites were able to block platelets aggregation induced by AA and collagen and the most active metabolite had even better potential in units of µM that the standard antiplatelet drug, acetylsalicylic acid. The mechanisms of action of these compounds included inhibition of platelet cyclooxygenase 1 and partly thromboxane A_2 synthase.
Summary/Conclusion: Some phenolic metabolites are able to interfere with platelet aggregation and could be responsible for antiplatelet effect of parent flavonoids; nevertheless, the main mechanism of action will be investigate in the further experiments as well as their *in vivo* potential.
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Biological Evaluation of the Therapeutic Effectiveness of Platelet Antiaggregants in Patients with Coronary Pathology

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Background: Today, cardiovascular disease is a real public health problem in the world. The thrombotic component is of paramount importance in the pathogenesis of acute coronary events, which is why antiplatelet therapy plays the role of a stone. angular in the secondary prevention of acute ischemic events. Despite the effectiveness of these molecules; recurrences of ischemic events remain frequent and dependent on a large interindividual variability. The concept of poor biological response has been proposed as one of the hypotheses for this recidivism.
Aims: To evaluate the platelet response to Aspirin and Clopidogrel in subjects with stents
Methods: A prospective and descriptive study was conducted over a period of 18 months in the hemobiology Center of the University Hospital of Annaba. It involved 81 patients with stents and put on aspirin and/or clopidogrel as a preventive measure. A measurement of platelet aggregation (aggregation with 200 µM of ADP and 15 mM of arachidonic acid) was carried out on a thromboAgrégometer TA_8V.
Results: The average age was 57 years old with extremes between 36 and 91 years old. The sex ratio was 2.5 with 58 men and 23 women. The indication for coronography with angioplasty was acute coronary syndrome in 90% of cases and myocardial ischemia in 5% of cases. 70% of patients were treated with active stents. The prevalence of biological resistance to platelet antiaggregants was 39.5% (n = 32): 12.3% (n = 10) for aspirin and 27% (n = 22) for clopidogrel. This prevalence is consistent with those described in most previous studies. This targeted biological monitoring has allowed these patients to benefit from an adaptive and effective therapeutic attitude. Indeed, an efficiency of 77% is observed in the cases resistant to Clopidogrel after increase of the therapeutic dose or change of the therapeutic molecule. An analysis of predictive factors such as diabetes, high blood pressure, kidney failure…was performed in these non-responder patients.
Summary/Conclusion: Several different therapeutic approaches can improve platelet inhibition in patients who maintain high reactivity with platelet antiaggregants therapy. New treatment modalities based on individual patient data will need to be investigated and tested to demonstrate their utility and applicability in clinical practice.

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Fluoxetine amplifies ticagrelor mediated protection during myocardial reperfusion injury

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Background: P2Y12 inhibitors like ticagrelor are routinely used in the acute treatment of myocardial infarction and have been described to limit myocardial ischemia and reperfusion injury, which is associated with an inflammatory response. We chose a model where we utilized fluoxetine (Flx) to deplete platelet serotonin because acute neutrophil triggered inflammation is dampened in these mice and analyzed the outcome after myocardial reperfusion injury.
Aims: The goal was to evaluate if and how excessive serotonin levels interfere with P2Y12 mediated cardioprotection.
Methods: Mice received Flx for 3 weeks. After administration of ticagrelor (100mg/kg loading; 50mg/kg twice afterwards), MI was induced for 30 minutes, followed by 24 hours of reperfusion. Heart function and infarct size was evaluated. Integrin expression on neutrophils was analyzed by flow cytometry.
Results: Fluoxetine treatment alone mildly reduced platelet aggregation while ticagrelor administration blocked ADP effects completely. Plasma Serotonin was increased in WT and ticagrelor treated mice (150±19 and 145±13 ng/mL,). Consequently, the complementary blockade of ADP-dependent platelet activation and serotonin release proved to reduce the infarct size even more effectively than either drug alone. In circulating neutrophils, Flx or ticagrelor alone reduced CD11b surface expression to a similar extent. The combination of both drugs, however, reduced neutrophil CD11b expression and platelet neutrophil complex formation by an additional 30-50%.
As a consequence of excessive serotonin levels during MI, twice as many neutrophils transmigrated into the affected heart tissue. Stimulation of human neutrophils with serotonin *in vitro* resulted in a spontaneous increase in CD11b surface expression (2 fold compared to vehicle control), and MPO release from granules (19±4 vs. 9±2 ng/mL in control samples). These effects could be completely blocked by addition of a protein transport inhibitor.
In line, patients with acute coronary syndrome, CD11b surface expression and plasmatic MPO concentration strongly correlated with serotonin levels in plasma (Pearson r=0,846 and r=0,6606, respectively).
Summary/Conclusion: Platelets, our data suggest, govern neutrophil responses in serotonin-dependent and -independent pathways. Wholesome platelet depletion, however, may offset the protection conferred by selective serotonin depletion and P2Y12 inhibition in exchange for bleeding complications and is not a viable option in practice. We therefore advocate for intervening in serotonin-neutrophil crosstalk which might provide novel anti-thromboinflammatory treatment options during myocardial reperfusion.

Platelets
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Board No. 221

Comparison of manual and automated paltelet count in patients with low platelets

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Background: Thrombocytopenia is any disorder in which there is an abnormally low amount of platelets. Many factors can cause a low platelet count, such as: decreased production in the bone marrow, increased damage or uses of platelets even if the bone marrow makes enough platelets, as well as increased hold of platelets by spleen or combination of the above factors. Manual platelet estimation is one of the methods used when automated platelet estimates are very low.
Aims: This study had aim to assess the reproducibility of manual platelet estimation following automated low platelet counts. In order to determine the comparasion between two methods, platelet counts were determined manually and with automated counters.
Methods: This study was performed by counting platelets manually using hemocytometer chamber as well as using automated platelet counter (Beckman Coulter DxH 500 hematology analyzer) for the same sample at the same time to give conclusive advice that will help in evaluation of patients with abnormal platelets count. **I**n this study we parallel tested samples with platelet counts less than 100 × 109/L with and compering with manually counting platelets. Beckman Coulter DxH 500 hematology analyzer use the impedance principle of cell counting which is based on the detection and measurement of changes in electrical resistance when a particle (such as a cell) is suspended in a conductive liquid and passes through a small aperture. As each cell goes through the aperture, it acts as an insulator and it momentarily increases the resistance of the electrical path between an external electrode and an internal electrode. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume. One advantage of automated profiling instruments is their ability to generate a mean platelet volume (MPV), which is unavailable through visual methods. The presence of predominantly larger platelets generates an elevated MPV value, which sometimes signals a regenerative bone marrow response to platelet consumption. Manual cell counts are performed using a hemacytometer, or counting chamber. In this procedure, whole blood, with EDTA as the anticoagulant, is diluted with 1% ammonium oxalate, which lyses the nonnucleated erythrocytes. To determine comparison between these two methods manually and by an automated counter was done by Passing Bablok and student t test. The study included 40 samples with platelet counts less than 100 × 10^9/L.
Results: The mediana platelets count estimated by the manual method was 69 ± 24.5 × 10^9 /L, while that estimated by the automated method was 74 ± 24,7× 10^9 /L**.** Statistically, there is no significant difference between the number of samples estimated with low count by the two methods (y=-2,47+0,9934x,r=0,981).
Summary/Conclusion: This study concluded that significant positive correlation is present between the manual and the automated counting methods of platelets and recommended that platelet count is not varied when done by manual or automated methods, but in every method, it should be accompanied platelet estimate, especially with abnormal counts.

Platelets
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Evaluation Of Heparin-Induced Multiple Electrode Aggregometry Method For The Diagnosis Of Heparin-Induced Thrombocytopenia Using 5b9 As A Reference Hit Igg Antibody

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Background: The diagnosis of Heparin-Induced Thrombocytopenia (HIT) is based on the clinical history and laboratory assays detecting antibodies specific to PF4-heparin complexes, but it often remains a challenge. Functional assays are performed on human fresh platelets using variable procedures, among which serotonin release assay (SRA) is considered as the gold standard method for HIT diagnosis. However, SRA is technically demanding, uses radiolabelled washed platelets (WP) and is thus restricted to expert laboratories. Platelet aggregation tests (PAT) performed on platelet-rich plasma (PRP) are more easily available but exhibit lower sensitivity for detecting HIT antibodies. Therefore, whole blood aggregometry achieved on the Multiplate® analyser has recently been proposed by the ISTH SSC as an alternative functional method (Morel-Kopp et al, J Thromb Haemost, 2016). However, the performances of this method are incompletely defined, in particular due to the lack of standardized reagents. Recently, we developed 5B9, a monoclonal IgG antibody to PF4/H that fully mimics the effects of human HIT antibodies (Kizlik-Masson et al, J Thromb Haemost, 2017), and which may serve as standard for HIT assays.
Aims: The aim of our study was therefore to analyze the sensitivity of Heparin Induced Multiple Electrode Aggregometry (HIMEA) by testing the effects of 5B9 in whole blood. Results obtained were compared to those of SRA and PAT.
Methods: The response to 5B9 of 16 donors tested with HIMEA, PAT and SRA was analyzed. The donors were randomly chosen, but their FcɣRIIa genotype was defined after all experiments. 5B9 was evaluated at 50, 20 and 10 µg/mL, without or with variable concentrations of unfractionated sodium heparin (UFH). The HIMEA protocol proposed by the ISTH SSC was adapted in order to optimize the procedure: NaCl 0.9%, usually pre-incubated with whole blood and patient plasma for 1 minute under stirring at 37°C before trigger of aggregation with heparin was replaced by Tyrode buffer (TB), to reduce measurement of non-specific impedance. In addition, we also reduced background signals by pre-incubation of WB (300 µl) with TB and heparin (150 µl) at 37°C for 6 minutes before adding 5B9 in TB (150 µl).
Results: When 5B9 was tested 50 µg/ml, platelet activation was clearly detected whatever the donor tested using the 3 functional assays (sensitivity or Ss of 100%). The sensitivity of PAT decreased (with 69% of responders) when 5B9 was tested at 20 µg/mL, while those of HIMEA and SRA remained excellent (100%). When the lowest concentration of 5B9 was tested (10 µg/mL), PAT was always negative, whereas HIMEA was positive with 8 out of 16 donors (Ss = 50%). In contrast, SRA was positive with the platelets from 15 of 16 donors (Ss = 94%). Interestingly, 7 of the 8 donors who were good responders to 10 µg/mL of 5B9 in HIMEA were either homozygous (RR) or heterozygous (HR) for the FcɣRIIA131R allele, versus 3 out of the 8 non-responder donors.
Summary/Conclusion: These data obtained with 5B9, a "reagent" allowing to test variable and well-defined concentrations of HIT IgG, confirm that HIMEA is a simple method, which is faster, and more sensitive than PAT for detecting platelet activating HIT antibodies. HIMEA also appeared less sensitive than SRA, but the selection of donors expressing the FcɣRIIA131R isoform on platelets, which are more sensitive in plasma or WB to HIT IgG (Rollin et al, Blood 2015), should improve the performances of the assay.

Platelets
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Assessment of light transmission aggregometry on the routine coagulation analyzer Siemens CS-2500 using CE-marked agonists from Hyphen Biomed

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Background: Platelet function testing is recommended in patients with symptoms evoking a primary hemostatic defect. Platelet function studies are also useful to assess platelet responses in patients treated with antiaggregant therapy. To date, light transmission aggregometry (LTA) is still considered as the "gold standard". However, it is only realized by few specialized laboratories, as the process is time-consuming and labour intensive. These challenges can be overcome by performing platelet aggregometry with an automated method on a routine coagulation analyzer.
Aims: The aim of our study was to compare and correlate results obtained from (i) a traditional manual LTA solution using platelet agonists provided from different manufacturers and realized in our Referent Center with (ii) a complete solution including the CS-2500 (Siemens, Munich, Germany) optimized automated system and CE-marked agonist reagents from Hyphen Biomed (Neuville-sur-Oise, France).
Methods: Blood samples were obtained from (i) patients presenting symptoms suggestive of a platelet disorder; (ii) patients referred to our laboratory for antiplatelet therapy tailoring; and (iii) patients for which Ristocetin Induced Platelet Aggregation (RIPA) was required. Platelet Rich Plasma (PRP) was assessed using a wide range of agonist concentrations: ADP, collagen Horm, ristocetin or arachidonic acid. The APACT-4004 aggregometer (Elitech, France) was used as the reference instrument with platelet agonists provided from different manufacturers. Normal volunteers PRP had been previously tested to determine reference values with this aggregometer. We evaluated the CS-2500 analyzer using dedicated software and CE-marked agonists from Hyphen Biomed. Correlation between the CS-2500 and the APACT-4004 was analyzed using the Passing and Bablok regression test and the Bland-Altman analysis.
Results: Platelet aggregometry studies were performed in 49 samples. Maximal aggregation response with ADP (0.5-10 µM), collagen (2 mg.mL-1), ristocetin (1.2 mg.mL-1) and arachidonic acid (1 mM) agonists showed significant correlation between the two aggregometers ($p<0.01$). However, we observed a more variable response using low doses of ADP (≤ 5 µM). Furthermore, we also noted discrepancies with low dose of ristocetin (0.625 mg.mL-1). Bland and Altman plots showed that 95% of the differences were located within acceptable range with most of the agonists, allowing to conclude that the two compared devices could be considered as consistent. In most of the cases with low platelet count in PRP, maximal aggregation profiles were also consistent between the two systems.
Summary/Conclusion: These data show that the CS-2500 is comparable to a stand-alone aggregometer in a clinical setting and has the advantages of a walk-away technology. The CS-2500 requires a small sample volume of PRP per agonist (140 µL) compared to the APACT-4004 (250 µL). This is particularly helpful for the investigation of pediatric patients. Aggregation with a PRP count of 150x109 L-1 was possible and showed a good correlation between the two instruments. As the CS-2500 is an open analytical system, protocols for additional agonists can also be used, allowing further investigation of specific patient samples. However, the semi-automated system has also some counterparts, such as: (i) running one sample at a time, (ii) taking extra-time to perform a full panel of agonists and (iii) the impossibility to view aggregation profile

Platelets
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Board No. 224

A novel rapid method of platelets and red blood cells permeabilization and staining for flow cytometry analysis.

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Background: Flow cytometry allows simultaneous analysis of multiple characteristics on a single cell. It essentially focuses on surface-expressed proteins and only few protocols are devoted to intracellular components. These protocols of sequential permeabilization and fixation are complex, time-consuming and lack standardization
Aims: To overcome these limitations, we characterized a new staining protocol (NSP) for intracellular and surface staining on different blood cell populations, with a particular attention paid on platelets and red blood cells (RBCs). This NSP is a two steps protocol without formaldehyde based on a new reagent that Immunotech is developing. We evaluated the effect of NSP on cell integrity, morphology and expression of major surface and intracellular markers.
Methods: Citrated-blood from healthy donors was treated as follow: **Step 1**: 20µl of diluted whole blood (1/20) were mixed with the permeabilization buffer (or PBS-BSA for control) for 4 min. **Step 2** - permeabilized samples were incubated with the different antibodies in the presence of the staining buffer for 15 min. Antibodies against surface and intracellular platelet (CD41, CD61, CD42a, CD62P and CD63) and RBC (CD235a, CD55, HbF and HbA1c) markers were used and an anti-CD45 for leukocytes. Samples were then analyzed by flow cytometry. In parallel, we studied platelet and RBCs morphologies using optical and scanning electron microscopy.
Results: Blood cell populations were discriminated according to forward scatter (FS)/side scatter (SS) signals and CD45-positive events were removed from the analysis. Platelets were further gated by CD61 staining. As expected for a permeabilization procedure, NSP significantly reduces blood cell counts (20%) but preserves their respective proportions. The use of detergent in NSP does not affect platelets' SS signal whereas it reduces that of RBCs suggesting it impacts cell structure.
This was further confirmed by brightfield and scanning electron microscopy. NSP-treated platelets maintain their resting discoid morphology with a minor reduction in diameter (11%). NSP-treated RBCs show more marked modifications as they adopt a spherical shape and a 42% diameter reduction. This does not require any modification in gating strategy and does not interfere with the discrimination of the different blood cell populations based on surface specific markers.
This novel technic gives a simultaneous access to all tested intracellular proteins. NSP allows the staining of CD62P and CD63 in resting-platelets, making possible to investigate platelet granules. The easy detection of HbF and HbA1c in RBCs highlights the potential of NSP to discriminate RBC sub-populations. Staining of intracellular markers was absent from control conditions.
Finally, NSP results in signal stability up to 48 hours after platelet staining and no benefit was found of an optional final 0.1% formaldehyde fixation step in terms of cellular integrity and staining intensity.
Summary/Conclusion: With the ability to detect both surface and intracellular antigens, a much shorter realization time without any wash, an absence of toxic formaldehyde and a better standardization, this two-step procedure represents an important improvement for the analysis of platelets and RBCs directly from whole blood. This new procedure can provide some avenues to the research on platelet functions and signalization.

Platelets
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Board No. 225

Standardisation of platelet sialylation quantification by flow cytometry and reference values in healthy subjects

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Background: The search of new biomarkers to improve management of thrombocytopenia has led to the study of platelet sialylation that was identified as a regulator of platelet clearance. Sialic acids are terminal sugar components of glycoproteins and glycolipids. During platelet ageing, sialic acid is removed by the desialylation process that leads to expose β-galactose residues considered as senescence antigens, influencing platelet clearance and life-span. This process has been shown to be involved in various diseases with thrombocytopenia such as allogenic hematopoietic stem cell transplantation (HSCT), providing new treatment's strategies. However, the translation of this biomarker to routine patient care applications remains limited.
Aims: Our aims were to determine the best conditions for a standardized measurement of platelet sialylation and the reference range in healthy subjects (HS). We also tested patients after HSCT.
Methods: Diluted EDTA-platelet rich plasma (PRP) was incubated with increasing concentration of Ricinus communis agglutin 1 (RCA) conjugated to fluorescein-isothiocyanate (FITC) and analysed on a flow cytometer. We used neuraminidase or β-lactose to test sensitivity and specificity. All results were normalized to platelet GPIbα to take into account platelet size (expressed in ratio $R^{RCA/GPIb\alpha}$). Intra-assay imprecision, pre-analytical stability at different temperature and impact of platelet count in PRP were determined as well as inter-assay variability using fixed platelets (from Siemens Healthcare Diagnostics) as internal control. The reference range were defined as the boundaries encompassing 95% of the 47 HS (23 men, 24 women) from the French Blood Bank Institute and were compared to 3 HSCT.
Results are presented as mean ± standard deviation. Non-parametric test of Kruskal-Wallis and Mann-Whitney were used for comparison. In all tests, a p value <0.05 was considered significant.
Results: Remarkably, at concentrations up to 250 µg/mL of RCA, no saturation was observed, whatever the incubation time. With 12.5 µg/ml RCA, neuraminidase treatment (allowing to remove sialic acid) led to an 8.4 ± 2.4 (n=3) fold increase in mean fluorescence intensity (MFI). In contrast, addition of β-lactose which compete with β-galactose exposed, decreased RCA binding (MFI 248 ± 98 vs 1805 ± 542, with and without β-lactose respectively, p=0.03, n=4) attesting the assay's specificity. Intra-assay coefficient of variation (CV) of RCA binding in a same assay was 7 to 12% (10 measurements in 4 HS). Platelet count adjusted at 10 G/L compared to PRP at 498 ± 53 G/L (n=3) did not lead to significant difference ($R^{RCA/GPIb\alpha}$ 0.150 ± 0.026 vs 0.143 ± 0.038, respectively). After whole blood storage 24 hours at 22°C, the ratio was stable (0.21 ± 0.14 at H0 vs 0.24 ± 0.17 at H24). However, storage at 4°C induced an increase of the ratio (0.71 ± 0.52, p=0.025). The inter-assay CV of fixed platelet was 17% (n=15). Reference values of $R^{RCA/GPIb\alpha}$ determined in these conditions (RCA 12.5 µg/ml, up to 24H at 22°C after blood sampling) were 0.07 to 0.28 (95% CI, mean 0.17 ± 0.05), with no relation to age, sex or ABO blood groups. As expected $R^{RCA/GPIb\alpha}$ was increased in the 3 thrombocytopenic HSCT (platelets: 60 ± 9 G/l; RCA: 0.31 ± 0.08, p=0.009).
Summary/Conclusion: This study demonstrates that our assay is stable, standardized and convenient for a routinely platelet sialylation measurement. Moreover, it is suitable for the detection of sialylation defects.

Platelets
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Board No. 226

Specific features of fluorophore sequestering in platelets allow more precise measurement of calcium concentration in cytosol

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Background: A rise in free calcium concentration in platelet cytosol governs platelet responces. Thus measurement of cytosolic calcium concentration is a valuable approach, used for investigation of platelet intracellular signalling. For anuclear platelets the only possible approach is the loading of cells with calcium sensitive fluorophores in AM-esterified form. Among the most used dyes are Fura-2, Fluo-3, Fura-Red and Calcium Green-1 (CG-1). Calcium sensitive dyes are prone to sequester in intracellular compartments in various cell types including platelets. Estimation of the impact of dye sequestering on experimental results will allow essential modifications to common approaches to measure platelet intracelluler signalling.
Aims: To investigate features of calcium fluorophore sequestering in platelets and its impact on cytosolic calcium measurement.
Methods: Blood was collected from healthy donors under the declaration of Helsinki. Platelets were washed as in Cazanave *et. al.* 2004 and loaded by 10 μM of dyes (Fura-2, Fura-RED, Fluo-3, CG-1) at 37ºC. For analysis of platelets suspensions, Cary Eclipse Fluorescence Spectrophotometer has been utilized. For investigation of single cell (spread on 100 ug/ml of Fibrinogen) emission spectrums Nikon A1R confocal microscope with spectral detection unit was used.
Results: Neither of the dyes produced a homogeneous fluorescence distribution within platelets: Fluo-3 produced 5-7 bright spots on an average, Fura-2 5 bright spots, Fura Red - 1-2 spots (405 nm excitation), 5-6 bright spots (488 nm). Distribution upon loading by CG 1 was the most homogeneous - 1 bright spot. In comparison to cytosolic dye fractions, the intensity of the bright spots was 2.3 (least, CG-1) - 5 (most, Fura-2, 488 nm excited Fura-Red). The impact on the fluorescence from the whole platelet was at least 25% increase for CG-1. Fura Red loaded cells excited at 488 nm more than 55% in comparison to cytosolic dye fractions. Cell viability was compromised: loading platelets with 10 μM Fluo-3 induced spontaneous transition to necrotic state (> 20% of the cells), while this effect was decreased significantly upon loading of cells by 2 μM of the dye (< 2%). Spectral analysis of the platelet suspension for all of the investigated dyes revealed that maximum intensity of the fluorescence was 7-10 nm shifted from the expected emission wavelengths (8 nm Fura-2, 7 nm Fluo-3, 10 nm Fura-RED, 7 nm CG 1). In single cells, this spectral shift was detected for bright spots (7-10 nm on an average), while fluorescence of the cytosolic dye was consistent with the standard emission maximum. Upon platelet incubation with mitochondrial un-coupler CCCP and/or 10 nm of thrombin, spectral shift in the sequestered dye fractions decreased for all of the fluorophores, while emission intensity remained the same.
Summary/Conclusion: Calcium sensitive dyes distribute in platelets non-uniformly and there is a significant contribution of the sequestered dye fractions to the signal from the whole cell. The spectra of bright spots are shifted 7-10 nm from the expected values as well as spectrum of the platelet suspension, obtained in spectrafluorimeter. Since CCCP and thrombin also shifted the spectra of the loaded (and de-esterified) dye, it can be speculated that most of the sequestered dye is localized in the mitochondria and, presumably, in platelet acidic stores. Platelet cytosolic calcium could be assessed more precisely by using more long-wave filters, that help avoid disturbing signal from the bright spots.

Platelets
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Board No. 227

Functional HIT testing based on flow cytometry-our experiences

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Background: Heparin-induced thrombocytopenia (HIT) is a condition induced by heparin / PF4 antibodies in heparin therapy. It causes thrombocytopenia and sometimes also arterial or venous thrombosis. Heparin is pathophysiologically bound to thrombocytes, releasing PF4 from the platelet granules. A heparin / PF4 complex is formed on the platelet surface, which causes the production of specific antibodies (most frequently IgG) in some individuals, followed by platelet activation, which releases components with procoagulant activity. Prevalence is approximately 0.5-5% of heparinized patients.
Laboratory diagnostics of HIT usually takes place in two steps. The first step is the evidence of presence of antibodies against the heparin-PF4 complex by some of the immunological assays. Subsequently, the positive test results for HIT are confirmed or excluded by a functional assay to demonstrate the ability of the present antibodies to activate thrombocytes. The definitive diagnosis of HIT is determined with a positive result of a functional test. Nevetheless, there is a significant group of patients (especially after cardiac surgery) who have antibodies present (positivity in immunoassays) but the functional test is negative and therefore they haven´t diagnosis of HIT.
Aims: The aim of the work was to point out the advantages, difficulties and possibilities of applying the HIT Alert functional test using flow cytometry.
Methods: Control samples of healthy donors were taken to 0.109 mol / l sodium citrate. Patient samples were taken into the tube without an anti-irritant. We analyzed the serum of 14 patients with HIT suspected, using the ID-PaGIA Heparin / PF4 (particle gel immunoassay). For samples with a positive result, the serum was analyzed by flow cytometry by a functional assay - the HITAlert kit.
Results: Analyzing 14 samples of patients with HIT suspected by the ID-PaGIA Heparin / PF4 immunological test, we received 13 positive results. Further testing of these samples with the HITAlert flow cytometer kit had 7 positive and 6 negative samples.
Summary/Conclusion: Based on our experience, using a functional HIT assay performed by flow cytometry, we can say that the method has its advantages (functional, relatively short analysis time, non-radioactive) and pitfalls (requires specialized workplaces with expertise and flow cytometry equipment).

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Contribution of the MAIPA Test in the Biological Exploration of ITP

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Background: Immune thrombocytopenia (ITP) is a common hematologic disorder characterized by isolated thrombocytopenia. ITP is an antibody-mediated condition in which autoimmune platelet destruction leads to low platelet counts and variable degrees of bleeding. It is a heterogeneous condition. The pathogenesis of ITP is complex, involving alterations in humoral and cellular immunity. ITP results in part from the presence of platelet antibodies.
Aims: to optimize our diagnostic algorithm for suspected ITP cases by detection of platelet antibodies.
Methods: A prospective and descriptive study was conducted over a period of 12 months in the hemobiology Center of the University Hospital of Annaba. It involved 35 patients with a suspected ITP. The biological diagnosis was made using NFS, blood smear, myelograms and the Monoclonal Antibody-Specific Immobilization of Platelet Antigens (MAIPA) assay.
Results: The platelet count of the ITP cases studied ranged from 9 G/L to 78 G/L. The myelogram revealed normal richness in non-dystrophic megakaryocytes. Hemorrhagic syndrome was observed in 42% of cases. Antiplatelet antibodies detected by MAIPA in our cohort were against the glycoproteins (GP) IIb/IIIa, IaIIa and Ib/IX. An anti-HPA-5b antibody was diagnosed in a case of ITP with major bleeding. Other anti-HLA class I antibodies have been detected in several cases.
The platelet count in the ITP cases with antiplatelets antibodies was significantly lower than in cases with negative MAIPA, which corresponds to the data of the literature; the platelet count is inversely proportional to the concentration of antibodies
Summary/Conclusion: Our experience suggests that MAIPA assays may be used to improve the diagnosis of ITP, should be performed before therapy, and could possibly become a guide for optimizing therapy towards a more personalized treatment of ITP.

Platelets
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Board No. 229

Long-term safety and efficacy of eltrombopag in pediatric chronic immune thrombocytopenia (cITP): An extension study of Russian patients previously enrolled in PETIT2

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Background: cITP is an acquired autoimmune disorder defined by low platelet counts for >12 months from diagnosis with continued risk of significant bleeding (Rodeghiero *et al. Blood* 2009). The randomized, multicenter, placebo-controlled PETIT (Bussel *et al. Lancet Haematol* 2015) and PETIT2 (Grainger *et al. Lancet* 2015) trials demonstrated that eltrombopag (EPAG), an oral thrombopoietin receptor agonist, is an effective and tolerable therapeutic option for pediatric patients with previously treated cITP. However, long-term data in pediatric patients are limited.
Aims: To evaluate the long-term safety and tolerability of EPAG in pediatric patients with previously treated cITP enrolled in a long-term extension of PETIT2 in four centers in Russia.
Methods: Patients aged ≥1–18 years previously enrolled in PETIT2 with clinical benefit from EPAG, were enrolled in the extension. Patients/guardians provided written informed consent. Screening was followed by a single-arm treatment period. Starting EPAG dose was based on the patient's dose at the end of PETIT2, and adjusted (max 75 mg/day) according to platelet count and the investigator's clinical judgement to maintain a safe hemostatic range (~50–200x10^9/L). Frequency and severity of adverse events (AEs) were recorded (graded using CTCAE version 4.03), and hematology and blood chemistry regularly monitored. Patients were considered to have completed the study and EPAG discontinued within 3 months once the patient reached 18 years of age or EPAG received local regulatory approval for pediatric cITP.
Results: Nine patients were enrolled (4 female; median 9 [range 4–15] years old); four (44.4%) completed the study and five (55.6%) discontinued (patient decision, n=2; lack of efficacy, n=2; AE [autoimmune hepatitis], n=1). All nine patients received EPAG for ≥3 months. The median exposure duration in the extension was 24.6 (range 3–48) months. EPAG was started on or escalated to 75 mg/day in eight patients. Eight patients experienced AEs, most commonly nasopharyngitis (n=3), epistaxis (n=2), and headache (n=2). Serious AEs were reported in three (33.3%) patients: autoimmune hepatitis (n=1), epistaxis (n=1), and scleral hemorrhage (n=1). None of the AEs or serious AEs were considered treatment related by the investigator. Autoimmune hepatitis occurred in a 6-year-old after ~3 months on EPAG, while on 75 mg/day. Laboratory tests at the time revealed Grade 3 alanine and aspartate aminotransferase (ALT/AST) elevations with normal bilirubin, but ALT/AST normalized over the next 3 weeks following discontinuation. Although platelet counts fluctuated, as expected with low patient numbers, they were generally maintained within a safe hemostatic range.
Summary/Conclusion: This open-label, Phase III extension study demonstrated the long-term safety of EPAG in pediatric cITP patients previously enrolled in PETIT2. Patients continued to receive benefit from EPAG. The safety profile of EPAG in the extension was consistent with that observed in the PETIT and PETIT2 trials and its known safety profile. Only one patient discontinued treatment because of an AE (autoimmune hepatitis), considered unrelated to EPAG by the investigator. There were no unexpected safety findings, indicating a favorable benefit–risk profile for EPAG in the long-term treatment of pediatric cITP patients.
NCT identifier: NCT02201290

Platelets
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Board No. 230

Autologous Platelet-Released Growth Factor and sexual dysfunction amendment: A Case series of successful improvement sexual dysfunction after pelvic irradiation

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Background: Sexual dysfunction is a common sequel to cancer treatment which affects the quality of life in women treated with pelvic radiotherapy.
Aims: The aim of this study was to evaluate the safety, symptom resolution and objective improvement the injection of autologous platelet released growth factor (APRGF) for treatment of sexual dysfunction in patients after pelvic irradiation.
Methods: This prospective case series study enrolled 10 cancer-free patients with sexual dysfunction who underwent pelvic radiotherapy at least 5 years ago. Each patient was received 1-2 cc APRFG within three weeks (3 times injection) period and all patients were re-evaluated at eight weeks and six months. CD34 IHC and Masson's trichrome staining were performed on vaginal biopsy section for angiogenesis and fibrosis (collagen sedimentation) respectively.
Results: Our results declared that APRFG injection was effective and symptoms were disappeared in the entire patients. Significant objective improvements in vaginal diameter (mean before injection, 6.5 cm vs 7.1 cm after injection) (p-value = 0.001) and also vaginal flexibility (mean before treatment, 0.72 cm vs 1.85 cm after injection) (P-value = 0.026) were observed. Characteristics of discharge before the injection in 60% of patients were included dry vagina and 40% had mild discharge but after injection 40% of patients had moderate and also 60% had mild and sufficient discharge (P-value= 0.190). Sexual satisfaction after the injection of APRFG was clinically difference and the entire patient had sexual satisfaction. In the patient's follow-up, none of them need to repeat the treatment.
Summary/Conclusion: To sum up, our patients reported better sexualdisfunction and show better vaginal function indexes after APRGF injection

Platelets
P280
Board No. 231

Optimised tools for evaluation of platelet function measured by impedance aggregometry

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Background: Previous studies with focus on thrombocytopenia indicate a strong association between platelet count and platelet function measured by impedance aggregometry. The association between platelet function and platelet count has attracted less attention at platelet counts in the normal range. However, this association may be highly significant and further assessment is warranted and may provide tools to improve evaluation of platelet function taking platelet count into consideration also at platelet counts in the normal range.
Aims: Firstly, the present study aimed to investigate the association between platelet function and platelet count in healthy individuals with platelet counts in the normal range combined with data from a model of thrombocytopenia with healthy platelet function. Secondly, we aimed to provide prediction intervals for healthy platelet function in relation to platelet count as a tool to optimise evaluation of platelet function.
Methods: Analyses were performed on raw data from three previously published studies from our group. Platelet function was measured by impedance aggregometry using four different agonists; collagen 3.2 µg/mL (COLtest), adenosine diphosphate (ADP) 6.5 µM (ADPtest), thrombin receptor activating peptide-6 (TRAP) 32 µM, and ristocetin 0.77 mg/mL (RISTOhigh). Thrombocytopenia with healthy platelet function was induced *in vitro* in 20 healthy volunteers employing a validated method with minimal manipulation of blood cells allowing whole blood assessment of platelet function (Platelets. 2016;27:295-300). The data from the thrombocytopenic range were combined with data from two studies on healthy individuals with platelet counts in the normal range using the agonists collagen, ADP and ristocetin (n=121, Thromb Res. 2012;130:420-3) and TRAP (n=125, Cytometry B Clin Cytom. 2018 May 23. doi: 10.1002/cyto.b.21642). Associations between platelet function and platelet count was investigated using linear regression analyses and 95% prediction intervals for platelet function in relation to platelet count were calculated.
Results: Linear regression analyses demonstrated strong positive associations between platelet function and platelet count across platelet counts in the thrombocytopenic as well as normal range: Collagen (R^2=0.72), ADP (R^2=0.70), TRAP (R^2=0.62), and ristocetin (R^2=0.81), all p-values < 0.001. 95% prediction intervals for platelet function in relation to platelet count were established in the platelet count range of 26 to 425 x 10^9/L.
Summary/Conclusion: The strong linear association between platelet function and platelet count previously established in thrombocytopenia also applies in the normal range. This finding underlines the importance of considering the platelet count when evaluating platelet function also at platelet counts in the normal range. Hence, 95% prediction intervals for platelet function in relation to platelet count were established and may provide a helpful tool for evaluation of platelet function by impedance aggregometry.

Platelets
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Board No. 232

Endothelial activation in acquired thrombotic thrombocytopenic purpura: mechanisms and correlation with disease severity

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Background: Thrombotic thrombocytopenic purpura (TTP) is a thrombotic micro-angiopathy (TMA) characterized by ischemic events affecting mainly the central nervous system and the myocardium, hemorrhages of various severity, mechanic haemolytic anaemia with schistocytes and deep thrombocytopenia. Acquired form of TTP (a-TTP) is characterized by the presence of anti-ADAMTS13 neutralizing auto-antibodies inducing an accumulation of circulating ultra-large von Willebrand Factor (VWF). ADAMTS13 deficient animals have however also revealed the importance of endothelial cell (EC) activation through UL-VWF release from Weibel-Palade Bodies (WPB) exocytosis.
Aims: We hypothesized that plasma from a-TTP patients contained activators of EC inducing Weibel-Palade bodies (WPB) exocytosis.
Methods: We studied the in vitro ability of a-TTP or control patients's plasma obtained by plasma exchange, to induce WPB exocytosis from dermal human micro-vascular EC (HMVEC-d) and the relationships with disease severity.
Results: We observed that plasma prospectively collected from 24 patients in acute phase a-TTP induced UL-VWF release and WPB exocytosis from ECs in a calcium (Ca^{++})-dependent mechanism and that this effect was correlated with disease severity and prognosis in 60 patients. We then looked for plasma factors present in a-TTP patients able to activate EC. We observed that 1) a-TTP plasma contained high concentrations of free heme and nucleosomes 2) free heme and nucleosomes but not complement, played a minor role in WPB exocytosis and 3) the IgG fraction from a-TTP plasma played a crucial role in Ca^{++}-dependent WBP exocytosis.
Summary/Conclusion: Our results confirm that EC activation leading to WPB degranulation and VWF exposure may constitute a central event in TTP pathogenic cascade and demonstrate the multifactorial mechanisms of EC activation.

Platelets
P282
Board No. 233

Effect of Direct Oral Anticoagulants on platelet reactivity

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Background: Since 2008, Direct Oral Anticoagulants (DOAC) are increasingly prescribed for the treatment and prevention of venous thromboembolic disease as an alternative to vitamin K antagonists and heparinoids. However, the effect of DOAC on arterial thromboembolic risk and platelet functions remains controversial.
Aims: We investigated *in vitro* the effect of two DOAC, namely rivaroxaban and dabigatran, on human platelet aggregation.
Methods: Citrated (3.2%) whole blood samples of six healthy fasting volunteers were spiked with rivaroxaban or dabigatran. Final plasma concentrations of DOAC were determined by specific anti-Xa or anti-IIa activity assays. Platelet aggregation was measured in platelet-rich plasma (PRP) by light transmission aggregometry (TA-8V aggregometer). Aggregation was induced by the addition of low doses of agonists allowing detection of either pro- or anti-aggregant effect: arachidonic acid (0.5 mM), ADP (2.5 µM), epinephrine (0.5 µM), collagen (0.8 µg/mL) and TRAP-6 (7.5 µM). Platelet aggregation secondary to endogenous thrombin generation was also evaluated by triggering coagulation in PRP with human recombinant tissue factor (TF; 0.5 pM) and $CaCl_2$ (10 mM) in the presence of H-Gly-Pro-Arg-Pro-OH (GPRP; 2 mg/mL) to prevent fibrin polymerisation. We also tested agglutination induced by ristocetin (1.2 mg/mL). Three parameters were analysed for each aggregation: maximum amplitude of platelet aggregation (%), lag time (sec) and velocity (%/sec). Results were expressed as mean ± SD or median (95% confidence interval).
Results: Platelet count in PRP (250 ± 75 G/L) had no effect on any of the studied parameters ($p > 0.05$). Whole blood from healthy volunteers was spiked with 3 concentrations of each DOAC. Respective concentrations further measured in plasma were of 37 (20-48), 149 (121-163) and 356 (284-396) ng/mL for rivaroxaban and 45 (39-51), 186 (169-213) and 351 (319-403) ng/mL for dabigatran. DOAC vehicle, i.e. 0.05% dimethyl sulfoxide, was devoid of any effect on the different parameters of platelet aggregation ($p > 0.05$). DOAC, at any of the concentrations tested, did not modify platelet aggregation parameters induced by low-dose of standard agonists (no significant effect on maximum amplitude, lag time or velocity, $p > 0.05$), nor the aggregation profiles (reversibility, number of phases and platelet shape change). Following coagulation activation, generated thrombin stimulated platelet aggregation, therefore increasing light transmission. A concentration dependent delay of aggregation resulting from thrombin generation was observed in the presence of DOAC. Dabigatran had a more important effect compared to rivaroxaban ($p < 0.001$). However, once activated, platelets eventually aggregated and the maximum amplitude was only decreased with the highest concentration of dabigatran ($p < 0.05$). Dabigatran but not rivaroxaban decreased the velocity at a concentration higher than 191 ng/mL ($p<0.001$).
Summary/Conclusion: Rivaroxaban and dabigatran, either at low or high concentrations, had no effect on platelet reactivity in PRP when stimulated with standard agonists. They delayed aggregation induced by thrombin generation in a concentration dependent manner. This aggregation depended on pro-coagulant platelet activity. Dabigatran had a more pronounced effect compared to rivaroxaban. Nevertheless, once activated, platelets aggregated almost similarly in the presence or absence of DOAC.

Platelets
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Board No. 234

Coagulopathy associated with Isolated Traumatic Brain Injury

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Background: According to recent studies, isolated traumatic brain injury (TBI) leads to coagulation disturbances including both hyper- and hypocoagulative states. It has been proven that TBI is associated with the development of secondary brain injuries with bleeding or ischemic complications that contribute to adverse events. Therefore, it can be assumed that hemostasis monitoring in the acute phase of TBI might help provide adequate prophylaxis to prevent coagulopathy and thus reduce the risk of secondary brain injuries.
Aims: /
Methods: /
Results: Clinical Case: Patient K., an 18-year-old white male, was transferred to our department from another hospital the day after experiencing a TBI (penetrating head injury, craniofacial trauma) due to a car accident. During admission, his Glasgow Coma Scale score was 7, and cerebrospinal fluid otorrhea was observed on his right side. Besides multiple facial bone fractures, head computed tomography revealed left and right frontal hematomas with no mass effect. The patient was followed up with a standard local protocol of acute care for TBI, including intensive care with invasive hemodynamic and intracranial monitoring, as well as management of intracranial complications. Platelet function analysis (PFA-100) and thromboelastography platelet mapping were used to assess platelet function, which detected platelet dysfunction. Fibrinogen function was also decreased according to a thromboelastography fibrinogen function analysis. Although the results of standard coagulation tests (activated partial thromboplastin time, international normalized ratio, and fibrinogen and platelet number) were normal, we initiated hemostatic therapy using tranexamic acid. The next day, a dynamic evaluation of hemostasis was performed since signs of hypocoagulation persisted. Although the results of standard coagulation tests were normal, hemostatic therapy was continued until day 3 at which time the TBI hemostasis system began to stabilize. After 10 days, patient was discharged, without any clinical signs of coagulopathy (normal platelet and fibrinogen function), as well as absence of hemorrhagic progression or appearance of ischemia foci on the brain computed tomography scan.
Summary/Conclusion: The pathophysiological mechanism underlying TBI-induced coagulopathy is not fully understood, but it is thought to involve platelet dysfunction. Unfortunately, standard coagulation tests are not sensitive to such changes. It can be assumed that an integral evaluation of hemostasis, as well as adequate treatment of coagulopathy after TBI, might prevent hemorrhagic and ischemic complications and thus improve neurological outcomes. Further studies on a larger scale are warranted to investigate this fascinating topic in medicine.

Platelets
P284
Board No. 235

Platelet function in preterm neonates

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Background: Preterm neonates have a higher bleeding tendency than term neonates. A reduced platelet function may be an essential contributor to this clinical challenge. Due to previous methodological limitations, the platelet function in preterm neonates is sparsely investigated. Our research unit has recently developed a flow cytometric method requiring only small volumes of blood, thereby allowing us to investigate the development of preterm neonatal platelet function in detail.
Aims: The present study aims to examine the development of platelet function in preterm neonates from gestational age (GA) 32 to 41 and to compare platelet function among preterm neonates reaching GA 38 to 41 with platelet function in neonates born at term. Furthermore, platelet function in peripheral- and umbilical cord blood will be compared in both preterm and term neonates.
Methods: Inclusion of 25 preterm neonates at GA 32+0 to 34+0 and 25 term neonates at GA 38+0 to 41+0 is ongoing. Umbilical cord- and venous blood is collected from both preterm and term neonates at birth and the preterm neonates are followed clinically and with blood samples at GA 36+4±2 and GA 38+0 to 41+0. Platelet function is analyzed by flow cytometry (Navios). Initially, the expression of glycoprotein (GP) Ia, IIa, IIb, IIIa and IX are determined and the expression of surface bound fibrinogen, CD63 and P-selectin are measured after the addition of collagen-related peptide (0.12 µg/ml), adenosine diphosphate (10.8 µM), thrombin-receptor-activating-peptide (28.5 µM) and arachidonic acid (0.58 mM). The Helsinki declaration is followed in all aspects, and parental informed consent is obtained prior to inclusion.
Results: The development of preterm neonatal platelet function is evaluated. Furthermore, the platelet function among preterm neonates reaching GA 38 to 41 is compared with platelet function of term neonates born at GA 38 to 41. Finally, we compare the platelet function in umbilical cord- and venous- blood obtained from preterm and term neonates at birth. Data is presently being collected and preliminary results will be ready for presentation at the congress.
Summary/Conclusion: We expect to clarify whether preterm neonatal platelet function changes the first weeks of life and how their platelet function at GA 38 to 41 compares to that of term neonates. Finally, we expect to demonstrate if platelet function in umbilical cord- and venous blood differ.

Platelets
P285
Board No. 236

No integrin deactivation assessed by fibrinogen binding to platelets was observed during reversible platelet aggregation induced by ADP

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Background: Reversible platelet aggregation is a phenomenon observed in both platelet suspension and PRP upon stimulation with ADP or a combination of serotonin and adrenalin in presence of calcium ions. Molecular mechanisms underlying the disaggregation process are not clear. One of the possible explanations of reversibility is inactivation of platelet integrins $\alpha_{IIb}\beta_3$ that leads to the release of fibrinogen.
Aims: To investigate dynamics of platelet aggregate formation and evolution of the state of integrins during ADP-induced platelet aggregation using a combination of mathematical modelling and flow cytometry approaches.
Methods: The platelet aggregation in response to ADP was observed on light transmission aggregometry. Aggregation of washed platelets were conducted in Tyrode's buffer (with albumin and calcium) supplemented with 300 μg/ml of human fibrinogen. For assessment of integrin activation, FITC-labeled human fibrinogen was added into the solution. The samples were analyzed by flow cytometry at different time points to assess aggregate size distribution and integrin function. To test experimental results a homogeneous mathematical model was used. Parameter values of the model were assessed automatically by means of several parameter estimation techniques implemented in COPASI software.
Results: As was previously shown (Cazenave et al. and Mustard et al.) platelet aggregation in response to any concentration of ADP in presence of calcium ions in the solutions was reversible. Flow cytometry analysis of the suspension for platelet rich plasma as well as for washed platelets shows that the transmittance of the platelet suspension correlates with the average size of aggregates in the solution. For weak activation of washed platelets in suspension, at the aggregation curve maximum, about 60% of platelets remained single, 25% were in small aggregates of 2-4 cells, and 15% were in large aggregates of 4-8 cells. These large aggregates continued to increase in size (up to 10-20 cells) even after the maximal aggregation, but disappeared over the further course of disaggregation. The level of fibrinogen binding to single platelets remained constant over the course of aggregation. For aggregates of size larger than two platelets, fibrinogen binding per platelet was higher by an order of magnitude. We developed a mathematical model of reversible platelet aggregation, which was able to describe reversible aggregation in response to ADP without assuming integrin inactivation. The aggregates in the model were formed either from single platelets or from smaller aggregates, and then large aggregates dissolved into smaller ones. The kinetic parameters for single platelet agglutination and aggregate stability increased with ADP increase. The estimated parameters of aggregate stability were much higher for PRP compared with washed platelets.
Summary/Conclusion: A series of transitions between the aggregates of different sizes rather than turning off of the integrins is behind the reversible aggregation phenomenon. The decrease in the fibrinogen binding is not observed in single platelets. It is observed for the platelet population as a whole, and is associated with disappearance of large aggregates, so it could be not the cause, but rather a consequence of disaggregation e.g. because of discontinued outside-in signaling. All parts of this study were supported by the Russian Science Foundation grant 17-74-20045

Platelets
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Board No. 237

Modulation of the platelet count by Romiplostim and inhibitors of LIM kinase in genetically-engineered von Willebrand disease type 2B mice: in vivo evaluation of haemostatic efficiency.

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Background: Von Willebrand disease type 2B (VWD2B) is characterized by gain of function mutations in the A1 domain of Von Willebrand Factor (VWF) and platelet abnormalities. Among VWD2B-related mutations, p.V1316M is associated with severe macrothrombocytopenia and platelet dysfunction. The macrothrombocytopenia is caused by the abnormality of the LIM Kinase/cofilin/actin pathway during megakaryocytopoiesis.
Aims: This study aims to characterize drug-induced modulation of platelet count in order to modulate the bleeding tendency in a murine model of genetically engineered von Willebrand disease type 2B (2B).
Methods: 2B and wild type (WT) mice received one subcutaneous injection of either Romiplostim (100µg/kg of body weight, agonist of thrombopoietin receptor) or LIM Kinase inhibitor (LIMKi), daily for 3 days (30mg/kg of body weight). Blood samples were analyzed for platelet count before and after treatment with Romiplostim (at day 4) or LIMKi (at day 7). The bleeding tendency was evaluated by two different bleeding times: tail clip and tail vein transaction (TVT). Platelet activation was analyzed in parallel *ex vivo* by flow cytometry.
Results: As previously described, 2B mice were thrombocytopenic (platelet count, 2B: 375 ± 113 G/L, n=40, WT: 872 ± 160 G/L, n=22). After Romiplostim treatment, platelet count was normalized in 2B mice and increased in WT mice (2B: 975 ± 348G/L, n = 15 *vs* WT: 2067 ± 495G/L, n = 12, $p \leq 0{,}001$). After LIMKi administration, platelet count increased in 2B mice but did not normalize (2B-LIMKi: 504 ± 116 G/L *vs* WT: 910 ± 78 G/L, n = 6, $p \leq 0{,}001$). However, both bleeding times in tail clip or TVT and blood loss were similar in 2B mice compared to untreated 2B mice after Romiplostim treatment. After LIMKi administration, no significant shortening of bleeding time was observed compared to untreated 2B mice. However, a subgroup with a decrease of the bleeding time (1^{st} subgroup 2B-LIMKi: 228 ± 113s, n = 7 *vs* 2^{nd} subgroup 2B-LIMKi: 1328 ± 388s, n= 6) and a decrease of blood loss (1^{st} subgroup 2B-LIMKi: 45 ± 36µL, n = 7 *vs* 2^{nd} subgroup 2B-LIMKi: 568 ± 183µL, n= 6) could be isolated. Interestingly, this subgroup has a bleeding phenotype comparable to WT mice in both bleeding time (1^{st} subgroup 2B-LIMKi: 228 ± 113s, n = 8 *vs* WT-LIMKi: 112 ± 20s, NS) and blood loss (1^{st} subgroup 2B-LIMKi: 45 ± 36µL, n = 8 *vs* WT-LIMKi: 79 ± 80µL, n= 3, NS). 2B mice treated with LIMKi had still a defect of platelet activation compared to WT mice, suggesting that LIMK is not likely involved in thrombocytopathy in VWD2B.
Summary/Conclusion: Our study shows that in 2B mice, the increase of the platelet count by Romiplostim or LIMKi is not sufficient to significantly and globally correct the haemorrhagic phenotype of these mice, suggesting a major role of platelet dysfunction in VWD-2B related bleeding tendency.

Platelets
P287
Board No. 238

Assessment of Platelet Aggregation with Automated Method Among Tunisian Healthy Blood Donors

Hadef Skouri*

Background: Automated method based upon Light Transmittance Aggregometry has been developed on Sysmex Automated Blood Coagulation Analyzers.
Aims: To assess normal ranges of platelet aggregation in response to different agonists among Tunisian blood donors.
Methods: Aggregation has been performed on CS-2100i analyzer. Plasma Rich Platelet from 130 voluntaries blood donors has been monitored for light transmission changes in response to ADP 10, 5, 2 and 1µM; Collagen 5, 2, 1 and 0.5µg/mL; Arachidonic acid 1 and 0.5µM; Epinephrine 10 and 5µM; and Ristocetin 1.2 and 0.6mg/mL.
Results: Aggregation percentage means obtained by high agonists' concentrations are 91.5% (SD 7.2), 91.1% (SD 6.8), 94.6% (SD 6.4), 90.7% (SD 7.6) and 91.7% (SD 7.2) with ADP, Arachidonic acid, Collagen, Epinephrine and Ristocetin, respectively. Second agonists dilutions showed similar results except for Ristocetin (0.6mg/mL) which dropped to 5.7%. Shape contraction and two waves' profiles has been obtained with Collagen and Epinephrine, respectively. Surprisingly, 34 blood donors (26%) showed defective aggregation in response to 0.5µM Arachidonic acid. Four out of these 34 did not respond to both concentrations of Arachidonic acid, and 12 had, simultaneously, a defect on 5µM Epinephrine response. One blood donor demonstrated concomitant aggregation defect to both concentrations of Arachidonic acid and Epinephrine. Two blood donors had defective aggregations with different ADP doses. In one case, there was an instable aggregation and the curve dropped rapidly (except agglutination with Ristocetin).
Summary/Conclusion: Automated aggregation approach on the Sysmex Automated Blood Coagulation Analyzer CS-2100i is an easy and rapid tool to overcome conventional methods' limitations. Platelet aggregation defects detected among Tunisian healthy blood donors may be considered as another feature of the hemostasis genetic polymorphism encountered in this Mediterranean population. These findings lead to evoke the real clinical relevance of theses abnormalities and the interference of these defects with the management of anti-aggregation therapy and the efficiency of platelet transfusion. Further studies are needed to highlight these issues.

Platelets
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Board No. 239

A novel GATA 1 mutation identified within one French family with thrombocytopenia and microcytosis

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Background: GATA 1 is a transcription factor essential for the differentiation of the megakaryocytic and erythroid precursors. Mutations in the gene coding for GATA 1 have been associated with X-linked dyserythropoietic anemia and thrombocytopenia. GATA 1 is characterized by the presence of two homologous zinc fingers. The N-terminal finger associates with a co-factor protein Friend of GATA 1 (FOG 1) to improve the stability of the interaction between GATA 1 and particularly complex or palindromic DNA sequences. The C-terminal finger binds with GATA-motif DNA sites. Several mutations have been described, concerning either the N-terminal or the C-terminal finger. We report a new deleterious mutation in one family within the N –terminal Finger.
Aims: .
Methods: .
Results: We present the case of a 9 years old boy with mild thrombocytopenia since childhood, discovered by easy bruising. He had neither mucosal bleeding nor dysmorphy. Serial blood investigations showed a platelet count ranging from 94 to 136 G/L with a normal MPV (9fl) and a microcytosis (75 fL) without anemia. The Duke bleeding test was 8 min. The occlusion time on PFA-100 was highly increased (epinephrine >300 sec., ADP=132 sec.) with normal Willebrand factor levels evoking a platelet dysfunction. However platelet function (agregometry) and platelet glycoprotein expression (flow cytometry) were normal. The prothrombin time and partial thromboplastin time were both normal. Thalassemia was ruled out by analysis of α globin genes and hemoglobin A_2 level (3%).
Family history revealed that the maternal grandfather had a thrombocytopenia (55 G/L platelets), and a microcytosis. An immune thrombocytopenia was ruled out after a bone marrow analysis showing a dysmyelopoisis in 2008. Given this likely X-linked thrombocytopenia associated with a dyserythropoiesis, we looked for a mutation on the *GATA 1* gene.
A novel mutation in exon 4 of GATA 1 (c.697A>G) was found in the child and his grandfather which was also detected, in a heterozygous state, in his mother and sister.
Summary/Conclusion: A novel GATA 1 mutation (c.697A>G) was identified within one French family with thrombocytopenia and microcytosis. In front of any inherited thrombocytopenia, it is important to check for abnormalities in other blood cells.
Furthers studies are necessary to better understand the pathogenic relevance of this mutation.

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Stormorken-Sjaastad-Langslet syndrome: a new family.

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Background: Stormorken syndrome is an inherited autosomal dominant disease first described in 1985. This disorder is characterized by thrombocytopenia, thrombocytopathy, miosis, asplenia, muscle fatigue, headache, short stature, dyslexia and ichtyosis. This syndrome is caused by a mutation in the STIM 1 gene. Stromal interaction molecule (STIM), spanning the endoplasmic reticulum (ER) membrane, is a dynamic coordinator of cellular calcium signals thanks to the Calcium release activated calcium channel (CRAC). Heterozygous missense mutation in the STIM1 gene (R304W) is identified in Stormorken syndrome. It causes a gain of function effect associated with an increase in store-operated calcium entry and resting calcium level. To date, only 13 patients in nine different families have been reported with this genetic pathology. Here we report a new family with Stormorken syndrome.
Aims: .
Methods: .
Results: We report the case of a 48 years old male patient presenting with a bleeding tendency since chilhood. A macrothrombocytopenia was discovered after orthopedic surgery and a bleeding requiring blood transfusion at the age of 17. Laboratory tests showed a mild thrombocytopenia (platelets count 110 G/L), with an increased mean platelet volume (12 fl), presence of Howell Jolly bodies, increased serum creatine kinase (3152 UI/L) and a prolonged occlusion time with Epinephrin (228 sec). Factor VIII, Willebrand antigen and activity were normal. A thrombocytopenia was found in his son and daughter at birth.
Clinically, the 3 patients presented striking miosis, functional asplenia, short stature and dyslexia.
The exon 7 STIM 1 sequencing showed that the patient (index case) was heterozygous for the c.910C>T; p.Arg304Trp mutation confirming the Stormorken syndrome. This mutation was also found in his children.
Summary/Conclusion: Stormorken syndrome is a rare condition that affects many body systems. In front of any hereditary thrombocytopenia, it is important to check for clinical abnormalities. The clinical examination is essential and can lead to the diagnosis like as in the case report.

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Imaging P2Y12-inhibited and non-inhibited platelets in the building thrombus under flow in vitro

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Background: Inhibition of the platelet ADP-receptor P2Y12 is a corner-stone in prevention of thrombotic events in adult acute coronary syndrome (ACS) patients. The combination of a short-lived metabolite and irreversible binding of the thienopyridines (P2Y12 antagonists) such as prasugrel should theoretically cause the formation of a fraction of new and non-inhibited platelets between each dose. With increased platelet turnover or poor patient compliance the portion of non-inhibited platelets will increase.
Aims: To explore the impact of the different mechanism of action of two P2Y12 antagonists, ticagrelor and prasugrel, on thrombus formation under flow in vitro and the potential impact of platelet turnover and compliance on thrombus formation.
Methods: The PDMS flow chamber was moulded on a template of photo- patterned SU-8 resist on a silicon wafer. A collagen strip was coated on the glass slide and a straight PDMS channel (height; 100 µm, width; 400 µm) was placed perpendicular over the collagen strip. The blood, anticoagulated with hirudin, was drawn through the flow chamber with a syringe pump. We used fluorophores to elucidate how different platelet populations contribute to thrombus formation in the flow chamber. Z-stack time-lapse images were captured with a wide-field 20x objective on a Zeiss Axio Observer Z1 with a LED- module and a Neo 5.5 sCMOS camera. Quantification of the subgroups and further analysis of thrombus dynamics, such as movement, stability and platelet location were determined with the use of our previously published method. A series of 8 experiments were performed for each donor. A control sample without any inhibitor added, three samples inhibited with ticagrelor at varying concentration (1.5, 0.4 and 0.2µM) and four samples containing PAM (3µM) with increasing fractions of non-inhibited platelets to mimic the formation of newly formed platelets.
The research, blood sampling from healthy volunteers and consent procedure was approved by the local ethical review board in Linköping.
Results: The impact of this non-inhibited platelet fraction on thrombus formation was evaluated in vitro under flow, with distinct labelling the inhibited and non-inhibited platelet fractions enabling their separate evaluation. Quantification of the number of labelled platelets for each fraction and their individual position within the forming thrombus was performed through image analysis. The results were compared with the direct acting and reversibly binding P2Y12 inhibitor ticagrelor which should inhibit all platelet equally. The addition of 20% or more of non-inhibited platelets to prasugrel inhibited platelets caused an increase in the total platelet accumulation comparable to the increase seen at lower concentrations of ticagrelor. The addition of the non-inhibited fraction to prasugrel inhibited platelets resulted in a shift in the distribution of inhibited and non-inhibited platelets, both in numbers and location within the forming thrombus, with a dominance of inhibited platelets which was more distinguishable at the surface and down-stream of the thrombus. Thus, the inhibited platelets are more prone to adhere and aggregate in zones of lower shear.
Summary/Conclusion: Accumulation of platelets increased with decreasing concentration of ticagrelor. For PAM increasing the non-inhibited platelet fraction caused increased accumulation and a shift in the thrombus composition with inhibited platelets in zones with lower shear.

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Adipocyte influences megakaryopoiesis: a link with obesity

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Background: Megakaryocytes (MKs) come from a complex and specialized hematopoietic progenitor maturation process within the bone marrow (BM) and give rise to *de novo* circulating platelets. BM microenvironment is composed of different cell types including a large number of adipocytes which medullar role is still ill defined. It has been shown that either marrow adipocytes in a steady state or high fat diet (HFD)-induced obesity that increase medullar adiposity have an impact on hematopoiesis, myelopoiesis and lymphopoiesis.
Aims: This study aims to analyze the influence of medullar adipocytes and diet-induced obesity on megakaryopoiesis and platelet production that still remain unknown.
Methods: To that aim, we set up an *in vitro* co-culture assay where freshly isolated MK progenitors (MKPs) are co-cultivated with adipocytes with no direct contact and obesity in mice is induced by a HFD.
Results: Our study shows that adipocytes support MK maturation by enhancing polyploidization and amplifying demarcation membrane system. MKs, in the other hand, are responsible for adipocyte delipidation and dedifferentiation. We show an unsuspected crosstalk between adipocytes and MKs with the existence of a lipid transfer from adipocytes to MKs to reinforce MK maturation. In a context of mouse obesity, increased medullar adiposity is associated to an enhanced MK nuclear and membrane maturation but disturb platelet production.
Summary/Conclusion: Thus, these findings demonstrate an unsuspected effect of adipocytes and obesity on MK homeostasis and platelet production with a bidirectional dialogue between adipocytes and MKs.

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MicroRNA and hyperaggregability of thrombocytes in sticky platelet syndrome- our first experiences

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Background: Sticky platelet syndrome (SPS) is referred to as platelet hyperaggregability triggered by low concentrations of platelet agonists adenosine diphosphate (ADP) and/or epinephrine (EPI), Aggregation with other inducers (collagen, arachidonic acid, ristocetin, and thrombin) remains normal. MicroRNAs (miRNAs) are small, non-coding RNA molecules that play an important role in posttranscriptional regulation of protein expression. More recently, the discoveries show that platelets are an abundant source of miRNAs, and that miRNA expression profiles within platelets correlate with platelet reactivity.
Aims: To determine a correlation between platelet function assay (platelet aggregation) and a miRNA determined by us, after induction with ADP and / or EPI low concentrations, in patients with SPS, and in healthy controls too.
Methods: In this study patients with SPS, and healthy controls will be included. Diagnosis of SPS is based on light transmission aggregometry (LTA), using low concentrations of ADP and/or EPI. To obtain pure samples of thrombocytes we use magnetic separation system. The cells to be separated, is first magnetically labeled with superparamagnetic microbeads. After magnetic labeling, cells are passed through a column which is placed in the strong permanent magnet of separator. Unlabeled cells pass through while magnetically labeled cell are retained within the column. Obtained pure thrombocytes samples are used for genetic analysis of miRNA, which include reverse transcription and real-time polymerase chain reaction (qPCR). For evaluation of our results a statistic analysis will be used.
Results: Our first experiences in platelet separation with magnetic separation system and preparation of platelet samples for next examinations seems to be successful. We obtained pure platelet samples, which will be used for further genetic analysis.
Summary/Conclusion: Many studies have shown the importance of miRNA not only in hematological diseases. The development of new technologies that would provide the conditions for faster extraction and direct analysis of miRNA should contribute to the diagnosis of disease, also SPS, but also to better understanding of the mechanisms of the genes in the platelets.
This work was supported by grants VEGA 1/0187/17, APVV 16-0020.

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Validation of a rapid von Willebrand factor propepide assay

Muriel Meiring*

Background: von Willebrand disease is the most common inherited bleeding disorder caused by a deficiency or defect in von Willebrand factor. The qualitative defects include type 1 von Willebrand disease (partial deficiency of von Willebrand factor) and type 3 von Willebrand disease (complete deficiency of von Willebrand factor). Type 1 von Willebrand disease is either due to decreased synthesis and secretion or increased clearance of von Willebrand factor. It is essential to diagnose individuals with an increased clearance rate of von Willebrand factor, as the treatment of these patients with 1-de-amino-8-D-arginine vasopressin is not effective. The ratio between the von Willebrand factor propeptide and the von Willebrand factor antigen is used to identify type 1 von Willebrand disease with increased clearance. Currently, there is only one commercial assay available to measure von Willebrand factor propeptide levels. This assay is not only too expensive to be used in developing countries but is also very time consuming.
Aims: With this research, we developed and validated a rapid assay to determine the von Willebrand factor propeptide levels in patients' plasma.
Methods: The commercial antibody pair CLB-Pro 35 and CLB-Pro 14.3 was used in an enzyme-linked immunosorbent assay. While the commercial assay uses two-hour incubations, our rapid assay uses 30-minute incubations, a time reduction of 78%. We compared our assay to the commercial assay using the plasma of 20 type 1 VWD patients. Two samples, the WHO 6th IS for FVIII/VWF in plasma and a known type 1 VWD patient with an increased VWFpp/VWF:Ag ratio were tested four times in duplicate each day for 5 days in order to determine the inter-assay and intra-assay precision.
Results: This rapid assay has an equal sensitivity of detecting 1.5625% VWF propeptide than the commercial assay. The intra- and inter-assay CV's of our assay were less than 10%, that is acceptable according to the Food and Drug Administration guideline of 2013 of less than 15%.
Summary/Conclusion: This rapid ELISA test has equal sensitivity, accuracy, and precision as the commercial ELISA kit method and can be used to diagnose patients with increased VWF clearance.

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Effect of plasma components on Staphylococcus aureus adhesion to tissues used for the right ventricular outflow tract (RVOT) revalvulation

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Background: RVOT reconstruction in congenital heart disease can be surgically done using in first line cryopreserved pulmonary homograft (CPH) and, as an alternative, xenografts such as the bovine jugular vein (BJV) conduit or stent-mounted valve. Despite this good therapeutic alternative recent clinical studies report an increased risk of infective endocarditis (IE) in BJV xenografts. These observations raise the question of why such valves are more prone to IE than homografts.
Aims: We investigate whether different graft tissues promote interactions with plasma components and therefore enhance the risk for *S.aureus* adhesion to valve tissue.
Methods: Similar tissue pieces prepared as for clinical use were incubated for 2h at 37 ^{o}C with 30 µg/ml of fluorescently labelled fibrinogen resuspended in human albumin. Then, *S. aureus* 8325-4 adhesion to the same tissues was assessed under flow conditions (10 min at 1000 s^{-1}) using a micro-parallel flow chamber after O/N tissue incubation at 4 ^{o}C with PBS, frozen human pooled plasma and human albumin or serum. Protein deposition was quantified by fluorescence microscopy and bacterial adhesion was evaluated by CFU counting on blood agar plates.
Results: Pericardium patch presented higher protein deposition ($P < 0.05$) compared to BJV and CPH. Although not significant, there is a slight trend to higher fibrinogen deposition on BJV tissue compared to CPH. After incubation with human plasma *S. aureus* adhesion to BJV increased significantly under flow conditions compared to the respective controls (human serum $P < 0.05$ and albumin $P < 0.01$).
Summary/Conclusion: We established straightforward method of visualization without need for tissue processing. Our results indicate that plasma protein deposition modulate differently *S. aureus* adhesion to the tested tissues, especially BJV. Therefore plasma proteins are a vital player facilitating risk of endovascular infection. Future studies will assess if platelet adhesion differs between those tissues, as well as focus on re-endothelialization of grafted tissues and how this affects bacterial adhesion and development of valvular infection.