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ABSTRACT BOOK

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Hypertensive complications of pregnancy and risk of venous thromboembolism: a population based cohort study in 2 million pregnant women

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Background: Preeclampsia, the most severe hypertensive complication of pregnancy, has been associated with an increased risk of venous thromboembolism (VTE) during pregnancy and the postpartum period. However, generalizability of these results is somewhat questionable, as most results mainly come from small case-control studies, and the long-term risk of VTE after hypertensive complications of pregnancy, i.e., outside pregnancy, has only been studied scarcely.
Aims: To investigate the risk of a first episode of VTE in women with a hypertensive disorder of pregnancy, both during this pregnancy and later in life in a large population based cohort study.
Methods: We conducted a nationwide cohort study on women with at least one pregnancy and studied their long-term first VTE risk by linking data from 1999-2012 from the Dutch perinatal registry (Perined) to that of anticoagulation clinics (>98%) in the Netherlands, where VTE is treated. We used Cox proportional hazard models to estimate hazard ratios (HR) and corresponding 95%CIs for the risk of VTE in women with hypertension during pregnancy, and in women with preeclampsia, and compared this with the risk in women with uncomplicated pregnancies (reference group).
Results: Out of 2 065 173 women with a first pregnancy 1 919 918 (93%) women were eligible for analyses. These women were followed for a median of 13.7 years for a total of 24 531 118 person years in which 5759 first VTEs occurred, at an incidence rate of 2.3 (95%CI 2.3-2.4) per 10 000 person years. On the short term, in the first pregnancy and three month postpartum period, the risk of VTE was higher in women with hypertension during pregnancy: HR 2.0 (95%CI 1.7-2.4) and highest among women with preeclampsia, HR 7.8 (95%CI 5.4-11.3), compared with the reference group. On the long term, during the complete follow-up period, women with hypertension during pregnancy and those with preeclampsia had a higher risk of VTE at some point during follow-up, as compared to women with uncomplicated pregnancies: HR 1.5 (95%CI 1.4-1.6) and HR 2.1 (95%CI 1.8-2.4) respectively. When excluding VTE events during pregnancy and three months postpartum, these HRs were 1.4 (95%CI 1.3-1.5) in women with hypertension during pregnancy and 1.6 (95%CI 1.4-2.0) in women with preeclampsia.
Summary/Conclusion: Hypertensive disorders during pregnancy and preeclampsia are associated with an increased risk of VTE both during this pregnancy and postpartum period, and in the 13 years after.

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In vitro and in vivo modulation of von Willebrand factor gene mutations with dominant-negative effect

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Background: von Willebrand factor (VWF) is a multimeric protein that undergoes dimerization and multimerization processes during its biosynthesis. Dominant-negative mutations are associated with severe von Willebrand disease phenotypes but remain difficult to identify.
Aims: To study and modulate the expression of VWF carrying dominant-negative mutations.
Methods: We created *in vitro* and *in vivo* transient models to express VWF carrying the p.P1127_C1948delinsR (del) and p.C2773R dominant-negative mutations affecting multimerization and dimerization processes, respectively. COS-1 cells were transiently co-transfected with plasmids for wild-type (wt) and mutant VWF expression. Similarly, *Vwf*-deficient mice were subjected to hydrodynamic gene transfer of both plasmids. VWF multimers were evaluated in conditioned media and mouse plasma.
Results: As expected, co-expression of pC2773R- with wt-VWF was associated with absence of high molecular weight multimers but normal VWF antigen levels in conditioned media.
Co-expression of del- & wt-VWF also resulted in severe reduction of VWF multimers and high-resolution gel enabled the separation of heteropolymers formed by wt- and del-VWF subunits. We next created plasmids for the expression of VWF carrying both gene defects (p.del/C2773R) in cells and mice. Noticeably the double-mutant VWF was unable to interact with wt monomers leading to abolition of the dominant-negative effect of the single defects and rescue of the multimer profile. The detrimental effect of the large deletion was also challenged *in vivo* by the administration of siRNA selectively directed against del-VWF. By interfering with the dominant-negative mechanism, the silencing treatment improved VWF antigen levels and multimer profile.
Summary/Conclusion: We established the first *in vivo* heterozygous mouse model of VWD associated with dominant-negative mutations. The association of other VWF mutations with del-VWF can be employed to investigate/antagonize their dominant-negative effect. An in vivo silencing approach was successfully applied to our von Willebrand disease mouse models.

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The use of in vitro clot lysis assay for predicting outcomes and safety in acute ischemic stroke patients undergoing intravenous thrombolysis

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Background: The outcome of intravenous thrombolysis using recombinant tissue plasminogen activator (rtPA) is favorable in only 33-35% of acute ischemic stroke (AIS) patients. In a subset of patients thrombolytic therapy is inefficient due to the failure of recanalization of the closed vessel. On the other hand, in approximately 6-8% of patients intracerebral hemorrhage develops as a potentially fatal side-effect. These complications cannot be foreseen at the initiation of therapy and their occurrence remains unexplained.
Aims: We tested whether an in vitro clot lysis assay performed before thrombolysis might predict therapy outcomes and safety.
Methods: In this prospective observational study, blood samples of 229 consecutive AIS patients, all undergoing i.v. thrombolysis by rtPA within 4.5 h of their symptom onset, were taken before thrombolysis. Platelet-poor-plasma was clotted using recombinant tissue factor, lysis was induced with rtPA. Clot formation and lysis was monitored by turbidimetry. In order to test the impact of neutrophil extracellular traps on lysis, assays were also performed in the presence of cell-free-DNA and histones. Curve analysis was performed by the ShinyApp software. Stroke severity was determined by NIHSS on admission. Therapy-associated intracerebral hemorrhage was classified according to ECASSII criteria. Short- and long-term outcomes were defined at 7 days and 3 months post-event according to the change in NIHSS and by the modified Rankin Scale, respectively. All patients or their relatives provided written informed consent.
Results: The median time to reach 50% lysis (50%CLT) was 41.3 (IQR:30.4-57.6) min in the total cohort, and became significantly prolonged in the presence of cell-free-DNA and histones (45.0 [IQR:32.0-64.0]min, $p<0.0001$). A dose-response relationship was observed between stroke severity and 50%CLT. Patients with favorable short-term outcome had significantly shorter 50%CLT in the presence of DNA and histones as compared to patients with poor outcomes (median: 43.5 [IQR:30.5-62.1] min vs. 48.8 [IQR:36.0-69.0] min; $p<0.05$). Clot lysis in the presence of cell-free-DNA and histones was significantly faster in patients who suffered post-lysis intracerebral hemorrhage as compared to those without such complications (median 50%CLT: 34.0 [IQR: 25.9-51.9] min vs. 46.7 [IQR: 32.6-65.5] min, respectively, $p<0.05$). Long-term outcomes showed no association with clot-lysis assay results.
Summary/Conclusion: Clot-lysis assay using pre-thrombolysis plasma of AIS patients might be useful to predict short-term outcomes and post-lysis intracranial hemorrhagic complications, particularly when the assay is supplemented with DNA and histones. Clot lysis assay however showed no association with long-term outcomes.
Funding: GINOP-2.3.2-15-2016-00043, NKFI-K120042

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Identification of a low shear stress-specific anti-thrombotic pathway in the microvasculature orchestrated by the endothelial transcription factor ERG

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Background: A crucial function of the healthy endothelium is to maintain an anti-thrombotic state *via* the regulation of pro-/anti-coagulant genes. Thrombomodulin (TM) is a key anti-thrombotic gene expressed in endothelial cells (EC) and is upregulated by shear stress (SS) *via* the flow-responsive transcription factor (TF) Krüppel-like factor2 (KLF2). Clinical evidence supports the concept that vascular bed-specific pathways balance local hemostasis, suggesting possible advantages in vascular bed-specific therapeutic approaches. However, the molecular basis for such specificity is poorly understood. The ETS-related gene ERG, a master TF expressed by all EC, has been shown to play a key role in multiple homeostatic processes; whether ERG also regulates hemostasis is unknown.
Aims: To investigate the potential role of ERG in protecting from thrombosis *via* the regulation of TM expression in EC.
Methods: We induced the chronic deletion of ERG in adult mice (6 weeks) specifically in EC (*Erg*$^{iEC\text{-}KO}$) using the i*Cdh5(PAC)-CreERT2* driver line. Analysis of the thombotic phenotype in mice was performed 30 days post-tamoxifen injection by histology, immunostaining (fibrinogen/CD41) and measurement of circulating biomarkers (platelet counts, fibrinogen, Thrombin-antithrombin (TAT) and D-dimer ELISA). To rescue TM *in vivo,* we performed a treatment (6 hours) with red blood cells-targeted thrombomodulin fusion protein (RBC-TM). To dissect ERG regulation of TM and its cooperation with KLF2 in driving TM, we used multiple *in vitro* approaches (Proximity ligation assay, ChIP-qPCR, transactivation assay). To investigate ERG regulation of TM under flow, we conducted experiments on HUVEC exposed to static, low (5 dynes/cm^2) and high SS (20 dynes/cm^2) conditions. ERG regulation of TM *in vivo* in different vascular beds was assessed by immunofluorescence (IF) microscopy and qPCR.
Results: Deletion of ERG in adult mice was associated with spontaneous thrombus formation in the liver, haemorrhages in liver/lung and signs of coagulopathy (increased TAT/D-dimer, decreased fibrinogen levels). qPCR analysis revealed that TM was the most consistently regulated ERG target gene in both *in vitro* and *in vivo* models. An acute treatment with RBC-TM in *Erg*$^{iEC\text{-}KO}$ mice was able to restore the TAT levels, an early biomarker of coagulopathy/thrombosis, showing that the prothrombotic phenotype in ERG-deficient mice is, at least, partly due to the loss of TM expression. *In vitro*, we show that ERG binds to and transactivates the TM promoter to drive TM expression in HUVEC. ERG also forms a nuclear complex with the TF KLF2, promotes KLF2 binding to the TM promoter and is required for KLF2's ability to transactivate TM, showing that ERG and KLF2 cooperate in regulating TM expression *in vitro*. qPCR analysis of TM in HUVEC exposed to different SS revealed that ERG regulates TM expression specifically in low SS conditions. In line with these data, IF for TM in tissues from *Erg*$^{iEC\text{-}KO}$ and control mice confirmed that ERG is required for the regulation of TM in microvasculature exposed to low SS (liver/lung) but is dispensable for TM regulation in blood vessels exposed to high SS (aorta/kidney capillaries).
Summary/Conclusion: This study identified a low SS-specific anti-thrombotic pathway in EC controlled by the TF ERG specifically in the microvasculature. This work introduces the concept that EC are able to use distinct transcriptional pathways in different vascular beds to prevent thrombus formation.

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Platelet-derived chemokines CCL5 and CXCL4 are rapidly internalized in endothelial cells

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Background: Interaction between platelets and the endothelium facilitates leukocyte arrest and subsequent transendothelial migration to sites of vascular inflammation. In turn, transmigrated leukocytes can stimulate the endothelium to produce substances that induce platelet activation. Activated platelets are known to release biomolecules from their granules, and the chemokines CCL5 and CXCL4, abundantly present in alpha-granules, can be deposited onto the endothelial cells where they play an important role in monocyte arrest to endothelial cells, which is an essential early step in the development of atherosclerosis.
Aims: In this study, we aimed to elucidate the mechanisms behind the leukocyte arresting properties of CCL5 and CXCL4 and the role of platelet-endothelial interplay in vascular inflammation. We focused on the localization of CCL5 and CXCL4 on the cell surface and their internalization to the endothelial cell interior.
Methods: HUVECs and the endothelial cell line EA.hy926 were incubated with recombinant human CCL5 or CXCL4 for up to 120 minutes. Cells were stained and analyzed with light-, confocal- or stimulated emission depletion (STED) microscopy. To quantify internalization, whole cell lysates and organelle-fractionated cells were analyzed using ELISA. Monocyte arrest was evaluated using laminar flow leukocyte adhesion assays.
Results: Both CCL5 and CXCL4 were rapidly internalized in endothelial cells (<10 min). Whereas CXCL4 remained partly presented on the cell surface, all of the CCL5 was internalized. The chemokines were endocytosed by a process dependent on dynamin and clathrin, as internalization was blocked by inhibitors of these molecules. Cell surface proteoglycans, chemokine binding polysaccharides, had a less definite role in the internalization process, as enzymatic cleavage of heparin- and chondroitin sulfate did not result in a decreased internalization of CCL5 and CXCL4. Combined incubation of CCL5 and CXCL4 with endothelial cells did not influence the internalization or the localization of either of the chemokines. Localization studies by confocal and super-resolution microscopy suggested that both CCL5 and CXCL4 partly have a nuclear localization which, in some cells, seem to be directed to the nucleoli. These visual observations were supported by cell fractionation experiments where chemokine accumulation was quantified in various cell compartments, which revealed a relatively high nuclear accumulation. Internalization of chemokines appears less in cells with an inflammatory phenotype, and monocyte-arrest is higher to endothelial cells that are incubated with CCL5 and CXCL4.
Summary/Conclusion: In summary, endothelial cells rapidly and actively internalize CCL5 and CXCL4 by clathrin and dynamin-dependent endocytosis, where the chemokines appear to be directed to the nucleus. These findings introduce a potential novel, non-canonical role of alpha-granule released chemokines in the cross-talk of activated platelets and endothelial cells, which could have implications for the mechanisms in which leukocytes are attracted to sites of inflammation.

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Combined effects of five prothrombotic genotypes and cancer on the risk of venous thromboembolism

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Background: Venous thromboembolism (VTE) is a frequent and severe complication in cancer. Several single nucleotide polymorphisms (SNPs) are associated with VTE-risk, and the risk of VTE has been shown to increase linearly with the combined number of risk alleles of five specific genotypes (5-SNP score). However, the role of these genotypes in cancer is scarcely studied.
Aims: To investigate the impact of a 5-SNP score on the risk of VTE in patients with and without cancer in a large population-based case-cohort study.
Methods: Cases with incident VTE (n=1493) and a randomly sampled sub-cohort (n=13072) were derived from the forth survey of the Tromsø study (1994-2012) and the second survey of the Nord-Trøndelag health (HUNT) study (1995-2008). DNA isolated from blood was genotyped for the following five SNPs: ABO (rs8176719), F5 (rs6025), F2 (rs1799963), FGG (rs2066865) and F11 (rs2036914). Participants with a previous history of cancer (n=573), missing information on risk alleles (n=170) or body mass index (n=80) were excluded. VTEs were considered cancer-related if they occurred 6 months prior to or within 2 years following a cancer diagnosis. Cox regression models were used to calculate hazard ratios (HRs) with 95% confidence intervals (CI) for VTE according to the combined number of risk alleles in the 5-SNP score (0-1, 2-3 and ≥4 alleles). The presence of biological interaction between cancer and the high-risk category of the 5-SNP score (i.e. ≥4 alleles) on VTE risk was assessed by calculating the relative excess risk due to interaction (RERI) and the attributable proportion (AP) with corresponding 95% CIs.
Results: During a median follow-up of 12.3 years, 1496 were diagnosed with cancer, of whom 232 experienced a cancer-related VTE. The VTE risk increased linearly (p<0.001) with the number of risk alleles in the 5-SNP score both among subjects without and with cancer. In cancer-free subjects, the HR was 2.17 (1.79-2.62) when those with ≥4 risk alleles were compared to those with 0-1 risk alleles. In cancer patients, the corresponding HR was 1.93 (95% CI 1.28-2.91) for ≥4 risk alleles versus 0-1 risk alleles. Cancer patients with 0-1 risk alleles had an 8-fold (HR 8.34, 95% CI 5.9-11.8) higher risk of VTE than cancer-free subjects with 0-1 risk alleles. The combination of cancer and ≥4 risk alleles displayed biological interaction (RERI: 6.7, 95% CI: 1.2-12.3), and yielded a 17-fold (HR 17.1, 95% CI 12.5-23.3) higher risk of VTE compared to cancer-free subjects with 0-1 risk alleles. The attributable proportion was 39% (95% CI 16-63%), indicating that 39% of the VTEs in the combined category (i.e. ≥4 risk alleles+cancer) were excess cases that could be attributed to the interaction.
Summary/Conclusion: The risk of VTE increased linearly with the number of prothrombotic risk alleles in subjects with and without cancer. A high number of prothrombotic risk alleles displayed a biological interaction with cancer on the risk of VTE. Our findings suggest that the 5-SNP score can be useful for identifying cancer patients at increased risk of VTE.

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ADAMTS13 conformation in the French cohort of child-onset acquired thrombotic thrombocytopenic purpura

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening thrombotic microangiopathy (TMA) related to a severe ADAMTS13 deficiency (activity <10%). This deficiency may be either congenital (cTTP, ~5% of all TTP cases) or acquired (aTTP, ~95% of all TTP cases). Child-onset TTP is also rare and represents less than 10% of all TTP cases (~1/3 of cTTP and ~2/3 of aTTP).
Recently, studies dedicated to ADAMTS13 have reported changes in its conformation through a disruption of the interaction between its spacer and CUB domains. In adult-onset idiopathic aTTP, ADAMTS13 conformation is specifically open at the acute phase of the disease and cryptic epitopes localized in the spacer domain of ADAMTS13 are exposed. In cTTP, *ADAMTS13* gene sequence variations may influence its conformation.
Aims: The aim of the current study was to determine ADAMTS13 conformation in child-onset aTTP during the acute phase of TTP and in remission.
Methods: Since 2000 all French patients with a clinical suspicion of TMA were included in the French registry for TMAs. From January 1st, 2000 till December 31st, 2018, we performed a cross-sectional analysis of the French TMA registry to identify child-onset aTTP patients (age <18 at the inaugural TTP episode), retrospectively. Clinical and biological data were collected after extensive analysis of medical records.
ADAMTS13 activity was measured by FRETS-VWF73 and anti-ADAMTS13 IgGs were titrated by ELISA (Technozym® ADAMTS13 INH ELISA, Technoclone, Austria) for diagnosis purposes. Child-onset aTTP, with plasma samples still available after diagnosis, were further investigated for ADAMTS13 antigen (home-made 3H9-ELISA) and conformation (home-made 1C4-ELISA).
Results: Forty-four child-onset aTTP were enrolled in this study. All patients exhibited an ADAMTS13 activity <10% in acute phase, by definition. Thirty-six patients were also tested in remission and exhibited ADAMTS13 activity ranging from 25 to 100%, except one patient whose ADAMTS13 remained <10%. Anti-ADAMTS13 IgGs were positive at diagnosis in 84% (n=37/44) of patients. We reported 59% (n=26/44) of idiopathic aTTP (almost systematically associated with the presence of anti-ADAMTS13 IgGs (26/26)) and 41% (n=18/44) of aTTP associated with other clinical contexts (auto-immune disease, infections …).
At the acute phase of aTTP, ADAMTS13 antigen was detectable (> 0.03 µg/mL) in 34% (n=15/44) of patients and ADAMTS13 conformation was open in 73% (n=11/15) of them. Anti-ADAMTS13 IgGs were positive in 87% (n=13/15) of these aTTP patients including 77% (n=10/13) with an open ADAMTS13 conformation.
In remission, ADAMTS13 antigen was detectable in all patients (36/36), anti-ADAMTS13 IgGs were positive in 39% (n=14/36) of them and ADAMTS13 conformation remained open in 42% (n=15/36) of patients. Interestingly, ADAMTS13 adopted an open conformation in the patient with an ADAMTS13 activity <10%, in 86% (n=6/7) of patients with an ADAMTS13 activity between 25 and 50%, in only 28% (n=8/28) of patients with an ADAMTS13 activity over 50%. Anti-ADAMTS13 IgGs were positive in 67% (n=10/15) of patients with an open ADAMTS13 conformation in remission.
Summary/Conclusion: At the acute phase of child-onset aTTP, ADAMTS13 conformation is open. During remission, ADAMTS13 remains open mostly when ADAMTS13 activity is still less than 50%. This indicates that subclinical aTTP is still present and a close monitoring is recommended to see if additional treatment is needed.

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The importance of the GPIba intracellular tail in VWF- and GPVI-mediated platelet signalling events

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Background: The platelet GPIba-VWF A1 domain interaction is essential for platelet tethering under high shear conditions. Under flow, VWF binding to GPIb-IX-V complex induces intracellular calcium fluxes and activation of tyrosine kinases that culminate in the activation of $a_{IIb}\beta_3$. Despite this, the GPIba-A1 signalling has been considered redundant for haemostasis. We recently demonstrated that human platelets are 'primed' (but not activated) following binding to VWF under flow and are able to recruit neutrophils via activated $a_{IIb}\beta_3$.
Aims: To evaluate the (patho)physiological relevance of VWF/flow-dependent signalling through GPIba.
Methods: *GPIba*$^{\Delta sig/\Delta sig}$ mice, in which the last 24 amino acids of the intracellular tail were deleted, were generated via CRISPR-Cas9 technology. Platelet function was assessed *ex vivo* by flow cytometry, platelet aggregometry, immunostaining, microfluidic assays, and *in vivo* by tail bleeding and thrombosis models.
Results: *GPIba*$^{\Delta sig/\Delta sig}$ mice exhibited mildly reduced platelet count (~80%) and slightly enlarged (~140%) platelets compared to *GPIba*$^{+/+}$ littermates. *GPIba*$^{\Delta sig/\Delta sig}$ mice exhibited normal haemostasis and normal platelet and fibrin accumulation in the laser-induced thrombosis model. Activation of *GPIba*$^{\Delta sig/\Delta sig}$ platelets with ADP and thrombin was unaffected; *GPIba*$^{\Delta sig/\Delta sig}$ platelets stimulated with collagen-related-peptide (CRP) exhibited markedly decreased P-selectin exposure and $a_{IIb}\beta_3$ activation. Consistent with this, reduced platelet spreading was observed on CRP, but not on fibrinogen. Upon CRP stimulation, *GPIba*$^{\Delta sig/\Delta sig}$ platelets displayed a significant decrease in tyrosine-phosphorylated proteins, including pSYK. Under high shear conditions, *GPIba*$^{\Delta sig/\Delta sig}$ platelets formed smaller aggregates on collagen-coated microchannels and exhibited increased rolling velocities on VWF A1-coated surfaces. Finally, *GPIba*$^{\Delta sig/\Delta sig}$ platelets primed under flow on VWF-A1 coated microchannels had a reduced ability to recruit neutrophils under low shear compared to *GPIba*$^{+/+}$ platelets.
Summary/Conclusion: We have generated a novel transgenic mouse with truncated GPIba cytoplasmic tail. Although the primary filamin binding site has been preserved, *GPIba*$^{\Delta sig/\Delta sig}$ mice have slightly lower platelet counts and modestly enlarged platelets. The defect observed in *GPIba*$^{\Delta sig/\Delta sig}$ platelets after CRP stimulation represents the first *in vivo* study corroborating *in vitro* data suggesting that the intracellular tail of GPIba (and its associated signalling machinery) is important for GPVI-dependent signalling. Our data from *ex vivo* flow assays showing that VWF-mediated capture of *GPIba*$^{\Delta sig/\Delta sig}$ platelets lead to inefficient recruitment of neutrophils under flow also suggest an important role for the GPIba intracellular tail to transduce signals downstream of the VWF A1-GPIba interaction. Additional thrombosis models and platelet signalling assays are underway to fully appreciate the physiological and pathophysiological role of the GPIba-A1 signalling.

FS 1.2

Platelets promote metastatic dissemination in mouse and human models in vivo and are associated with reduced survival in lung adenocarcinoma patients

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Background: Lung cancer is the leading cause of cancer mortality globally, and approximately 50% of patients present with metastatic disease. in non-small-cell lung cancer (NSCLC) elevated platelet count and indicators of platelet activation are associated with poor survival, however, the role of platelets in NSCLC, and its major subtypes, lung adenocarcinoma (ADC) and squamous-cell carcinoma (SqCC), is not fully understood.
Aims: We explored platelet roles *in vivo* and *in vitro* using mouse and human models of ADC and SqCC. Using NSCLC patient data, we assessed the association between thrombocytosis and prognosis in NSCLC subtypes.
Methods: ADC cell lines derived from tumours arising in mice carrying mutations in K-Ras and TP53 were intravenously transplanted to model metastasis *in vivo*. The role of platelets was evaluated using genetically engineered thrombocytopenic mice (<25% of wild type (WT)), or pharmacological inhibition of platelet function. A novel thrombocytopenic NOD-*scid*-IL2rγ^{null} (NSG) mouse was created by introducing a null mutation in c-Mpl on an NSG background (NSG *Mpl*$^{-/-}$) for use in human cell transplantations. Additionally, data comprising 686 NSCLC patients from the Peter MacCallum Cancer Centre Thoracic Malignancies Cohort Study was retrospectively analysed to identify the significance of thrombocytosis (defined as ≥400x10^9 platelets/L).
Results: *In vivo*, thrombocytopenic mice exhibited a survival advantage following intravenous (IV) transplant of murine ADC cells, compared to WT. Similarly, NSG *Mpl*$^{-/-}$ mice had reduced metastasis following IV transplant of human ADC cell lines (A549 and NCI-H358), compared to control. Conversely,transplant of a human SqCC cell line (NCI-H520) into NSG and NSG *Mpl*$^{-/-}$, resulted in no difference in metastasis compared to control.
In vitro, ADC cell invasiveness increased following co-incubation with platelets or extracts from activated platelets, and prior exposure to platelets increased metastatic potential of ADC cells following *iv* transplant in WT mice. In ADC tumour-bearing mice, we measured a two-fold increase in soluble P-selectin compared to healthy controls, demonstrating the presence of platelet activation. Inhibiting activation with clopidogrel resulted in reduced metastatic burden and increased survival.
Analysis of NSCLC patients implicated thrombocytosis as predictive of poor survival in patients with metastatic (Stage IV) disease. Interestingly, individual analysis of two major NSCLC subtypes, ADC and SqCC, revealed thrombocytosis was associated with reduced survival only in metastatic ADC.
Summary/Conclusion: Here, we highlight a divergence between NSCLC subtypes in their relationship with platelets. We also demonstrate a link between platelets and ADC metastasis, providing an opportunity for exploration of anti-platelet agents combined with conventional lung cancer therapies to increase treatment efficacy.

FS 1.3

The impact of second cancer on the risk of cancer associated venous thromboembolism; a Danish nationwide study

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Background: Venous thromboembolism (VTE) is a frequent and severe complication to cancer. An increasing proportion of the population live with indolent/chronic cancer, some of these patients become exposed to more than one cancer.
Aims: To investigate the risk of VTE according to exposure to second cancer.
Methods: We studied all Danish patients diagnosed with a first breast, prostate, lung, or colorectal cancer from 1995 through 2017 by linkage of data from the Danish National Cancer Registry to the Danish National Patient Registry. Exposure was prospective diagnosis of a second cancer. Second cancer was defined as a cancer (ICD-10: CC00-96, except non-melanoma skin cancer, CC43) not previously fully registered in the Danish National Cancer Registry with an IDC-10 group code different from the first registered cancer. The second cancers were classified as associated with very high VTE risk, high VTE risk or low VTE risk. The patients changed status from unexposed to exposed at the diagnosis date of the second cancer. Study entry was the diagnosis date of a first breast, prostate, lung or colorectal cancer, the patients were followed until VTE, emigration, death or last date of follow-up for VTE (December 31, 2017), whichever came first. Cumulative incidences of VTE were calculated according to time-varying exposure to second cancer with delayed entry (i.e. estimates are given for time since first cancer diagnosis as patients change exposure prospectively), death was treated as competing risk. Hazard ratios for VTE were estimated in multivariate Cox proportional regression models stratified by time since first cancer diagnosis.
Results: In total 309 079 patients were diagnosed with one of the four studied first cancer types from 1995 through 2017, 28.0% (86 632) had breast cancer, 20.8% (64 381) prostate cancer, 25.2% (77 791) lung and 26.0% (80 275) had colorectal cancer. Mean age at study entry was 68.2 years (SD: 12.0 years), 52% (160 669) were women. A second cancer was registered for 7.8% (6 744) of those with breast cancer, 7.9% (5 069) with prostate cancer, 2.5% (1 930) with lung cancer and 7.9% (6 347) with colorectal cancer. Seven percent (1431) of the second caners were classified as very high VTE risk cancers, 77.6% (15 592) as high VTE risk cancers and 15.3% (3067) as low risk caners. In total 14 962 VTEs were observed in the study period, 6.6% (994) were diagnosed after exposure to a second cancer.
The one, two and four - year cumulative incidences of VTE in unexposed cancer patients were 2.08 % (95% CI, 2.03-2.14), 2.84% (95% CI, 2.74-2.90), and 4.00% (96% CI, 3.99-4.15), respectively. In patients exposed to a second cancer, the corresponding cumulative incidences of VTE were 4.05 (95% CI, 3.34-4.92) one year after the first cancer diagnosis, 5.83 (95% CI, 5.02-6.77) two years after the first cancer diagnosis and 9.25 (95% CI, 8.31-10.28) four years after the first cancer diagnosis. The HRs of VTE in patients exposed to very high VTE risk second cancers, high VTE risk second primary cancers and low VTE risk second primary cancers were 2.6 (95% CI, 1.6-4.3), 1.7 (95% CI, 1.2-2.4) and 1.1 (95% CI, 0.7-1.7) compared with unexposed patients, respectively.
Summary/Conclusion: Exposure to a second cancer was associated with a markedly higher risk of VTE in patients with a first breast, prostate, lung or colorectal cancer.

FS 4.2

Characterisation of citrullinated TFPI and truncated TFPI constructs by PAD4 in model and plasma systems

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Background: Tissue factor pathway inhibitor (TFPI) is an important regulator in hemostasis. Decreased concentrations of TFPI have been reported as a risk factor for thrombosis and complete TFPI deficiency is lethal. TFPI is also reported to be an important link between inflammation and thrombosis. Neutrophil extracellular traps (NETs) formed by NETosis can bind TFPI which can then be cleaved and inactivated by neutrophil elastase (NE) causing local thrombus formation. In neutrophils, the enzyme peptide arginine deiminase 4 (PAD4) is central to citrullination of histones prior to the externalization of DNA during NETosis. In this study, we provide evidence that PAD4 regulates the activity of TFPI by posttranslational modification of its functional arginines into citrulline.
Aims: To study the effect of citrullination of TFPI and various TFPI constructs on their functional activity on FXa or thrombin generation.
Methods: Citrullination of TFPI by neutrophil protein PAD4 was studied in a model system (FXa inhibition) and in plasma system (thrombin generation). Various TFPI constructs, Kunitz (K) domains K1K2, K2, and TFPI1-161 were used to study the effects of citrullination on inhibition of FXa. LC-MS was used to locate the specific sites of citrullination.
Results: This study shows that PAD4 very efficiently citrullinates full lenght TFPI. Very low concentrations of PAD4 were sufficient (K_i 0.4 nM) to abolish FXa inhibition by TFPI. Citrullination is calcium-ion, time- and concentration-dependent. The truncated variants K1K2 and TFPI 1-161 and the isolated K2 domain were citrullinated less efficiently by PAD4 than TFPI, implying the presence of specific binding sites for PAD4 at the C-terminus of TFPI. The presence of phospholipids inhibited the citrullination reaction, an effect only seen for TFPI and not for all the other TFPI variants. Thus, the presence of the C-terminus in TFPI appears to be favorable for citrullination by PAD4.
Thrombin generation in TFPI-deficient plasma triggered with TF or Russell's viper venom (RVV)-X showed almost complete absence of anticoagulant activity of citrullinated TFPI.
Summary/Conclusion: TFPI is very sensitive to citrullination by PAD4. Citrullinated TFPI has lost its ability to inhibit FXa. This process might play a role in the increased thrombosis risk associated with inflammation. Further experiments are needed to determine the physiologic or pathologic relevance of this process

FS 4.3

Molecular, biological and clinical characterization of variants affecting the c-terminal end of antithrombin

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Background: The analysis of natural mutants of serpins, and antithrombin in particular, has helped to identify functional and structural domains, although all these studies have been done with single nucleotide variants. The C-terminal end (C-term) of antithrombin has no evident functional role, but it has been suggested to be a relevant structural domain that may be crucial for the folding and secretion of this anticoagulant.
Aims: We aim to characterize molecular, biological and clinical consequences of different variants affecting the C-term of antithrombin.
Methods: From 340 unrelated cases with antithrombin deficiency recruited during 22 years (1998-2019) we selected cases with genetic variants in C–term (Val432-Lys464), all in exon 7 not affecting the reactive centre loop. Plasma antithrombin was characterized by functional and antigenic methods, including western blot using different electrophoretic conditions. A recombinant model of eukaryotic expression was used for selected natural and artificial variants with different C-term.
Results: We identified 9 genetic variants affecting the C-term of antithrombin, 5 missense and 4 small deletions in 14 unrelated cases (14/340: 4.1%). Two mutations were recurrent (p.Arg445Serfs*17; p.Pro439Thr). Six caused type I deficiency with severe clinical phenotype (early and recurrent thrombosis) while 3, all missense, caused type II pleiotropic deficiency with increase of the latent form.
As two natural frameshift mutations, both creating frameshift+1 (p.Arg445Serfs*17, p.Ile444Metfs*18), might generate variant antithrombins with similar size than the wild type, but with different aminoacid sequence, and there are other natural variants with the same frameshift but different extension of the aberrant C-term, we evaluated the consequences of 10 different frameshift+1 mutations on protein size, secretion, conformation and anticoagulant activity in the recombinant model. Frameshift+1 mutations affecting Val432-Phe440, despite of having a long aberrant C-term, were efficiently secreted although they had no anticoagulant activity as they have latent conformation. Intriguingly, frameshift+1 mutations after Phe440, despite having shorter aberrant C-term, had a severe impaired secretion, had no anticoagulant activity and formed disulphide linked complexes. A common aberrant Proline shared by the 5 variants efficiently secreted was chosen as a candidate to explain this difference. The p.Leu441Pro mutation in the p.Arg445Lysfs*19 variant rescued the secretion of the variant antithrombin.
Summary/Conclusion: Mutations in the C-term of antithrombin, which are relatively frequent, generate a considerable biological and clinical heterogeneity strongly related with the localization of the mutation, and support a key structural relevance of the C-term of antithrombin, and potentially all serpins. Our study shows that a completely aberrant C-term caused by frameshift+1 mutations may be secreted although without anticoagulant activity. This may be explained because the stop codon appeared at similar position than that of the wild type, and a Cys involved in intramolecular disulphide linkage (Cys462) was conserved. However the resulting protein is folded to polymers if the frameshift takes place after Phe440 and to latent if located before. The generation of a Pro441 in mutants located between Val432-Phe440 probably allows a torsion that impairs the polymerization of the variant antithrombin, improving its secretion.
FUNDING: PI18/00598(ISCIII);19873/GERM/15(F. Séneca).

FS 3.2

In Silico Peptide Design: From Molecular Mechanism to Novel Therapeutic Treatments for Inflammatory Diseases

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Background: Protein-protein interactions (PPIs) are involved in the pathogenesis of various diseases. For example, interactions between the platelet-borne chemokine CCL5 and the neutrophil-derived protein HNP1 can lead to monocyte adhesion, an essential mechanism that causes acute and chronic inflammatory diseases (Alard J. E. et al., *Sci. Transl. Med*., 2015). The direct interactions between extracellular histone H4 and membranes can trigger arterial tissue damage which can consequently promote atherosclerosis (Silvestre-Roig C. et al., *Nature*, 2019). Thus, interrupt the interactions between these proteins represents a promising strategy to develop novel therapeutic treatments for inflammatory diseases.
Aims: In this work, we present a generic approach utilized to develop novel peptidic inhibitors to disrupt PPIs (CCL5-HNP1 and Histone H4-membrane interactions), starting from applications of *in silico* methods to investigate PPIs and peptide design to experimental tests of potential hit compounds at different levels (from *in vitro* to animal models).
Methods: Similar strategies were applied to develop inhibitors to disrupt CCL5-HNP1 and Histone H4-membrane interactions. We first have applied different *in silico* methods (e.g. molecular docking and molecular dynamics simulation) to investigate the interactions and identify a likely binding mode between CCL5 and HNP1 (Wichapong K. et al., *J. Med. Chem.*, 2016) as well as for Histone H4-membrane interactions. The 3D structures of protein-protein complexes represent a good starting point to serve for inhibitor design (Wichapong K. et al., *Future Med. Chem.*, 2019). Thus, the derived results (key interacting residues and the targeted protein-protein complexes) were utilized for rational peptide design. The designed peptides were then prioritized by their binding free energy with the targeted proteins (CCL5 and histone H4) and were selected for further synthesis and following by various experimental tests.
Results: To investigate the potential of peptide candidates to interrupt CCL5-HNP1 interactions, the deigned peptides were first tested in the flow chamber assay to test their potential to disrupt the monocyte adhesion, mediated by CCL5-HNP1 complex formation. The most potent peptide, called "**SKY peptide"** (RRYGT**SKY**Q), was selected for further *in vivo* experiments and it shows high potential to reduce monocyte adhesion in a mouse model of myocardial infarction. Similarity, to identify the potent inhibitor to prevent histone H4-mediated cell lysis, the candidate peptides were first tested *in vitro* cell viability assay. The most potent inhibitor, **HIPe** (**H**istone **I**nhibitory **Pe**ptide), was chosen to investigate its ability to prevent histone H4-mediated cell lysis. Results revealed that the HIPe can disrupt the interactions between histone H4 and membranes and shows therapeutic benefits in mouse model of atherosclerosis.
Summary/Conclusion: We have applied *in silico* methods to successfully develop novel peptidic inhibitors targeting different classes of proteins, a globular protein (CCL5-HNP1 complex) and disordered protein (histone H4). In both cases, around 10 peptides extracted from more than billion candidates were synthesized and experimental tested. Thus, the approach presented here is a fast approach which can accelerate the drug discovery process. More importantly, by application of computational methods as shown here we can significantly reduce time and cost required in drug development process.

FS 3.3

Targeting CXCL1-deposition on the vessel wall with a tick-derived protein

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Background: Chemokine CXCL1 plays an important role during early atherogenesis as it is responsible for monocyte arrest on activated endothelium [1,2]. Evasin-3, a chemokine-binding protein produced by ticks, selectively binds to CXCL1 [3] and could therefore be used for targeting atherogenesis for molecular imaging or even therapeutic treatment of this disease.
Aims: The aim of this study is to investigate the CXCL1-targeting potential of Evasin-3 using chemotaxis assays and to study its mode of action through NMR spectroscopy.
Methods: Evasin-3 and CXCL1 and derivatives thereof were produced via either solid-phase peptide synthesis (SPPS) and native chemical ligation (NCL) or recombinant expression in *E.coli* for NMR studies. Subsequently, the binding potential of fluorescently labeled CXCL1 and Evasin-3 was determined on human endothelial cells (HMVECs) grown under shear stress. Incubation HMVECs under static conditions or in the presence of an excess of non-labeled CXCL1 served as control experiments. The affinity of Evasin-3 for CXCL1 was determined with Surface Plasmon Resonance (SPR). Structural determinants of CXCL1/Evasin-3 complex formation was studied using solution NMR spectroscopy.
Results: Evasin-3, CXCL1, and derivatives of both were successfully synthesized using Boc-based solid-phase peptide synthesis (SPPS) and native chemical ligation (NCL). Evasin-3 showed targeting towards shear stress-cultured ECs, whereas almost no EC-targeting was observed under static culture conditions. From migration experiments it appeared that Evasin-3 could efficiently block neutrophil migration towards chemoattractant CXCL1. Additionally, Evasin-3 significantly decreased neutrophil adhesion towards LPA-activated ECs compared to non-activated ECs. NMR data demonstrated that Evasin-3 disrupted the receptor-binding potential of CXCL1, whereas glycosaminoglycan (GAG)-binding was only partially affected. Ex vivo two-photon laser scanning microscopy (TPLSM) experiments showed that fluorophore-labeled Evasin-3 only binds to LPA-activated mouse carotids.
Summary/Conclusion: Chemically synthesized Evasin-3 binds selectively to chemokine CXCL1 and may decelerate atherosclerotic lesion progression.
Supported by The Netherlands Organisation for Scientific Research (VIDI-723.013.009 and Aspasia-015.010.005 to I.D.)

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FS 2.2

A fully-human bispecific antibody for the treatment of hemophilia A

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Background: Loss of blood coagulation Factor VIII (F.VIII) results in the bleeding disorder, hemophilia A. The current standard of care for hemophilia A patients routinely involves replacement of F.VIII either as prophylaxis or on-demand. However, approximately 30 % of hemophilia A patients taking F.VIII develop inhibitory antibodies against the replacement F.VIII, blocking its action. Additionally, venous infusion of F.VIII is burdensome especially in young patients. Recently, a novel treatment for hemophilia A involving the humanized bispecific antibody (BiAb) Hemlibra, which mimics the action of F.VIII, was developed. Successful approval of Hemlibra provides new therapeutic options for patients.
Aims: Integrating Kymab's IntelliSelect® Transgenic and IntelliSelect® Bispecific platforms, we sought to develop a fully-human F.VIII common light chain (CLC) bispecific antibody that can mimic the action of F.VIII ex vivo.
Methods: Kymab IntelliSelect® Transgenic mice were immunized with Factor IX (F.IX) or Factor X (F.X) and isolated arms were combinatorially expressed as 2-heavy-2-light (2H2L) BiAbs. Functionally active BiAbs were identified using a high-throughput chromogenic FXase assay. We identified a F.IX arm which demonstrated high FXase activity when paired with a variety of different F.X arms. To generate a common light chain (CLC) BiAb, we focused on a CLC BiAb based on the light chain isolated from this promiscuous F.IX arm. To identify Factor X antibodies able to pair with the F.IX CLC, IntelliSelect® transgenic mice solely expressing the isolated F.IX CLC were generated, immunized with F.X and F.X specific antibodies recovered. The isolated F.X arms were then expressed as CLC BiAbs, together with the heavy and light chains of the selected F.IX arm and re-screened by functional assays. Antibody sequences of biologically active BiAbs were further optimized using deep mining of next generation sequencing (NGS) data using Kymab's IntelliSelect® Bioinformatics platform. Coupled with a minimal mutagenesis strategy, this optimization identified variants with elevated hemostatic activity resulting in a lead BiAb, KY1049. KY1049 was purified using Protein A and cation ion-exchange chromatography (cIEX). The purified BiAb was then characterized using a combination of in vitro and ex vivo hemostatic assays including chromogenic FXase (FXase), activated partial thromboplastin time (aPTT) and thrombin generation assay (TGA).
Results: More than 8000 2H2L BiAbs were initially screened, followed by the analysis of more than 400 CLC F.X arms by chromogenic FXase to identify functionally active CLC BiAbs. Further optimization to iteratively and combinatorially optimize the F.IX/F.X BiAb was carried out resulting in a potent BiAb, KY1049. KY1049 demonstrates a dose-dependent reduction in clotting time (aPTT) and an increase in thrombin generation (TGA), thereby functionally restoring the hemostatic activity of F.VIII-depleted plasma. KY1049 can be purified using standard purification processes and is identified as a single species by mass spectrometry. KY1049 simultaneously binds F.IX and F.X by surface plasmon resonance (SPR).
Summary/Conclusion: KY1049, developed using Kymab's IntelliSelect® Bispecific platform, is a potent F.VIII mimetic BiAb with comparable hemostatic activities to a sequence-identical analogue of Hemlibra. KY1049 is a fully-human F.VIII mimetic CLC BiAb in which both heavy chains naturally bind a cognate CLC.

FS 2.3

Emicizumab demonstrates long term efficacy and tolerability in a broad population of persons with haemophilia A (PwHA) with or without FVIII inhibitors: pooled data from four HAVEN studies

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Background: Emicizumab is a subcutaneously administered, bispecific monoclonal antibody that bridges activated factor (F)IXa and FX to replace the function of missing activated FVIII and restore haemostasis in PwHA.
Aims: To evaluate the long-term efficacy and safety of emicizumab in PwHA across the four phase III studies: HAVEN 1 (NCT02622321), HAVEN 2 (NCT02795767), HAVEN 3 (NCT02847637), and HAVEN 4 (NCT03020160).
Methods: The studies enrolled paediatric (<12 years old) and adolescent/adult (≥12 years old) PwHA, with or without FVIII inhibitors; informed consent/assent was obtained. Emicizumab prophylaxis was administered 1.5 mg/kg weekly, 3 mg/kg every 2 weeks, or 6 mg/kg every 4 weeks following four loading doses of 3 mg/kg per week. All participants assigned to receive emicizumab were included, and data analysed by study or pooled across studies.
Results: Overall, 400 participants in HAVEN 1, 2, 3, and 4 (n=113, 88, 151, and 48, respectively) were included in the efficacy analysis, with a median efficacy period of 82.4 weeks (with 77% of participants treated for ≥74 weeks). The model-based annualized treated bleed rate (ABR; derived via negative binomial regression) was 1.5 (95% CI 1.20–1.84). The mean ABR (95% CI) decreased across consecutive 24-week treatment intervals: 1–24 weeks, 1.9 (0.2–7.1); 25–48 weeks, 0.8 (0.0–5.2); 49–72 weeks, 0.8 (0.0–5.2); and 73–96 weeks, 0.3 (0.0–4.4). Mean ABRs over time were consistent between studies, in children and in adults with or without FVIII inhibitors. In all studies, the proportion of participants with zero treated bleeds increased over time (1–24 weeks, 70.8% [95% CI, 66.1–75.3]; 25–48 weeks, 79.4% [74.8–83.5]; 49–72 weeks, 82.7% [77.8–87.0]; and 73–96 weeks, 88.6% [81.3–93.8]) and the proportion with 0–3 treated bleeds approached 100% within a year and was then maintained. Across studies, over 87% of participants had no treated joint bleeds in each treatment interval from Week 25 and over 92% had no spontaneous bleeds. No death, thrombotic, or thrombotic microangiopathy events occurred in these trials beyond those reported in HAVEN 1 at the respective primary analysis (Oldenburg et al. NEJM, 2017). Emicizumab continued to be well tolerated, with no participants discontinuing due to adverse events beyond the five previously described (Oldenburg et al. NEJM, 2017; Young et al. ASH, 2018; Mahlangu et al. NEJM, 2018; Pipe et al. WFH 2018).
Summary/Conclusion: Emicizumab maintained low bleed rates and favourable safety and tolerability long term in a broad population of PwHA regardless of age, FVIII inhibitor status, or dosing regimen. Mean ABR decreased and the proportion of participants with zero joint or spontaneous bleeds increased across each treatment interval from Week 25. No new safety concerns were identified.
Funding: The HAVEN studies were sponsored by F. Hoffmann-La Roche Ltd and Chugai Pharmaceutical Co., Ltd. Writing assistance was provided Gardiner-Caldwell Communications, and funded by F. Hoffmann-La Roche Ltd.

OS 1.1

The effect of thrombophilic risk factors on VTE risk in orthopaedic surgical patients: results of a large population-based case-control study

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Background: Patients undergoing surgery have a high risk of Venous Thromboembolism (VTE), in particular following major orthopaedic surgery. To prevent VTE, the majority of these patients need to be treated with thromboprophylaxis. Patients with thrombophilic risk factors undergoing orthopaedic surgery, such as factor V Leiden mutation, non-O blood type and prothrombin mutation are at increased risk of VTE. However, the size of this risk is not well known.
Aims: The main objective of this study was to evaluate the effect of thrombophilic factors on VTE risk following orthopaedic surgery.
Methods: Data from a large population-based case–control study (the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis [MEGA] study) on the aetiology of venous thrombosis were used. Odds ratios (ORs) with 95% confidence intervals (CIs), adjusted for age, sex and body mass index (ORadj) were calculated for patients undergoing any orthopaedic intervention. ORs and adjusted ORs of possible interactions between orthopaedic surgery and additional thrombophilic risk factors (factor V Leiden mutation, prothrombin GS20210A mutation, non-O blood type and elevated plasma levels of factor VIII [>150mg/dl]) were calculated.
Results: Of 4.721 cases and 5.638 controls, 263 cases and 94 controls underwent an orthopaedic operation (within 1 year) for an ORadj of 3.74 (95%CI 2.91-4.80). Patients with thrombophilia who underwent an orthopaedic operation had a 16-fold (ORadj 16.95 (95%CI 9.23-31.15) increased risk as compared with those patients who had no biological nor genetic risk factor and did not undergo any orthopaedic operation. Patients with high levels of factor VIII or patients with factor V Leiden mutation had a 18-fold and 17-fold increased risk following surgery, respectively. Patients with non-O blood type had an 11-fold increased risk following surgery.
Summary/Conclusion: An increased VTE risk was found due to a joint effect between thrombophilic risk factors and orthopaedic surgery. This information may aid orthopaedic surgeons decide on administration of thromboprophylaxis. Our findings may ask for intensified thromboprophylaxis strategies in patients with thrombophilia, although additional studies are warranted.

OS 1.2

Plasma levels of a complement factor H related protein are associated with VTE

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Background: In venous thromboembolism (VTE), comprising both deep vein thrombosis (DVT) and pulmonary embolism (PE), there is a need for clinical biomarkers for both risk prediction and diagnosis. The only clinically used biomarker today, D-dimer, is used primarily for excluding a VTE diagnosis in low-risk patients. In medium to high risk patients, diagnostic imaging procedures are necessary to verify diagnosis, which can be difficult in acute situations, increasing the risk of delayed or missed diagnosis.
Aims: To identify and further characterise novel plasma biomarkers with potential clinical application for VTE diagnosis.
Methods: As part of the Venous Thrombo-Embolism BIOmarker Study (VEBIOS), we performed an affinity proteomics-based discovery screening of 408 proteins in plasma in a cohort of patients presenting to the Emergency Room (VEBIOS ER) with suspected VTE (48+48 cases and controls). Following target validation and development of quantitative dual binder immunoassays, plasma levels of candidate proteins of interest were measured in additional cohorts as follows: 1) VEBIOS Coagulation (144 cases sampled 1-3 months after stopping treatment for a first VTE, 140 controls); 2) FARIVE study (580 cases with first VTE sampled at diagnosis, 589 controls), 3) the MARTHA study (1398 cases with history of VTE); 3) the RETROVE study (315 cases sampled ≥6 months after VTE, 357 controls); 4) the Swedish DFW-VTE (47 cases and 111 controls sampled in ER with suspected VTE), 5) a nested case control study derived from the 4thsurvey of the population based Tromsø Study (416 subjects with incident VTE, 848 matched controls). Ethical approval was obtained in all studies. Testing for association of protein levels with VTE was performed using logistic regression with protein concentration treated as continuous variable, or divided in tertiles to estimate odds ratios (OR) with 95% confidence intervals. All analyses were adjusted for age and sex. Functional *in vitro* platelet activation assays were performed using freshly isolated human platelets.
Results: In VEBIOS, compared to the 1sttertile, the 3rdtertile plasma concentrations of a complement factor-H related (CFHR) family member protein was associated with increased VTE risk, both at diagnosis (OR 9.05 [2.93-31.6], p=2.51e-04) and at baseline after stopping anticoagulant treatment following a first VTE (OR 2.51 [1.39-4.63], p= 2.68e-03). The associations replicated in the FARIVE, RETROVE and DFW-VTE studies. Functional *in vitro* experiments indicated that increased levels of the CFHR protein augments platelet response to activating agonists.
Summary/Conclusion: Plasma levels of a CFHR protein was associated with VTE both at diagnosis and at baseline. As the CFHR family of proteins is described to have a regulatory function in complement activation via the alternative pathway, our results could indicate this pathway to have a role in VTE development.
This work was conducted with support from the K. G. Jebsen Foundation, Erling-Persson Family Foundation, Swedish Heart and Lung Foundation, Stockholm City Council (SLL, ALF and HMT), and GENMED (ANR-10-LABX-0013) «EPIDEMIOM-VTE» Chair of Excellence from the University of Bordeaux

OS 1.3

Thrombosis risk after switching oral contraceptive type

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Background: Combined oral contraceptives (COCs) increase the risk of venous thrombosis (VT) 2 to 4-fold, depending on the type of COC. This risk is highest in the first 3-12 months of use (starters-effect), due to redistribution of clotting factors and attrition of susceptibles. The effect of switching between different COCs on VT risk is yet unknown. Potentially, this could induce a renewed starters-effect.
Aims: We aim to study the effect of switching COC type on VT risk.
Methods: We conducted a cohort study using data from the Dutch Foundation for Pharmaceutical Statistics (SFK) including women <50 years starting COC use between 2002-2016. Using Poisson regression, we calculated incidence rates (IRs) per month after starting or switching COC, per type of COC and per type of switch, up to a total follow-up of 365 days. As we do not have information of COC use prior to 2000, starters were defined as women who did not use COC in the two years prior to starting their current COC. When comparing the risk over time after switching with that after starting with a similar COC, we can assess whether the risk in switchers changes only because a different risk associated COC preparation is initiated or whether there is a new starters effect. As the database does not provide definite diagnoses of VT, we used prescriptions of oral anticoagulants as a proxy for VT. In this abstract, we present preliminary results, based on prescription dates which were rounded to the first of a month. We are currently repeating the analysis on our newly acquired data in which the exact prescription dates are available.
Results: Information was available on 5 076 214 women starting COC and 138 966 women switching between COCs. In total 2606 VT events occurred among women using COC. Due to this low number of VT events among women who used certain COCs, we grouped the COCs into generations (second, third and newer generation).
Our preliminary results show that the IR of VT among switchers resembled or was even higher than that of a starters-effect of the same COC generation.
Using the data containing exact prescription dates, we will be able to calculate IRs after switching COC more accurately. Results of these analyses will be available to present at the ECTH meeting in October 2019.
Summary/Conclusion: Switching from one to another contraceptive generation may induce a renewed starters-effect.

OS 1.4

Impact of dietary intake of marine n-3 polyunsaturated fatty acids on surgery as a trigger for venous thromboembolism: results from a case-crossover study

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Background: Previous Studies have shown that intake of marine polyunsaturated fatty acids (n-3 PUFAs) is associated with reduced risk of VTE. However, the impact of n-3 PUFAs intake on triggers for VTE is unexplored. Recognized triggers for VTE include major surgery, immobilization, infection and hospitalization. A general challenge in exploring the relationship between dietary intake of n-3 PUFAs and future risk of VTE in observational studies is confounding. Therefore, we conducted a case-crossover study, a design where each case serves as its own control.
Aims: To assess the impact of dietary intake of marine n-3 PUFAs on major triggers for VTE in a case-crossover study.
Methods: We recruited 445 patients with a first-lifetime VTE from the fourth (1994-95) and the sixth (2007-08) surveys of the Tromsø Study, in a population-based case-crossover study. Hence, confounding by persisting individual characteristics such as age, sex, life-style and chronic comorbidities were controlled for through the study design. The total weekly n-3 PUFAs intake was calculated for each participant based on complete questionnaires on marine food intake and fish oil supplements at the surveys. A hazard period was defined as the 90 days preceding the incident VTE event, which was compared to four preceding and consecutive 90-day control periods. We used conditional logistic regression to calculate regression coefficients and corresponding odd ratios (ORs) with 95% confidence intervals (CIs) for the presence of major triggers preceding the VTE event according to tertiles (T) of n-3 PUFAs intake (T1: <8.1, T2: 8.1-28.9, T3: >28.9 g/wk).
Results: Among the 445 cases, 288 (65%) had one or more triggers within the hazard period compared to 172 (10%) in the control periods, yielding high ORs for all triggers: OR for surgery: 7.6 (5.2-11.1), OR for immobilization: 115.7 (42.7-313.4), OR for infection: 22.8 (14.8-35.1), and OR for hospitalization: 16.3 (11.3-236.5). For surgery, we observed a protective dose-response effect between the total intake of n-3 PUFAs and incident VTE (T1; OR 11.8, 95% CI 6.1-22.6), T2; OR 8.1, 95% CI 4.2-15.7, T3; OR=4.1, 95% CI 2.1-8.2, p for trend < 0.001). The difference between tertile 1 and 3 was statistical significant (p= 0.03). There were no evident influence of n-3 PUFAs intake and immobilization, infection and hospitalization as triggers for VTE.
Summary/Conclusion: Our findings suggest that high intake of n-3 PUFAs had a protective effect on major surgery as trigger for VTE. Future interventional studies should investigate whether intake of marine n-3 PUFAs prior to surgery would protect against surgery-related VTE.

OS 1.5

Splanchnic vein thrombosis and antithrombin deficiency

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Background: Thrombosis of the splanchnic veins (SVT) is a rare disorder (5/10.000) with high morbi-mortality rate that can lead to severe complications such as portal hypertension or intestinal ischemia, particularly when delayed diagnosis or incorrect management. Thrombophilia is the most frequent cause of SVT. Accordingly, there are reports of cases with SVT carrying congenital antithrombin deficiency (CATD), the most severe thrombophilia mainly caused by mutations in *SERPINC1* and with lower rate by disorders of N-glycosylation. However, to our knowledge there is no study evaluating SVT in cohorts of patients with CATD. On the other hand, the diagnosis of CATD in patients with SVT can be complicated by the potential hepatic failure derived from thrombosis.
Aims: i) To define the prevalence of SVT in CATD; ii) to improve the diagnostic methods of CATD in patients with SVT.
Methods: The prevalence of SVT was retrospectively evaluated in 715 patients with CATD from 347 unrelated families. Moreover, we studied 89 cases with SVT in whom a CATD had been ruled out by routinely methods. In all cases, functional (anti-FXa and anti-FIIa assays using chromogenic methods), antigenic (ELISA and Western blot), and genetic (Sequencing and MPLA of exons and flanking regions of *SERPINC1*) analysis of antithrombin were carried out.
Results: 16/715 cases with CATD (2%) presented SVT as the first thrombotic event. The majority of cases (63%) had type I deficiency caused by a serious defect in *SERPINC1*, mainly frameshift due to small in/dels. Three new *SERPINC1* defects were identified. In two families, two relatives developed SVT.
21/89 cases (24%) with SVT from the second cohort presented antithrombin deficiency by functional and immunological methods (<80%), although they also had deficiency of other hepatic proteins (protein C or S). Sequencing of *SERPINC1* in these cases identified two variants: 1) p.Ala156Ala (MAF: 0.00002). The absence of segregation of this defect with deficiency in relatives ruled out a functional effect. 2) p.Gly199Arg (MAF: 0.00003), which affects a residue highly conserved and causes a type II deficiency (Anti-FIIa: 65%; anti-FXa: 70%, Antigen: 100%) with the presence of a variant antithrombin in circulation. The directed search of two *SERPINC1* mild pathogenic variants that are not detected by functional methods (antithrombin Cambridge II -p.Ala416Ser- and Dublin -p.Val30Glu-) identified one SVT case carrying the Dublin variant.
Two cases with SVT, one of each cohort, presented an N-glycosylation disorder identified by electrophoretic and HPLC analysis of different plasma proteins.
Summary/Conclusion: The splanchnic veins are a relatively frequent location of thrombotic events in CATD patients, especially type I. These results recommend performing the necessary diagnostic tests of SVT in carriers, just by the first abdominal symptoms. The combined deficiency of other hepatic proteins in a high proportion of cases with SVT may mask CATD. This and the existence of pathogenic mutations hidden to functional methods recommend the molecular study of *SERPINC1* for the diagnosis of this severe thrombophilia in certain cases with SVT. Finally, our study confirms the serious thrombotic risk associated with N-glycosylation disorders that can cause deficiency of antithrombin and SVT.
PI18/00598 (ISCIII&FEDER); 19873/GERM/15 (Fundación Séneca)

OS 1.6

Combined effect between genetic risk factors and pregnancy/postpartum period on the risk of venous thrombosis

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Background: Previous studies have shown that there is a synergistic effect of genetic risk factors [i.e, the prothrombin 20210A mutation (FII) and factor V Leiden (FVL)] and pregnancy/postpartum period on the risk of venous thrombosis (VT). However, information regarding the combined effect between SNPs in genes ABO (rs8176719) and γ Fibrinogen (FGG, rs2066865) and pregnancy/postpartum status on the risk of VT is lacking.
Aims: To assess the combined effect of ABO-rs8176719 or FGG-rs2066865 and pregnancy/postpartum on the risk of VT. Furthermore, we assess the combined effect between a genetic risk score comprising of rs8176719, rs2066865, FVL, and PT20210A and pregnancy/postpartum on the risk of VT.
Methods: Analyses were performed in the Multiple Environmental and Genetic Assessment of risk factors for VT (MEGA) study, a large population-based case-control study on risk factors for a first VT. For the current analysis, we included women aged 18–50 years. Women taking either oral contraceptives or hormone replacement therapy at the time of the thrombotic event were excluded. Information on pregnancy and postpartum period was obtained via self-administered questionnaires. Postpartum period was defined as the index date within three months after delivery. In total 413 cases and 833 controls were included in the study. FVL and PT20210A were measured in all participants; ABO-rs8176719 was measured in 397 patients and 806 controls; FGG-rs2066865 in 207 patients and 529 controls. We calculate odds ratios (OR) adjusted for age.
Results: The median age was 40.2 years (IQ range 33.6-45.4). Within the cases, 142 subjects had been pregnant in the last three months before the index event (34.4% of the cases), compared with 66 controls (7.9%). We observed a synergistic effect between ABO-rs8176719 and pregnancy/postpartum [compared with non-pregnant/postpartum, wildtype ABO-rs8176719: pregnancy/postpartum alone OR 4.3 (95CI: 2.6-7.1), ABO-rs8176719 carrier alone OR 1.7 (95CI: 1.3-2.3), combined effect OR 14.8 (95CI: 8.9-24.5)]. The combined effect for pregnancy/postpartum and FGG-rs2066865 was less pronounced (OR for pregnant/postpartum carriers of FGG-rs2066865 compared with non-pregnant/postpartum, wildtype FGG-rs2066865: 5.37; 95% CI 2.9-9.9).
We analyzed the distribution of the eight risk alleles of the four SNPs included in the genetic risk model including participants with all four SNPs available (206 patients and 527 controls): 18.3% had no risk alleles (reference OR), 39% carried 1 risk allele (OR 1.48), 24.8% carried 2 risk alleles (OR 2.1), 14.7% carried 3 risk alleles (OR 1.9) and the maximum number of risk alleles was 4 in 3.1% of the study population (OR 4.6).
Based on our data, we considered the median distribution of risk alleles as the cut-off to evaluate the risk of VT using this genetic model (i.e., ≥2 risk allele versus ≤1 risk allele). Using this genetic model, we again observed a synergistic effect between genetic risk factors and pregnancy/postpartum [pregnancy/postpartum alone OR 4.5 (95CI: 2.5-8.2), ≥2 risk alleles alone OR 1.6 (95CI: 1.1-2.3), combined effect OR 11.5 (95CI: 5.5-23.9)].
Summary/Conclusion: Women carrying genetic risk factors have a high risk of developing pregnancy/postpartum VT. Especially ABO-rs8176719 may be important to consider when assessing VT risk during pregnancy.

OS 2.1

Surgical experience from four phase III studies (HAVEN 1–4) of emicizumab in persons with haemophilia A (PwHA) with or without FVIII inhibitors

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Background: Emicizumab is a subcutaneously administered, bispecific, humanized monoclonal antibody that bridges activated factor (F)IXa and FX to replace the function of missing activated FVIII and restore haemostasis in PwHA.
Aims: To present our experience of surgical procedures in pooled data from four phase III studies: HAVEN 1 (NCT02622321), HAVEN 2 (NCT02795767), HAVEN 3 (NCT02847637), and HAVEN 4 (NCT03020160).
Methods: The studies enrolled PwHA of all ages, with or without FVIII inhibitors; informed consent/assent was obtained. Participants requiring minor or unplanned major surgery were managed per the investigator's discretion. We analysed perioperative use of prophylactic coagulation factor (FVIII or bypassing agents), as well as the frequency and management of post-operative bleeds.
Results: Across the HAVEN studies, 214 minor and 19 major surgeries were performed in 113 and 19 participants, respectively. The majority of minor surgeries were dental (n=63, 29.4%) and central venous access device (CVAD; n=34, 15.9%) procedures. The majority of minor surgeries (n=141; 65.9%) were managed without use of prophylactic coagulation factor; of these, 128 (90.8%) did not result in treated post-operative bleeds. Of the 73 (34.1%) procedures managed with prophylactic coagulation factor, 64 (87.7%) did not result in treated post-operative bleeds. Treated post-operative bleeds occurred most commonly following dental procedures, managed either with (5/22) or without (9/41) prophylactic coagulation factor. Among minor surgeries of PwHA with inhibitors who were given prophylaxis, 17.9% (19/106) involved recombinant (r)FVIIa and one each involved activated prothrombin complex concentrate (aPCC) or standard FVIII. Among minor surgeries of PwHA without inhibitors who were given prophylaxis, 43.5% (47/108) involved standard FVIII and 4.6% (5/108) with long-acting FVIII.
Of the 19 major surgeries, 16 (84.2%) were managed with prophylactic coagulation factor; only one of which resulted in a treated post-operative bleed. Of the three major surgeries managed without prophylactic coagulation factor, no post-operative bleeding occurred. Among major surgeries of PwHA with inhibitors who had prophylaxis, rFVIIa was used for 72.7% (8/11), aPCC for 9.1% (1/11) and standard FVIII for 9.1% (1/11). Among major surgeries of PwHA without inhibitors, prophylaxis was with standard FVIII for 62.5% (5/8) and long-acting FVIII for 25.0% (2/8). Importantly, no procedure resulted in death, thrombosis, FVIII inhibitor development, or unexpected bleed.
Summary/Conclusion: Emicizumab alone provides good haemostatic coverage for patients undergoing minor surgeries. The majority of minor procedures were performed without prophylactic coagulation factor, and of these, >90% did not result in a treated post-operative bleed.
Funding: The HAVEN studies were sponsored by F. Hoffmann-La Roche Ltd and Chugai Pharmaceutical Co., Ltd. Writing assistance was provided Gardiner-Caldwell Communications, and funded by F. Hoffmann-La Roche Ltd.

OS 2.2

Functional investigation of a CD36 variant in patients with an inherited bleeding disorder

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Background: Inherited thrombocytopenia (IT) is comprised of a group of hereditary disorders characterised by reduced platelet counts as the main feature, and often with abnormal variable bleeding diathesis. CD36, also known as platelet glycoprotein 4 (GPIV), is an integral membrane protein and a major receptor which binds thrombospondin, collagen and oxidized low-density lipoprotein among others. CD36 has several functions of clinical significance such as roles in malaria, angiogenesis, thrombocytopenia, obesity and cancer, but precise mechanisms are still unclear.
Aims: This study aimed to investigate the functional role of a nonsense sequence variant in CD36 in two siblings with an inherited bleeding disorder.
Methods: Exome sequencing data were analysed for two siblings with a history of bleeding and low platelet counts. Platelet lysates were used to perform Western blot. To gain insight into the functional consequences of this variant, site direct mutagenesis was carried out on WT CD36 to generate mutant versions (with the stop gain truncated version of CD36) of the CD36 expression construct and transfected into Jurkat T cells.
Results: Exome sequencing analysis identified a heterozygous stop gain variant, leading to a truncated protein in CD36. Western blotting of platelet lysates demonstrated a truncated CD36 protein in the two siblings compared to control samples. To elucidate the effect of the CD36 variant on downstream signalling pathways, a nuclear factor of activated T-cells (NFAT) transcriptional reporter assay was performed.
Summary/Conclusion: Investigation of the effect of downstream signalling pathways has given novel insights into how a genetic variant of CD36 in patients with an inherited thrombocytopenia affects CD36 function.

OS 2.3

Antithrombotic therapy with an anti-glycoprotein VI humanized Fab does not increase the risk of inflammation-associated bleeding in mice

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Background: Glycoprotein VI (GPVI), the main platelet receptor for collagen, has emerged as a new target for anti-thrombotic therapy because its blocking inhibits platelet aggregation and arterial thrombosis without increasing bleeding time after trauma. However, recent studies have indicated that GPVI contributes to prevention of inflammation-induced bleeding in the skin and lungs, thus suggesting that GPVI inhibition might increase the risk of bleeding in those organs upon inflammation.
Aims: Here, we investigated the risk of inflammation-induced bleeding associated with GPVI inhibition by Act017 (Acticor Biotech), an antithrombotic drug candidate currently in phase 2 clinical trial.

Methods: After confirming the antithrombotic activity of Act017 in a model of occlusive thrombosis in the mouse carotid artery, mice humanized for GPVI (GPVIh mice) were treated with Act017 at antithrombotic dose and subjected to either immune complex-mediated skin inflammation or LPS inhalation-induced lung inflammation. Bleeding at the reaction site was quantified by measurement of skin hemoglobin content or hemoglobin concentration in bronchoalveolar lavage fluid.

Results: The bleeding phenotype of control and Act017-treated GPVIh mice in these models was compared to that of mice treated with a platelet-depleting antibody, or with partial (GPVI+/-) or complete (GPVI-/-) deficiency in GPVI. GPVIh mice treated with Act017 and GPVI+/- mice, which have half the normal platelet surface level of GPVI, did not bleed during immune complex-mediated skin inflammation. This was in contrast to the petechial skin bleeding at the reaction site observed in platelet-depleted and GPVI-/- mice. Absence of skin bleeding in GPVI+/- and Act017-treated GPVIh mice was not due to altered neutrophil recruitment as this was comparable to that in control GPVIh and GPVI+/+ mice. Only mice immunodepleted of platelets bled in the LPS inhalation-induced lung inflammation model.
Summary/Conclusion: Our results show that, in contrast to platelet depletion or GPVI deficiency, treatment with the anti-GPVI humanized Fab Act017 used at antithrombotic dose does not increase the risk of inflammation-associated bleeding.

OS 2.4

Modulation of alternative splicing of the F5 gene using morpholino antisense oligonucleotides

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Background: Coagulation factor V (FV) is a liver-derived multi-domain (A1-A2-B-A3-C1-C2) protein encoded by the *F5* gene. Recently, a low-abundance splicing variant of FV has been identified. This so-called FV-short variant originates from alternative splicing of exon 13 and lacks 702 amino acids within the B domain. Both activated FV and FV-short express procoagulant activity as cofactors of factor Xa (FXa) in prothrombin activation. However, FV-short also binds tissue factor pathway inhibitor (TFPIa) with high affinity, stabilizing it in the circulation and potently enhancing its anti-FXa activity. Accordingly, genetic mutations that up-regulate FV-short expression have been associated with markedly elevated TFPIa levels and a bleeding tendency (East Texas and Amsterdam bleeding disorders). These bleeding disorders may be amenable to "splicing modulation therapy" using specific antisense oligonucleotides that decrease the relative expression of FV-short.
Aims: To design and test specific morpholino antisense oligonucleotides able to decrease the relative expression of FV-short, as a potential "molecular therapy" for the East Texas and Amsterdam bleeding disorders.
Methods: 25-nucleotide morpholino antisense oligonucleotides targeting the donor (MAO-5') and acceptor (MAO-3') splice sites of the FV-short-specific intron were designed and tested on a liver cell line (HepG2) that naturally expresses FV and FV-short. MAOs (0-20 μM) were delivered to the cells using EndoPorter Reagent. Forty-eight hours later, total RNA was isolated from untreated and treated cells and reverse-transcribed with a *F5*-specific primer. FV and FV-short mRNAs were amplified using transcript-specific primers and PCR products were analyzed by agarose gel electrophoresis. Band intensities were evaluated by densitometry.
Results: HepG2 cells expressed 2-3 orders of magnitude more FV than FV-short mRNA (as estimated by qPCR). Cell treatment with MAO-5' and MAO-3' decreased FV-short mRNA expression in a dose-dependent manner, whereas treatment with a control MAO with a random sequence hardly affected FV-short expression. A 1:1 mixture of MAO-5' and MAO-3' had a more potent effect than either MAO used individually. According to semi-quantitative evaluation of agarose gel bands by densitometry, FV-short expression at the highest treatment concentration (20 μM) was decreased by ~70% when MAO-5' or MAO-3' were used individually and by ~80% when they were used in combination. A more reliable real-time qPCR protocol for the quantification of FV and FV-short transcripts is currently under development.
Summary/Conclusion: We have designed specific MAOs that decrease the relative expression of FV-short mRNA in HepG2 cells. Once fully validated, these molecules may form the basis for a "molecular therapy" for the East Texas and Amsterdam bleeding disorders.

OS 2.5

Functional characterization of FV deficiency in Norway: Effects of F5 mutations on secretion, endoplasmic reticulum stress, apoptosis, and activated protein C sensitivity

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Background: Coagulation factor V **(**FV) deficiency is a rare autosomal recessive bleeding disorder (1:1 mill). Since the disease was discovered in a Norwegian patient by Paul Owren in 1943, no Norwegian cases have been reported. We have now characterized the *F5* gene mutations in five additional FV deficient patients in Norway. We found combinations (homozygous or compound heterozygous) of three *F5* gene mutations; FV-p.H1803R, FV-p.Y1997C, and FV-p.P2098L, where the second mutation was novel, and the last was identical to Owren's patient. The bleeding phenotypes ranged from asymptomatic to severe.
Aims: To study the molecular mechanisms underlying the FV deficiency in these patients by *in vivo* (in blood) and *in vitro* studies of the missense mutations. In addition, we investigated whether the *F5* mutations could be Norwegian founder mutations.
Methods: FV antigen/activity was measured by ELISA/one-stage clotting assay. Thromboplastin-initiated thrombin generation (TG)+/-activated protein C (APC) was measured. HEK293T and CHO-K1 cells were transfected with a plasmid containing the wild-type FV (FVwt) or mutated FV. Intracellular localization was studied by confocal immunofluorescence microscopy. *F5* mRNA, endoplasmic reticulum (ER) stress, unfolded protein response (UPR) markers and chaperons were determined by qRT-PCR, luciferase reporter systems, and Western blot, respectively. The effects on apoptosis were studied by a cell death ELISA. The frequency of the mutations was explored in different populations.
Results: In the patients the FV antigen/activity ranged from 3 to < 1 %. The lag time (TG) was always prolonged but the peak varied from not detectable to similar to the normozygotes. Two patients, without increased bleeding tendency, were homozygous for the FV-p.H1803R mutation. They had similar peak as the normozygotes, but a reduced APC sensitivity. We found the FV-p.H1803R to be more prevalent in the Norwegian population than in other populations. In *in-vitro* studies the FV protein (antigen) secretion was severely impaired, with subsequent reduced FV activity in the cell media, for the FV-p.Y1997C and FV-p.P2098L mutations, but not for the FV-p.H1803R. Intracellular co-localization analysis indicated that these two FV mutations were retained in the ER which led to increased ER stress and subsequent activation of UPR markers, and also to increased expression of the ER chaperon BiP, compared with the FVwt. Apoptosis was increased in cells expressing FV-p.H1803R and FV-p.P2098L.
Summary/Conclusion: Five type I FV deficient patients with inherited homozygous or compound heterozygous mutations had very low FV antigen/activity but the bleeding phenotype ranged from asymptomatic to severe. The peak reflected the bleeding symptoms, and the homozygotes with FV-p.H1803R had normal peak, but reduced APC sensitivity. FV-p.Y1997C and FV-p.P2098L were not secreted from the cells, possibly due to protein misfolding. Retention in ER induced ER stress, and increased expression of ER chaperons. Chaperon-like compounds, which stabilize protein folding and reduce ER stress, should be explored as potential therapeutic options.

OS 2.6

Evaluation of different therapeutic strategies to manage the rare coagulation defect due to enhanced protein C activity induced by the thrombomodulin c.1611C>Ap.Cys537Stop mutation

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Background: A family with unexplained bleeding was identified as having a rare bleeding disorder, caused by elevated soluble thrombomodulin (sTM). A nonsense mutation in the TM gene, THBD, (c.1611C>A p.Cys537Stop) was associated with elevated levels of sTM in plasma and inhibition of thrombin generation.
Aims: To characterise the effect of different therapeutic strategies on the defect caused by the TM c.1611C>A, p.Cys537Stop mutation.
Methods: Platelet poor plasma (PPP) and platelet-rich plasma (PRP) samples were obtained from a female patient undergoing lithotripsy. Samples were taken prior and after administration of 2 units of platelets. Thrombin generation (TG) was triggered using 1 pM tissue factor and measured using the Calibrated Automated Thrombogram (CAT). Samples were spiked with 0.5 U/ml activated prothrombin complex concentrate (APCC), 1 µg/ml activated Factor VII (FVIIa), 50 x 109platelets/litre from a healthy donor or 0.3 mg/ml HAPC1575 monoclonal antibody to activated protein C (APC). Thrombomodulin antigen was quantified using a commercial kit (Abcam).
Results: The patient had sTM levels approximately 130 x higher than normal controls, 372.6 vs.2.89 ng/ml respectively. The endogenous thrombin potential (ETP) in PPP was decreased 3.8-fold compared to the healthy control. Infusion of platelets abrogated the difference in TG. A similar 1.72-fold increase in ETP was observed with FVIIa spiking in both patient and control plasma. APCC enhanced ETP in patient and control plasma, 5.2- and 1.6-fold respectively. Incorporation of the HAPC1575 antibody to inhibit APC activity restored TG parameters to within the normal assay range (ETP 689.84 nM.min vs.1889.35 nM.min). HAPC1575 had no effect on the control plasma. In PRP, TG was 1.3-fold lower compared to control plasma, and spiking with additional control platelets abolished the difference.
Summary/Conclusion: Increased sTM results in enhanced activated protein C generation which reduces TG presumably by degradation of activated Factor V and VIII. Our results indicate that treatment with platelet and FVIIa concentrates may be beneficial for management of this rare bleeding disorder. Administration of APCC also increases thrombin generation but to supranormal levels which may increase risk of thrombosis. The beneficial effect of platelets is unexplained and requires further investigation.

OS 3.1

Repetitive genomic sequences and their role in antithrombin deficiency

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Background: Repetitive non coding sequences such as LINE, SINE and HERV, cover 40% of the human genome. They are mobile DNA elements that may be active (retrotransposonic) or not active (transposonic). Their mobility and homology make them able to trigger pathogenic genetic rearrangements, as it has been described in some diseases as duchenne, thalassemia or cancer (Haig H. Mobile DNA in Health and Disease. NEJM 2017;377:361-70).
The high proportion of LINE or SINE sequences surrounding *SERPINC1*, the gene coding antithrombin (AT), and the fact that 5% of cases with AT deficiency have gross structural variants, let us think that rearrangements implying these mobile elements could be involved in the structural alterations found in *SERPINC1*.
Aims: Characterization of the extension and breakpoint sequence of the structural variants found in *SERPINC1* in order to clarify the role of repetitive sequences.
Methods: Sixteen patients with AT deficiency caused by structural variants detected mostly by MLPA were studied. The characterization of the structural variants was performed by Long Range-PCR (LR-PCR), massive sequencing (Illumina), CGH array (CytoScan® HD) and/or nanopore sequencing (MinION, ONT)
Results: Five patients had whole gene deletion (one was *de novo*), eight had partial deletions and three partial duplications in *SERPINC1*.
LR-PCR was able to detect the breakpoint in 6 cases. SINE sequences implied in their breakpoint was detected in 4 of them. CGH array was performed in 8 cases where LR-PCR was not informative. The extension of the deletions detected ranged between 2 and 30 genes. The relative positions of the breakpoints provided by the CGH array suggested the implication of LINE sequences in all of them. In order to validate these results and to know the exact position of the breakpoint, nanopore sequencing was performed in 3 cases. LINE and SINE sequences in their breakpoints were validated. In addition, CGH array showed an interesting result: all of the 8 patients with structural variants studied, shared three Copy Number Variants (CNV). The analysis of these CNV in 13,000 genomes in general population of UK and in patients with AT deficiency but with punctual mutations in *SERPINC1* confirmed that the CNV Gain 22q11.22 was specific for structural variants in *SERPINC1* (it was only detected in 3/13,000 genomes).
Summary/Conclusion: This study, which combines different and novel molecular diagnostic methodologies on the analysis of the widest cohort of structural variants in *SERPINC1* causing AT deficiency, shows the important role of mobile repetitive sequences (LINE or SINE) in genetic rearrangements. They are shown as *hotspots* for the gain or loss of genetic material leading to AT deficiency and its consequent risk of thrombosis, as we have observed in one case with 2MB *de novo* deletion. The identification of a specific CNV in chromosome 22 associated with these alterations, suggests the activation of retrotrasposonic activity could provoke the rearrangement of LINE or SINE sequences in different genomic localizations. These rearrangements could imply *SERPINC1* gene most probably due to the high concentration of LINE and SINE sequences surrounding this gene. **PI18/00598 (ISCIII& FEDER); 19873/GERM/15 (Fundación Séneca).**

OS 3.2

Fine mapping of anti-spacer autoantibodies from immune-mediated thrombotic thrombocytopenic purpura patients using ADAMTS13/ADAMTS1 spacer hybrids

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Background: Epitope mapping of anti-ADAMTS13 autoantibodies from immune-mediated thrombotic thrombocytopenic purpura (iTTP) patients has shown that 97-100% of patients have autoantibodies against the spacer domain of ADAMTS13. The R568/F592/R660/Y661/Y665 epitope in the spacer domain has been well studied and was shown to be targeted in the majority of iTTP patients. However, anti-spacer antibodies targeting other epitopes in the spacer domain have been identified but less is known about their frequency and precise epitopes.
Aims: Fine mapping of anti-spacer autoantibodies from iTTP patients by using ADAMTS13/ADAMTS1 spacer hybrids to get a better insight into the immunogenic epitopes in the spacer domain.
Methods: A library of 16 full length ADAMTS13/ADAMTS1 spacer hybrids was created: 15 hybrids (named A-O), in which 5-10 amino acids of the spacer domain of ADAMTS13 were exchanged for the corresponding region of ADAMTS1 and one hybrid in which the complete spacer domain of ADAMTS13 was exchanged for the one of ADAMTS1 (TS1-swap). The correct folding of the produced ADAMTS13/ADAMTS1 spacer hybrids was studied in ELISA using monoclonal anti-ADAMTS13 antibodies recognizing conformational epitopes. A pilot study was performed using plasma of nine acute iTTP patients known to contain anti-spacer ADAMTS13 autoantibodies. Fine mapping of anti-spacer ADAMTS13 autoantibodies was done in ELISA using the ADAMTS13/ADAMTS1 spacer hybrids.
Results: Twelve of the 16 ADAMTS13/ADAMTS1 hybrids were efficiently secreted and correctly folded and were used for fine mapping of anti-spacer autoantibodies in 9 iTTP patients (4 hybrids were not secreted and were hence excluded (hybrids D, H, I and K)). A decreased binding of anti-spacer autoantibodies towards hybrids E, F, G and M compromising amino acid regions 588-592, 593-601, 602-610, 657-666, respectively, was observed in all 9 iTTP patient plasmas, indicating that those regions contain amino acids that are involved in the epitopes of anti-spacer autoantibodies. In contrast, in none of the 9 iTTP patient plasmas, a reduced binding of the anti-spacer autoantibodies towards hybrids A (556-563) and L (649-656), was observed implying that those regions are not involved in the epitopes of their anti-spacer autoantibodies. Other regions (hybrids B, C, N and O, containing amino acid regions 564-571, 572-579, 667-676, 677-685, respectively) also contained epitopes for anti-spacer antibodies but were not targeted in all 9 iTTP patients. Spacer autoantibody profiles were the same in patients TTP03 and TTP17 with epitopes in hybrids CEFGJM, and in patients TTP09 and TTP10 with epitopes in the EFGM hybrids. Patients TTP01 (CEFGJMN hybrids), TTP02 (EFGM), TTP11 (EFGJMNO), TTP13 (BCEFGJMNO) and TTP15 (CEFGJMNO) had unique epitope profiles.
Summary/Conclusion: The fine mapping of anti-spacer autoantibodies in iTTP patients using the 12 ADAMTS13/ADAMTS1 spacer hybrids showed that almost the entire spacer domain harbors binding sites for anti-spacer ADAMTS13 autoantibodies. Additionally, the reduced binding of anti-spacer autoantibodies to hybrid M (657-666) confirmed previous reports showing that all iTTP patients indeed have anti-spacer autoantibodies that target the R660/Y661/Y665 residues in the spacer domain. Finally, patients can have overlapping anti-spacer antibody profiles.

OS 3.3

Partial rescue of naturally occurring active site factor X variants through decreased inhibition by tissue factor pathway inhibitor and antithrombin

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Background: Factor (F)X is a serine protease which plays a central role in the coagulation cascade. It is activated by tissue factor (TF)/FVIIa into FXa. FXa is the serine protease component of the prothrombinase complex, the only known physiological activator of prothrombin. FX Nottingham (A404T) and Taunton (R405G) are two naturally occurring mutations, associated with moderate bleeding in affected individuals.
Aims: We aimed to functionally characterise FX Nottingham and Taunton using recombinant proteins.
Methods: Recombinant wild-type (WT) FX, FX Nottingham and FX Taunton were expressed and purified. Their activities and inhibition were investigated in plasma using prothrombin time (PT) and real time thrombin generation assays. Using detailed pure-component assays, the kinetic parameters for their activation by TF/FVIIa and peptide substrate and prothrombin cleavage were determined. Their inhibition by the natural inhibitors tissue factor pathway inhibitor (TFPI) and antithrombin was evaluated in FXa inhibition assays.
Results: The PT times in FX-depleted plasma supplemented with FX Nottingham and Taunton were greatly increased compared to WT FX (104.4 and 55.5 sec compared to 13.7 sec for WT). In agreement with these results, detailed kinetic investigations of activated variants in the prothrombinase complex showed reduced k_{cat}/K_m, ~50-fold and ~5-fold, respectively for both peptidyl substrate and prothrombin cleavage, explaining the prolonged PT times. The substituted residues are located in the protease domain Na^+-binding loop, which is known to be important for the activity of FXa. Both FXa Nottingham and Taunton showed reduced affinity for Na^+, demonstrated by cation titration, suggesting structural changes around the Na^+-binding loop and also providing an explanation for their reduced activities. However, when investigated further in plasma-based thrombin generation assays, the variants demonstrated only small differences in activities compared to WT FX when the assays were triggered with 1pM tissue factor (TF), but large reductions at 10pM TF. A potential explanation for these discrepancies could be reduced inhibition of FXa by its natural inhibitors since also inhibition is strongly associated with Na^+-binding. Blocking of tissue factor pathway inhibitor (TFPI) and antithrombin (AT) anticoagulant activities with inhibitory antibodies substantially increased thrombin generation in plasma supplemented with WT FX, while having much less effect in plasma supplemented with the variants. The severely reduced inhibition of both FXa Nottingham and Taunton by TFPI and AT was confirmed in pure-component FXa inhibition assays, suggesting reduced inhibition as well as activity of both variants.
Summary/Conclusion: FX Nottingham and Taunton both display decreased proteolytic activity. However, their reduced activity in plasma triggered by low TF can be partially rescued by decreased inhibition by the natural FXa inhibitors, TFPI and AT. These cases illustrate the delicate balance between procoagulant and anticoagulant pathways and how they can be simultaneously influenced by genetic mutations.

OS 3.4

Thrombosis and genetics: is p.Arg541Trp the future Breizh prothrombin?

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Background: Venous thrombosis related to heritable thrombophilia has been deeply studied since the first descriptions of familial tendency in thrombo-embolic disease. However, a large part of its heritability still remains unravelled, even after the genome-wide association study era. Whole exome sequencing might be a valuable strategy to better qualify this missing heritability.
Aims: To better understand venous thrombosis mechanisms by discovering new causal genes or variants in a large family, which contains several cases of severe deep venous thrombosis (DVT) or pulmonary embolism (PE).
Methods: We included families from probands with DVT or PE, and at least two of their relatives from first to fourth degree. Thrombosis had to be unprovoked, i.e. without major clinical risk factor nor acquired or known hereditary thrombophilia, with a diagnosis confirmed by ultrasonography for DVT, scintigraphy or tomodensitometry for PE. In one family, we used whole exome sequencing (WES) in selected cases and controls to determine variants of interest. We applied different filters to analyse the variants, such as allele frequency below 1 ‰, impact high or moderate, link with 9 HPO codes and 54 genes related with PE or DVT, and heterozygote status for cases. Next, we used targeted sequencing in the other family members to restrain the list of variants. Finally, we performed polymerase chain reaction – high resolution melting (PCR-HRM) to search for the last variants in two groups: one gathering all cases of the families included in the study, the other constituted by unprovoked but sporadic cases of PE or DVT.
Results: Forty families were included, gathering 140 cases. One of the family (F342) regrouped 23 persons, with 9 cases. Thrombosis occurred at young age, were often recurrent and severe. We selected five distant cases and three controls for WES. After annotation, we obtained 483 542 variants, but only 2 remained after filtering strategy. One of them was c.1621C > T, a missense variant of F2, which has never been reported in public databases. The other was a variant of TKFC, with no obvious link with thrombosis. The F2 variant was shared by all of the cases after targeted Sanger sequencing. Only 1 among 14 controls was heterozygote for the variant. The group of familial DVT and PE cases regrouped 128 cases: 1 sample was not analysed due to low DNA concentration. The other gathered 158 cases: 11 samples had too low volume to be used. We did not detect any heterozygote among these groups by PCR – HRM.
Summary/Conclusion: Here we report a new private variant in F2 gene, which seems to be associated with venous thrombosis. Several prothrombotic F2 variants have been reported: all of them affect Arg596 and confer antithrombin resistance by a modification of exosite I, which normally directly interacts with antithrombin. In our study, the protein is impacted in Arg541, in exosite II. This region interacts with glycosaminoglycans, which is essential to improve thrombin inhibition by antithrombin and interacts with thrombomodulin and protein C. Further functional tests are currently in progress to determine the effect of this new Breizh variant.

OS 3.5

Comparison of the procoagulant activity of extracellular vesicles obtained from cellular monolayers and spheroids

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Background: Cancer-associated thrombosis (CAT) is a major cause of morbidity and the second leading cause of death in cancer patients, annually causing the mortality of one million patients worldwide. Tissue factor (TF) is the main activator of blood coagulation and is associated with thrombosis and tumor progression. TF may be released into the blood circulation incorporated within cancer cell-derived extracellular vesicles (EVs), a mechanism that can be induced following the activation of protease-activated receptor (PAR)-2. Two-dimensional cell cultures have been widely used in research for more than hundred years. However, these cultures cannot fully reproduce the tumor microenvironment, therefore making it inaccurate in measuring cellular responses. Three-dimensional spheroid models are an interesting alternative to reduce animal use in research, and to improve the reproducibility of pathophysiologic studies.
Aims: In this study, we investigated the influence of two-dimensional (monolayer) and three-dimensional (spheroid) tumor cell culture methods, and co-culture of breast tumor cells and cancer-associated fibroblasts (CAF), on the level of EVs release and the associated TF activity.
Methods: Spheroids of Hs578t human breast cancer and CAF cell line were generated using 10,000 cell/well which were seeded out in non-adherent round bottom 96-wells plates. Parallel sets of cell monolayers were prepared alongside using identical number of cells, seeded in adherent flat bottom 96-wells plates. The density of EVs released into the media were measured after 2h by evaluating the concentration of the phosphatidylserine (PS), using the Zymuphen MP assay kit. In addition, the concentration of the released TF antigen was analyzed using the Quantikine human TF-ELISA kit. Finally, cell samples were activated using a PAR2-activating peptide (SLIGKV; 20 μM) for 30 min and the release of EVs and the TF content examined.
Results: The Hs578t cell line showed a higher level of TF expression compared with CAF as determined by ELISA. The release of EVs from resting Hs578t was found to be 7-fold higher in monolayers compared to the spheroid cultures. This was also associated with an 11-fold higher TF antigen release compared to the spheroids. Activation of the cells with PAR2-AP resulted in a significant increase in the release of EVs from the monolayers (from 5 ± 1 nM to 9 ± 1 nM; $p < 0.01$), but no significant increase was observed in the spheroids (from 4 ± 0.5 nM to 5 ± 0.4 nM). The incubation of cells with PAR2-AP also increased the amount of TF antigen released from the monolayers (from 57 ± 14 pg/mL to 98 ± 7 pg/mL; $p < 0.001$), but was not significant in the spheroid cultures (from 11 ± 4 pg/mL to 16 ± 7 pg/mL). The co-culture spheroid (50% Hs578t/50% CAF) resulted in an increased diameter (669,8 μm ± 40,2) when compared with mono-culture spheroid of Hs578t (486,4 μm ± 20,8) or CAFs (495,7 μm ± 34,5), and also an increase in EVs and TF antigen released.
Summary/Conclusion: Taken together, our results demonstrate that monolayer cell cultures are capable of releasing greater amounts of EVs and associated TF than spheroid cultures. The levels of released EVs and associated TF are more strongly amplified following PAR2 activation in the monolayer cultures. Moreover, co-cultures of tumor and stroma spheroids resulted in larger spheroids, able to release higher amounts of EVs and TF. This work was funded by ISTH-EHA Training Fellowship.

OS 3.6

The critical role of tissue factor pathway inhibitor (TFPI) under flow and in vivo

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Background: Tissue factor pathway inhibitor (TFPI) is a Kunitz-type inhibitor that is essential for haemostatic regulation. It functions through inhibition of the initiating TF-FVIIa complex in a FXa-dependent manner. TFPI(α) contains three tandemly arranged Kunitz (K) domains where K1 and K2 inhibits the TF-FVIIa and FXa respectively, while K3 binds to its cofactor protein S. Full length TFPI circulates in plasma at very low concentrations (~0.25nM), meaning that current static plasma-based assays underestimate the importance of TFPI due to depletion of the inhibitor. In both humans and mice, complete TFPI-deficiency is embryonic lethal making the study of TFPI *in vivo* challenging, and has limited the analysis of its *in vivo* role in thrombosis.
Aims: To develop a novel assay to monitor TFPI function under flow, that diminishes the effects of TFPI depletion, and to determine the contribution of TFPI to thrombosis *in vivo*.
Methods: For *in vitro* flow assays, citrated whole blood was perfused over VWF and TF coated microchannels to capture platelets. Thereafter, recalcified plasma supplemented with corn trypsin inhibitor and fluorescently-labelled fibrinogen was perfused at low shear, and real-time fibrin deposition was monitored. Recombinant TFPI or function blocking anti-TFPI and anti-protein S antibodies were added to analyse the role of TFPI in this model. To determine the role of TFPI *in vivo,* inhibitory anti-murine TFPI or isotype control antibodies were injected into mice prior to analysis of laser induced thrombus formation.
Results: We found the addition of recombinant TFPI in our novel flow-based assay markedly delayed the onset of fibrin formation and simultaneously reducing the total fibrin deposition. Conversely, inhibition of endogenous plasma TFPI significantly shortened the time to initiation and increased the amount of fibrin deposited, thus revealing the importance of the plasma TFPI pool. Similarly, we observed an increase in fibrin deposition when plasma was incubated with inhibitory anti-protein S antibodies. *In vivo*, inhibition of TFPI in mice lead to a profound increase in fibrin deposition at the site of injury, revealing the importance of TF-dependent coagulation in this model.
Summary/Conclusion: Using a novel *ex vivo* flow assay, we demonstrate a clear and profound concentration dependent effect of TFPI upon fibrin formation and deposition in human plasma. This reveals the significant role of TFPI in haemostasis that has previously been suggested by static assays, but perhaps underestimated due to rapid TFPI depletion. Our data also highlight how more subtle changes in TFPI concentration may influence clot formation *in vivo* and further demonstrates the important regulatory role of cofactor protein S in TFPI function. Finally, we show for the first time, that TFPI deficiency *in vivo* leads to a profound increase in fibrin deposition in the laser-induced thrombosis model.

OS 4.1

NOX1 inhibitors suppress collagen-stimulated platelet activation in vitro and endothelial damage-induced carotid occlusion in vivo with no effect on haemostasis

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Background: The regulation of platelets by oxidants is critical for vascular health and may explain thrombotic complications in cardiovascular diseases. In our previous study, we found the NADPH oxidase family of enzymes, NOX1 as the main source of oxygen radical generation in response to collagen stimulation, while NOX2 showed to be the critical driver during thrombin stimulation. This study suggested the possibility of selectively inhibiting platelet agonists by targeting either NOX1, offering new opportunities for the development of disease specific antiplatelet drugs with limited bleeding side effects by selectively targeting NOXs.
Aims: The aim of this study was to test a novel NOX1 inhibitor, 2APT-PD6 with better pharmacodynamics profiles (i.e. limited bleeding side effects) for its anti-platelet function without compromising the overall haemostatic response.
Methods: To test its effect on platelet activation in vitro, washed platelets were obtained from healthy human blood and pre-treated for 10 minutes with either control DMSO or the lab synthesised NOX1 inhibitor, 2APT-PD6. Platelet aggregation studies were performed using a chronolog aggregometer. The functional relevance of 2APT-PD6 on thrombus formation was tested using whole blood treated with DMSO or 2APT-PD6 and flown through collagen-coated microchips using the ExiGo pump. The ferric chlorideinduced thrombosis model was used to determine the total time to occlusion in genetically modified mice, NOX1-/- and NOX2-/-. Furthermore, to validate the NOX1 inhibitor 2APT-PD6 as a potential antithrombotic agent, C57BL/6 mice were fed with DMSO or 2APT-PD6 for 2 days. Surgery was performed to isolate the carotid artery and ferric chloride (5%) was applied to induce injury. Thrombosis was assessed by measuring the total time to occlusion using a Doppler flow probe. Finally bleeding assay was performed in mice by tail tip amputation, immersing the tail in saline at 37 °C, continuously monitoring bleeding patterns and measuring bleeding volume.
Results: The NOX1 inhibitor 2APT-PD6 selectively inhibited collagen- but not thrombin-induced platelet activation in vitro, in a concentration dependant manner. Furthermore, whole blood thrombus formation on collagen was significantly reduced in 2APT-PD6 compared to DMSO treated. NOX1 but not NOX2-deficient mice demonstrated a delay in thrombotic growth after ferric chlorideinduced injury to the carotid artery. To determine the antithrombotic effects of 2APT-PD6 in vivo, C57BL/6 mice were fed with either the inhibitor or DMSO as control. After 2 days, mice were anaesthetised and carotid artery injury was performed using ferric chloride. Thrombus formation was indicated by reductions in Doppler blood flow and total time to occlusion was recorded. There was a significant delay in occlusion time in 2APT-PD6 treated compared to control (p
Summary/Conclusion: The results of this study highlights a novel NADPH 1 oxidase inhibitor, 2APT-PD6 as an antiplatelet agent in reducing arterial thrombosis and venous thrombosis in patients with cardiovascular disease.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

OS 4.2

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. Lung collagen content was assessed by hydroxyproline levels. 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 after bleomycin instillation compared to the control group. Lung collagen content plateaued 6 days after bleomycin administration compared to control mice. Additionally, von Willebrand factor (vWF) expression is increased in lung after bleomycin instillation. Thrombocytopenic (TP) mice challenged with bleomycin died prematurely 6 days after instillation. To further identify platelet mechanisms in bleomycin-induced lung fibrosis, transgenic mice lacking the GPIba subunit of the platelet receptor for von Willebrand factor, hIL4R/GPIba mice are used. A similar profile is observed with hIL4R/GPIba mice compared to TP mice. Remarkably, hemoglobin level was significantly higher in the BAL of bleomycin-induced TP mice and hIL4R/GPIba mice compared to bleomycin-induced mice with normal platelet counts. Conversely, mice deficient for the glycoprotein VI, the collagen receptor, seem to be protected after bleomycin instillation. However, bleomycin-challenged mice lacking the thrombin receptor PAR4 showed a similar survival rate than WT mice.
Summary/Conclusion: We here showed that platelets play a protective role during lung fibrosis. We uncovered that GPIba, but not PAR4 and GPVI 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.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

OS 4.3

Platelet integrin alphaIIbbeta3 and phosphatidylserine are vital in extracellular vesicle release from activated platelets

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Background: Circulating extracellular vesicles (EVs) are considered messengers in cell-to-cell communication through delivering of cargo, e.g. chemokines, to target cells. The majority (70%) of EVs are derived from platelets from which they are released by activation with various platelet stimuli. Interestingly, platelets from Glanzmann patients have reduced levels of platelet-derived EV (PEV) release. These platelets lack functional $a_{IIb}\beta_3$ integrins, indicating that $a_{IIb}\beta_3$ integrin is critical in vesicle release. However, the mechanism behind PEV release still remains to be clarified. Taken into account that PEVs contain chemokines that originate from platelet a-granules, the signalling pathways of a-granule- and PEV-release might overlap.
Aims: This study investigates the functional role of integrin $a_{IIb}\beta_3$ and downstream signalling events in PEV release in relation to a-granule content release (chemokines CCL5 and CXCL4).
Methods: Isolated platelets from healthy volunteers were activated with convulxin or thrombin. PEVs and chemokines were isolated and analysed. A panel of pharmacologic inhibitors was used to interfere in specific signalling pathways. PEV release was quantified by nanoparticle tracking analysis and by using a prothrombinase-based assay, based on membrane phosphatidylserine (PS) content. In addition, shedding of PEVs was visualised in real-time by confocal microscopy. Platelet activation status was measured by multicolour flow cytometry, and chemokine release was determined by immuno-assays.
Results: Glycoprotein VI receptor activation with convulxin resulted in a robust PEV release, when compared to platelet activation with thrombin. Kinetic measurements indicated that PEV release followed the pattern of secondary integrin closure (after activation) and of PS exposure. On the other hand, prevention of $a_{IIb}\beta_3$ activation with eptifibatide or tirofiban dramatically suppressed PEV release in activated platelets. Similarly, inhibition of actin cytoskeleton rearrangements with cytochalasin D decreased PEV release, whereas inhibition of signalling targets downstream of integrins showed no such effect. In addition, a-granule content release was only decreased after integrin inhibition.
Summary/Conclusion: Taken together, these data indicate that EV release in platelets relies on signalling events subsequent to integrin activation followed by integrin closure, and PS exposure. Additionally, filopodia formation plays a vital role in PEV release. However, it does not appear that a-granule release and PEV release have a common signalling pathway downstream of the $a_{IIb}\beta_3$ integrin.

OS 4.4

Deep Phenotyping of Platelet Sensitivity and Response Capacity

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Background: Platelet activation underpins both hemostasis and thrombotic disease and techniques that measure platelet function are used to diagnose platelet-based bleeding disorders and assess anti-thrombotic drug efficacy. Platelet responsiveness varies between individuals within healthy populations and is also affected by lifestyle and disease. Anti-platelet drugs are used to supress platelet functional response, but drug efficacy varies across populations. Better methods to assess platelet function in large cohorts would enhance our ability to identify factors that alter platelet function in health and disease and determine a donor's susceptibility to anti-platelet medication.

Aims: To develop a reproducible, consistent high-throughput method to analyse platelet function in large cohorts of healthy blood donors or patients that can assist in donor stratification.
Methods: Platelet rich plasma from 50 donors was processed using a high-throughput 96 well plate assay adapted for flow-cytometry to detect markers of platelet activation (P-selectin exposure and fibrinogen binding) in response to five agonists (ADP, CRP-XL, TRAP-6, epinephrine and U46619) at multiple concentrations. An open source R package (developed specifically to facilitate analysis of the output of this assay) automated identification of dirty data, summarisation of results and clustering of responses into distinct phenotypic groups.
Results: The high-throughput assay enabled quantitation of both responsiveness to concentrations of agonist (Sensitivity) and magnitude of the maximal response (Response Capacity). These measures were found to be independent variables, it being possible for samples to have a high sensitivity to an agonist with a low response capacity and vice versa. Samples also exhibited differential Sensitivity and Response Capacity to different agonists. Machine learning approaches (unsupervised hierarchical clustering) divided participants into distinct sub-populations displaying combinations of high to low Response Capacity and Sensitivity (fibrinogen and P-selectin) to specific agonists
Summary/Conclusion: We have utilised a reproducible high-dimensional assay to measure platelet function in a large group of healthy donors. Use of the assay is facilitated by the availability of an open access bespoke R package (PlaFun) that automates data analysis. The outputs from 50 healthy donors reveals that measures of platelet Sensitivity and Response Capacity are distinct. Machine learning approaches allow us to cluster data into distinct platelet function phenotypes that exist as stable subgroups within the healthy population.

OS 4.5

Novel platelet-neutrophil interaction between activated αIIbβ3 and SLC44A2 mediates NETosis under flow

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Background: Platelet-leukocyte interactions are important in diverse pathophysiological settings from infection to DVT. Previously characterised interactions require full platelet activation (e.g. via P-selectin, CD40L) and/or leukocyte activation (e.g. via Mac-1). However, platelets captured by von Willebrand factor (VWF) under flow also bind leukocytes. We hypothesized that, under flow, GpIba attachment to VWF A1 domain 'primes' platelets, leading to novel platelet-leukocyte interactions.
Aims: To characterise the interaction between VWF-'primed' platelets and leukocytes under flow.
Methods: VWF, the isolated A1 domain or purified activated $a_{IIb}\beta_3$were captured onto microchannels. Labelled leukocytes and platelets from whole or plasma-free blood were perfused at defined shear rates ($50s^{-1}$to $1000s^{-1}$) and recorded in real-time.
Results: Binding of GPIba to VWF A1 under flow 'primed' platelets causing intracellular Ca^{2+} release and $a_{IIb}\beta_3$ activation. VWF-'primed' platelets captured neutrophils and T cells (but not monocytes and B cells) under low shear. Leukocyte capture preferentially occurred in regions of turbulent flow as opposed to laminar flow. Leukocyte binding to 'primed' platelets was independent of P-selectin, but significantly reduced by $a_{IIb}\beta_3$ blockade. Channels coated with activated $a_{IIb}\beta_3$ captured neutrophils under low shear, leading to phenotypic changes, Ca^{2+} release and formation of neutrophil extracellular traps (NETs). NETosis was dependent on shear, intracellular Ca^{2+} release and the NADPH-oxidase pathway. Neutrophil binding to activated $a_{IIb}\beta_3$ was inhibited by blockade of the SLC44A2 receptor. SLC44A2-transfected HEK293T cells bound VWF-'primed' platelets and activated $a_{IIb}\beta_3$, in a manner that could be blocked by SLC44A2 or $a_{IIb}\beta_3$ inhibition. A SNP in *SLC44A2* (rs2288904, G>A, M.A.F.-0.22) encoding R154Q substitution was recently shown to be protective against DVT and stroke. HEK293T cells transfected with *SLC44A2* rs2288904-A exhibited a significant reduction in the ability to bind VWF-'primed' platelets or activated $a_{IIb}\beta_3$. Neutrophils collected from an individual homozygous for the *SLC44A2* rs2288904-A allele had a significantly reduced capacity to interact with VWF-'primed' platelets.
Summary/Conclusion: GPIba binding to VWF under flow 'primes' (but does not activate) platelets, leading to $a_{IIb}\beta_3$ activation. For the first time, we show that activated $a_{IIb}\beta_3$ directly binds neutrophils via SLC44A2, triggering NETosis under flow. We propose that the rs2288904-A SNP in *SLC44A2* diminishes the platelet-leukocyte interactions involved in the pathogenesis/development of DVT. Targeting this interaction may provide a novel therapeutic strategy to protect against DVT.

OS 4.6

Towards a quantitative ex vivo assessment of the innate immune system participation in thrombus formation

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Background: Thrombus growth is influenced by multiple factors: circulating blood cells, coagulation system condition, vascular permeability, inflammation. Upon vessel wall injury subendothelial collagens and laminins become exposed, leading to platelet activation and, as a result, P-selectin exposure and chemokine release. This state leads to leukocyte activation, arrest and adhesion to the site of vascular injury. In particular, a platelet monolayer has been shown to provide a surface for leukocyte capturing, rolling, arrest and firm adhesion. A common way to observe thrombus growth are parallel-plate flow chambers.
Aims: Assessment of platelet - leukocyte interaction in thrombus formation under *ex vivo* conditions.
Methods: Adult healthy donors (18-35 years old; under cell count control; n=30) were included in the research. Whole blood was loaded with DiOC6, AnnexinV, CD66b-, CD11b-conjugated antibodies within 3 hours after blood collection. The samples of healthy donors were incubated with myeloperoxidase (MPO), lipopolysaccharides (LPS), fucoidan and lactoferrin 5 min prior to microscopy experiments (inverted Nikon Eclipse Ti-E and confocal Z1) with parallel-plate flow chambers coated with fibrillar collagen type I. Python 3.6 was utilized for data analysis.
Results: Leukocytes were identified as CD66b-positive cells. Alternatively, a diffused DiOC6 staining was utilized as a cheaper method for leukocyte identification. Leukocytes arrest and adhesion to collagen or platelets were observed only within low shear rate and in the presence of calcium ions (hirudinated or heparinized blood, in citrated blood leukocytes were only capable of arrest, but not crawling). Furthermore, at these conditions leukocyte crawling through thrombi was observed. Highly spread leukocytes were observed more frequently in heparinated blood in comparison with a hirudinated blood. Alternatively, slowly crawling around and inside thrombi leukocytes were more frequently observed in hirudinated blood. Both in heparinated and in hirudinated blood procoagulant platelets were attached to the areas of high CD66b-antibody fluorescence intensity. CD11b-staining also colocalized with the sites of adhesion to procoagulant platelets. Thus, we suggest, that leukocyte LFA-integrins play the major role in carrying procoagulant platelets. Based on this finding we developed the following criteria for hemostasis-innate immune system response assessment: thrombus area, number, velocity and morphological phenotypes of leukocytes (actively moving leukocytes, highly spread actively moving, motionless highly spread cells with distinct mitochondria). Thrombus area increased 4 times by 25th minute from the start, representing initial platelet count. Incubation with MPO, lactoferrin or fucoidan lead to smaller thrombi area and increased number of leukocytes per observation area. Incubation of blood samples with LPS lead to 40-60% increase in the fraction of spread leukocytes and an insignificant increase in thrombus area.
Summary/Conclusion: Parallel-plate flow chambers could be used for observation of an interplay between the systems of innate immunity and hemostasis. Leukocyte amount, velocity and their morphological changes during thrombi formation could be appropriate criteria for the assessment of platelet-leukocyte interactions.

OS 5.1

Factor XII proline rich domain is essential for contact activation

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Background: Contact activation is the process in which coagulation factor (F)XII is auto-activated upon exposure to a charged surface. Activated FXII (FXIIa) initiates coagulation via the intrinsic pathway by activating FXI with critical implications for thrombosis.
Aims: To identify and characterize the FXII structure that mediates FXII auto-activation and its functional and diagnostic implications.
Methods: Using a systematic approach, we generated 19 FXII deletion mutants lacking different protein structures of the FXII heavy chain. FXII deletion mutants were analyzed for their ability to support contact activation *in vitro* in clotting assays, thrombin generation, and FXIIa formation, and *in vivo* in a mouse model for thrombosis. Polyclonal and monoclonal antibodies were generated against parts of the proline-rich domain of FXII and used to establish antibody-based diagnostic coagulation tests.
Results: Clotting and real time thrombin generation triggered by kaolin and polyphosphates were defective in FXII deficient plasma. Reconstitution of FXII deficient plasma with full size FXII and all FXII deletion mutants with the exception of variants lacking the C-terminal part of the proline-rich domain of FXII (PR3) restored defective contact-induced clotting and thrombin generation. Kaolin and polyphosphates failed to cleave and activate FXII mutants lacking PR3 (FXII_ ΔPR3). In contrast, the protease plasma kallikrein readily activated FXII_ΔPR3. In plasma, recombinant PR3 peptide conjugated to maltose-binding protein competed with FXII for surface finding and interfered with auto-activation by polyphosphates. *F12*$^{-/-}$ mice were protected in a FeCl3-induced mouse model for arterial thrombosis. Reconstitution of *F12*$^{-/-}$ mice with full size FXII but not with FXII_ ΔPR3 conferred susceptibility for thrombosis.
Polyclonal and monoclonal antibodies against PR3 stimulated FXII auto-activation and coagulation in a dose-dependent manner, in the absence of a charged surface. These antibodies synergistically enhanced polyphosphate-driven coagulation in plasma.
Controlled FXII activation by monoclonal anti-PR3 antibody was established to standardize commercially available aPTT reagents. Furthermore, antibody-triggered FXII activation was used to generate novel diagnostic coagulation assays that accurately determined low plasma levels of FVIII and FIX in hemophilic plasma samples, and plasma levels of novel therapeutic target FXI.
Summary/Conclusion: We demonstrated *in vitro* and *in vivo* that PR3 is crucial for FXII contact activation and that antibodies directed against PR3 induce contact activation in the absence of a charged surface. A monoclonal antibody against PR3 stoichiometrically activates FXII and allows for improved diagnostic assays.

OS 5.2

High molecular weight kininogen but not factor XII deficiency protects against acetaminophen-induced hepatotoxicity in mice

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Background: Acetaminophen (APAP) overdose is a common cause of acute liver failure (ALF). The acute phase is marked by hepatotoxicity, inflammation and activation of coagulation, followed by multi-organ damage. In animal models, inhibition of the tissue factor/factor VIIa (FVIIa) pathway attenuates APAP-induced liver injury, but could pose a bleeding risk as a therapeutic strategy in humans. Activation of FXII leads to two events: propagation of coagulation by activation of FXI (intrinsic pathway) and activation of plasma prekallikrein to kallikrein, with subsequent cleavage of high molecular weight kininogen (HK) into bradykinin and cleaved HK fragments (cHKF) (contact pathway).
Aims: We investigated whether the intrinsic coagulation pathway contributes to the coagulation-mediated pathology observed in a mouse model of APAP-induced ALF. We hypothesized that deficiency of FXII and/or FXI would attenuate coagulation and reduce hepatotoxicity without affecting hemostasis. Furthermore, we postulated that FXII deficiency might provide additional benefit by preventing contact pathway activation and reducing inflammation.
Methods: FXII, FXI, prekallkrein and HK deficient mice and their respective WT controls were given 400 mg/kg APAP or sterile saline by intraperitoneal injection. Six and 24 hours later blood and livers were collected. Plasma levels of alanine transaminase (ALT- marker of liver injury), thrombin-antithrombin (TAT) complexes, plasmin-a2 antiplasmin (PAP) complexes and interleukin-6 were analyzed. Liver sections were analyzed for hepatocellular necrosis. BK ELISA, western blot, and mass spectrometry were used to identify HK cleavage.
Results: Plasma levels of ALT, TAT and IL-6, and liver injury scores were significantly increased in APAP-challenged mice compared to saline injected WT mice at both 6 and 24 hours. Surprisingly, neither FXII, FXI, nor prekallikrein deficiency had statistically significant effects on any of these parameters in APAP-challenged mice at either time point. Despite the lack of a significant effect on APAP-induced coagulation activation (plasma TAT levels [mean±SEM]: 72.9±8.2 in $HK^{+/+}$ vs 70.8±4.3µg/L in $HK^{-/-}$), plasma levels of ALT were significantly reduced in $HK^{-/-}$ mice (n=23-34) compared to $HK^{+/+}$ (n=19-31) controls at both 6 (1278±184 vs 1850±235 U/L; $p<0.05$) and 24 hours (1546±302 vs 3857±493 U/L; $p<0.01$) after APAP administration. HK deficiency also reduced plasma levels of IL-6 (75.8%; $p<0.001$), number of infiltrating neutrophils in liver (59.8%; $p<0.001$) and liver necrotic area (28.1%; $p<0.001$) 24 hours after APAP challenge. Injection of HK protein into APAP-treated $HK^{-/-}$ mice completely reversed the protective effects of HK deficiency. Plasminogen deficiency ptotects against APAP-induced injury. Consistently, APAP administration increased plasma levels of active tissue plasminogen activator (19.5 fold; $p<0.01$) and PAP complexes (64.4 fold; $p<0.01$), indicative of plasmin generation. Western blotting analysis, BK ELISA, and mass spectrometry analysis demonstrated that plasmin efficiently cleaves HK in a buffer system as well as in mouse and human plasma resulting in the release of BK and generation of cHKF.
Summary/Conclusion: Extrinsic but not intrinsic coagulation pathway drives the coagulation-mediated pathologies associated with APAP-induced ALF in mice. Furthermore, plasmin, rather than FXIIa-dependent generation of kallikrein, may contribute to HK cleavage and downstream hepatotoxicity in APAP-challenged mice independently of thrombin generation.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

OS 5.3

uPA-mediated plasminogen activation is enhanced by polyphosphate

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Background: Polyphosphate (polyP) is a biomolecule stored in platelet dense granules and released upon activation. We have previously shown that polyP, delays polymerisation of fibrin and consequently, alters the structure and mechanical properties of the fibrin network. PolyP attenuates binding of tissue plasminogen activator (tPA) and plasminogen to fibrin, thereby down-regulating tPA-mediated fibrinolysis. In contrast, we have also shown that polyP augments plasminogen activation by factor XIIa (FXIIa). Here we determine the influence of polyP on urokinase (uPA)-mediated plasminogen activation and fibrinolysis.
Aims: To investigate the influence of polyP on uPA-mediated plasminogen activation and fibrinolysis.
Methods: Binding of proteins to biotinylated-polyP was quantified using streptavidin-coated plates and appropriate chromogenic substrates for tPA, uPA and plasmin (S2288, CS-61, S2251) or horse radish peroxidase linked antibodies to FXII or plasminogen. Fibrin clots were formed with fibrinogen, Glu- or Lys-plasminogen, tPA or uPA ± polyP by initiating clotting with thrombin and $CaCl_2$. Fibrinolysis was monitored by change in absorbance at 340 nm and plasmin generation quantified using the fluorogenic plasmin substrate D-Val-Leu-Lys 7-Amido-4-methylcoumarin. Fibrinolysis of clots containing fibrinogen (9% was DyLight 488-labelled) and plasminogen (20% was DyLight 633-labelled) ± polyP was visualised in real time by confocal microscopy.
Results: PolyP bound with high affinity to uPA (K_d 9.8 nM), FXII (K_d 7.8 nM) and FXIIa (4.0 nM). The affinity of the interaction with tPA (K_d 245.3 nM) was considerably lower and no binding to plasmin was observed. PolyP significantly accelerated the rate of uPA-mediated plasmin generation (4.0 ± 0.3 vs. 0.8 ± 0.1 pM/s). Subsequently, fibrinolysis was enhanced by 1.7-fold by the presence of poyP ($t_{1/2}$ 64.5 ± 1.7 min vs. 108.1 ± 3.8 min). These effects are consistent to those observed with FXIIa-mediated plasminogen activation. Enhancement of uPA-mediated lysis required polyP of a minimum chain length of 60 – 100 phosphate residues, consistent with the size found in platelets. The effect of polyP on uPA was highly concentration dependent, suggestive of a template mechanism. Consistent with the clot lysis assays, real-time analysis of fibrinolysis demonstrated that polyP enhances uPA-mediated lysis 1.7-fold while delaying tPA-mediated lysis 1.5-fold. PolyP binds both glu- and Lys-plasminogen and co-localises within fibrin dense areas of the clot. The rate of uPA-mediated plasmin generation and fibrinolysis is augmented by increasing concentrations of Glu- or Lys- plasminogen (0 – 1 µM). At high concentrations of Glu-plasminogen polyP was unable to further accelerate uPA-mediated lysis. In contrast, the concentration of plasminogen did not affect attenuation of tPA-mediated lysis by polyP. PolyP shortened the lag time for uPA-mediated plasmin generation from Glu-plasminogen. This suggests that this biomolecule potentially enhances conversion of Glu to lys-plasminogen thereby augmenting plasmin generation.
Summary/Conclusion: PolyP binds with high affinity to uPA and enhances plasmin generation and fibrinolysis, potentially by accelerating conversion of Glu-plasminogen to the more readily activated form, Lys-plasminogen.

OS 6.1

Endothelial cells derived from induced pluripotent stem cells have the potential to model von Willebrand Disease

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Background: Von Willebrand disease (VWD) is the most common congenital bleeding disorder, characterised by a deficiency and/or dysfunction of von Willebrand factor (VWF). Existing cellular approaches to study the effect of VWF variants include the ectopic expression of VWF in immortalised non-endothelial cell lines, and human umbilical vein endothelial cells (HUVECs) or patient-derived blood outgrowth endothelial cells (BOECs). Immortalised lines, such as HEK293, may lack some of the cellular machinery required for VWF synthesis and release. BOECs are difficult to extract from some donors, and like HUVECs, have a finite capacity for passage and expansion. Given these limitations, we explored whether the differentiation of human induced pluripotent stem cells (hiPSCs) to endothelial cells (iECs) could be an alternative in vitro model for determining the effect of variants of unknown significance found in VWD patients.
Aims:
1. To develop an efficient method for obtaining iECs from hiPSCs
2. To demonstrate that this approach can be used to study the cellular distribution and release of VWF
Methods: We merged existing methods [1,2] to develop a ten day protocol for the differentiation of hiPSCs to iECs. Mesoderm was induced by the inhibition of glycogen synthase kinase 3-beta with CHIR99021. Endothelium was then specified by the use of vascular endothelial growth factor, followed by enrichment with magnetic cell selection for vascular endothelial cell cadherin (VECAD). Endothelial identity was assessed by flow cytometry. Immunofluorescence and Western blotting were used to detect VWF expression; localisation was carried out with confocal microscopy. Barrier function was assessed by measuring electrical impedance of the cell monolayer.
Results: Five established hiPSC lines were tested: expression of VECAD varied between 1 and 90% at day 10. HPSI1113i-qolg_3 [3] (Qolg) was the most reliable cell line; these cells were 94% positive for VECAD following purification and expressed three other endothelial cell markers: CD34 (100%), platelet endothelial cell adhesion molecule (79%), and vascular endothelial growth factor receptor 2 (98%). For every Qolg hiPSC initially seeded, between 2.7 and 5.0 iECs were obtained, of which 50-82% expressed VWF, typically in a punctate pattern with a peri-nuclear distribution. Stimulation with the agonists adrenaline and thrombin resulted in an increase in VWF secretion and a corresponding decrease in the cell lysate. Thrombin treatment also produced a transient reduction in monolayer impedance consistent with the expected increase in vascular permeability in response to this agonist.
Summary/Conclusion: The iECs derived using this protocol produced VWF and responded to physiologically relevant agonists known to stimulate Weibel-Palade body release. The major challenge was the refractory nature of some hiPSC lines to differentiation stimuli. Further work is underway to localise VWF to specific cellular compartments and assess the response to therapeutically relevant agonists, such as desmopressin. The potential of iECs as a model for VWD will be further explored by generating patient-specific hiPSCs and by using genome editing to study putative aetiological variants in well-characterised hiPSC lines.

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Funding
M.S. is supported by a MRC Clinical Research Training Fellowship (MR/R002363/1).

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

OS 6.2

Acquired von willebrand syndrome associated with monoclonal gammopathy

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Background: Acquired Von Willebrand Syndrome (AVWS) is a rare disease that can be associated with monoclonal gammopathy (MG).
Aims: The aim of this study was to evaluate if, in patients with AVWS and MG, there is a correlation between the quantity and type of the MG and the severity of AVWS.
Methods: Clinical charts of all patients evaluated for AVWS between 1990 and 2019 were reviewed. Levels of MG, FVIII and VWF (antigen and ristocetin cofactor) were measured at onset. Relations between quantitative variables (MG and FVIII/VWF levels) were evaluated using the Pearson correlation. In case of VWF:Ag <12 IU/dL and VWF:RCo <6 IU/dL, a mean value of 6 IU/dL and 3 IU/dl were considered.
Results: In the selected timeframe 45 patients were diagnosed with AVWS (22 associated with MG). Median age at diagnosis was 66.5 years (range 39-90), 14 patients were males and 8 females. MG was associated with IgG monoclonal gammopathy of undetermined significance (MGUS) in 14 patients (64%), IgM MGUS in 5 (23%), Waldenstrom macroglobulinemia in 1 (4%), IgG + IgM in 2 (9%). Median concentration of MG was 0.41 g/dL (range 0.1-4.5). Median levels and range of FVIII:C, VWF:Ag and VWF:RCo were respectively 19 IU/dL (5-85), 13 IU/dL (5-99) and 4 IU/dL (3-47). Levels of FVIII:C and VWF (Ag and RCo) were similar in the following subgroups: males and females, quantity of MG (<=0.40g/dL or >0.40g/dL) and kappa or lambda MG. Levels of FVIII:C and VWF were higher in the subgroup of patients >60 years (n=13) with a median FVIII:C 25 IU/dL (range 10-85) and a median VWF:RCo 6 IU/dL (range 3-47), compared to the subgroup <=60 years (n= 9, median FVIII:C 15 IU/dL, range 5-42, median VWF:RCo 3 IU/dL, range 3-25) and in the subgroup of patients with IgM MG (n=6) with a median FVIII:C 36 IU/dL (12-43) and a median VWF:RCo 8IU/dL (3-47), compared to patients with IgG MG (n=14, median FVIII:C 19 IU/dL, range 5-85, median VWF:RCo 3 IU/dL, range 3-38).
Summary/Conclusion: Levels of FVIII:C and VWF are similar in patients of different sex, kappa or lambda MG and high or low quantity of MG. Higher levels of FVIII:C and VWF were found in older patients and in case of IgM MG. These data support the hypothesis that in patients with AVWS associated to MG the severity of the disease (in terms of FVIII and VWF levels) is associated with the type and affinity of the antibody, rather then its quantity.

OS 6.3

Determination of prevalence and features of main VWF synonymous variants of the CRMW database

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Background: von Willebrand disease (VWD), the most common congenital bleeding disorder, is caused by a genetic defect in the von Willebrand factor gene (VWF). Considering that the clinical expression is very variable between patients, VWF genotyping is valuable for the diagnosis and the investigation of the molecular etiology. A challenging part is the characterization of synonymous variants, especially the determination of their impact.
Aims: The aim of this work is to determine the prevalence and features of the main VWF synonymous variants in the French VWD reference center database (Centre de Référence de la Maladie de Willebrand, CRMW).
Methods: We realized a descriptive work from the CRMW database. In June 2019, it includes 3762 patients in total.
Results: In the CRMW database, 3037 patients on 3762 had a full characterization with genotyping. Synonymous variants were described in 99 patients on 3037 (23 different variants). Three variants are described more than the other: p.Cys1130= (c.3390C>T, exon 26) described in 39 patients from 17 unrelated families, p.Val510= (c.1530G>A, exon 13) described in 28 patients from 15 unrelated families, mostly from Martinique, and p.Thr1086= (c.3258C>T, exon 25) described in 12 patients from 7 unrelated families. The variant p.Cys1130= is probably pathogenic, it's located at the beginning of exon 26 and always associated with exon 26 skipping and type 1 VWD. It isn't described in GnomAD database (0/77544 alleles). The variant p.Val510= is associated with type 2A (IIE) VWD and perfectly segregates with the disease. It's not described in the Leiden Open Variation Database (LOVD) and the minor allelic frequency (MAF) is almost null in GnomAD (1/248288 alleles). These 2 variants are most of the time isolated and no other pathogenic variant is found in the VWF. The variant p.Thr1086= is always associated with another variant (7 times with p.Val510=). It seems to be likely benign as its described like this in the LOVD and it has a high MAF in GnomAD (10.3% in African population, 2477/27320 alleles). For the 2 variants the most described in our cohort above, the challenge remains to elucidate their pathogenic mechanism. It could alter the splicing itself by creating new acceptor splice site or donor splice site (dss), or affect the splicing regulation if they are located in exon splice enhancers. It could even interact with a deep intronic variant and create aberrant splicing. In silico analysis is not sufficient to confirm a pathogenic effect, even if it provides valuable information. Functional assays are necessary to determine the pathogenic impact, like mRNA study or minigene that analyze the effect of splice site variants, either exonic or intronic. Some synonymous variants have been described in the literature as disease causing: p.Gly2352= (described in 1 family in the CRMW) by creating a dss (GnomAD MAF 2/250226) and p.Ser182= (not described in the CRMW) leading to exon 6 skipping (GnomAD MAF 361/282860).
Summary/Conclusion: The pathogenic effect is still hard to determine for synonymous variants in VWD. It remains a challenge to distinguish pathogenic variants from rare polymorphisms, especially when the synonymous variant segregates with the disease and/or with another pathogenic non-synonymous variant. Functional studies are essential to prove the pathogenic effect of a variant but they are hardly feasible to study the huge number of variants of uncertain significance that can be found in the VWF gene.

OS 7.1

TLR3 promotes venous thrombosis through neutrophil recruitment

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Background: Venous thromboembolism (VTE), which includes deep vein thrombosis and pulmonary embolism remains the third most common cause of mortality among all cardiovascular diseases. Surgery and trauma are among the most prevalent risk factors associated with venous thrombosis. They share with acute inflammation, an increase in endothelial and platelet activation raising the possibility that factors derived from the inflamed endothelium and/or activated platelets might contribute to the propagation of the thrombotic response. Moreover, studies supported intertwined roles of innate inflammatory systems and thrombosis in the pathogenesis of VTE. Toll-like receptors (TLRs) localized on vascular cell surface or in intracellular compartments play pivotal roles in the innate immune system by detecting « pathologic ligands » and transducing signals into a prothrombotic state. Studies suggest that TLR3 recognizes endogenous extracellular RNA (eRNA) that are generated during tissue damage and inflammation.
Aims: We hypothesize that eRNA and TLR3 promote venous thrombosis development after vessel injury.
Methods: The ferric chloride (FeCl3) model was used to induce venous thrombosis in mice. WT or TLR3 deficient (-/-) mice received an i.v. injection of either vehicle, a specific fluorescent probe for RNA (Syto RNA Select), RNase1, poly(I:C) or RNA extracted from murine endothelial cells (eRNA). Human umbilical vein endothelial cells (HUVECs) were treated with siRNA directed against TLR3 or an IL-8 receptor (CXCR2) antagonist.
Results: Using a cell-permeant nucleic acid stain, we demonstrated that FeCl3 promoted RNA release in vivo and thus increased the RNA content in the thrombus. Therefore, RNase 1 treatment reduced thrombus size compared to mice injected with vehicle. In contrast, eRNA and poly (I:C) increased thrombus size in WT mice whereas no modifications were observed in $TLR3^{-/-}$ mice. Poly (I:C) and eRNA treatments bolstered neutrophil infiltration in WT but not in $TLR3^{-/-}$ mice. Similarly, the expression of citrullinated histone 3 (CitH3), a neutrophil extracellular traps (NETs) biomarker, was heightened by poly (I:C) and eRNA in WT but not in $TLR3^{-/-}$ mice. In vitro, eRNA stimulated CXCL5 mRNA expression, a murine isoform of human IL-8, in WT but not in $TLR3^{-/-}$ endothelial cells. ELISA analysis corroborated that CXCL5 expression is induced by eRNA via TLR3 in endothelial cells. To confirm these data, human endothelial cells treated with poly (I:C) were transfected with siRNA targeting TLR3. This contributes to the abolition of neutrophil recruitment as compared with control siRNA. The administration of an IL-8 receptor (CXCR2) antagonist to the human endothelial cells abrogated neutrophil infiltration.
Summary/Conclusion: These results suggest that eRNA through TLR3 enhances neutrophil recruitment and NET formation leading to venous thrombosis.

OS 7.2

CTL-2 is a VWF receptor involved in neutrophil activation

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Background: Venous Thrombosis (VT) is a multifactorial disease. Two recent genome wide association studies linked the *SLC44A2* rs2288904 (461G>A) polymorphism with VT in humans. This single nucleotide polymorphism leads to HNA-3a (human neutrophil antigen-3a) or HNA-3b epitope expression by the choline transporter like protein-2 (CTL-2). HNA-3b is associated with a 30% decreased risk of VT. CTL-2 is a ubiquitous transmembrane protein and has been identified as a receptor for Von Willebrand factor (VWF). Neutrophil activation and VWF are also critical during VT. VWF interaction with CTL-2 has been shown to be followed by the formation of a trimolecular complex formed by CTL-2/MAC-1 (macrophage antigen 1) and VWF. Integrin clustering is a process that can be observed following "inside-out" signaling, which suggests that VWF engagement through CTL-2 on neutrophils may induce neutrophil activation.
Aims: We wanted to describe how the presence of CTL-2 on neutrophils can modulate their activation and adhesion on VWF and to determine the proteins involved under dynamic conditions. We also aimed to show how the polymorphism of interest linking CTL-2 to VT could affect these effects.
Methods: Neutrophils homozygous for the HNA-3a- or the HNA-3b-coding allele purified from healthy consenting blood donors were perfused in flow chambers at venous shear rates (100s^{-1}). Different matrices including VWF, and different blocking antibodies were tested. Neutrophil activation under VWF and LPS challenge was also evaluated at various cell concentrations in different flow conditions
Results: : We found that HNA-3a expression was required for CTL-2-mediated neutrophil adhesion to VWF at low shear rates as HNA-3b-expressing neutrophils were unable to adhere to VWF in the same experimental conditions. This adhesion was found to be matrix specific and A1-VWF-dependent as inhibited by A1-specific blocking antibodies. We observed that a part of this adhesion was calcium-dependent and that beta 2 integrins were also involved. The adhesion of HNA-3a expressing neutrophils to VWF was enhanced when neutrophils were activated with LPS prior to perfusion when compared with activated neutrophils expressing HNA-3b. Interestingly we observed that specific disturbed shear conditions can act as a potent "second hit" on HNA-3a expressing neutrophils. In such conditions, neutrophils perfused on VWF can get highly activated forming aggregates of neutrophil extracellular traps (NETs). This phenomenon was also HNA-3a specific, matrix dependent, and was greatly enhanced by cell concentration and/or by LPS activation prior to infusion.
Summary/Conclusion: These results suggest that engagement of CTL-2/HNA-3a from neutrophils with VWF at venous shear rate can induce their adhesion and their activation. This interaction can induce integrin activation and thus consolidate neutrophil adhesion to VWF under such shear rates. We observed that adding to these neutrophils a "second hit" that can be associated with VT can potentiate this process and even result in NET formation. Neutrophils expressing CTL-2/HNA-3b not being associated with these observations, these results could thus explain the association between the HNA-3b epitope and the reduced risk for VT in humans.
This work was supported by a grant from the French Agency for National Research and by a grant from the Lefoulon-Delalande foundation

OS 7.3

DNAse-dependent but NETs-independent pathways of thrombus formation in vivo

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Background: The contribution of NETs in thrombus formation has been intensively documented in both arterial and venous thrombosis in mice. These observations were, however, mainly based on the inhibition of thrombus formation following the treatment of mice by DNAse-1 or using different deficient mice, such as PAD4 null mice, in which neutrophil activation and secretion could also be affected. We previously demonstrated that neutrophils play a key role in the initiation of the TF-dependent activation of the blood coagulation cascade leading to thrombus formation following a laser-induced injury (Darbousset R. et al. Blood 2012 and Blood 2014). In this model, thrombus formation is independent of GPVI and thrombin, ADP and ATP constitute the main platelet and neutrophil agonists leading to thrombus formation.
Aims: The goal of our study was to determine the contribution of NETs in arterial thrombus formation following a laser-induced injury.
Methods: The contribution of NETs in vivo was determined by treatment of mice with 100U of DNAse-1 using intravital microscopy. Thrombi were fixed and transmission electron microscopy experiments were performed. In vitro, NETs formation, platelet aggregation and neutrophil activation were studied in different conditions.
Results: Treatment of mice by 100 U of DNAse-1 significantly inhibited thrombus formation induced by a dye laser. *In vivo*, DNase-1 reduced thrombus formation in the seconds following the injury. *In vitro*, formation of NETs occurred in 2 to 3h following activation of neutrophils by TNF-alpha, IL6 or PAF. This kinetics was independent of the agonist used. Electron microscopy of the thrombus formed revealed that neutrophils present at the site of injury induced by a dye laser did not formed NETs. To understand this apparent discrepancy, we determined if DNAse-1, which cleave phosphodiester linkages, may hydrolyze ATP and ADP*in vitro*. As suspected, addition of 25U of DNAse-1 was sufficient enough to hydrolyze ATP and ADP, decreasing the concentration of ATP by 25% in 5 minutes. Aggregation of platelets induced by ADP and activation of neutrophils by ATP were both also significantly reduced.
Summary/Conclusion: Following a laser-induced injury, neutrophils but not NETs are involved in thrombus formation. Treatment by DNAse-1 induces the hydrolysis of ATP and ADP, affecting activation of platelets and neutrophils, leading to the inhibition of thrombus formation *in vivo*.

OS 8.1

Allelic polymorphism of platelet glycoprotein genes GPIa AND GPIIb as a possible predictive marker of response to therapy in patients with primary immune thrombocytopenia

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Background: Primary immune thrombocytopenia (ITP) is characterized by variability of both clinical course (in particular, severity of hemorrhagic syndrome) and response to distinct therapy options. To date, the choice of therapeutic approach to ITP treatment is empirical and often based on the experience of clinician. It is assumed, that allelic polymorphism (AP) of some genes coding for glycoproteins (GP), involved in regulation of immune response or/and platelets' functional activity, can be associated with different response to ITP treatment, which provides an opportunity for individual approach to therapy.
Aims: To investigate association between the effectiveness of certain methods of ITP treatment and the features of AP of *GPIa* and *GPIIb* genes in the groups of patients with different response to corticosteroids (CS) in the first line, thrombopoietin receptor agonists (aTPOr) and splenectomy (SE) in the second line of therapy.
Methods: A total of 81 patients with primary ITP were involved in the study, all of them received 1-st line treatment with CS, then 37 (46%) patients received aTPOr and 22 (27%) underwent SE in the 2-nd line. In respect with the effectiveness of each therapy option, we divided the patients to 3 following groups: "achievement of response", "no response" and "durable response". In all patients, we analyzed DNA polymorphism of *GPIa* (A1648G) and *GPIIb* (T2622G) genes by PCR-RFLP. The differences in genotype frequencies between the patient groups with distinct response to treatment were assessed by Fisher's exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated by using the Graph Pad Prism 5.0 software.
Results: The frequency of heterozygous *GPIIb* 2622TG genotype in patients with durable response to CS was more than 2-fold increased when compared to those with response (72.2% vs. 30.9% respectively; OR=5.8, 95% CI: 1.7-19.7, p=0.005). Homozygotes for the *GPIa* 1648A allele were much more prevalent among the patients who responded to aTPOr (87.5% vs. 20.0% in the no response group; OR=28.0, 95% CI: 2.5-317.9, p=0.005). Moreover, all patients with durable response to SE possessed *GPIa* 1648AA genotype, whereas in the group of patients with no response the proportion of such persons was only 44% (OR=33.0, 95% CI: 1.5-722.5, p=0.005).
Summary/Conclusion: Genotype *GPIIb* 2622TG is associated with durable response to CS in the 1-st line treatment of ITP patients. The presence of *GPIa* 1648AA variant could predict the effectiveness of aTPOr or SE in the 2-nd line of therapy.

OS 8.2

Platelet biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis

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Background: Alzheimer's disease (AD) is the most common form of dementia and it is characterized by progressive impairment of memory and cognitive function. The main neuropathological changes of AD consist of extracellular accumulation of amyloid-A, intracellular deposition of neurofibrillary tangles, neuronal loss, and synaptic dysfunction. Due to the lack of blood biomarkers, the diagnosis of AD is still challenging. Indeed, the only biomarkers currently available (i.e. amyloid-A, total tau and phosphorylated-tau181) are measured in the cerebrospinal fluid (CSF) and therefore obtainable from an invasive procedure. Over the last decades, platelets have been considered a suitable peripheral model to study the metabolic mechanisms occurring in the central nervous system in AD and some platelet-derived factors have been proposed as possible peripheral biomarkers. However, despite the growing interest in the relationship between platelets and AD, only few studies aimed to evaluate different platelets biomarkers as reliable diagnostic tools.
Aims: The purpose of this meta-analysis is to provide evidence about the association of platelets-related biomarkers and AD. Platelets biomarkers may represent a non-invasive alternative to CSF collection with the view of providing clinicians with new laboratory parameters in the diagnosis of AD.
Methods: According to the PRISMA guidelines, we conducted a systematic review and meta-analysis on six pre-selected promising and reliable platelet biomarkers [ADAM10, mono-amino oxidase B (MAO-B), phospholipase A2, cytochrome C oxidase, serotonin and the ratio between high molecular weight (HMW) and low molecular weight (LMW) tau protein] in subjects with AD. Papers published until May 2019 indexed in Medline (PubMed), Web of Science and Scopus were reviewed by two independent assessors. No restriction on publication date or design of the study was made. Statistical analysis was performed using Review Manager 5.3 software. Standardized mean differences (SMDs) and 95% confidence intervals (CIs) were calculated for each study and the pooled estimates were computed according to a random effect model.
Results: In our search, we included 25 studies among the identified 225 citations. Platelets biomarkers were compared between AD and healthy donors in all studies. Diagnosis of AD was mainly established according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria. Patients with AD showed lower platelet levels of ADAM10 (SMD: -2.97; 95% CI: -5.14, -0.81) and cytochrome C oxidase (SMD: -0.96; 95% CI: -1.46, -0.47) compared to controls. Conversely, significantly higher platelet levels of MAO-B (SMD: 1.08; 95% CI: 0.83, 1.83) and of HMW/LMW tau protein (SMD: 0.93; 95% CI: 0.53, 1.33) were detected in AD compared with controls. No association was observed between AD and platelet serotonin (SMD: -0.66; 95% CI: -2.16, 0.84) and phospholipase A2 (SMD: -0.62; 95% CI: -2.53, 1.30).
Summary/Conclusion: Our findings indicate a notable association between platelet biomarkers and AD, suggesting that platelets should be considered as a source of potential biomarkers in the suspect of AD. However, further studies investigating the predictive role of these markers in comparison with those measured in CSF are necessary.

OS 8.3

Effect of antiplatelet agents on bacterial induced platelet aggregation

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Background: Platelets are the cornerstone of hemostasis. Activation of platelets by any inducer in an exaggerated manner results in their aggregation, leading to deleterious effects. *Staphylococcus aureus*and *Streptococcus sanguinis*, through their membrane motifs interact with platelets inducing their activation and aggregation. These events are observed in several situations such as infectious endocarditis or sepsis. This triggering of platelet aggregation by bacteria results in several complications such as thrombus formation. Despite platelet involvement, no antiplatelet therapy is currently recommended for this indication.
Aims: The aim of this study was to evaluate the effect of major antiplatelet agents on platelet aggregation induced by these two bacterial pathogens.
Methods: Blood samples were collected from healthy donors, Platelet rich plasma were treated *in vitro*by different antiplatelet drugs (Aspirin, Ticagrelor, Tirofiban). Three bacterial strains of *Staphylococcus aureus (P6142, P2188 and P6141)* and*Streptococcus sanguinis (P8633, P760 and P2574)* were studied. Platelet aggregation induced by bacteria with or without anti-platelet drugs was evaluated by Light Transmission Aggregometry (Helena, France). P-selectin expressed by platelets was evaluated by flow cytometry under the different conditions of infection and platelet treatment (Navios, Coulter). Ultrastructures of platelet-bacterial aggregates under different conditions were analyzed by Scanning electron microscopy.
Results: The six strains tested showed significant platelet aggregation. Antiplatelet drugs showed different effect depending on strains. Thus, in case of *S. aureus,*between the two drugs taken orally and on long term, ticagrelor showed the highest inhibitory effect in aggregometry (**P6142**: 10.89% p=0.005. **P2188**: 5.08%; p<0.001. **P6141**: 17.99%; p=0.019 vs untreated, infected platelets), and the lower P-selectin expression (**P6142**: p= 0.003 vs untreated, infected platelets). For *S. sanguinis*, it's the combination (aspirin and ticagrelor) which showed the highest inhibitory effect (**P8633**: 20%; p=0.018. **P760**: 3.45%; p=0.002. **P2754**: 9.05%; p<0.001 vs untreated, infected platelets) and the lower P-selectin expression (**P8633**: p=0.014vs untreated, infected platelets). Despite an ever-higher response to ticagrelor in the case of *S. aureus*and to combination in the case of *S. sanguinis*, a significantly different sensitivity between strains of the same species was noted for all antiplatelet drugs. Ultrastructure analysis showed several differences between *S. aureus*and *S. sanguinis*-platelet clots. These differencesconcern the presence of filaments and bacterial bags in the case of *S. aureus*and the organization in amorphous cluster with loss of cellular integrity in the case of *S. sanguinis*.
Summary/Conclusion: Our study demonstrated that antiplatelet agents inhibit platelet aggregation induced by bacteria in a strain-dependent manner. These results indicate the need to take the incriminated strain into consideration for optimal antiplatelet therapy.

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OS 9.1

Increased mortality risk after venous thrombosis: MEGA follow-up study

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Background: Patients with venous thrombosis (VT) have an increased risk of subsequent death, even years after its diagnosis, but the underlying pathophysiology is unclear.
Aims: We aimed to find clues as to why patients with VT have a long-term increased mortality risk compared with the normal population.
Methods: Data from the Multiple Environmental and Genetic Assessment (MEGA) of risk factors for VT follow-up study were used. In total, 4350 patients with VT and 5635 controls were followed between 1999 and 2009. Baseline characteristics were defined and hazard ratios (HRs) for mortality with 95% confidence intervals (CIs) were calculated for patients versus controls. Adjustments were made for age, sex, overweight/obesity, smoking, diabetes, major illness, surgery, hospitalization, number of hospital visits, anticoagulant therapy and factor VIII (FVIII) activity levels. Information about these factors was collected from the MEGA self-reported questionnaires and Statistics Netherlands (CBS) and FVIII was measured using a commercially available ELISA.
Results: During a maximum follow-up time of 7 years, 124 deaths occurred in the controls and 489 in the patients. The mortality rate was 0.46 per 100 person-years (95%CI, 0.38-0.54) in controls and 2.09 (95%CI, 1.91-2.28) in patients. Distribution of age and sex was comparable in patients (mean age 49 years, 46% men) and controls (mean age 48 years, 46% men). At time of VT, patients presented more major illness compared with controls, 22% versus 12%, respectively. Mean level of FVIII activity in patients was 141 IU/dL compared with 113 IU/dL in controls. Sex, age, obesity and diabetes were associated with increased FVIII activity levels in controls. Other underlying risk factors only presented a weak (at most) association with FVIII and smoking was associated with a 6 IU/dL lower FVIII level compared with non-smoking. Increased FVIII levels in controls were associated with mortality: every IU/dL increase in FVIII led to a 1.011-fold (95%CI, 1.008-1.015) increased risk for mortality. Adjustments for confounders (age, sex, overweight/obesity, smoking, diabetes, major illness, surgery, hospitalization and number of hospital visits) did not influence this risk (1.010; 95%CI 1.006-1.015). The HR for mortality after VT in patients compared with controls was 4.74 (95%CI, 3.89-5.77). After adjustment for all measurable confounders except for FVIII this risk attenuated to 3.02 (95%CI, 2.37-3.85). The HR for mortality in patients versus controls in whom blood testing took place was 2.72 (95%CI, 1.90-3.89). After adjustment for all measurable confounders except FVIII this risk attenuated to 1.99 (95%CI, 1.36-2.93). This risk attenuated even further to 1.45 (95%CI, 0.97-2.18) when we adjusted for all measurable confounders plus FVIII.
Summary/Conclusion: The association between VT and subsequent mortality could partially be explained by underlying known confounding factors like age, sex and comorbidity. Most of these underlying comorbidities were not directly associated with increased FVIII levels. FVIII was associated with mortality independent of VTE. Adjustment for FVIII levels attenuated the HR for mortality in patients with VT. Future studies should focus on how the associations between FVIII and mortality can be explained.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

OS 9.2

Thromboembolic and Bleeding Complications in Patients with Atrial Fibrillation and Liver Cirrhosis – a Population-based Cohort Study.

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Background: Atrial fibrillation and atrial flutter (AFF) are risk factors for ischemic stroke. Several conditions such as diabetes and kidney diseases increase the thromboembolic risk in patients with AFF, but it is unclear whether liver cirrhosis affects this association.
Aims: To examine the risk of thromboembolic and bleeding complications in AFF patients with and without liver cirrhosis.
Methods: We conducted a population-based cohort study using Danish nationwide health registries. We identified all patients with a first-time AFF diagnosis during 1995–2015. AFF patients were categorized according to presence or absence of a history of liver cirrhosis. We computed crude incidence rates per 1000 person years and hazard ratios (HRs) based on Cox regression adjusting for age, CHA_2DS_2VASc score, and Charlson Comorbidity Index score.
Results: We identified 273,225 patients with a hospital-based AFF diagnoses. Of those, 1,463 had a history of liver cirrhosis. During 1 year of follow-up, patients with liver cirrhosis had higher incidence rates of hemorrhagic stroke, subdural hemorrhage and gastrointestinal hemorrhage. We found no substantial differences in the rates of ischemic stroke, myocardial infarction and venous thromboembolic events. Patients with liver cirrhosis were at increased risk of ischemic stroke (HR: 1.77 [95% confidence interval (CI): 1.39-2.24]) and hemorrhagic stroke (HR: 1.55 [95% CI: 0.86-2.80] as well as venous thromboembolic event, subdural hemorrhage and gastrointestinal hemorrhage (2-fold, 3-fold and 3.5-fold), during 1 year of follow-up. We found no increased risk of myocardial infarction.
During 2-5 years of follow-up, patients with liver cirrhosis had higher incidence rates of ischemic stroke, venous thromboembolic events, subdural hemorrhage and gastrointestinal hemorrhage, but not of myocardial infarction and hemorrhagic stroke. During 2-5 years of follow-up, patients with liver cirrhosis were at increased risk of myocardial infarction venous thromboembolic events and gastrointestinal hemorrhage (2-fold, 1.5-fold and 4-fold). We found no increased risk of ischemic stroke, hemorrhagic stroke or subdural hemorrhage.
Summary/Conclusion: In patients with AFF, liver cirrhosis was associated with an increased risk of ischemic stroke, venous thromboembolism, hemorrhagic stroke, subdural hemorrhage and gastrointestinal hemorrhage during 1 year of follow-up. During 2-5 years of follow-up liver cirrhosis was associated with an increased risk of myocardial infarction, venous thromboembolic events and gastrointestinal hemorrhage.

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OS 9.3

Rosuvastatin use decreases FVIII levels by mechanisms not associated with the cholesterol-lowering effect of the drug: results from the START trial

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Background: The STAtins Reduce Thrombophilia (START) trial has shown that rosuvastatin downregulates coagulation in patients with a prior venous thromboembolism (VTE) through a decrease in factor (F)VIII levels. Whether or not this pleiotropic effect of rosuvastatin differs from the cholesterol-lowering effect, which takes place in hepatocytes, is not answered.
Aims: In order to provide insight into the mechanisms by which rosuvastatin affects coagulation, we evaluated whether rosuvastatin affects levels of factor IX, a liver-derived coagulation factor, and whether cholesterol-lowering and FVIII-lowering effects of rosuvastatin are associated.
Methods: After anticoagulation withdrawal, patients with VTE were randomized to rosuvastatin at 20mg/day for 4 weeks or no intervention. Levels of FVIII activity, FIX activity and cholesterol were assessed at baseline and at end of study. Analyses were done by intention to treat and regression models were adjusted for age and sex. For the cholesterol analysis, we considered rosuvastatin users only because cholesterol levels are markedly reduced in statin users.
Results: The study comprised 247 patients, 126 rosuvastatin users and 121 non-users. At baseline, the mean age was 58 years, 61% were men, 19% had unprovoked VTE. Factor IX levels did not change from baseline to the end of the study neither in rosuvastatin users (mean change 1.53 IU/mL; 95% CI, -2.25 to 4.62) nor in non-users (mean change 1.19 IU/mL; 95% CI, -2.25 to 4.62). The mean difference in FIX change between groups was 0.79 IU/mL (95% CI, -3.90 to 5.13). The between-group difference in FVIII change was -7.16 IU/mL (95%CI, -12.42 to -1.89), as previously published, and remained at -7.31 IU/mL (95%CI, -12.19 to -2.44) after adjustment for FIX levels. Among rosuvastatin users, the change in FVIII levels was not associated with changes in cholesterol levels (Beta 0.18; 95%CI, -5.84 to 6.2; R^2 Linear <0.0001).
Summary/Conclusion: We demonstrated that rosuvastatin use does not affect the levels of the liver-derived FIX. Moreover, changes in FIX and cholesterol levels did not modify the effect of rosuvastatin on lowering FVIII levels. Our results suggest that the pleiotropic effect of rosuvastatin on coagulation is independent of the intended cholesterol-lowering effect. Our results also suggest that this pleiotropic effect probably occurs at a site different from the liver.

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OS 9.4

Survival after cancer-related venous thrombosis: the Scandinavian Thrombosis and Cancer Study (STAC)

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Background: Cancer patients have an increased risk of venous thrombosis (VT). Cancer patients who develop venous thrombosis are reported to have poorer survival than those without thrombosis.
Aims: To investigate the impact of VT on survival of cancer patients in a general population.
Methods: We used data from the Scandinavian Thrombosis and Cancer (STAC) cohort, a population-based cohort including 144,952 subjects without previous VT or cancer. During follow-up, incident cancer and VT were registered. 'Cancer-related VT' was defined as VT diagnosed in patients with overt or occult cancer (i.e., cancer diagnosed within 1 year after VT). Survival of subjects without cancer or VT ('disease-free subjects') was compared with survival of subjects diagnosed with cancer and cancer-related VT during follow-up. Cox-regression models with cancer and VT as time-varying exposure were performed to calculate hazard ratios (HR) for death. Sub-analyses were performed across various cancer types and stages and type of VT (i.e., deep vein thrombosis (DVT) or pulmonary embolism (PE)).
Results: During follow-up (mean 11.7 years) 14,621 subjects developed cancer and 2,444 developed VT. There were 567 cancer-related VTs (454 with overt, 113 with occult cancer). The mortality rates (per 100 person-years) for disease-free [BS1] subjects, subjects with VT only, with cancer only and with cancer-related VT were 0.63 (95%CI 0.62-0.64), 5.0 (95%CI 4.5-5.5), 11.6 (95%CI 11.3-11.9) and 45.3 (95%CI 41.0-49.9), respectively. Compared with disease-free subjects, patients with cancer-related VT had a 25.9-fold (95%CI 23.4-28.6) increased risk of death. Compared with patients with cancer only, the risk of death for patients with cancer-related VT was increased 2.7-fold (95% CI 2.5-3.0). Within each cancer type, the occurrence of VT increased the risk of death 1.9 to 12.2-fold among all cancer types studied.
Summary/Conclusion: In a general population, cancer patients with related VT had a much higher risk of death than patients with cancer only, and this effect was seen within all cancer types studied.

OS 10.1

The VWF variant D1472H affects VWF binding to ristocetin in vitro: the usefulness of measuring VWF activity with the Innovance® VWF:GpIbM assay to avoid VWD genotyping

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Background: The diagnosis of von Willebrand disease (VWD) relies on measurements of von Willebrand factor antigen (VWF:Ag) and functional activity. Until now, the gold standard assay for measuring VWF functional activity is the ristocetin cofactor activity (VWF:Rco), that quantifies the binding of VWF to platelets through the surface glycoprotein receptor GpIb, induced by ristocetin. A qualitative defect (type 2 VWD) is suspected when the ratio VWF:Rco/VWF:Ag is inferior to 0.7. However, some polymorphisms, in particular the variant D1472H, can affect this assay *in vitro* and falsely decrease VWF:Rco by interacting with ristocetin. Other tests are available to measure VWF activity, as the Innovance® VWF:GpIbM assay, that does not use ristocetin. This test is using latex particles coated with an antibody against GPIb. A recombinant GPIb including two gain-of-function mutations is added and allows VWF-GpIb binding without ristocetin. The platelet agglutination is detected turbidimetrically. Thus, there is no interferences with these polymorphisms (like D1472H).
Aims: The aim of this work was to reevaluate the biological criterias for inclusion in the French VWD reference center (Centre de Référence de la Maladie de Willebrand, CRMW) and define a new algorithm for inclusion to avoid useless explorations and in particular genotyping, introducing VWF:GpIbM as a selection criteria.
Methods: The CRMW database includes in June 2019 3762 patients in total. 3037 patients had a full characterization with genotyping, including 2764 patients with full VWD phenotypes, 84 healthy relateds and 189 whose VWD was finally excluded. Evaluating centralized potential inclusions, we noticed that some patients had a VWF:Ag >30% and a VWF:Rco/VWF:Ag ratio <0.7, leaving them in a grey area for VWD diagnosis. We looked for genotype correlation and VWD classification for these patients in order to evaluate the misleading impact of VWF:Rco as a trigger for genotyping.
Results: In the CRMW database, we found 1047 patients suspected to have type 2 VWD but with biological measurements in the grey area (VWF:Rco/VWF:Ag ratio <0.7 and VWF:Ag >30%). On these 1047 patients, 227 presented the variant D1472H, including 47 without any other pathogenic variant associated. VWD was finally excluded for these 47 patients after genotyping. We also observed that 34 patients on the 1047 had no pathogenic variant (neither D1472H) described after sequencing the whole *VWF* coding sequence with next-generation sequencing. For this reason, VWD was also excluded for these 34 patients after genotyping. Genotyping remains long and costly and it could have been avoided if the Innovance® VWF:GpIbM assay has been performed at once before genotyping. The VWF:GpIbM/VWF:Ag ratio would probably have been normal (>0.7) for the 47 patients with the variant D1472H, and maybe even for the 34 patients with no variant described, saving 81 *VWF* genotyping.
Summary/Conclusion: We elaborated a new algorithm for CRMW inclusion in Lille University Hospital: for patients with a VWF:Ag >30% and a VWF:Rco/VWF:Ag ratio <0.7 ("grey area"), we realize the Innovance® VWF:GpIbM assay. Patient are included in CRMW only if the VWF:GpIbM/VWF:Ag ratio is also <0.7, thus the genotyping can be performed. When the ratio is >0.7, patients are excluded from CRMW.

OS 10.2

Neither a structured questionnaire at pre-anesthesia visit nor the prescription of routine hemostatic tests have acceptable performances to detect hemostatic abnormalities before surgery

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Background: Identifying patients with bleeding risk before surgery remains challenging. Several studies have demonstrated the very low efficacy of routine pre-operative PT, aPTT, platelet count (PC) for the prediction of bleeding risk. In 2011 several professional societies recommended AGAINST the systematic prescription of routine hemostasis tests and advocated their replacement by an un-validated structured questionnaire.
Aims: The aim of our study was to investigate the diagnostic accuracy of a standardized questionnaire at the pre-anesthesia visit in order to detect patients with a bleeding risk requiring further hemostatic investigations.
Methods: We conducted a multicenter study in 7 French academic hospitals. Inclusion criteria were patients scheduled for surgical interventions (except for cardiac, vascular/thoracic, hepatic surgeries and obstetrics). Exclusion criteria were previously documented risk of bleeding and anti-thrombotic medication.
All patients completed the questionnaire and underwent PT, aPTT, PC, and vWF activity and antigen, factors VIII, IX and XI, Platelet Function Analyzer (PFA) and when required, factors II, V, X and VII, and hemostasis consultation. We compared the diagnostic performance of the two strategies: standardized questionnaire versus routine hemostatic tests.
Results: From January 2015 to January 2018 year, we identified 16/1405 (1.14%) patients with hemostatic abnormalities potentially associated with bleeding risk: 11/1405 (0.78%) with von Willebrand disease, 2 with hepatic impairment, 1 with moderate FXI deficiency,1 with moderate hemophilia A and 1 with platelet defect.
Sensitivity of the questionnaire (score≥2) was 50% (IC 95%:[25-75]) and the specificity 87% (IC 95%:[85-89]). Sensitivity and specificity of the routine hemostatic tests strategy were 75% (IC 95%:[48-93]) and 51% (IC 95%:[48-54]), respectively. Concordance between the 2 strategies was very low (kappa=0.04).
Summary/Conclusion: In conclusion, prevalence of hemostatic abnormalities was of 1.14% in this cohort of 1405 patients. The standardized questionnaire at the pre-anesthesia visit as well as the routine hemostatic tests strategy have poor performances to detect an increased risk of bleeding.

OS 10.3

The effect of direct oral anticoagulants on bleeding and blood product usage in adults undergoing major cardiac surgery compared with warfarin and control groups

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Background: Excessive bleeding is a major complication of cardiac surgery requiring blood transfusion and affecting patient outcomes. Current guidelines suggest the duration direct acting oral anticoagulant (DOAC) discontinuation and/or renal function may influence bleeding and blood product usage, but are based on studies including non-cardiac cases and lack control or Warfarin patients for comparison. Although contrary to current guidelines, it is common practice to perform coagulation screens (Prothrombin time [PT] and activated partial thromboplastin [APTT]) prior to surgery. Whilst such tests have poor Negative Predictive Value and sensitivity in predicting DOAC concentrations, their utility as predictors for bleeding and blood product use in major cardiac surgery has not been assessed.
Aims: To assess:
1) Anticoagulant and antiplatelet (AP) therapy,
2) The duration of DOAC discontinuation or renal function and associated routine coagulation tests
as predictors of bleeding events and blood product usage in adults undergoing major cardiac surgery.
Methods: Retrospective single centre observational study at a UK tertiary cardiothoracic surgery centre. All patients >18years undergoing cardiac surgery from 01.01.15 to 31.10.2018 were considered for inclusion. Patients with missing information or known bleeding disorder were excluded. Data were extracted from Clinical Data Warehouse and patient electronic records. Comparisons were made between each treatment group (Warfarin, DOAC and DOAC + AP) vs control and between Warfarin and DOAC patients. Warfarin was discontinued 5 days prior to surgery and appropriate bridging given. P2Y12 inhibitors were stopped 5-7 days before and Aspirin was continued until the day of surgery.
Results: Of 3325 patients having major cardiac surgery, 2928 were included for analysis. Patients on Warfarin or DOAC were older than controls: 72.5yrs (37–81yrs) and 72yrs (38–83yrs) respectively vs 67yrs (15-94yrs), p=0.046 and <0.001. Median DOAC discontinuation was 5 days (range 1-22 days) for DOAC and 5 days (2-7) for DOAC + AP. There were no differences in bleeding (assessed by haemoglobin fall, volume of red cell salvage and 24-hr surgical drain volume) in patients on DOAC vs controls or Warfarin vs DOAC or Warfarin vs controls. However, patients on Warfarin received more Platelets, Cryoprecipitate and fresh frozen plasma (FFP) vs control: 50%, 25% and 37.5% vs 28%, 5.4% and 19.4%, P=0.006, <0.001 and 0.011, and more Cryoprecipitate and FFP vs DOAC patients: 25% and 37.5% vs 7.8% and 18.8%, P=0.036 and 0.042. There was no difference in the use of blood products in patients on DOAC or DOAC + AP vs controls. Neither the discontinuation of DOAC duration nor the creatinine clearance influenced these findings. Abnormal APTT but not PT predicted increased FFP use but not bleeding during surgery.
Summary/Conclusion: Discontinuation of DOAC for 5 days +/- AP or renal impairment, is sufficient to prevent an increase in bleeding or blood product use among adults undergoing major cardiac surgery. Despite no difference in bleeding compared to patients on DOAC or controls, patients on Warfarin received more FFP and cryoprecipitate. This may reflect surgeons' bias/perception of warfarin. Routine coagulation tests have little predictive value although prolonged APTT was associated with increased FFP use.

OS 10.4

Evaluation of Provision of external quality assurance for thromboelastography cartridge based systems:TEG6 and Rotem Sigma

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Background: UK National External Quality Assessment Scheme for Blood Coagulation (NEQAS BC) has been providing External Quality Assessment (EQA) for 5 years for the TEG 5000 device (Haemonetics Ltd) and the Rotem delta device (Werfen Ltd).
Aims: NEQAS BC aimed to establish whether a similar approach to EQA could be used for the new TEG6 and Rotem Sigma (both cartridge based systems).
Methods: Over a period of 1 year, 3 different lyophilised plasma samples - 1; normal plasma, 2; normal plasma spiked with apixaban and 3; normal plasma spiked with heparin- were distributed to up to 26 TEG6 and 37 Rotem Sigma users together with disposable pipettes and pre-measured diluent to reconstitute the samples. Users were asked to test for 30 minutes and to record measures of speed of clot formation - R time, K time and angle (TEG 6), and CT, CFT and angle (Rotem Sigma); and clot size – MA (TEG 6) and A5, A10 and A20 (Rotem Sigma).
TEG 6 users were asked to return results for the 4 citrated sample channels -CK (with kaolin), CRT (rapid TEG with kaolin and tissue factor), CKH (with kaolin and heparinase) and CFF (with platelet inhibitor to allow determination of fibrinogen effect). Rotem Sigma cartridges are of 2 types -Trauma and Heparin. Users were asked to return results for the Fibtem (with platelet inhibition to assess the effect of fibrinogen), Extem (extrinsic pathway activation),and Intem (intrinsic pathway activation) for both Trauma and Heparin cartridges and Heptem (with heparin inhibitor) -Heparin cartridges only and Aptem (with fibrinolysis inhibitor) – Trauma cartridges only.
Results: TEG 6 results: For the 3 samples, the median R time results in minutes (min) for the 4 channels, were 1)normal sample CK 12.4min, CRT 0.3min, CKH 14.5min and CFF 48.6min. 2) apixaban sample CK 15.5min, CRT 0.4min, CKH 14.2min and CFF 32.3min. 3) heparin sample CK >30 min, CRT 0.6 min, CKH 7.4 min and CFF 36.5 min. The precision of these tests varied from 3.5% to 35.7%.
Rotem Sigma results: For the 3 samples, the CT median results in seconds (sec) were 1)normal sample Fibtem 92 sec, Extem 92 sec, Intem 233 sec, Heptem 239.5 sec and Aptem 91sec . 2) apixaban sample, Fibtem 222.5 sec Extem 249.5 sec, Intem 330 sec, Heptem 330 sec and Aptem 243.5 sec. 3) heparin sample Fibtem 118 sec, Extem 127.5 sec, Intem 452 sec, Heptem 223 sec and Aptem 107sec . The precision of these tests varied from 4.7% to 36.8%.
Summary/Conclusion: Provision of EQA material is possible for these cartridge based systems using lyophilised plasma samples. Participants on the whole gave the results expected for these samples but there is still a lot of variation between results with high percentage CVs.

OS 11.1

Cells of the macrophage lineage secrete factor (F)XIII-A into the plasma

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Background: FXIII-A is secreted by an unconventional pathway and may not be released from all cell-types within which it is synthesized. Studies in thrombocytopenic mice and in a mouse floxed in the *F13a1* gene have indicated that platelet factor 4 (Pf4)-expressing resident tissue macrophages secrete plasma FXIII-A. Resident FXIII-A-expressing macrophages co-express CD163.
Aims: To confirm the myeloid origin of plasma FXIII-A and to identify the macrophage subpopulation involved.
Methods: Plasma FXIII-A was assayed by cross-linking activity and tissue FXIII-A mRNA by qPCR in (i) monocytopenic colony stimulating factor deficient (*Csf-1*$^{op/op}$) mice and *Csf-1*$^{op/+}$ littermates and (ii) in irradiated *F13a1*$^{+/+}$ and *F13a1*$^{-/-}$ mice 10 weeks after transplantation of strain-matched *F13a1*$^{-/-}$ or *F13a1*$^{+/+}$ total bone marrow (BM) respectively. Irradiated *F13a1*$^{-/-}$ mice were also transplanted with cultured *F13a1*$^{+/+}$ BM-derived macrophages or with monocyte-enriched or monocyte-depleted *F13a1*$^{+/+}$ BM cell fractions obtained by immune depletion. Flow cytometry confirmed that the BM monocytes were CD45^{+}/CD11b^{+}/Ly6C^{+}. Cultured macrophages and cell fractions were supplemented with supportive *F13a1*$^{-/-}$ total BM to promote survival, while maintaining a 5-fold excess of candidate *F13a1*$^{+/+}$ cells.
Results: Levels of plasma FXIII-A and tissue FXIII-A mRNA in *Csf-1*$^{op/op}$ and *Csf-1*$^{op/+}$ mice were 20±9% (n=7) and 70±15% (n=5) respectively of levels in strain-matched controls. Normalised levels of mRNAs encoding in the heart (H), aorta (A) and lungs (L) of *Csf-1*$^{op/op}$ mice were reduced to the following levels relative to wild-type mice: FXIII-A 1%(H), 5%(A), 14%(L); CD163 2%(H), 4%(A), 1%(L); Pf4 7%(H), 9%(A), 36%(L); CD11b 8%(H), 9%(A), 57%(L). Transplant of *F13a1*$^{+/+}$ total BM into *F13a1*$^{-/-}$ mice restored plasma FXIII-A to 81±12% (n=7) of WT, while transplant of *F13a1*$^{-/-}$ BM into *F13a1*$^{+/+}$ mice decreased plasma FXIII-A levels to 40±18% (n=7) of WT. Transplant of either cultured macrophages (0.5x10^{6}, n=16) or BM monocytes (0.5x10^{6}, n=7) failed to restore plasma FXIII-A. However, monocyte-depleted BM restored plasma FXIII-A to 81% of WT (n=3).
Summary/Conclusion: The decrease in plasma FXIII-A in monocytopenic, but not thrombocytopenic, mice confirms that plasma FXIII-A derives from myeloid cells. The corresponding decreases in mRNAs encoding FXIII-A and macrophage markers exclude the possibility that plasma FXIII-A was depleted as a response to osteoporosis or other pathology in the *Csf-1*$^{op/op}$ mice, but do not further identify the subpopulation concerned. The decrease in plasma FXIII-A following transfer of *F13a1*$^{-/-}$ BM into *F13a1*$^{+/+}$ mice confirms that irradiation destroys, and BMT replaces, the physiologically relevant cell type. This excludes the possibility that the reconstitution of plasma FXIII-A by *F13a*$^{+/+}$ BM transplanted into *F13a1*$^{-/-}$ mice establishes an ectopic population of FXIII-A secretory cells. The ability of CD11b-antigen depleted BM, but not of CD11b-positive BM monocytes, to reconstitute plasma FXIII-A following BMT, suggests that the secretory cells arise primarily through a CD11b-negative lineage. This agrees with our previous finding that expression of the CD11b-cre transgene only weakly reduces plasma FXIII-A in the *F13a1*$^{flox/flox}$ mouse.

OS 11.2

Acute ischemic stroke thrombi have an outer shell that impairs fibrinolysis

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Background: Thrombi responsible for large vessel occlusion (LVO) in the setting of acute ischemic stroke (AIS) are characterized by a low recanalization rate after intravenous thrombolysis.
Aims: To test whether AIS thrombi have inherent common features that limit their susceptibility to thrombolysis, we analyzed the composition and ultrastructural organization of AIS thrombi causing LVO.
Methods: A total of 199 endovascular thrombectomy-retrieved thrombi were analyzedby immunohistology, scanning electron microscopy (SEM), and subjected to ex vivothrombolysis assay. The relationship between thrombus organization and thrombolysis resistance was further investigated in vitrousing thrombus produced by recalcification of citrated whole blood.
Results: SEM and immunohistology analyses revealed that, although AIS thrombus composition and organization was highly heterogeneous. AIS thrombi shared a common remarkable structural feature in the form of an outer shell made of densely compacted thrombus components including fibrin, von Willebrand factor, and aggregated platelets. In vitro thrombosis experiments using human blood indicated that platelets were essential to the formation of the thrombus outer shell. Finally, in both AIS and in vitro thrombi,the thrombus outer shell showed a decreased susceptibility to t-PA-mediated thrombolysis as compared to the thrombus inner core.
Summary/Conclusion: Irrespective of their etiology and despite their heterogeneity, intracranial thrombi causing LVO have a core-shell structure that influences their susceptibility to thrombolysis.

OS 11.3

Role of the fibrin αC region in mechanical strength, resistance to fibrinolysis and formation of a stable whole blood clot

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Background: The fibrinogen αC-region contains important binding sites for FXIII, α2-antiplasmin and plasminogen, and is comprised of a globular domain (αC-domain) and a connector region. We have previously shown that truncations of the αC-region alters fibrin clot structure in purified systems. Clots made from purified α390 fibrinogen (lacking the αC-domain) demonstrated reduced maximum absorbance compared to wild type (WT), and microscopy showed a denser clot structure with numerous branches. Clots made from purified α220 fibrinogen (deficient in the entire αC-region) had a porous structure with short stunted fibres, but a similar maximum absorbance to WT. However, the relative contributions of the connector and the αC-domain to alterations in whole blood clot structure and function are unknown.
Aims: To understand the influence of the αC-domain and connector on clot lysis, mechanics and whole blood clot contraction.
Methods: Recombinant fibrinogens WT, α390 and α220 were produced in CHO cells, truncations were confirmed by SDS-PAGE and native PAGE. The clotability of the recombinant protein was 97% for the WT, 94% for α390 and 89% for α220. Fibrinolysis was studied through turbidity, and clot mechanics by magnetic microrheology. *Ex-vivo* investigations of clot contraction, red blood cell and platelet incorporation were performed using whole blood from fibrinogen$^{-/-}$ mice reconstituted with recombinant fibrinogens as well as rotational thromboelastometry (ROTEM) and scanning electron microscopy. Interactions between fibrinogen and platelets activated with various agonists was achieved by flow cytometry. Results are shown as mean ±SD.
Results: WT clots showed a 50% lysis time of 74±5 min (n=4), which was similar for α390 (69±12 min, n=4), but much faster for α220 (18±4 min, n=4; p=<0.0001). Clot mechanics for α220 could not be studied without FXIII, as clots were exceptionally weak. With FXIII, α220 clots showed markedly reduced storage modulus (G') (0.05±0.06 Pa, n=4) compared to WT (2.40±0.36 Pa, n=4), in contrast to α390 which showed an increase (3.50±0.74 Pa, n=4). No difference in clot weight, contraction, red blood cell or platelet incorporation was observed comparing WT and α390, while α220 showed no platelet-driven contraction, and no visible clots after 120 min. ROTEM displayed reduced clot firmness for both truncations α390 24±8.5 mm (n=3) and α220 7±2 mm (n=3) whereas the WT was 46±6 mm (n=3). Clotting time was increased for α220 (426±111 seconds n=3) compared to WT (65±18 seconds n=3). Scanning electron microscopy of whole blood α220 clots demonstrated the predominance of cells with limited fibrin. There was no difference in binding of the truncated fibrinogens to platelets compared to WT, suggesting that the results observed were due to alterations in fibrin clot structure.
Summary/Conclusion: Our results show that without αC-domains and connector, fibrin is extremely weak, loses resistance to fibrinolysis and does not support stable clot formation in whole blood. These data indicate that the fibrinogen αC-connector provides critical mechanical strength, stabilises the fibrin network and supports normal whole blood clot formation.

OS 11.4

A newly established murine model shows that fibrin γ-chain cross-linking protects against clot embolization

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Background: Activated factor XIII cross-links fibrin α- and γ-chains, and incorporates fibrinolysis inhibitors into the clot, to increase resistance to mechanical strain and proteolytic degradation respectively. The fibrin γ-chain crosslinking sites are highly conserved. The role of fibrin clot mechanical properties during thrombosis and thromboembolism *in-vivo* is unknown. We have developed a new genetically modified mouse model with mutations in the fibrinogen γ-chains, which were previously shown to abolish fibrin γ-γ cross-linking in human recombinant fibrinogen.
Aims: To investigate the role of fibrin γ-γ cross-linking on clot stability *in-vivo*.
Methods: Genetically modified FGG3X mice were designed and produced by mutating the conserved fibrinogen γ-chain cross-linking residues (γQ423N/Q424N/K431R). Clot formation was analysed *ex-vivo* by ROTEM and *in-vivo* by intravital microscopy using a $FeCl_3$ femoral vein injury model, after injection of AlexaFluor^{488}-fibrinogen for fluorescence labelling of thrombi. A new *in-vivo* model for pulmonary embolism was developed based on $FeCl_3$ injury to the inferior vena cava of mice injected with AlexaFluor^{647}-fibrinogen, followed by *ex-vivo* quantification of the fluorescent emboli by light-sheet microscopy of the lungs. We also developed a new *in-vivo* model for stroke, similar to the pulmonary embolism model, except that the left carotid artery was subjected to $FeCl_3$ injury and brain fluorescent emboli were quantified *ex-vivo*.
Results: Compared to WT (C57BL/6), FGG3X mice showed no differences in growth, blood cell counts, fibrinogen concentration, FXIII activity and tail bleeding time, but were unable to form γ-γ cross-links whilst α-chain cross-linking was unaffected as confirmed by SDS-PAGE analysis. ROTEM analysis of whole blood showed that clotting and lysis times were not significantly different. However, maximum clot firmness was significantly lower in FGG3X mice compared to WT (40.6±1.1 vs 64.6±1.0 mm; n=8, $p<0.001$), indicating a reduction in clot elastic modulus of 37%. Intravital microscopy showed FGG3X mice exhibited a significantly larger number of breakdown events, compared to WT, during thrombus formation (2.1±0.1 vs 1.0±0.3 events/mouse; n=8, $p<0.01$). *Ex-vivo* light-sheet microscopy analysis of the lungs extracted after sacrifice showed a 33% increase in emboli count for FGG3X mice, compared to WT (1924±115 vs 1288±49 emboli; n=8, $p<0.001$). Additionally, preliminary data on brain embolization, resulting from $FeCl_3$ application to the left carotid artery, also showed a 32% increase in emboli count in FGG3X mice, compared to WT (208±9 vs 157±11 emboli; n=4, $p<0.01$).
Summary/Conclusion: Characterisation of this new genetically modified FGG3X murine model of impaired fibrin cross-linking shows that γ-γ cross-linking plays a key role in clot stability during thrombus formation *in-vivo*, and protects against embolization in both veins and arteries. These findings indicate that improved γ-γ cross-linking may help to prevent thrombo-embolic diseases such as pulmonary embolism.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

OS 12.1

An endothelial-enriched G protein-coupled receptor regulates tissue factor expression

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Background: Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), is a serious disease with a population-based incidence rate of 1:1000. The endothelial cells (EC), constituting the inner layer of the vessels, have a central role in regulating coagulation. EC dysfunction is implicated in VTE. A G protein-coupled receptor (*GPCRX*) has previously been identified by our group as an EC-enriched gene. Preliminary unpublished works have shown that the *GPCRX* rs77779514 tends to associate with VTE risk in the MARseille THrombosis Association (MARTHA) case-control study and that this polymorphism was associated with lower *GPCRX* mRNA in EC.
Aims: To study *GPCRX* function and to investigate its potential role in VTE.
Methods: Human umbilical vein EC were treated with siRNA targeting *GPCRX* (*GPCRX* siRNA-EC) or a scrambled control, with or without subsequent addition of the inflammatory cytokine tumour necrosis factor alpha (TNF). Resultant effects were measured using mass spectrometry, qPCR, western blot, flow cytometry and in-house developed *in vitro* coagulation assays.
Results: Mass spectrometry analysis revealed higher tissue factor (F3) expression in *GPCRX* siRNA-EC, relative to control-EC, 24h after TNF stimulation. Measurement of *F3* mRNA over a time course following TNF stimulation revealed the initial increase (0-4h) was comparable between samples, but this upregulation was prolonged in *GPCRX* siRNA-EC, relative to control-EC. At these later time points, increased thrombin generation in plasma and fibrin deposition from flowing whole blood, was observed on *GPCRX* siRNA-EC, compared to controls. The presence of F3 function-blocking antibodies abolished this effect.
Summary/Conclusion: Our results indicate that *GPCRX* is involved in tissue factor regulation and that reduced *GPCRX* expression could induce a more pro-coagulant cellular environment.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

OS 12.2

Evaluation of a novel mouse model of abdominal aortic aneurysm to study platelet mechanisms of the proinflammatory thrombus.

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Background: Abdominal Aortic Aneurysm (AAA) is defined as a permanent localized dilation in the arterial wall with a diameter >50% and characterized by thinning and weakness of the vascular wall. In humans, aneurysms are associated with intramural thrombus and are prone to rupture and often result in death. Thrombi from abdominal aortic aneurysm (AAA) patients are highly enriched in leukocytes and in bacteria, eg. Porphyromonas gingivalis (Pg). This leukocyte-rich thrombus is considered the driving force in vessel wall rupture leading to death. Beyond their role in aneurysmal thrombus formation, platelets support leukocyte recruitment and interact with bacteria. This cross-talk is an important feature in thrombo-inflammatory vascular disease
Aims: To study the role of platelets in the aneurysmal thrombus formation
Methods: AAA was induced by applying an elastase-soaked filter paper on the infrarenal abdominal aorta of wild-type mice. Platelet adhesion and leukocyte recruitment to the vessel wall were analyzed by intravital microscopy, the presence of thrombi was quantified by immunohistology at early and late time points. At day 7 and 14, platelet activation was analysed by flow cytometry.
Results: In elastase-treated WT mice, we observed, by intravital microscopy, an early recruitment of platelets and leukocytes to the damaged vessel wall. The diameter of the aorta was increased two-fold compared to sham mice and histological analysis did not reveal thrombus formation. In contrast, elastase-treated WT mice injected with Pg, immunohistology showed large thrombi in the dilated vessel wall which were enriched with platelets, leukocytes and neutrophil extracellular traps. In addition, histology analysis showed thrombosis occurs in 70% of mice at day 7 and in 50% of mice at 14. At day 7 and 14, flow cytometry analysis shows that platelets are in a preactivated state as well as increased levels of platelet-leukocyte aggregates in elastase-treated WT mice injected with Pg compared to sham
Summary/Conclusion: Here we establish a novel mouse model of abdominal aortic aneurysm allowing us (i) to study platelet activation mechanisms in the initiation and progression of AAA and (ii) test efficient anti-platelet therapies in AAA.

OS 12.3

Thrombin in complex with dabigatran can still activate PAR-1 on endothelial cells

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Background: Direct oral anticoagulants (DOACs) are the preferred choice of anticoagulant treatment for stroke prevention in newly diagnosed patients with atrial fibrillation (AF). During AF, stasis of the blood within the left atrial appendage can trigger coagulation and clot formation, thereby increasing the risk for stroke. In addition, AF progresses due to atrial scar tissue formation, causing a vicious circle of enhanced remodeling, more AF and increased risk for heart failure. We hypothesize that the AF-induced bouts of ischemia/reperfusion trigger inflammation, platelet activation and coagulation inducing a hyper-coagulant environment which can mediate microvascular destabilization through protease-activated receptor (PAR) signaling. Inhibition upstream in the coagulation cascade at the level of FXa with rivaroxaban directly inhibits FXa, blocks PAR-2 signaling of the endothelium and prevents thrombin formation. Inhibition at the level of thrombin with dabigatran or hirudin, permits the formation of FXa, but inhibits thrombin activity and potentially PAR-1 mediated EC-signaling. Dabigatran is a direct, reversible thrombin inhibitor that binds to the active site of thrombin, but not to exosites I or II, whereas hirudin is an irreversible inhibitor, that blocks both the active site and exosite I. These different modalities may have differential effects on EC-barrier function, mediated by PAR-signaling.
Aims: Study the potential role for different DOACs in preventing PAR-mediated microvascular destabilization.
Methods: Loss of endothelial cell-cell contact, initiating microvascular destabilization, was measured using Electric Cell-Substrate Impedance Sensing (ECIS). To study the role of coagulation, platelet-free plasma (PFP) was incubated on an endothelial cell (EC)-monolayer treated with tumor necrosis factor a (TNFa), leading to the expression of TF. Coagulation inhibitors were added to assess the role of coagulation factors Xa (rivaroxaban, 10 µM) or thrombin/IIa (dabigatran, 10 µM or hirudin, 1 µM) on microvascular destabilization, while monitoring their anticoagulant efficiency with a thrombin-activity assay. To investigate the contribution of thrombin's exosite I, we developed a thrombin-binding peptide (D50-S64), mimicking the hirudin-like domain of endothelial PAR-1.
Results: Incubation of ECs with PFP resulted in maximal thrombin activity and barrier loss. Inhibition with rivaroxaban, dabigatran and hirudin completely blocked thrombin-activity. Addition of rivaroxaban or hirudin to the plasma retained optimal barrier function, while in the presence of dabigatran, the endothelial barrier formed initially, but was lost after 20-30 minutes. Addition of PAR-1 derived D50-S64 peptide to dabigatran anti-coagulated plasma restored optimal EC-barrier, in a concentration dependent manner up to the level of hirudin.
Summary/Conclusion: This study shows that preventing the formation of thrombin with a specific FXa-DOAC such as rivaroxaban is more favorable than inhibiting in situ formed thrombin with a FIIa-DOAC such as dabigatran that only blocks the active site. This leaves thrombin's exosite I available for interaction with PAR-1 on the endothelium.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

OS 12.4

The endothelial-enriched protein KANK3 regulates cell motility and tissue factor expression

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Background: The endothelium is the innermost layer of all blood vessels. Endothelial cells (EC) play a central role in the regulation of vascular processes, such as coagulation, inflammation and angiogenesis. Proteins with EC restricted expression (´EC-enriched´) tend to be critical for these functions. We previously identified *KANK3* as a body-wide EC-enriched transcript. The protein encoded by *KANK3* is poorly charaterised and its function is currently unknown.
Aims: To investigate the role of KANK3 in EC function.
Methods: Human umbilical vein EC were transfected with siRNAs targeting KANK3 (*KANK3* siRNA-EC) or a scrambled control, with or without subsequent addition of the inflammatory cytokine tumour necrosis factor alpha (TNF). Expression of coagulation-related EC genes and cell proliferation/migration was measured using qPCR and an *in vitro* scratch assay, respectively.
Results: In *KANK3* siRNA-EC, baseline and TNF-induced expression of tissue factor (*F3*) mRNA was increased, whilst expression of tissue factor pathway inhibitor (*TFPI*) mRNA was decreased, compared to controls. In wound healing assays, *KANK3* siRNA-EC had a faster gap closure rate, in both serum-starved and non-starved conditions, compared to controls.
Summary/Conclusion: KANK3 could have a role in the regulation of coagulation through the modification of EC tissue factor and TFPI. In addition, KANK3 could be important for the regulation of cell migration. If, and how, these roles are linked requires further investigation.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-001

Molecular genetic investigations in Hereditary Hemorrhagic Telangiectasia in Hungary; identification of a founder mutation

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Background: Hereditary hemorrhagic telangiectasia (HHT; Osler-Rendu-Weber syndrome) is an autosomal dominant vascular disease characterized by the presence of epistaxis, mucocutaneous telangiectases and visceral arteriovenous malformations (AVMs). Mutations in the genes for endoglin (*ENG*), the activin receptor-like kinase 1 (*ACVRL1*) and *SMAD4*, which encode proteins of the transforming growth factor-beta superfamily are responsible for the disease. Most recently, other candidate genes are under investigation. Approximately 85 % of HHT cases have heterozygous family-specific mutations either in the *ENG* or *ACVRL1* genes, causing HHT type 1 and 2, respectively. Clinical diagnosis of the disease is based on the four Curacao criteria.
Aims: Our aims were to identify causative mutations of HHT in Hungary, to examine if common mutations exist and to establish a feasible diagnostic algorithm.
Methods: Genetic analysis was performed in HHT index patients and their relatives (total n=109) between 2012 and January 2019. All exons and flanking intronic regions of *ENG*, *ACVRL1* and *SMAD4* were determined by direct fluorescent sequencing. Next-generation sequencing (NGS) was performed in 15 cases, where causative mutations in ENG, ACVRL1 or SMAD4 were not found. Genotyping of markers D12S1677, D12S85, D12S2196, D12S1712, D12S270, rs2071219, rs706815 and rs706816 around *ACVRL1* gene was performed in 14 carriers of the *ACVRL1* c.625+1 G>C and 50 healthy controls to ascertain the possibility of a founder effect.
Results: The mutation detection rate was 50% among the 109 HHT index patients, 50% of patients were detected with ENG (16 known and 6 novel), 48% with *ACVRL1* (3 known and 4 novel) mutations, 1-1% with *SMAD4* and RASA1 mutations (1-1 novel mutation). A novel *ACVRL1* c.625+1 G>C mutation was detected in 6 apparently unrelated HHT families. Haplotype analysis of the above-mentioned genetic markers suggested a founder effect. The genealogical analysis revealed that the possible common ancestors were married in 1779. Using the NGS method, instead of confirming the diagnosis of HHT Haemophilia A and von Willebrand disease type 2N have been detected in two patients, respectively.
Summary/Conclusion: The genetic background of HHT is heterogeneous in this geographical region. Identifying a founder effect in HHT is very useful because the clinical appearance of the disease is much more predictable and simplifies the diagnostic algorithm, which is well suited for diagnosing patients. NGS method can explore the genetic background of unclear bleeding cases.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-002

Risk of minor and major bleeding in patients who switched from vitamin K antagonists to direct oral anticoagulants

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Background: Patients treated with vitamin K antagonists (VKAs) switch to direct oral anticoagulants (DOACs) with increasing frequency. Bleeding is the most frequent and important complication of anticoagulant treatment. Identifying predictors for bleeding on DOACs might lead to improved strategies to prevent bleeding.
Aims: To determine predictors for minor and major bleeding among patients who switched from VKA to DOAC.
Methods: A questionnaire was sent to 2920 former VKA patients from three anticoagulation clinics in the Netherlands who switched to a DOAC between 1-1-2016 and 31-12-2017. Minor bleeding was defined as any bleed (e.g. superficial skin bleeding, nose bleed) that did not require hospital treatment. Major bleeding was defined as any bleed that required hospital treatment. Non-adherence was also studied and identified for patients deviating from the prescribed regimen (*i.e.* who indicate to sometimes forget to take DOAC as prescribed). Odds ratios (OR) and 95% confidence intervals (95%CI) for predictors of bleeding were estimated by logistic regression and adjusted for age and sex where applicable (aOR).
Results: 1399 Patients (48%) responded; 60% were men (n=816), 76% (n=1068) had atrial fibrillation, mean age was 74 years, 31% (n=432) used rivaroxaban, 27% (n=379) used apixaban, 29% (n=404) used dabigatran, 12% (n=164) used edoxaban. 291 patients (21%) experienced a minor bleeding during follow-up. There were 60 patients (4%) who experienced a major bleeding over a median of 199 days of treatment. Several studied predictors overlapped with predictors used in the CHA2DS2-VASc score, such as increasing age, female sex and congestive heart failure. Increasing age (aOR 1.88; 95%CI 1.27-2.79 for patients >75 years vs <65 years), female sex (aOR 1.44; 95%CI 1.10-1.88), and congestive heart failure (aOR 1.43; 95%CI 1.05-1.94) were associated with an increased risk of minor bleeding. A risk factor for bleeding included in the HAS-BLED score, *i.e.* kidney disease, was also associated with an increased risk of minor bleeding (aOR 2.17; 95%CI 0.90-5.25). Other characteristics identified as risk factors for minor bleeding were (history of) cancer (aOR 1.27; 95%CI 0.85-1.91), once daily DOAC use (aOR 1.41; 95%CI 1.08-1.84 vs twice daily), and non-adherence to DOAC (aOR 1.93; 95%CI 1.32-2.81). Hypertension and switching to a DOAC on doctor's initiative (vs own initiative) were also studied, and were not associated with minor bleeding risk. Diabetes seemed associated with less minor bleeding (aOR 0.77; 95%CI 0.52-1.14). For major bleeding most point estimates were about the same, except for DOAC non-adherence (aOR 0.79; 95%CI 0.30-2.03), kidney disease (aOR 0.99; 95%CI 0.48-2.06), and doctor's initiative for switching to DOAC (aOR 2.63; 95%CI 1.03-6.70), but confidence intervals were wide.
Summary/Conclusion: In this study, many clinical variables that are also used in prediction scores such as the CHA2DS2-VASc and HAS-BLED score (increased age, female sex, history of congestive heart failure, kidney disease) were associated with an increased risk of bleeding, as well as once daily DOAC use. This information could be used to improve the safety of DOAC use, for example by more frequent follow-up or lowering DOAC dose for patients with the highest risk of bleeding.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-003

Reversal of apixaban anticoagulation by andexanet alfa in healthy subjects: a subgroup analysis of the reversal activity using an optimized anti-FXa activity assay vs commercial anti-FXa assays

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Background: Andexanet alfa (AnXa) is a modified human FXa protein approved in the US and EU for patients treated with apixaban (Apix) or rivaroxaban, when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding. AnXa binds to FXa inhibitors with high affinity and reduces their anticoagulant activities as demonstrated by the reversal of pharmacodynamic markers in healthy subjects (anti-FXa activity, unbound inhibitor concentration and thrombin generation) and >80% hemostatic efficacy in bleeding patients taking FXa inhibitors. While measuring anti-FXa activity is not required for AnXa treatment, proper assays must be used for testing AnXa-containing samples when they are performed. Current commercial anti-FXa assays, both FXa inhibitor-specific and LMWHs, are unsuitable for measuring the reversal activity of AnXa due to high sample dilutions (e.g.,1:44 with STA-Liquid Anti-Xa), which cause dissociation of the inhibitor from AnXa (due to reversible binding). This results in substantial underestimation of the reversal activity of AnXa and erroneously elevated anti-FXa levels in laboratory tests.
Aims: To evaluate the effect of sample dilutions on Apix anti-FXa activity in plasma samples from healthy subjects following AnXa treatment, using both an optimized and a commercial anti-FXa assays.
Methods: Healthy subjects were treated with oral Apix (5 mg, twice/day) for 5.5 days followed by intravenous (IV) administration of AnXa on Day 6, 3-hours post the last Apix dose (~Cmax) (Siegal et al, Blood Adv, 2017). Reversal of apixaban anticoagulant activity was assessed by an optimized anti-FXa assay (96-well format, manual), using neat plasma and modified reagents ratios from the Coamatic Heparin kit (DiaPharma) with Apix calibrator, which limits the overall sample dilution to ~1:2, thereby preserving the AnXa-inhibitor complex. In parallel, samples from three cohorts treated with AnXa/placebo IV bolus (90, 210, 420 mg) were tested on an automated analyzer using STA-Rotachrom LMWH Anti-Xa (IU/mL) (Stago). STA-Liquid Anti-Xa (ng/mL) (Stago), before and after assay modifications to reduce sample dilutions, were performed with Apix calibrator.
Results: In the Phase 2 studies with Apix in healthy subjects, AnXa demonstrated dose-dependent and ~95% reversal of Apix anti-FXa activity using the optimized assay (manual). The anti-FXa activity (ng/mL) showed good linear correlations with both total (r^2=0.883) and unbound (r^2=0.712) Apix concentrations before AnXa treatment, and unbound Apix concentration after AnXa treatment.
Before AnXa treatment, Rotachrom Anti-Xa (IU/mL) showed good linear correlation with Apix plasma concentration (r^2=0.941). Conversely, for samples obtained after AnXa treatment, an increase in anti-FXa activity was observed with Rotachrom Anti-Xa (IU/mL) compared to ~95% reversal by the optimized assay (manual). Similar increase in anti-FXa level was also observed using STA-Liquid Anti-Xa and Apix calibrator with AnXa samples. Modifications of STA-Liquid Anti-Xa (ng/mL) using neat plasma and modified reagents ratios demonstrated good reversal activity with AnXa-containing samples.
Summary/Conclusion: Before AnXa treatment, anti-FXa levels may be estimated using commercial assays. For AnXa-containing samples after AnXa treatment, modified anti-FXa assays must be used to minimize sample dilutions, which can be performed manually or on automated analyzers. Further optimizations on automated analyzers are underway in collaboration with various manufacturers.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-004

The influence of glomerular filtration rate on bleeding and mortality risk during hospitalization – multi centar cohort

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Background: Renal dysfunction may occur in pulmonary embolism settings due to haemodynamic disturbances. Acute renal dysfunction in hospital settings may be associated with adverse events and in pulmonary embolism with higher bleeding rate. The aim of our study was the investigation of the predictive value of renal dysfunction on intrahospital regarding bleeding and mortality risk in pulmonary embolism patients hospitalized in intensive care units of six university hospitals.
Aims: Ths prospective cohort study, comprised 665 consecutive patients with APE confirmed using MDCT-PA. All patients underwent echocardiography examination on admission and blood samples were collected for Tni, BNP and creatinine assays.
Methods: This prospective cohort study, comprised 665 consecutive patients with APE confirmed using MDCT-PA. All patients underwent echocardiography examination on admission and blood samples were collected for Tni, BNP and creatinine assays.
Results: Based on estimated glomerular filtration rate (GFR), patients were divided into three groups: the first group with the GFR < 30ml/min, the second with GFR 30-60 ml/min, and third with GFR >60 ml/min. There was a statistically significant distribution among the groups regarding the risk of pulmonary embolism ($p<0.0001$). During hospitalization in the first group overall death was recorded in 28 (45.9%), in the second in 42 (18.9%), and in the third in 30 (7.9%) ($p<0.0001$). Pulmonary embolism as a cause of death was recorded in the first group in 18 (29.5%), in the second in 25 (11.3%) and in the third in 17(4.5%) patients ($p<0.0001$). Fatal bleeding was recorded in the first group in 1(1.6%), in the second in 1 (0.5%) and in the third group in 3 (0.8%) patients ($p<0.05$). There were no significant differences regarding major bleeding between groups (p 0.126). A multivariate analysis showed that age and comorbidities, hemodynamic status, TnI and GFR strongly influenced overall death as well as death due to pulmonary embolism, while the anticoagulation therapy influenced the fatal bleeding rate. Administration of enoxaparin and nadroparin on admission were associated with higher overall mortality ($p<0.001$) and pulmonary embolism regarding mortality ($p<0.05$, $p<0.001$) respectively. ESC guided thrombolysis was associated with a higher rate of overall and pulmonary embolism mortality rate ($p<0.001$, $p<0.005$) respectively, as well as higher rate of major bleeding ($p<0.001$). Slow protocol rTPA was not connected with overall and pulmonary embolism mortality rate and major bleeding.
Summary/Conclusion: In patients with pulmonary embolism, the estimation of GFR is crucial in order to predict adverse events and to prevent the bleeding complications caused by inappropriate dosage of anticoagulation and thrombolytic drugs

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-005

DOAC Filter®: a useful tool to monitor unfractionated heparin anti-Xa activity after periprocedural interruption of anti-Xa direct oral anticoagulants

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Background: Although biological monitoring of direct oral anticoagulants (DOAC) is not required, these molecules affect laboratory coagulation tests that can be useful to other diagnoses or monitoring of other anticoagulants. Hence, when therapy with anti-Xa DOAC is bridged with unfractionated heparin (UFH), DOAC-induced interferences impede proper UFH monitoring. DOAC Filter® (Diagnostica Stago, Asnières, France) is currently evaluated to neutralize DOAC-induced interferences on laboratory routine coagulation tests but nothing is know about its effects on UFH anti-Xa activity. Neutralizing DOAC anti-Xa activity *ex vivo* without neutralization of UFH would allow the monitoring of UFH with anti-Xa activity after DOAC interruption when periprocedural bridging is required.
Aims: To assess the effects of DOAC Filter® on UFH anti-Xa activity.
Methods: Control pool plasma spiked with 5 increasing concentrations of UFH (0.2 to 1.5 UI/ml) and frozen plasma samples from 6 patients who were treated by UFH were tested. 600 µl of plasma were transferred into DOAC Filter® and centrifuged 15 minutes at 300g at room temperature. Anti-Xa activity calibrated for UFH (Heparin LRT, Hyphen Biomed, La Neuville sur Oise, France) was measured before and after filtration. Activated partial prothrombin time (aPTT), prothrombin time (PT) and fibrinogen (Clauss method) were also evaluated before and after filtration by DOAC filter®.
Results: As expected, UFH spiked control pool samples caused dose-dependent prolongation of aPTT (median >150 sec, range 51.8 to >150) and UFH anti-Xa activity (median 0,73 UI/ml, range 0,27 to 1.64). PT was decreased for anti-Xa >1.0 UI/ml only and no effect on fibrinogen was observed. After filtration of UFH spiked plasma with DOAC Filter®, aPTT (median >150 sec, range 57.9 to >150) and UFH anti-Xa activity (median 0.81 UI/ml, range 0.29 to 1,81) remained unchanged.
Plasma from patients treated by UFH had a dose-dependent prolongation of aPTT (median >150 sec, range 37,1 to >150) and anti-Xa activity (median 1,0 UI/ml, range 0,47 to 2.28). Filtration of patient plasma samples with DOAC Filter® had no significant effect on aPTT (median 106.5 sec, range 35 to >150) and anti-Xa activity (median 1.13 UI/ml, range 0.48 to 2,39).
Summary/Conclusion: DOAC Filter® was not able to neutralize UFH anti-Xa activity in both UFH spiked plasma and patient plasma receiving UFH. DOAC Filter® could be a useful tool allowing proprer monitoring of UFH anti-Xa activity in patients treated by anti-Xa DOAC and requiring periprocedural bridging with UFH.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-006

Living Well with Haemophilia: The everyday experience of haemophilia in Europe in 2018

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Background: Despite encouraging developments in treatment in recent years, people living with haemophilia (PwH) still carry burdens of the disease on a day-to-day basis.
Aims: The aim of the present study was to explore the everyday life of PwH, including their beliefs and experiences related to their condition, their treatment, and their personal ways of managing the condition.
Methods: In depth interviews were held with 51 PwH A and B in 5 European countries (Italy, Germany, Spain, UK, Ireland), with a range of ages and treatment regimens. Interviews included the wider social ecology of each PwH, including friends, family, and caregivers. In addition, health care professionals (HCPs) at haemophilia treatment centres (HTCs) were interviewed and observed with and without patients. Historical and disease area context was provided prior to the initiation of interviews by five haemophilia experts (practitioners and researchers) to help frame the research design. Data were collected through semi-structured interviews, written exercises, facilitated group dialogues, and on-site observations of PwH interactions with friends, family, and HCPs. The combined data were analysed using various approaches (e.g. inductive and deductive analysis, needs mapping, and clustering exercises).
Results: Forty-two PwH A and nine PwH B, between 1.5 and 82 years old, were included in the study; along with 18 HCPs at 7 HTCs. Thirty (59%) used standard half-life factor treatment, fifteen (29%) were on extended half-life factor treatment, and six (12%) used non-factor therapy. Two PwH interviewed used on-demand treatment; 49 used prophylaxis. The vast majority of PwH reported that they regularly experienced bleeds, pain, and/or a variety of other burdens related to their condition. The most commonly reported burdens included: refraining from certain activities (78%); struggling with injection (53%); and limiting travel and movement (33%). Nearly half (48%) reported regular bleeds and/or joint pain, despite self-reported adherence to prophylaxis treatment. To ease burdens and pain, 39% of PwH created personalised adaptations to care. Four summary insights about the lived experience of haemophilia and its treatment emerged from the combined analysis: 1. PwH experienced a 'perceived normality' around the disease, in spite of frequent limitations; 2. PwH were primarily treated to achieve a baseline level of stability, rather than to overcome limitations imposed by haemophilia; 3. PwH developed their own mental models and personalised care adaptations to navigate uncertainty around treatment; and 4. PwH faced specific challenges depending on their life stage, without necessarily receiving care tailored to each stage.
Summary/Conclusion: Despite improvements in treatment and adherence, PwH experience regular bleeds and restrictions in their daily life. Since many PwH have built a narrative of normalcy around this way of living, the study points to a need to demonstrate and treat for a way of living with expanded possibilities. Given the personal care adaptations and specific life stage challenges, the insights further indicate an unmet need for more flexible and personalized approach to treatment. Such an approach could help reduce challenges facing PwH, their families, and the healthcare system, and building further on this research would prove valuable.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-007

The impact of specimen volume and haemolysis on routine coagulation test results: reducing the recollection burden in a high-throughput laboratory

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Background: The inability to process haemolysed and underfilled specimens has been a perennial problem in routine coagulation testing causing delays, inconvenience and increased costs. While there has always been a degree of ambiguity surrounding the true impact of these pre-analytical variables on test results, the general consensus is that they should not be tested. The controversy has been amplified in recent years with studies suggesting that some test results from haemolysed and underfilled specimens may in fact be reportable. However to date, these findings have not been incorporated into best practice guidelines.
Aims: This study evaluated the effect of both haemolysis and inadequate volume on routine coagulation testing to provide laboratories with more scope to reduce the number of recollected specimens than the best practice guidelines currently allow.
Methods: From November 2016 to March 2017 at Wellington Hospital New Zealand, the Prothrombin Time (PT), Activated Partial Prothrombin Time (APTT) and Fibrinogen results of the original rejected haemolysed / underfilled specimens were compared with those of the recollected specimens. Specimens were rejected if they were >10% underfilled or contained >0.40 g/L free Haemoglobin (Hb). All testing was performed using photo-optical measurements on a Sysmex® CS-2100i coagulation analyser. Statistical analysis was performed using regression analysis and 2-tailed paired t tests.
Results: A total of 83 consecutive paired patient specimens included in the study were either underfilled (n = 41) or haemolysed (n= 42). In volume studies, we showed that accurate results are still achieved for PT if a specimen is >73% filled ($R2 = 0.9879$, $y = 0.9964x$; $P < 0.05$), APTT if a specimen is >79% filled ($R2 = 0.9665$, $y = 0.9752x$; $P < 0.05$) and fibrinogen if the specimen is >59% filled ($R2 = 0.9587$, $y = 0.977x$; $P < 0.05$). It should be noted however that our data was limited below a fill volume of 80%. In haemolysis studies, there was no statistically significant difference in PT results ($R2 = 0.9879$, $y = 0.9637x$; $P < 0.05$) or fibrinogen results ($R2 = 0.9607$, $y = 1.0162x$; $P < 0.05$) at any level of haemolysis. APTTs showed a statistically significant difference overall ($R2 = 0.87$, $y = 0.9684x$; $P < 0.05$), however the only clinically meaningful differences were observed in three paired samples with >1 g/L free Hb. Incorporation of these findings into our processes at Wellington Hospital reduced the number of recollected specimens by approximately 90%.
Summary/Conclusion: This study has validated a minimal fill volume of 80% and a maximum haemolysis level of 1 g/L free Hb or PT, APTT and Fibrinogen. Evidence-based acceptance criteria was developed using these findings resulting in significant cost savings, improved turnaround times and increased satisfaction across clinical and allied health teams. This review offers a clinically appropriate solution to a problem which has always hindered routine coagulation testing.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-008

A new fully automated FXIII activity assay

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Background: Fully automated testing of coagulation factors is advantageous in many aspects and becomes a given in many laboratories. Assays for testing FXIII activity are currently only available as manual methods. Therefore we have developed an automatable assay using a new detection module on a fully automated coagulation analyser. As FXIII plays a pivotal role in the terminal phase of blood coagulation by cross-liking the fibrin network and therefore stabilizing the formation of mature blood clots. Deficiency of FXIII occurs rarely but activity levels found ≤ 30% may be associated with severe bleeding.
Aims: The aim of the study was to develop an easy to run assay for the determination of FXIII activity using a new module on a fully automated coagulation analyser.
Methods: The assay principle is based on the use of a highly sensitive fluorogenic substrate in combination with a thrombin reagent.
FXIII is activated by thrombin into FXIIIa. At the same time, thrombin converts fibrinogen into fibrin. The clotting is prevented by an aggregation inhibiting peptide. FXIIIa cleaves a dark quenching molecule from the side chain of a modified peptide incorporating glycine methyl ester. Subsequently, the fluorescence of an N-terminal coupled dye increases and can be monitored on-line.
Results: A linear calibration curve ranging from 0-80% could be established using SSCLOT4 plasma ($R^2 \geq 0.9$). Intra assay precision for normal plasma (78%) and abnormal plasma (38%) was ≤4%. Day-to-day precision for normal and abnormal plasma was ≤5%. Recovery tested with different dilutions of SSCLOT4 plasma was between 95% and 105% using both dilutions in assay buffer and FXIII depleted plasma. A method comparison was performed with FXIII samples covering the whole assay range including FXIII samples ≤5% using a turbidimetric FXIII antigen assay. FXIII activity levels measured were comparable with the antigen concentration ($r \geq 0.9$). Reagent stability was ≥ 8h on board and ≥ 24h at 2…8°C .
Summary/Conclusion: This study has demonstrated that measuring FXIII activity levels with this fluorogenic assay is an appropriate method for determining this critical factor in the stabilisation of blood clots. The assay is designed to be automatable on a new fully automated coagulation analyzer (under development).

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-009

Dosage of factor IX in persons with Hemophilia B treated with recombinant FIX linked to an IgG Fc domain: Comparison of 3 methods in a French hospital

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Background: Management of persons with hemophilia B (PwHB) is currently moving ahead with extended half-life product, i.e. recombinant FIX linked to an IgG Fc domain (rFIX-Fc, ALPROLIX®, Sobi). Ensuring optimal therapy is challenging due to discrepancies between FIX dosages using one-stage-assay (OSA) and chromogenic-stage-assay (CSA).
Aims: Compare two OSA methods and one CSA method (considered as the reference method) for the dosage of FIX in PwHB receiving rFIX-Fc.
Methods: A monocentric study was conducted in Bicetre haemostasis laboratory. FIX was measured in 183 samples originating from 21 PwHB using CSA (RoxFactorIX®, Rossix, CS-5100® Siemens) and OSA methods: STA-CKPrest®/STA-ImmunodefIX® (CK) and STA-CEPHASCREEN®/STA-ImmunodefIX® (CFS) on a STARMax® device (Stago). Statistical analyses included comparison using Deming regression and Bland-Altman analysis.
Results: Bland-Altman analysis found a systematic negative bias between CFS (-3.76) and CK (-9.71) compared to CSA. Comparison between methods, using Deming regression, confirmed a systematic underestimation of CFS (-19% on average) and CK (-39% on average) versus CSA. Deming regression slope and intercept were computed at 0.61 (CI95% slope=[0.59;0.64]) and 0.36 (CI95% intercept=[-0.17;0.90]) respectively for CK, and at 0.81 (CI95% slope=[0.78;0.83]) and 1.18 (CI95% intercept=[0.59;1.77] respectively for CFS. Relative difference between CK and CSA ranged 47-52% whatever FIX levels. Relative difference between CFS and CSA ranged 18-20% for FIX ranging 10-110IU/dL (absolute difference 3.7-8.84IU/dL) and reached 51% for FIX ranging 0-10IU/dL (absolute difference 1.54IU/dL).
Summary/Conclusion: Our study showed that, when monitoring FIX levels in PwHB receiving rFIX-Fc using OSA methods 1/CK should be avoided, 2/CFS is not reliable for FIX< 10IU/dL, 3/CFS is in agreement with CSA for FIX ranging 10-110IU/dL with a relative error around 20%, which is commonly tolerated in the monitoring of anti-hemophilic factors. Therefore, CFS, which is an inexpensive, easy and all-day long available method, can confidently be used for PwHB receiving rFIX-Fc for values commonly used to adjust treatment.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-010

Evaluation of a new extraction thromboplastin reagent with ISI close to 1.0 for the measurement of prothrombin time, international normalized ratio and factor assays.

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Background: A new prothrombin time (PT) reagent (STA-NeoPTimal) was tested on a STA R Max2 analyzer.
Aims: The aim of this study was to compare the performance of this new reagent with three other PT reagents (Readiplastin, STA-Neoplastine R (NeoR) and STA-Neoplastine CI Plus (CI+)) some being from recombinant origin and other from extraction.
Methods: All the experiments were performed on a STA R Max2 analyzer.
Repeatability and reproducibility were tested for the 4 reagents using 2 levels of lyophilized commercial control plasmas (PT and factor II, V, VII and X assays). Heparin sensitivity was assessed by spiking normal plasma with unfractionated heparin or enoxaparin at 0, 0.5,1.0,1.2, 1.5 and/or 1.8 IU/ml. Sensitivity to direct oral anticoagulants was compared by testing plasmas from patients receiving dabigatran (n=12), rivaroxaban (n=13), apixaban (n=17) or edoxaban (n=1). Comparison of the 4 reagents (PT and factor II, V, VII and X assays) was also performed using different normal (n=20) and abnormal plasmas (VKA patients (n=47), preoperative patients (n=23) and liver failure patients (n=12))
Results: CVs for within run precision (normal level) ranged from 0.9% (NeoPTimal) to 1.2% (Readiplastin). CVs for within run precision (pathological level) ranged from 0.8% (CI+) to 1.5% (NeoPTimal).
CVs for between run precision (normal level) were 1.9% (NeoR) and 2.3% (NeoPTimal). CVs for between run precision (pathological level) were 1.5% (NeoR) and 4.5% (NeoPTimal).
All reagents suffered minimum interference with LMWH and UFH.
For preoperative patients and liver failure patients, NeoR and CI+ showed good correlation with Readiplastin (r= 0.93) but NeoPTimal had decreased performance (r=0.89).
For VKA patients, all reagents demonstrated good correlation with Readiplastin (R: NeoPTimal 0.98, NeoR: 0.998 and CI+: 0.99). However, there are significant differences (difference of more than 0.5 INR units) between methods for samples within the therapeutic and above the therapeutic ranges. Readiplastin appeared to give higher INR values than CI+ and NeoPTimal. For NeoPTimal, a difference of more than 0.5 INR units was observed in 13 samples.
On the same VKA patients, NeoPTimal, NeoR and CI+ showed excellent correlations in comparison to Readiplastin for factor II, V, VII and X assays (R>0.9) except for factor V assay (NeoR: 0.88, CI+: 0.86)
Poor responsiveness of the PT to DOAC concentrations was observed. There is no significant difference between the sensitivity of the 4 thromboplastins. A normal PT(%) or PT (ratio) cannot exclude DOAC anticoagulant activity. For concentrations of DOAC < 50ng/ml (n=18), PT(%) and PT (ratio) were never above the cut-off (1.2 or 75%) with the NeoPTimal and CI+.
Summary/Conclusion: NeoPTimal showed comparable performance relative to Readiplastin, CI+ and NeoR. It is suitable for VKA control, detection of factors II, V, VII, X deficiency and assessment of liver disease coagulopathy. However, for patients receiving VKA, there are significant differences between methods for samples within the therapeutic and above the therapeutic ranges, which claims for good interaction between laboratory and clinicians to establish new cut-offs. Poor responsiveness of the PT to low DOAC concentrations was expected and confirmed for all reagents, meaning that PT assay is not sufficient to detect DOAC treatment in emergency situations.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-011

Importance of classification and genetic tests in the diagnosis and differential diagnosis of von Willebrand disease type 2N

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Background: Von Willebrand disease (VWD) is an inherited bleeding disorder caused by a dysfunction of primary hemostasis. Type 2 VWD includes a wide range of qualitative abnormalities of von Willebrand factor (VWF) resulting in different subtypes. Type 2N is characterized by markedly decreased VWF affinity for coagulation factor VIII (FVIII), while all other functions of VWF are usually normal. Considering treatment, differentiation of Type 2N VWD from Haemophilia A is very important.
Aims: We present cases with type 2N VWD and Haemophilia A to demonstrate the importance of provident laboratory investigation for exact diagnosis.
Methods: Coagulation and platelet function screening tests, initial (immunological and functional assays of VWF, FVIII activity) and classification VWD tests including VWF:FVIII binding ELISA assay and DNA sequencing were performed.
Results: Patient 1 is an 8-year-old male patient with APTT prolongation due to low (5%) FVIII activity. In his case the normal VWF:FVIII binding assay result ruled out type 2N VWD. With molecular genetic test hemizygous p.A723T mutation was found in F8 gene, which confirms the diagnosis as Hemophilia A. Patient 2 and 3 are 9-, and 10-year-old girls without bleeding symptoms, in their cases hemostasis screening tests were carried out before tonsillectomy and prolonged APTT was detected due to low FVIII activity (22% and 19%, respectively). VWF levels and VWF activities were in the reference range. Their extremely low VWF:FVIII binding assay results verified type 2N VWD. DNA sequence analysis showed that patient 2 carries homozygous p.R854Q mutation in exon 20, while this mutation was identified in heterozygous state in patient 3 who also displayed the p.Y795C mutation in heterozygous form in exon 18 of the VWF gene. Patient 4 is a 14-year-old boy whose anamnesis showed massive bleeding following tonsillectomy. Among the screening tests PFA-100 closure time with collagen-epinephrine cartridge and APTT were prolonged. His FVIII activity was 17%, while VWF level and VWF activity were slightly decreased. The low VWF:FVIII binding assay result (11%) verified type 2N VWD. The patient carries heterozygous p.R854Q mutation in exon 20 (paternal allele), and also displays heterozygous p.L757Vfs*22 deletion in exon 17 and heterozygous p.R852Q mutation in exon 20 (maternal allele) of the VWF gene. These molecular genetic test results prove that this patient is compound heterozygote for a mutation in the region of the gene coding for the FVIII binding domain and has a VWF null allele. His mother's VWF level and activity were below the reference range. Siblings of the proband are healthy, however they are carriers of the paternal allele.
Summary/Conclusion: These cases demonstrate that despite normal VWF immunological and functional assay results the patient may suffer from rare VWD subtype 2N. However even this subgroup is heterogeneous and abnormality of these test results does not exclude the Normandy variant. Classification VWD tests followed by molecular genetic investigation have to be performed for proper diagnosis.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-012

Developing an Assay for Anti-Emicizumab Antibodies in Patients with Haemophilia A

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Background: Haemophilia A is an X-linked bleeding disorder. Patients have a deficiency in clotting factor VIII meaning they are at high risk for uncontrollable haemorrhages, particularly within joints. Conventional haemophilia treatment involves recombinant factor VIII as a replacement therapy, both on-demand and prophylactically. Unfortunately, approximately a third of patients develop antibodies against the factor VIII protein (inhibitors), rendering the treatment ineffective. For patients with inhibitors, there are fewer treatment options to control bleeding episodes. Emicizumab (Hemlibra®) is a humanised, bispecific monoclonal antibody, which acts as a mimetic for factor VIII function so can therefore be used to successfully treat haemophilia A patients with inhibitors. It was commissioned for use within the NHS in July 2018. However, based on the HAVEN clinical trials, emicizumab has been found to be immunogenic resulting in 14 patients producing antidrug antibodies (ADAs), some with drug-neutralising potential. Currently, there is no commercially available test for anti-emicizumab ADAs which may have life-altering consequences for patients with haemophilia A who are unknowingly using a failing prophylaxis.
Aims: This project aims to develop the reagents for a diagnostic assay to rapidly assess whether patients treated with emicizumab are producing ADAs.
Methods: Recombinant proteins (GloBody™, *patent pending*) consisting of each single chain variable fragment of emicizumab with a dual nanoluciferase enzyme between the heavy and light chain were produced. The GloBody™ bind to any circulating ADAs against either specificity of emicizumab. The ADA-GloBody™ complexes are captured on protein G agarose beads and emit luminescence given a positive result. 6 serum samples of haemophilia A cases treated with emicizumab were then tested for ADAs compared to negative controls.
Results: In the case of the tested samples, no significant difference in measured luminescence was seen between 5 of the samples treated with emicizumab versus the wild-type serum. This likely indicates that these patients are not producing ADAs against emicizumab. One patient had a significant increase in measured luminescence over the negative controls ($p=0.016$), suggestive of ADAs against the FIXa region of emicizumab. The lack of a positive control for the assay does however limit the impact of this result. A storage stock of the GloBody™ reagent was created meaning that further patient samples can be tested providing a fuller clinical picture for their management.
Summary/Conclusion: The reagents for an anti-emicizumab ADA assay were successfully developed and a storage stock of GloBody™ was created for future use. The assay was initially trialled on 6 haemophilia A patients treated with emicizumab, a majority of whom show no signs of producing ADAs against emicizumab. In future, the development of a positive control for the assay will further supplement the data obtained from testing serum samples.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-013

Evaluation of the analytical performances of new apixaban & rivaroxaban low range anti-FXa activity assays on STA-R & STA-Compact Max after reversal by andexanet alpha

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Background: Portola Pharmaceuticals has developed andexanet alfa (AnXa) to reverse direct factor-Xa inhibitors (DXaI). AnXa has been approved in the US and EU for patients treated with apixaban or rivaroxaban, when reversal of anticoagulation is needed due to life-threatening or uncontrolled-bleeding. While it is not required to assess anticoagulation after administration of AnXa for clinical-decision making, if measured, the regular anti-FXa assays need to be modified to accurately measure its reversal activity, due to the high sample dilution used in the existing assays.
Aims: To evaluate the analytical performances of two modified apixaban and rivaroxaban anti-FXa assays for measuring residual anti-FXa levels in presence of AnXa in spiked samples on STA-R® and STA-Compact® Max using STA®-Liquid Anti-Xa with STA®-Apixaban and STA®-Rivaroxaban calibrators.
Methods: Assay optimizations were performed using commercial STA®-Liquid Anti-Xa with neat plasma samples to minimize dilution effect on AnXa reversal activity.
Five levels of normal pooled plasma spiked with apixaban or rivaroxaban and three levels of AnXa were used. Automated calibration and quality control (QC) recovery were performed with STA®-Apixaban, STA®-Rivaroxaban calibrators and STA®-Calibrator level 0, Pool-Norm or reference frozen pool as diluents. Two STA-R® and one STA-Compact® Max were used.
- Limit of detection (LoD) determined on STA-R® using DXaI-spiked plasmas.
- Within-run (n=21) and day-to-day precision (10 series) determined on STA-R® using 2 levels of QC and 8 levels of apixaban or rivaroxaban-plasma reversed by AnXa.
Results: - LoD is 4 ng/mL for apixaban and 6 ng/mL for rivaroxaban.
- Within-run CVs are less than 5%.
- Day-today maximum CV of 10% is observed with QC and AnXa-containing samples.
- Good agreement between Stago modified assays and Portola microplate assay using spiked samples.
Modified STA-Liquid Anti-Xa assays can be easily installed on STA-R® and STA-Compact® Max. STA®-Calibrator level 0, Pool-Norm or reference frozen pool can be used as diluent with minimal plasma dilution to perform a low range calibration curve. Maximal absolute difference between diluents is 8 ng/mL.
Summary/Conclusion: These new fully automated "reversal" set-ups have been developed to measure DXaI from 6 to 90ng/mL in apixaban or rivaroxaban-patient samples following AnXa-treatment. Results obtained with spiked plasmas show good agreement compared to the reference method.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-014

Acquired FXIII deficiency due to anti-FXIII-A autoantibody; unusual laboratory and clinical characteristics

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Background: Acquired hemorrhagic diathesis due to anti-factor XIII (FXIII) autoantibody is a rare, but severe disorder with a high number of fatal outcome. In a review of 2018 (Muszbek et al. J Thromb Haemost 2018;16:822-32) 47 well characterized cases with antibodies against FXIII-A and a single one with anti-FXIII-B were described and new approaches were proposed for characterization of the autoantibody.
Aims: To diagnose and characterize the autoantibody that after a minor trauma caused huge muscular hematoma on each thigh of a 67-year-old female without previous bleeding history and to follow the clinical course of the patient.
Methods: FXIII activity was measured by two commercial ammonia release assays the Berichrom and Technochrom FXIII assays. The latter assay was used with and without blank subtraction. FXIII antigens were determined by ELISA (Katona et al. Thromb Haemost. 2000;83:268-73, J Immunol Methods 2001;258 :127-35) and by Western blotting. The cross-linking of fibrin was evaluated by SDS PAGE. The inhibition of FXIII activity of normal plasma by the addition of the patient's plasma was expressed in Bethesda units (BU). The inhibitory activity of the patient's IgG was expressed as 50% inhibition (IC50) of plasma FXIII. The binding affinity of the patient's IgG to FXIII-A was measured by surface plasmon resonance (SPR). The effect of the patient's IgG on thrombin and Ca2+ induced FXIII activation and on activated FXIII (FXIIIa) was investigated as described earlier (Pénzes et al. J Thromb Haemost 2016;14:1517-20).
Results: 17% and 18.5% FXIII activity was measured in the patient's plasma by the Berichrom and Technochrom FXIII assay without blank compensation. The Technochrom kit also contained blank reagent and after blank subtraction the FXIII activity was <1%. The considerable overestimation of FXIII activity was very likely due to higher plasma ammonia level. The cross-linking of fibrin γ-, and a-chains was completely blocked. High titer (74 BU) of anti-FXIII autoantibody was measured by the Nijmegen-Bethesda assay. An IC50 of 74.0 µg patient's IgG /mL confirmed the high inhibitory activity. The antibody showed high affinity toward FXIII-A2 (Kd 2.77±0.66 x10^{-9} M). was <0.5% FXIII-A2 and FXIII-A2B2 antigens were measured in the plasma by ELISA, but interestingly FXIII-A could be well detected by Western blotting. The autoantibody did not affect the cleavage of FXIII-A by thrombin; its main effect was the inhibition of Ca2+-induced activation. To a lesser extent it also inhibited the transglutaminase activity of activated FXIII. Eradication therapy and occasional FXIII replacement decreased the BU titer but FXIII activity remained low and the patient suffered large spontaneous hematoma of diaphragm pillars and the rectus abdominis muscle. Surprisingly, four months after her last hemorrhagic symptom, she presented a femoral deep vein thrombosis and pulmonary embolism which required a vena cava filter.
Summary/Conclusion: 1/ Detailed characterization of an anti-FXIII-A autoantibody was demonstrated. 2/ Blank compensation is required to obtain adequate FXIII activity values. 3/ Due to the patient's interfering antibodies immunoassays might not give valid FXIII antigen level. 4/ The patient having anti-FXIII autoantibody is not fully protected against venous thromboembolism.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-015

Outcome of major haemorrhage in a tertiary cardiothoracic centre in patients with activated major haemorrhage protocol vs non-activated protocol

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Background: Major haemorrhage (MH) is a significant cause of mortality and morbidity. A Major Haemorrhage protocol (MHP) should be implemented in every hospital to reduce delays in provision of blood products and improve patient outcomes. We hypothesised that patients who experienced a true MH but did not triggered activation of the MPH would have a worse clinical outcome than patients with activated MHP.
Aims: 1. To assess whether non-activation of MHP can affect patient outcome
2. To identify potential variables which affect patient outcome other than activation of MHP following a MH.
Methods: A retrospective single centre observational cohort study in a major cardiothoracic centre in the UK from January 2016 to December 2018. MH was defined as patients who received more than 5 units of RBCs in less than 4 hours, or more than 10 units in 24 hours as per National Comparative Audit of Major haemorrhage 2018. Patients were identified from hospital transfusion database. Patients who did not meet National Comparative Audit definitions and those age <16 years were excluded from further analysis.
Results: 132 patients were identified as MH (23 had activated MHP and 109 non-activated MHP). There were no differences in age, sex, baseline haemoglobin, platelet count, coagulation screen or renal function between the two groups. However, patients with activated MHP had significantly higher mortality rates at 24hrs and 30 days compared to patients with non-activated MHP (p=0.003, p=0.044 respectively). There were no significant differences in the mortality at 24 hrs (p=0.11) or at 30 days (p=0.17) between patients receiving or not receiving anticoagulants prior to MH. There was no significant difference seen in baseline haemoglobin or platelet count in survivors and deceased at 24hrs or 30days.
Higher fibrinogen was associated with increased mortality at 30 days but not at 24hrs. Despite the MHP recommendations, only 26% patients received tranexamic acid. Mortality was higher at 30 days but not at 24hrs in those who received tranexamic acid. Total number of blood products usage was significantly higher in patients who died both at 24hrs and 30 days than survivors. The total number of red blood cell (RBC) and platelet units received were higher in deceased patients than survivors but no difference was seen in the number of FFP units. There was a higher mortality when patients received a disproportionally higher number of RBC units compared to FFP without following current recommendation of RBC: FFP ratio of 1:1.
Summary/Conclusion: Patients with activated MHP had significantly higher mortality at both 24 hrs and 30days. This, and the fact that they received more red cells and platelets (opposite of any survivor bias), suggests they were correctly recognised as having more severe bleeding. There was a higher mortality when patients received a disproportionally higher number of RBC units compared to FFP counter to the MHP and current recommendations of RBC: FFP ratio of 1:1. Dilutional coagulopathy may therefore contribute to mortality although elevated fibrinogen at baseline was not protective and was associated with higher mortality at 30-days.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-016

Gastrointestinal bleeding in patients with bleeding disorders

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Background: Gastrointestinal (GI) bleeding is a well-recognised complication of bleeding disorders. While less common than other sites of bleeding, it has a significant mortality rate of 10%.
Newcastle-upon-Tyne Hospitals (NUTH) conduct an annual audit of the management of upper GI bleeding at the Royal Victoria Infirmary (RVI). However, GI bleeding occurring specifically in patients with bleeding disorders is less well researched.
Aims: To carry out a quality improvement project; analysing the incidence, presentation and management of GI bleeding in patients with bleeding disorders at the RVI.
To compare this data to the 2018 NUTH audit.
Methods: All patients under the care of the RVI for their bleeding disorder, who were admitted to hospital with a GI bleed between 1st January 2017- 31st December 2018 were included.
Data was collected retrospectively from electronic medical records, discharge summaries and transfusion laboratory records.
Patient demographics, admission details, admitting bloods, endoscopy findings, treatment (inpatient and four weeks post discharge) and readmission rates were analysed.
Findings were compared to the 2018 NUTH audit.
Results: 15 admissions for GI bleeds occurred in 10 patients over the 2 year period; 3/10 patients had more than one admission.
Type of bleeding disorders: 6 patients had Von Willebrand Disease and 4 had Haemophilia A.
The median age of patients was 53, averaging younger than the patients in the NUTH audit (71), and there was a 1:4 female: male ratio.
Melaena was the most common presenting symptom and the median length of admission was 7 days.
Of the haemoglobin (Hb) results available, 11/14 were low on admission and 13/14 Hb had dropped from patients baseline.
For 3/15 admissions, endoscopies were not carried out with acceptable reasons.
70% of the endoscopies were carried out within 24 hours which is less that the 81% in the NUTH audit. The most common finding on endoscopy between patients was 'no source of bleeding found' in 4/12 (33%) of scopes, compared to NUTH's 19%. 100% of the scopes carried out after 24 hours found not active source of bleeding.
13/15 admissions received factor replacement as an inpatient and 47% required ongoing replacement as outpatient or home therapy post-discharge.
3 patients were readmitted within 4 weeks with recurrent GI bleeds (1 following self-discharge). These patients either had a significant co-morbidity or were found to be on medication known to increase the risk of GI bleeds, or both. 2 patients had ongoing symptoms post-discharge but were not readmitted.
Summary/Conclusion: GI bleeding is occurring in patients with 'mild' bleeding disorders and at a younger age than those in the NUTH upper GI bleed audit.
Endoscopies for patients with bleeding disorders are carried out later and are less likely to find a source of bleeding; this may be because patients are more likely to receive treatment earlier in their admission.
Ongoing treatment and readmission is common in this patient group, and co-morbidities can increase the likelihood of this.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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P-017

Adaptation and comparison of the technical performance of two chromogenic FVIII assay reagents

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Background: The use of chromogenic methods of factor VIII (FVIII) assays is increasingly widely used for hemophilia A (HA) diagnostic and treatment monitoring. The French group of haemostasis and thrombosis (GFHT) recommend the use of a chromogenic method for laboratory monitoring of substituted HA (1). Some coagulation machines, including that using viscosimetric (mechanical) detection, are widely represented in France, but the supplier does not propose a complete adaptation of the assays of FVIII chromogen, in particular with the FVIII bovine reagent that may be necessary for laboratory monitoring of HA with inhibitors and/or patients treated with emicizumab.
Aims: The goal of the study is to assess analytics performances of a chromogenic FVIII activity reagent A in comparison with reagent B on a viscosimetric detection coagulometer.
Methods: Validation of methods was realized according to the supplier on the coagulometer STAR MAX® (STAGO). Repeatability and reproducibility were assessed with quality internal controls (QC) with normal level (NC), low level (LC) and very low level of control (LC 10: LC diluted 10 fold). Contamination, linearity, detection limit and quantification limit were assessed too. Analytics performances of reagents were assessed, comparing results of 49 samples, mostly from HA patients receiving efmorofocog alpha, for whom a pharmacokinetic (PK) was realized. ECAT foundation controls assays (European external quality evaluation) were performed.
Results: For reagent A (Siemens) repeatability was assessed on LC10 (n=30), LC (n=25) and NC (n=30), with a coefficient of variation respectively at 3.74%, 5.25% and 5.38%. For reagent B (TriniCHROM) repeatability was assessed on LC10 (n=10), LC (n=9) and NC (n=10), with a coefficient of variation respectively at 14.56%, 1.33% and 4.49%.
According to Ricos tables (2), repeatability are not compliant for both reagents for some levels of controls (CV>4.8%).
For reagent A (Siemens) reproductibility was assessed on LC10 (n=12), LC (n=12) and NC (n=15), with a coefficient of variation respectively at 6.50%, 9.04% and 10.78%. For reagent B (TriniCHROM) reproductibility was assessed on LC10 (n=34), LC (n=29) and NC (n=29), with a coefficient of variation respectively at 30.0%, 11.4% and 11.7%.
According to Ricos table (2), reproducibility data are compliant for reagent A (CV<19.1%) but not for reagent B for the LC10.
Linearity was checked for both reagents which are compliant. Comparison of both reagents was realized from 49 samples or QC. Both methods are correlated with a correlation coefficient of 0,983 (slope 0.99, intercept 2.76) and a mean bias of -5.11 %. No systematic error was observed. PK are not significantly different between the two methods. ECAT results were compliant for the two methods.
Summary/Conclusion: Analytics performances of chromogenic reagent A and reagent B are good. Ricos acceptable criterias, wich regard chronometric FVIII assay are difficult to extrapolate to chromogenic assay. Both reagents are acceptable for substituted HA monitoring and PK evaluation. However, probably due to a single polynomial curve which may has limitations for very low values detection, precaution must be taken for sample of severe or moderate HA diagnostic.. The chromogenic reagent A has however a better CV (reproducibility and repeatability) on very low values (LC10) on our viscosimetric coagulometer.
(1) Lasne D *et al.* Ann Biol Clin (Paris). 2019 Feb 1;77(1):53-65.
(2) https://www.westgard.com/biodatabase1.htm

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-018

Frequency of large deletions and duplications of F8 gene in hemophilia A patients from Serbia

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Background: Large genomic rearrangements, deletions and duplications in *F8* gene, are cause of hemophilia A (HA) in 1.4-6% of patients. These mutations usually produce a severe HA phenotype and approximately 41% of hemophiliacs, carrying a large deletions, develop FVIII inhibitors. Due to guidelines, in patients with severe HA, the identification of these structural variants is recommended if the status for recurrent *F8* inversions of intron 22 and 1 showed as negative.
Aims: During the investigation of molecular-genetic base of HA patients from Serbia, this study was create to determine frequency of large deletions and duplications of *F8* gene in Serbian hemophiliac population.
Methods: After the detection of recurrent HA inversions in 70 severe HA patients, for those with negative status, 37/70 patients, MLPA analysis was obtain in order to define presence of large deletion and duplication of *F8* gene.
DNA samples used for investigation were isolated from peripheral blood. The multiplex ligation dependent probe amplification analyses (MLPA) was handle with 50ng of template DNA and PCR products were separated on ABI 3500 capillary sequencer.
Results: In our cohort of 70 HA patients, after screening for intron 22 and 1 inversions, 37 patients with negative status were undertaken for MLPA analyses. Large genomic rearrangements were found in 5/70 (7%) hemophiliacs, four of them males and one female with severe HA. Deletions were detected in two cases: deletion of exon 7 and deletion of 1-22 exons in female patient. Duplications was presented in three male genotypes: duplication of exons 6-20, duplication of exons 2-22 and duplication of exons 24-26 of *F8* gene. According to morphology of founded mutations, different mechanisms of their origin could be predict (result of intrachromosomal recombination, result of second event in *F8* gen, etc). Clinical follow up showed presence of FVIII inhibitor in one patient with deletion of exon 7. Family members of probands (two mothers and two sisters) were also analyzed for carrier status. Positive status was obtained in one mother and one sister, and all families got adequate genetic counseling.
Summary/Conclusion: Defined frequency of large deletion and duplication in *F8* gene, in studied cohort of HA patients from Serbia, was in line with literature data. The results also confirm the importance of large sequence variants detection in HA patients and their families and using of MLPA as a method of choice for probands as well as for carrier status detection.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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P-019

Management of cardiovascular patients with hemorrhoidal bleeding under antiplatelet or anticoagulant therapy

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Background: Hemorrhoids is frequent complicating condition in a lot of patients cardiovascular patients. Regular antiplatelet/anticoagulant therapy able to increase a risk of recurrent hemorrhoidal bleeding in them. The discussed challenge is how to reach a balance between risks of hemorroidal bleeding and cardiovascular thrombotic complications.
Aims: The study was aimed to find an influence of anticoagulant/antiplatelet therapy on recurrent hemorrhoidal bleedings in patients with cardiovascular diseases and then to build medicamental and surgical algorithm of hemorrhoid treatment.
Methods: From January 2018 to March 2019 we studied cardiovascular 80 patients complicated chronic combined hemorrhoids (2-4 stages). The patients have been taken antiplatelet (ASA, Clopidogrel) and/or anticoagulant therapy (vitamin K antagonists, NOACs). All patients were tested with lab tests, anoscopy and colonoscopy. Hemorrhoidal bleedings as their intensity and frequency, and life quality were analysed. Basing on that 41 patients with recurrent hemorrhoidal bleeding consisted Group 1, and 39 patients with rare hemorrhoidal bleeding represented Group 2. Patients of Group 1 were underwent Doppler guided hemorrhoid artery ligation with recto-anal repair (HAL-RAR) with no interrupting antithrombotic therapy, whereas antithrombotic therapy was rejected for 3-7 days in order to implementation of hemorrhoidectomy by Milligan-Morgan in Group 2.
Results: Antiplatelet/anticoagulant therapy has increased the intensity but not frequency of hemorrhoidal bleedingin 68 %. In Group 1 the bridge-therapy with LMWH was used in 12 patients with warfarin; in one patient NOAC apixaban was rejected 24 hours before surgery and restarted 12 hour after. No bleeding or thrombotic complications were observed postoperatively in this Group. All of these patients have been denying recurrent hemorrhoidal bleeding as well.
In the early postoperative period hemorrhagic complications requiring rectal revision and hemostatic sponge tamponing have been noted in 2 patients of Group 2. One patient had double antiplatelet therapy (ASA plus clopidogrel) and underwent surgery with Hb 112 g/L; PLT $105*10^9$/l; APTT 38,04 sec; PT 17 sec; fibrinogen 4.9 g/L. During rectal revision blood leak detected under a suture was stopped by hemostatic sponge and tamponing with 3% hydrogen peroxide solution. The second patient who had bridge-therapy from warfarin to LMWH developed minor bleeding from puncture points of mucous lining. His hemostasis parameters belonged references ranges in general (Hb 96 g/l; PLT $422*10^9$/l; APTT 36.04 sec; INR 1.8; fibrinogen 4.9 g/l). Reliable surgical hemostasis was reached by rectal tamponing for 1.5 days.
Summary/Conclusion: The HAL-RAR as a minimally invasive surgical method did not require interrupting antithrombotic therapy, and the one have not affected systemic hemostasis. However we recognize that bridge-therapy may be neededin some cases. The most critical point associated with this method is the meticulous surgical hemostasis.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-020

Chromogenic Factor VIII in Haemophilia A plasma of patients with emicizumab

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Background: Factor VIII activity (FVIII:C) assays of samples containing recombinant FVIII or the new bispecific antibody emicizumab (Haemlibra) can be associated with differences in FVIII recovery in vitro between one-stage-clotting-assays and chromogenic assays. Chromogenic assays designed with human proteins, offer the possibility to measure the activity and recovery of this new drug in patient samples.
To monitor FVIII-INH titers with Nijmegen-Bethesda-Assays in haemophilic patients treated with emicuzumab, it was shown that only chromogenic assays with bovine proteins can be used.
Aims: Aim of this study was to investigate if chromogenic assay containing both, human and bovine proteins, can be used to measure the recovery of emicizumab in patient samples or the assay can be used in FVIII-INH determination by Nijmegen-Bethesda-Assay.
Methods: Plasma from Haemophilia-A patients was used for spiking with Refacto, Fanhdi and emicizumab. The FVIII activity was determined with one-stage-clotting-assay and a chromogenic assay with human FIXa and bovine FX protein. Both assays were calibrated with reference plasma prepared from normal pooled plasma. FVIII-INH was determined by modified Nijmegen-Bethesda-Assay.
Results: The regression parameters of the chromogenic FVIII activity determination of Haemophilia-A sample spiked with increasing concentrations of Fanhdi and Refacto showed a good correlation with a slope of 0.91 and r2= 0.9951 for Fandhi and slope 0.71 and r2 = 0.995 for Refacto. As expected the one-stage-clotting-assay underestimates Refacto in patient plasma, as shown by a slope of 0.4.
Chromogenic FVIII assay showed no significant sensitivity to emicizumab concentrations within the expected therapeutic range and recovery of FVIII-INH titer was 100±5%.
Summary/Conclusion: Chromogenic assay with both human FIXa and bovine FX protein can be used for therapy monitoring of full length and truncated FVIII preparation but is insensitive to emicizumab concentrations in therapeutic range. FVIII-INH titer in chromogenic Nijmegen-Bethesda-Assay is insensitive to emicizumab.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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P-021

Case study: Laboratory experience of severe Haemophilia A inhibitor patients treated with Emicizumab

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Background: There has been a number of recent developments in the clinical options available for treatment of Haemophilia A with key benefits for patients including improved quality of life with less treatment days. In the case of patients with Factor VIII inhibitors treatment has always been clinically challenging in ensuring effectiveness. The pharmaceutical development of an antibody that directly binds factor IXa to factor X without the need for presence of factor VIII has provided a significant advance in the treatment of Haemophilia A patients with inhibitors.
Aims: To assess effect of Emicizumab therapy on results of local diagnostic assays and use in assessment of effectiveness of treatment and potential bleeding risk.
Methods: Assays were performed on citrated patient plasma Pre and Post Emicizumab over a 5 month period. Activated Partial Thromboplastin Time (APTT) was performed using TCoag TriniCLOT APTT HS. One-stage factor VIIIC assay was performed using TCoag TriniCLOT APTT HS, Technoclone FVIII deficient plasma and TCoag reference plasma. Chromogenic factor VIII assays was performed using Technoclone chromogenic FVIII:C assay using human IX and bovine X reagents and Siemens chromogenic FVIII:C assay using bovine IX and X reagents. Specific one-stage assay for Emicizumab was established using R^2 Diagnostics calibrator. All clotting factor assays were performed on the Destiny Max analyser (Stago). Rotational thromboelastometry was performed on the ROTEM measuring EXTEM and INTEM. Thrombin generation was performed using the CAT analyser (Thermo) and Thrombinoscope reagents.
Results: As expected APTT results significantly shorted into the normal range with associated high increases in one-stage factor VIII with a weak correlation between results. Post treatment analysis using ROTEM showed a significant INTEM improvement in Clot Time (CT) and Clot Formation Time (CFT) and Endogenous Thrombin Potential (ETP) and Peak Thrombin using thrombin generation which improved and stabilised by Week 3 of therapy. Both chromogenic assays gave patient FVIIIC levels <1.0iu/dL and one-stage assay for measuring Emicizumab detected circulating levels.
Summary/Conclusion: In summary, effect of Emicizumab on local diagnostic assays has been characterised with APTT and one-stage factor VIIIC results providing evidence of active circulating drug. Chromogenic FVIII assay results using a bovine source of factor X were not affected by circulating levels of Emicizumab. Thromboelastometry and thrombin generation may be a useful diagnostic tool to access effectiveness of individual patient treatment.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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P-022

Calibrated automated thrombogram values in pediatric craniosynostosis surgery

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Background: Craniosynostosis is the premature closure of skull sutures in young children. Surgical correction is required at an early age to release increased intracranial pressure and to normalize cranial shape. During surgery massive blood loss occurs as well as dilutional coagulopathy. Haemostasis is usually monitored by standard coagulation assays such as the prothrombin time (PT) and activated partial thromboplastin time (APTT), although they only partly reflect haemostasis. In contrast, analysis of thrombin generation describes all phases of the haemostatic process and might provide a better assessment of global haemostasis. Thrombin generation in infants undergoing craniosynostosis surgery has never been described.
Aims: To evaluate thrombin generation using calibrated automated thrombography (CAT) and other coagulation tests, such as PT, APTT, and rotational thromboelastometry (ROTEM) FIBTEM, in infants undergoing craniosynostosis surgery.
Methods: This study is an add-on study of a double-blind, placebo-controlled, randomized trial investigating the effect of fibrinogen concentrate on total blood loss and number of transfusions needed in infants undergoing craniosynostosis surgery. In these children blood samples for coagulation studies were obtained before surgery immediately after induction of anesthesia, and during surgery after administration of crystalloids and colloids but before transfusions of red blood cells, plasma and platelets.
Results: 108 patients were included in our study, with 54 in the fibrinogen group and 54 in the placebo group. Baseline characteristics were similar in both groups. Endogenous thrombin potential (ETP) and peak height significantly decreased during surgery in the placebo group, but not in the fibrinogen group (ETP 1115±174 nM*min at baseline to 1114±124 nM*min during surgery (p=0.81)(fibrinogen group) vs. 1096±155 nM*min at baseline to 987±137 nM*min during surgery (p<0.001)(placebo group); peak height 163±43 nM at baseline to 157±28 nM during surgery (p=0.16)(fibrinogen group) vs. 168±40 nM at baseline to 144±31 nM during surgery (p<0.001)(placebo group)). FIBTEM-maximum clot firmness (MCF) decreased significantly during surgery in both groups, but remained higher in the fibrinogen group than in the placebo group during surgery (FIBTEM-MCF 9.0 (7.0-12.0)mm (fibrinogen group) vs. 5.0 (4.0-8.8)mm (placebo group)(p<0.001)). APTT and PT prolonged significantly during surgery in both groups; PT levels during surgery were significantly longer in the placebo group than in the fibrinogen group (PT 12.1 (11.6-12.6)sec (fibrinogen group) vs. 12.6 (11.9-13.7)sec (placebo group)(p=0.003)). Hemoglobin levels decreased significantly during surgery in both groups, but were not significantly different between groups. No significant differences had been found in total blood loss and number of transfusions needed between both groups (total blood loss 637 (541-830)ml (fibrinogen group) vs. 650 (542-819)ml (placebo group)(p=0.72); red blood cells 279 (210-392)ml (fibrinogen group) vs. 263 (223-350)ml (placebo group)(p=0.28)).
Summary/Conclusion: In children undergoing surgery for craniosynostosis, administration of fibrinogen concentrate seems to improve global haemostasis as reflected by thrombin generation and ROTEM results. However, this had no influence on the total blood loss and number of transfusions needed between both groups.

ECTH 2019
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P-023

A multicenter experience with idarucizumab "in real world" as reversal anticoagulation of dabigatrán

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Background: Idarucizumab is the first reversal agent approved for dabigatran. and works by binding to the drug compound to neutralize its effect in major bleeding and emergency surgeries. However there is not patients reported outcomes of using idarucizumab "in real world".
Aims: Analyze the use of idarucizumab as reversal agent of dabigatran in current clinical practice in the hospitals of Aragón (Spain) from 2016 ut to now.
Methods: From January-16 to date, 23 patients were registered in this multicenter retrospective study. We analyzed clinical variables and laboratory tests; efficacy, safety and thrombogenic capacity of the idarucizumab and related mortality with its use.
Results: The indications of dabigatran in patients were non-valvular auricular fibrillation in 22 patients and aortic valve prosthesis in 1 pt. The indications of idarucizumab were emergency surgeries in 16 pts, major bleeding in 6 pts and 1 pt with severe renal failure requiring dialysis received the drug off-label. Time from the last dose of dabigatran to the administration of idarucizumab was <12h in 15 patients. Surgeries requiring reversal anticoagulation were 1 splenic rupture, 2 aortic dissections, 1 cardiac transplant, 1 cholecystectomy, 1 stroke fibrinolysis, 1 nephrectomy, 3 lower limb thrombectomies, 1 stroke thrombectomy, 1 ankle osteosynthesis, 1 femur fracture, 1 left nephrostomy, 1 strangulated umbilical hernia and 1 drainage subdural hematoma. One patient was at renal failure (clearance creatinine <30 ml/min). Activated partial thromboplastin time (aPTT) and prothrombin time (PT) were prolonged in 15 patients prior to idarucizumab infusion. Two hemorrhagic complications related with surgery: retrocardiac hematoma and cerebral hemorrhage, were registered. The use of the drug in major bleeding was 4 pts with intracerebral hemorrhage, 1 with retroperitoneal hemorrhage, 1 lower gastrointestinal bleeding and 1 hematuria. aPTT and PT were prolonged, and one patient was at renal failure. Neither adverse effects or thrombotic events were identified. Six patients died in relation with intraventricular hemorrhage, heart transplant complications, septic shock and multi-organic failure respectively.
Summary/Conclusion: Our series includes 23 patients treated with idarucizumab to reverse the anticoagulation effect of dabigatran in hospitals of Aragon. In our experience "in real world" idarucizumab reversed immediately, completely, sustainably and safely the anticoagulation with dabigatran. The implementation of multidisciplinary protocols would optimize the cost / effectiveness ratio of idarucizumab in the approved clinical indications.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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P-024

Correlation of the parameters of thromboelastogram with the results of coagulation tests in hemophilia A patients with prophylaxis

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Background: In order to control the treatment of haemophilia patients, classical coagulation methods (APTT, FVIII (IX)) and global tests are used. One of the tests for a comprehensive coagulation assessment is thromboelastography (TEG), which can provide immediate results and reflect the hemostatic status of the patient during therapy. Some of the parameters obtained from the TEG can be compared with the coagulation tests since they describe similar processes of coagulation.
Aims: To carry out a correlation analysis of TEG parameters with the parameters of coagulation tests to determine the role of TEG in the control of prophylactic treatment and the development of inhibitors in patients with severe haemophilia A.
Methods: 9 haemophilia A patients were treated with 45 ± 5 IU / kg of FVIII of body weight twice a week prophylactically an incremental recovery test (IR) at a dose of 60 ± 5 IU / kg body weight was performed. Prior to and after administration, TEG was performed and the PT, APTT, fibrinogen, FVIII, FVIII:Ag, vWF:Ag and the platelet count were measured. The possible dependencies of 10 TEG parameters (R, K, a-Angle, MA, TMA, SI, SP, G, LY30, TPI / c) and coagulation tests results were analyzed.
Results: Significant direct correlation of the R and SP on the PT and APTT was found (r> 0,71) prior to the administration of the FVIII concentrate. The negative average correlation between R and FVIII:Ag (r = - 0,56) was established. The elongation of the PT and APTT and the decrease in the activity of FVIII:Ag causes a decrease in the total hemostatic potential of the SI in the direction of hypocoagulation (r = 0,75). Prior to administration of the calculated dose, the concentration of MA, TMA and a-Angel inclination strongly and directly depends on the level of vWF:Ag and platelet count (in all cases r> 0,7). After administration of the FVIII concentrate in haemophilia A patients, a significant positive correlation between the response time R and the APTT (r = 0,64) was found. The growth of the CI coagulation index is associated with an increase in the level of FVIII: Ag (r = 0,75) and a shortening of the APTT (r = - 0,76). Other dependencies between TEG parameters and hemostatic tests have not been established.
Summary/Conclusion: Correlation of TEG parameters and results of traditional coagulation tests characterized similar processes of coagulation and was revealed in haemophilia A patients on prophylactic treatment. For R, SI, SP depending on the values of the PT, APTT, FVIII: Ag, the end point is coagulation. MA, TMA, a-Angel depend on the level of vWF: Ag and platelet count which reflects the initiation of haemostasis and the strength of clot. TEG fully reflects the changes in hemostasis, so thromboelastography can be successfully applied to control the treatment and the development of FVIII (IX) inhibitors in hemophilia patients.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-025

A new fully automated ADAMTS-13 activity assay

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Background: ADAMTS-13 activity is a unique marker for thrombotic thrombocytopenic purpura (TTP) which helps differentiate TTP patients from those suffering other more common thrombotic microangiopathies (TMA) and also less frequent conditions such as hemolytic uremic syndrome. Treatment for TTP patients includes plasma exchange. However, for most TMA patients, PEX is an unnecessary and expensive treatment.
In routine, it may take an external laboratory between days and weeks to return the results of ADAMTS-13 activity. The preventive treatment initiated for a high risk TTP patient amounts to thousands of dollars. Moreover, routinely available ADAMTS-13 diagnostic is essential for the monitoring of TTP treatment and control of relapse rates. Thus, an in-house laboratory delivering results on the same day, spares the cost of unnecessary treatments and improves the overall outcome of the patient care.
Aims: The aim of this study was to develop a fully automated ADAMTS-13 activity assay.
Methods: The assay principle is based on a modified FRETS-VWF73 Substrate. ADAMTS-13 from the diluted plasma sample cleaves the substrate in a dose dependent manner. The generated fluorescent signal is proportional to the activity of ADAMTS-13 signal.
Results: The newly developed assay covered an assay range 0.0-1.0IU/ml with calibrators directly traceable to the WHO standard. The LLoQ of the assay was determined well below 0.01IU/mL. The ULoQ was tested up to 2.0IU/mL. In a method comparison with the chromogenic ELISA plasma samples covering the whole assay range were analyzed. The regression between the two assays exhibited a good correlation (r>0.90) with an intercept <0.01 IU/mL.
Summary/Conclusion: This feasibility study demonstrates that this developed assay enables the accurate assessment of ADAMTS-13 activity and also the presence of antigenic inhibitors. The assay is designed to be automatable on a new fully automated coagulation analyzer.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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P-026

Investigating a potential new bleeding mechanism through novel, atypical GPIb-IX-V receptor complex mutants

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Background: The GPIb-IX-V receptor complex is essential in the initial tethering of platelets to the site of vascular injury, via binding to von Willebrand factor(1). Mutations to any one of the proteins that form the complex are associated with Bernard-Soulier syndrome, typically an autosomal recessive disorder that results in macrothrombocytopenia, reduced platelet surface expression of GPIb-IX-V, and a range of bleeding symptoms(2).
A cohort of patients with mild, unexplained bleeding have been recruited to the Genotyping and Phenotyping of Platelets (GAPP) project, and whole exome sequencing has uncovered a number of *GP1BA* and *GP1BB* sequence variants which are atypical, in that complex expression is retained on the platelet surface(3). There are also observed examples of autosomal dominant inheritance within the cohort. It is hypothesised that these sequence variants may have a novel, previously uncharacterised effect on the receptor which is distinct from Bernard-Soulier syndrome, leading to a mild bleeding disorder.
Aims: This project aims to identify a range of novel, atypical sequence variants within the GPIb-IX-V complex and investigation the effects on these variants on receptor structure, localisation and function.
This technically integrated approach will combine platelet function testing, advanced microscopy and structural investigation in an effort to characterise a novel mechanism that contributes to bleeding.
Methods: In line with the GAPP project workflow, *GP1BA* and *GP1BB* sequence variants were identified using whole exome sequencing. Potentially pathogenic variants were identified with *in silico* analyses. Other, previously established bleeding disorders were ruled out via a panel of investigations which indicated normal cell-surface expression of GPIb-IX-V and normal aggregation responses. These variants of interest are to be introduced into human cell lines via CRISPR/Cas9 gene editing for localisation and functional studies.
Results: Thus far, several *GP1BA* and *GP1BB* variants of interest have been identified. These are all novel and monoallelic with previously unseen localisation within their protein sequence, which may suggest a previously uncharacterised bleeding mechanism of disease.
Summary/Conclusion: Given the novelty of these sequence variants, both in incidence and nature, it is hoped that multi-faceted investigation of their consequences will elucidate a new mechanism by which GPIb-IX-V mutation may lead to disease/the presentation of bleeding symptoms.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-027

Rare bleeding disorders in southern Iran: revisited and updated data

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Background: Rare bleeding disorders(RBDs) include deficiency of coagulation factor I, II, V, VII, X, XI, XIII and combined Factor VIII & V. The inheritance is generally autosomal recessive pattern with incidence rate of one in 500000 for factor VII deficiency to one in 2 million for factor II and factor XIII deficiencies. Periodic epidemiological studies render to improve knowledge towards this rare disorders, specially in countries like Iran where consanguinity marriage is common.
Aims: The updated frequency of diagnosed patients with RBDs is detremined in Fars provine, southern Iran, until end of 2018. The phenotypic pattern as well as the treatment options of RBDS are also evaluated.
Methods: In this cross-sectinal study, all demographic, clinical and paraclinical data of patients (n=120) who have been registered as RBDs at Shiraz hemophilia comprehensive treatment center were evaluated and analyzed with SPSS version23 software. A designed questinanaire adapted from www.rbdd.org/pro-rbdd is used to gather all required information.
Results: The mean age of the patients was 23.9 years (range, 2–86 years).Sixty three patients were Female and 57 were male. Near half of the patients (44.2%) were from Shiraz, capital of Fars province.The rate of consanguinity marriage was 61.7% among RBDs.The patients were referred to hospital because of different reasons including bleeding events (66.7%) , kinship with proband screening (15%), surgery (10%) and 8.3 % for confirmed diagnosis. The most common RBDs were FVII deficiency (32.5%) and the rarest one was FII deficiency (1.7%).The prevalence of factor X, XI, XIII,V and fibrinogen deficencies was 18.3%, 15%, 11.7%, 11.7% and 7.1% respectively. The most prevalent bleeding events were epistaxis (28.3%), hematoma (21.7 %), menorrhagia (20.6%), oral cavity bleeds (15.8%) and hemarthrosis (15.8%) .The most intra cranial hemorrhage (ICH) has occurred in the patients with FXIII (21.4%), FI (10%) and FVII (7.7%) deficiencies. Hematoma was the most common symptom in FI and FXIII deficiencies with the same frequency (50%). Menorrhagia was also observed in all types of RBDs.
Summary/Conclusion: The distribution of RBDs is somewhat different in southern Iran compared to what was reported in other parts of the world.The frequency of Factor X (18.3%), XIII (11.7%) and XI (15%) are different versus worldwide prevalence of 8%, 6.5% and 26.5% respectively.Therefore, premarital screening might be applicable in areas where consanguineous marriages and frequency of RBDs are common.The bleeding manifestations patterns were observed similar to other reported data, although in our study, a significant higher frequency of hematoma in FI and FXIII deficiencies were recorded. Based on our results, risk of ICH should be taken into account in patients with FXIII, FI and FVII deficiencies and specific management of menorrhagia in women with all types of RBDs is essential.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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P-028

Treatment with recombinant Von Willebrand factor (VWF) in type 2 Von Willebrand patient for a total knee replacement (TKR) and comparison with a precedent TKR with plasma-derived VWF concentrate

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Background: We report the case of a 64-year-old women with von willebrand disease (VWD) type 2 who was referred for a right knee replacement. She was diagnosed with VWD at the age of 44 because of menorrhagia, excessive bleedings after her two deliveries and red cell packed transfusions for rupture of an ovarian cyst. As she had a poor response to desmopressin she had been treated with plasma-derived Von Willebrand factor concentrate (PDVWFC) sometimes in association with factor VIII (FVIII) for several surgical procedures: right ankle arthrodesis, abdominal plasty, and left knee replacement.
Aims: Laboratory tests revaled FVIIIC: 61 %, VWF antigen (VWF:Ag) :31 % % (normal 50–150%), and VWF activity as measured by glycoprotein 1 activity (VWF:GP1bM; Innovance®, Siemens): 5% and prolonged PFA® test >300 secondes with both cartridges (PFA-200®, Siemens®), VWF:GP1bM/VWF:Ag ratio 0.16. In previous laboratory tests, VWF activity measured by ristocetin cofactor activity (VWF:RCo) was observed less than 10% (normal 50–150%), Multimers study showed loss of high (H) and intermediate (I) molecular weight multimers (MWM) consistent with type 2 VWD, probably a type 2M disease.
Methods: As FVIII was in normal range before surgery (61%), we decided to treat her with recombinant von willebrand (rVWF) concentrate (vonicog alfa) alone for this surgical procedure. She received 44 UI/kg one hour before the procedure, followed with 29 UI/kg infusion 12 hours later.
Results: In constrast with what is seen with PDVWFC, we observed a normalization of the PFA tests correlated with VWF and multimeric profile. VWF GP1bM/VWFAg ratio was 1.18, 1h after 3900 UI and 1.07 before re-infusion (day 1, 12h) and H and I MWM were present. From day 1 to day 8, VWF GP1bM/VWFAg ratio correlate with the presence of HMWM, The ratio VWF Ag/VWF:Gp1bM was in agrement with multimeric profile during all the perfusion of rVWF. With PDVWFC VWF:RCoVWFAg ratio remain at 0.5 or lower. She received four other perfusions of 2600UI of rVWF during the next 7 following days. VWF antigen or activity remained >100% up to day 3. When we compared laboratory results with those observed during the preceeding knee replacement five years ago, a 1.9 UI/kg increased in VWF activity measured with VWF:RCo with PDVWFC was observed compared to a 3 UI/kg increased for VWF activity with VWF:GP1bM method with rVWF was observed. With VWF:Ag mesurement she had 3 UI/kg increase with PDVWFC and 2 UI/Kg increase with rVWF.
Summary/Conclusion: Low molecular heparin was administrated to prevent thrombo-embolic disease during 35 days following surgery no bleed was reported and the evolution was uneventful Screening for antibody anti-VWF was negative 3 weeks after last perfusion. This case suggests that because of an improved multimeric profile, rVWFC may offer a better profile. In addition, laboratory management of patients receiving rVWFC will be easier than for PDVWFC therapy, because laboratory measurment of VWF:Gp1bM seems to be more reliable and PFA 100 analyzer could be useful to monitor the treatement. Further studies or case report are needed to confirm our results.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Unexplainable acute bleeding due to acquired factor XIII deficiency induced hyperfibrinolysis in patients with chronic phase of chronic myeloid leukaemia– three case reports

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Background: Chronic myeloid leukaemia (CML) is a common myeloproliferative neoplasm with an incidence of 1 – 2 cases per 100000 adults[1,2].It is characterized by fusion of Abelson (ABL1) gene on chromosome 9 at breakpoint cluster region (BCR) gene on chromosome 22. This results in expression of BCR-ABL oncoprotein which activates tyrosine kinase that promotes leukaemogenesis[1]. The natural course of CML defines three phases - the chronic, accelerated and blast. Latter two phases contain higher blast count and bleeding in these could occur due to marrow infiltration and thrombocytopenia. Bleeding in chronic phase with<05 % blast count is rare, and, if occurs, it is described due to platelet function defect, disseminated intravascular coagulation or acquired von Willibrand disease[3,4]. However, bleeding due to an acquired deficiency of isolated coagulation factor has not been reported. Herein we report 3 cases of acute bleeding, due to isolated factor X111 deficiency induced hyperfibrinolysis in the chronic phase of CML, and how they were managed.
Aims: To highlight and discuss pathophysiological aspects pertaining to rear occurrence of acute bleeding due to acquired factor XIII deficiency in the chronic phase of chronic myeloid leukaemia.
Methods: The clinical findings and results of laboratory investigations, including primary coagulation profile, ROTEM test and factor XIII assay of three cases of the unexplainable bleeding chronic phase of chronic myeloid leukaemia were analysed.
Results: All three cases showed normal primary coagulation profiles [PT, APTT, TT], higher maximum lysis at both EXTEM and INTEM with normal APTEM indicating hyper-fibrinolysis suggestive of factor XIII deficiency which has been confirmed by factor XIII assay. The possibilities of platelet function defect, acquired von Willibrand syndrome, dysfibrinogenemia and disseminated intravascular coagulation were excluded by normal maximum clot firmness (MCF) in the INTEM and EXTEM, normal von Willibrand factor antigen and recof activity (vW profile), clauss fibrinogen activity and normal D dimer levels.
Summary/Conclusion: Acute bleeding is rare in the chronic phase of CML. If bleeding occurs, it is described due to a part of disseminated intravascular coagulation or platelet dysfunction. However, unexplainable bleeding due to acquired and isolated coagulation factor X111 deficiency induced hyperfibrinolysis has not been reported in the chronic phase of CML. And such bleeding manifestations are linked to the high impact of morbidity and mortality. The diagnosis of acquired coagulopathy depends on haemostatic assessment beyond first level coagulation investigations. The management of acquired coagulopathy depends on the specific coagulation factor replacement and concurrent targeted therapy for CML.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

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Inhibitor against coagulation Factor XIII: A case report

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Background: Deficiency of Coagulation factor XIII (FXIII) is one of the rare bleeding disorders with incidence of 1:2,000,000 births. Umbilical cord bleeding, delay of wound healing, intracerebral hemorrhage (ICH), ecchymosis and hematomas are common clinical manifestations among these patients. Prophylactic therapy is recommended to reduce the risk of bleeding including ICH.
Aims: This study outlines a case of FXIII deficiency that developed an autoantibody after repeated exposures to plasma-derived FXIII concentrate.
Methods: A 3-year-old female was diagnosed with severe congenital FXIII deficiency (c.1262-1263ins(ACGC)(p.H422Rfs*8) in F13A1 gene) following bleeding from the tongue when she was 2-year-old. The patient was assigned to get prophylaxis with plasma-derived FXIII concentrate (10-15 U/Kg/ month) after her diagnosis was confirmed. She was referred to hemophilia comprehensive treatment center with ecchymosis and hematoma after one-year prophylaxis regimen. The dose of prophylaxis was elevated to 25-30 U/kg monthly, however, no improvement was observed. Therefore, evaluation of inhibitor against FXIII was scheduled. Mixing study with normal pooled plasma was performed and 5M Urea was used to evaluate the stability of formed clot which was abnormal after 24 hours. In the next step, a serial dilution of patient plasma was prepared and was mixed with NPP. Then, clot solubility with 5M Urea test was done for each dilution. The clot that was seen after 24 hours in 1/8 dilution had a denser structure in comparison to 1/4 and in the same way 1/4 vs 1/2 dilution.
Results: The laboratory investigation revealed the presence of inhibitor in this patient but due to lack of commercial FXIII activity assay kit, titration of FXIII inhibitor was not possible. New prophylaxis with high dose plasma-derived FXIII concentrate (37-40 U/Kg, every month) has been scheduled for the patient which was satisfactory following six months treatment. No sign of significant bleeding has been observed during this new prophylactic regimen after that.
Summary/Conclusion: Inhibitor against congenital FXIII deficiency is rare and should be taken into consideration when a patient does not respond to conventional on-demand or prophylaxis regimens. It seems higher doses of FXIII concentrate can work to control bleeding events and should be applied as a first optional treatment as we have seen in our case report. Also, availability of commercial kit plays an essential role to evaluate the titer of inhibitor and follow up of these patients.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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P-031

Systematic review of the risk of bleeding in patients with renal insufficiency treated with tinzaparin

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Background: The progression of renal failure is associated with an increase in the risk of bleeding. The elimination half-life of low molecular weight heparins (LMWH) increases as the glomerular filtration rate (GFR) decreases. Some LMWH recommend a dose adjustment for patients with a GFR<30 ml/min. However, the risk of bleeding should be minimized but not at the expense of increasing the risk of recurrence.
Aims: The aim of this study is to analyze the risk of bleeding in patients with venous thromboembolism treated with tinzaparin.
Methods: A PubMed, EMBASE, BIOSIS and International Pharmaceutical search was conducted. Articles and abstracts containing information on bleeding complications in patients with renal insufficiency treated with full doses of tinzaparin were selected.
These studies were able to discriminate, in the tinzaparin arm, groups with GFR<30 ml/min compared to GFR>30 ml/min or GFR<60 ml/min compared to GFR> 60 ml/min. The articles were excluded if the patients were dialyzed or the bleeding could not be assigned to any GFR degree. Overall, 8 studies (4 randomized clinical trial and 4 prospective observational studies) with 1831 patients were included.
Results: Major bleeding (MB):
Six (3.7%) out of 161 patients with GFR<30 ml/min and 25(3.4%) out of 732 patients with GFR>30 ml/min had MB (p=0.81) in 6 studies. Among the 1016 patients (5 studies) with GFR<60 ml/min vs GFR>60 ml/min, the risk of MB was 2.1% and 3.5% respectively (p=0.25). Selecting two studies that include patients with active cancer, 4(4.4%) out of 91 patients with GFR<60 ml/min and 21(3.8%) out of 550 patients with GFR>60 ml/min had MB [1.34 (95% CI 0.45–3.92)p=0.6].
Clinically relevant bleeding (CRB):
Eleven (8.5%) out of 129 patients with GFR<30 ml/min and 24(5.8%) out of 779 patients with GFR>30 ml/min had CRB in 4 studies [1.65 (95% CI 0.82-3.29)p=0.16]. Among the 278 patients with GFR<60 ml/min and 783 with GFR>60 ml/min (4 studies), 22(7.9%) and 71(9.1%) had CRB respectively [1.11 (95% CI 0.67-1.89)p=0.67].
Summary/Conclusion: This systematic review of clinical studies does not show a significantly higher risk of major hemorrhage nor clinically relevant bleeding to tinzaparin in patients with moderate and severe renal failure.
Tinzaparin is the largest LMWH and clearance is less dependent on the kidney function. Therefore, the available evidence indicates that tinzaparin does not accumulate above GFR>20 ml/min.
Reducing the doses of tinzaparin in these patients may not reduce the risk of bleeding. The risk/benefit balance should be individualized in these patients.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Perioperative management of patients with rare bleeding disorders

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Background: Rare bleeding disorders (RBD) are uncommon disorders with a wide clinical and analytical variability, because of this, there is no solid evidence in the perioperative management of patients with these coagulopathies
Aims: The objective is this study is to describe the experience of our unit in perioperative management of patients with RBD
Methods: We included 23 patients with RBD who underwent at least one surgery from December-18 to May-19. The clinical characteristics of the patients, the type of surgery, perioperative haemostatic treatment and the result and complications of surgery were analyzed
Results: We reviewed 26 surgical procedures performed on 23 patients with RBD (13 women and 10 men). Nine procedures (39%) involved major surgery. The majority of the patients (12) had FVII deficit (6 FXI, 2 FV, 2 FX and 1 FXII). All had a mild-moderate factor deficit, except 2 patients with a severe FXI deficit. Eleven patients had a surgical history before diagnosis of RBD with no record of hemorrhagic complications. The surgeries performed were, 5 ENT, 6 gynecological, 5 general, 3 trauma, 3 urological, 2 ophthalmological and 2 cardiovascular surgeries
Two patients didn't receive treatment. The rest of the patients received tranexamic acid alone or associated with PFC (2 caesarean in a patient with mild FXI deficit, 1 ovarian puncture in patient with mild FX deficit, 1 valve replacement and 1 thoracic aneurysm in one with severe FXI deficit and 1 hemicolectomy in patient with severe FXI deficit). A patient with mild FVII deficit received rFVII (phacoemulsification with intraocular lens replacement)
With this hemostatic protocol, only one patient presented a serious postoperative hemorrhagic complication (compressive hematoma in the neck that required surgical drainage and transfusion of blood products). Only 1 patient with a colon neoplasm required postoperative prophylactic antithrombotic treatment. Hospital stay was not prolonged due to hemorrhagic complications related to surgery, except in the only case of postoperative hemorrhage
Summary/Conclusion: Perioperative management in RBD reported an anecdotal incidence of hemorrhagic complications in relation to surgery. The treatment, adapted to the hemorrhagic / thrombotic risk of antifibrinolytic drugs (oral / iv) in the mild forms did not report any hemorrhagic / thrombotic complications. Like Siboni et al, we did not observe hemorrhagic complications in the 3 orthopedic surgeries. There were no deaths or cerebral hemorrhages in the perioperative period

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Desmopressin for reversal of Antiplatelet drugs in Stroke due to Haemorrhage (DASH): rationale and design of a phase II double blind randomised controlled trial

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Background: Intracerebral haemorrhage caused ~3 million deaths worldwide in 2015. Two-thirds of survivors are left dependent on others. Roughly one third of patients are taking antiplatelet drugs at the time of intracerebral haemorrhage in high-income countries, and this proportion has been increasing over time. Pre-stroke antiplatelet drug use is associated with a 27% relative increase in one-month case fatality compared to patients not using antithrombotic drugs. Platelet transfusion, tranexamic acid, and recombinant activated factor VII do not seem to improve outcome after antiplatelet-associated intracerebral haemorrhage, but desmopressin has seemed promising in non-randomised pilot studies.
Aims: We aim to assess the feasibility of screening, checking the eligibility, approaching, randomising, administering desmopressin or placebo, and completing follow-up for patients with antiplatelet associated intracerebral haemorrhage.
Methods: We aim to include 50 patients within 12 hours of spontaneous intracerebral haemorrhage onset, associated with oral antiplatelet drug(s) use in the preceding seven days. Exclusion criteria are: known secondary cause for intracerebral haemorrhage; risk of fluid retention associated with desmopressin; significant hypotension (systolic blood pressure < 90 mmHg); known drug-eluting coronary artery stent in previous three months; allergy to desmopressin; pre-stroke dependency; Glasgow coma scale < 5; pre-morbid life expectancy < 3 months; and pregnant or breastfeeding at randomisation. Patients will be randomised (1:1) to receive intravenous desmopressin 20 ug in 50 ml Sodium Chloride 0.9% infused over 20 minutes or matching placebo. We will mask participants, relatives, researchers and outcome assessors to treatment allocation. Feasibility outcomes include proportion of patients approached being randomised, number of patients receiving allocated treatment, rate of recruitment, and adherence to treatment and follow up. Secondary outcomes include change in intracerebral haemorrhage volume at 24 hours; hyponatraemia at 24 hours, length of hospital stay, discharge destination, early mortality < 28 days, death or dependency at day 90, mortality up to day 90, serious adverse events (including thromboembolic events) up to day 90; disability (Barthel index, day 90), quality of life (EuroQol, day 90), cognition (telephone MMSE day 90), and health economic assessment (EQ-5D). Baseline platelet dysfunction will be measured and correlated with response to desmopressin; and change in factor VIII, Von Willebrand factor (VWF) antigen and VWF activity will be assessed one hour after administration of desmopressin.
Results: *
Summary/Conclusion: This is a feasibility trial, which will inform the design of a definitive trial.
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ECTH 2019
European Congress on Thrombosis and Haemostasis
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P-034

Management of anticoagulant therapy in pacemaker implantation. retrospective study in an intensive care unit.

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Background: The management of anticoagulants in the periprocedural period is characterized by being very heterogeneous. Sometimes, the balance between hemorrhagic risk and thrombotic risk is complex. it is important to standardize the management of these patients.
Aims: Primary objectives:
- Thromboembolic events (TE) in patients who have been suspended anticoagulant therapy for pacemaker implantation.
- Appearance of severe post-procedure haemorrhagic complications in those patients who underwent bridge therapy (TP).
Secondary objectives:
- Demographics of anticoagulated patients who required pacemaker implantation.
- Average times of withdrawal and reintroduction of anticoagulant therapy.
Methods: It is a retrospective descriptive study of patients on anticoagulant treatment who had a pacemaker implanted in the last 6 years (2013-2018).
417 patients had a pacemaker implant. 124 were receiving anticoagulant therapy (29.7%). 37 women and 88 men. The average age was 81.1 years. 86 patients were treated with acenocoumarol (69.3%). 34 patients underwent bridge therapy. 38 patients were treated with DOAC (30.7%).
Results: An episode of TIA appears in a patient with low TE risk.
There were no severe hemorrhagic complications secondary to the intervention.
The main reason for anticoagulation was atrial fibrillation with 110 patients, followed by mechanical valves, 10, venous thromboembolism, 3, and hereditary thrombophilia 1.
Stratifying them according to thromboembolic risk: 14 patients high risk (11.3%), 44 moderate risk (35.5%) and 66 low risk (53.2%).
The treatment was removed 2.9 days before on average, and it was reintroduced 2.5 days later. The mean time without anticoagulation was 4.4 days.
Summary/Conclusion: There was a thromboembolic complication in the entire sample, appearing in a patient with low TE risk. There were no serious hemorrhagic complications (reoperation, transfusion ...). These data give information about the performance in our Unit in this period of time, however, it is necessary to unify criteria not only among the different centers, but within the same Unit.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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P-035

An unusual presentation of Clopidogrel side effects

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Background: Clopidogrel, a platelet $P2Y_{12}$ receptor blocker is used to treat cardiovascular events, i.e. acute coronary syndrome, stroke and peripheral vascular disease. The common side effects are gastrointestinal disorders (diarrhea, abdominal pain, dyspepsia) and bleeding tendency. Rare side effects inculde a broad spectrum of different disorders e.g. severe allergic reactions, liver and kidney dysfunction and musculoskeletal bleedings.
Aims: To describe a clinical case with excessive musculoskeletal bleeding during the treatment with Clopidogrel.
Methods: Review of medical records.
Results: A 79-year old man was admittet to the hospital due to shortness of breath and right leg pain. CT angiography was normal and ultrasound scanning revealed an intramuscular hematoma with the size of 8,0 x 2,0 cm in musculus vastus medialis of the right leg. Blood hemoglobin was lowered to 5,0 mmol/l (reference range 8,3-10,5 mmol/l). The patient was taken tab. Clopidogrel 75 mg x 1 daily due to previous stroke 2 years ago. The treatment was well-tolerated without side effects. Neither other anticoagulant was concomitantly taken, nor Clopidogrel was overdosed. The patient denied any trauma or intake of herbal supplements, which could potentiate action of Clopidogrel. The Clopidogrel treatment was paused for 10 days.
A week after the Clopidogrel treatment was restarted the patient was suffering from a pain in the left leg. Further investigation has shown lowered blood hemoglobin to 4,9 mmol/l. CT scanning revealed an intramuscular hematoma with the size of 9,0 x 3,5 x 5,4 cm in left gluteal area. Any trauma, changes in patient's usual treatment or overdose of Clopidogrel was denied.
The patient was tested for primary and secondary hemostasis disorders but all laboratory results were within reference range or above, e.g. plasma coagulation factor VIII activity and plasma von Willebrand factor concentration and activity. The patient could not exclude the fact, that he possibly bought tab. Clopidogrel from other pharmaceutical company than he used to buy it before bleeding episodes.
Clopidogrel treatment was stopped, and the patient was prescribed tab. Aspirin 75 mg x 1 daily. No bleeding episodes were observed.
Summary/Conclusion: Clopidogrel treatment caused an excessive musculoskeletal bleeding despite long term therapy, and it is an unusual observation. It could be explained by the fact, that side effects of the same drug can vary between different pharmaceutical companies.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Subgaleal hematoma in infant with severe hemophilia A

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Background: Hemophilia A is a genetic disorder caused by missing or defective factor VIII, a clotting protein. Mutations in F8 gene have been described as cause of hemophilia A. The disorder is clinically heterogenous with variable severity, depending on the plasma levels of coagulation factor VIII. Subgaleal hemorrhage is a rare but potentially serious complikation that occurs when blood acccumulates outside o the baby' skull. It is caused by trauma to the head especially during to the delivery. It is results in the severing of emissary veins, located betwen the dural sinuses that cover the skull and the scalp.
Aims: Here we report a case of nine month infant with masive subgaleal hemorrhage after head trauma. pending on the plasma levels of coagulation factor VIII.
Methods: Retrospective analises of medical history.
Results: A full term male neonate was born at 39 weeks gestation by spontaneous vaginal delivery, with birth weight of 3250 g and birth height of 50 cm. The first signs and symptoms of severe hemophilia presenting in second day of life after prophylactic intramuscular dose of vitamin K. Diagnosis of severe hemophila A was established, recombinant factor VIII was administrated and therapy was continuated with prophylaxis with recombinant factor VIII once a week.
At the age of nine months, after head trauma, the infant had swelling in the forehead area. The working diagnosis of subgaleal hemorrhagie was made. We started immediately with 50 IJ/kg of recombinant FVIII every 8 hours, parenteral antibiotics and hydration. On second day infant became pale and developed severe anemia (Hgb 74 g/l, Hct 18%). He received repeated transfusions of packed red cells. Head CT reveiled normal finding. Therapy was continuated after complet resolutiuon of subcutaneous oedema
Summary/Conclusion: Infants diagnosed with subgaleal haemathoma must be treated immediately in order to prevent any further damage.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Thrombosis in haemophilia – how could it happen?

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Background: Despite the defective synthesis of coagulation factor VIII (FVIII) in haemophilia A, it does not prevent affected individuals from the thrombotic complications. When compared with general population, the presumed ten-year risk is even significantly increased in hemophiliacs (6.7 % vs. 8.9 %), showing unfavourable cardiovascular event risk in subjects with haemophilia.
Aims: To present the possibility and circumstances of the coincidence of bleeding and thrombotic disorder.
Methods: The authors present the case report of the patient with moderate haemophilia A, who was a smoker with further prothrombotic risk factors, such as arterial hypertension, hypercholesterolaemia, inherited thrombophilic disorder and immobilization due to the presence haemophilic arthropathy, treated with on demand with FVIII concentrate.
Results: The computed tomography showed an irregular thrombus present almost in whole extent of the abdominal part of aorta. Combination of antithrombotic drugs and changes in lifestyle led to diminishing of his symptoms.
Summary/Conclusion: The life span of haemophiliacs is closer to that of general population. Thus, the coexistence of haemophilia and thrombosis should be studied preferentially in older patients with haemophilia and cardiovascular risk factors. The treatment of thrombotic episodes in haemophiliacs is challenging especially in the situations when antithrombotic management or surgerical intervention is required. Thus, prominent question is how to optimalize concomitant antithrombotic and substitution therapy in individuals with the coexistence of opposing haemostatic disorders.
Key words: haemophilia, thrombotic complications, treatment
Acknowledgement: Authors thank the support of the projects of the Scientific Grant Agency (Vega) 1/0168/16 and Vega 1/0549/19. Informed consent of the patient was obtained.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Thrombotic complications and bleeding disorders of haemostasis

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Background: Bleeding disorders do not prevent the patients from the risk of the thrombosis development. Factors to the thrombosis are the arterial hypertension, advanced atherosclerosis, smoking, immobilization, trauma, advanced age, surgery, pregnancy, hormonal treatment, coincidence of the thrombophilia, substitution treatment and the use of by-passing drugs.
Aims: To discuss the issue of the coincidence of bleeding and thrombosis.
Methods: The authors present the case reports of the subjects with bleeding disorders of haemostasis and simultaneous thrombosis treated at our National centre of haemostasis and thrombosis and manifest the challenging clinical management. They discuss their risk factors, dagnosis and treatment.
Results: In the described cases, the antithrombotic treatment was administered without any rethrombosis.
Summary/Conclusion: Patients with bleeding disorders may develop acquired preventable or non-preventable prothrombotic risk factors, which are comparable with those present in general population. Unfortunately, currently, there is a lack of studies contributing to the preparation of the guidelines for these clinical situations. Thus, the combination of the substitution and antithrombotic treatment should be individualized and based on the actual results and clinical state of the patient.
Key words: bleeding disorders, prothrombotic risk factors, management
Acknowledgement: Authors thank the support of the projects of the Scientific Grant Agency (Vega) 1/0168/16 and Vega 1/0549/19. Informed consent of the patient was obtained.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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P-040

The risk of a first venous thrombosis associated with elevated D-dimer and thrombin generation in elderly

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Background: D-dimer and parameters of the thrombin generation assay are associated with the risk of a first venous thrombosis (VT) in young and middle-aged populations.
Aims: We investigated whether D-dimer levels and parameters of the thrombin generation assay (lag-time, time-to-peak, peak thrombin, endogenous thrombin potential (ETP), and velocity index) are associated with increased risk of a first VT in those aged 70 and older.
Methods: The AT-AGE study, a two-center case-control study performed in Vermont, USA and Leiden, the Netherlands, included 401 cases with a first deep venous thrombosis or pulmonary embolism and 431 controls, all >70 years. 215 Patients and 358 controls provided a blood sample (for cases, while not on anticoagulation and >1 year after the VT) and had a valid measurement of both D-dimer and thrombin generation. D-dimer was assayed with the Vidas D-dimer (BioMérieux, Basingstoke, UK). Thrombin generation was determined using the thrombin generation assay of the Calibrated Automated Thrombogram (Diagnostica Stago, Asinères, France). Thrombin generation was initiated using assay reagents according to the manufacturer's specifications (tissue factor (low concentration) and phospholipids) and tested in duplicate.
To assess the risk of VT, odds ratios (OR) with 95% confidence intervals (CI) were calculated using logistic regression analysis after stratification of the thrombin generation parameters in quartiles based on the distribution of the controls. Risk estimates were adjusted for age, sex, body mass index, study center, and smoking.
Results: All thrombin generation parameters except lag-time were associated with increased risk of VT. The risk associated with peak, time-to-peak, ETP, and velocity index increased in a dose dependent manner when comparing the 2nd, 3rd and 4th quartile with the lowest quartile as a reference (for time-to-peak comparing the 1st, 2nd, and 3rd quartile with the highest quartile as a reference). Comparing the 4th with the 1st quartile (for time-to-peak comparing the 1st with the 4th quartile), risk estimates were: peak: 7.8 (95CI: 4.0-15.0), time-to-peak: 2.0 (95CI: 1.2-3.3), ETP: 9.1 (95CI: 4.4-18.9), velocity index: 11.5 (95CI: 5.7-23.3). D-dimer was also associated with the risk of a first venous thrombosis. Comparing the highest quartile (>848 ng/ml) with the lowest (<338 ng/ml), the risk was 7.7 fold increased (95CI: 4.0-14.8). In a combined analysis with ETP and D-dimer, both stratified at the 50th percentile, the risk was highest when both D-dimer and ETP were elevated; compared with ETP
Summary/Conclusion: In the elderly, thrombin generation parameters (except lag time) and D-dimer were strongly associated with the risk of a first VT. The risk was especially high when both ETP and D-dimer levels were elevated.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

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Hypertension is associated with lower risk of venous thromboembolism in men

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Background: Somewhat surprisingly, a meta-analysis showed an association between higher systolic blood pressure and lower risk of venous thromboembolism (VTE). The importance of cardiovascular risk factors varies between men and women, resulting in differences in health outcomes.
Aims: To investigate the association between hypertension, systolic blood pressure (SBP), diastolic blood pressure (DBP), and risk of first-time VTE in the total population and in men and women separately.
Methods: We performed a prospective, population-based cohort study in northern Sweden. Study participants were 108,025 persons (51% women) aged 30 to 60 years without previous VTE who participated in a health examination between 1985 and 2014. At the baseline health examinations, weight, height, SBP, and DBP were measured, and participants answered a questionnaire regarding e.g. smoking, education level and medication use. Data on cancer was obtained from the Swedish Cancer Registry. Participants were followed until a VTE event, death, emigration, or the study end on September 5, 2014. Potential first-time VTE events were identified using the Swedish National Patient Registry and Cause of Death Registry and then validated by review of medical records and radiology reports.
Cox proportional hazards regression was used to study the association between hypertension, SBP, DBP and risk of VTE. All analyses were adjusted for age, body mass index, smoking, education level and cancer. Analyses of men and women combined were also adjusted for sex. The study was approved by the Regional Ethics Review Board, Umeå, Sweden, and all participants gave written informed consent to participate in research.
Results: During the follow-up period (1,496,669 person-years), 2,054 participants (46% women) had a first-time VTE event with a median time-to-event of 12.6 years. The mean age at inclusion was 46 years, and 31% of men and 25% of women had hypertension. In the total population, there was an association between hypertension (hazard ratio [HR] 0.86; 95% confidence interval [CI] 0.78–0.95) and lower risk of VTE. There was a multiplicative interaction between hypertension and sex in relation to risk of VTE (*P*-value 0.03). Separate analyses for men and women were performed.
In men, there was an association between hypertension and lower risk of VTE (HR 0.75; 95% CI 0.66–0.86). There were also statistically significant associations between SBP (HR 0.92; 95% CI 0.89–0.96 per 10 mm Hg), DBP (HR 0.93; 95% CI 0.87–0.99 per 10 mm Hg) and lower risk of VTE in men.
In women, there was no association between hypertension (HR 1.02; 95% CI 0.87–1.18), SBP, DBP and risk of VTE.
Summary/Conclusion: Men with hypertension had a substantially lower risk of VTE. This finding is further supported by the associations between higher SBP, DBP and lower risk of VTE. In women, no association between hypertension, blood pressure and risk of VTE was seen. The present study also highlights the importance of studying risk factors for VTE in men and women separately.

ECTH 2019
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The association of a procoagulant state with venous thrombosis in patients with lower-leg injury

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Background: Patients with lower-leg injury treated with cast immobilization have a 56-fold increased risk of venous thrombosis (VT) in first three months as opposed to the general population. The exact mechanism of VT in these patients is unclear. In a previous study we found that lower-leg trauma leads towards a procoagulant state, which is associated with severity of injury.
Aims: To determine whether increased levels of procoagulant factors are associated with a higher risk of venous thrombosis in patients with lower-leg injury.
Methods: Patients with lower-leg injury, who participated in the POT-CAST (Prevention Of Thrombosis after Lower Leg Plaster Cast) trial and provided a blood sample, were included. Blood samples were taken shortly after trauma, before patients received cast immobilization or underwent surgery. Patients who developed VT (cases) in the first three months were compared to patients who did not (controls). The following procoagulant factors were measured: factors (F)VIII, IX and XI activity, fibrinogen (Fib), von Willebrand Factor antigen (vWF), and D-dimer. Information on risk factors for VT were obtained by questionnaires. Coagulation factor levels were expressed as mean with standard deviation, while D-dimer was expressed as median with interquartile range due its skewed distribution. Unadjusted mean differences, and mean ratio for D-dimer (due to natural logarithmic retransformation), were calculated with 95% confidence intervals (95%CI). Logistic regression models were used to calculate odd ratios (ORs) as an estimate for the relative risk of VT, according to the following cut-offs for the coagulation factor levels in the controls: <75th percentile (reference), 75-90th percentile and >90th percentile. The ORs were adjusted for age and sex.
Results: In total, 1352 patients (23 cases, 1329 controls) were eligible for analyses. Cases had the following mean procoagulant factor levels: FVIII 178.9% (SD 51.6%), FIX 142.6% (SD 30.1%), FXI 128.5% (SD 21.5%), vWF 197.4% (SD 37.9%), Fib 322.0 mg/dL (SD 82.0 mg/dL). Median D-dimer in cases was 449.7 ng/mL (IQR 260.8 to 1233.4 ng/mL). Controls had lower mean procoagulant factor levels except for Fib, with the following mean differences: FVIII 29.99 (95%CI 12.92 to 47.06), FIX 6.65 (95%CI -3.66 to 16.97), FXI 11.09 (95%CI 1.30 to 20.88), vWF 39.44 (95%CI 16.82 to 62.05), Fib -18.47 (95%CI -58.73 to 21.80). D-dimer was also lower in controls, expressed in mean ratio: 0.71 (95%CI 0.47 to 1.08). FVIII and vWF were clearly associated with the risk of VT, expressed in ORs. FVIII: P75-90 OR 3.6 (95%CI 1.4 to 9.2), P90 OR 2.3 (95%CI 0.7 to 7.6). vWF: P75-90 OR 4.2 (95%CI 1.6 to 11.0), P90 OR 4.2 (95%CI 1.4 to 12.4). The other factors were not significantly associated with VT risk.
Summary/Conclusion: A procoagulant state in patients with lower-leg injury is associated with an increased risk of VT. FVIII and vWF showed the strongest association.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Is the Caprini score a useful tool to predict venous thrombosis in orthopedic patients?

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Background: Patients who undergo orthopaedic surgery have an increased risk of Venous Thromboembolism (VTE). The size of this risk depends on factors such as the type of surgery, patient characteristics and post-surgical care. In order to help physicians to identify patients who are at low- or high-risk of VTE, several risk assessment models (RAM) have been published. For general surgical patients, the Caprini score is well known and extensively validated. For orthopaedic surgery however, the predictive performance of this score is not well known and therefore, it is currently unclear whether this score can be used in practice to stratify patients in low- or high-risk categories.
Aims: The purpose of this study was to validate the Caprini score in patients undergoing (any) orthopaedic operation.
Methods: Data from a large population-based case–control study (the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis [MEGA] study) on the etiology of venous thrombosis were used. Cases and controls completed a questionnaire on risk factors for VTE which information was used to calculate the Caprini score. Missing data were imputed by multiple imputation. Odds Ratios (OR) with their 95% Confidence Intervals (95%CI) were calculated for VTE risk for several risk Caprini score categories (with Caprini score 0-2 as reference). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculate for several dichotomized cut-off scores. The overall discriminative predictive performance was assessed by estimating the Area Under the Curve with a Receiver Operating Characteristic.
Results: Of 4.721 cases and 5.638 controls, 263 cases and 94 controls underwent an orthopedic intervention. A total of 20.9% (55/263) of cases and 41.5% (39/94) of controls were classified in the lowest risk group (Caprini <5 points). Patients with a Caprini score >11 points had a six-fold (OR 6.3, CI 95% 1.7-22.9) increased risk of VTE and patients with a score of 9-10 had a three-fold increased risk (OR 3.5, CI 95% 1.2-10.3), as compared to patients with a Caprini score of 0-2 points. The discriminative performance was moderate with an AUC of 0.64 (CI 95% 0.58-0.71). Using a cut-off >5 points to stratify patients at high risk of VTE, the sensitivity was 79%, the specificity was 41% and the PPV 2.3%.
Summary/Conclusion: The Caprini score may have some use to predict the risk of VTE in orthopaedic surgical patients. However, its predictive performance was estimated to be moderate.

ECTH 2019
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Coagulation factor V is associated with immune response and tumor lymphocyte recruitment in breast cancer

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Background:
Coagulation abnormalities are common in cancer patients and increase the risk for blood clots. Moreover, components of the tissue factor coagulation pathway have been shown to support mechanisms that promote tumor progression and metastasis. The coagulation factor (F) V is an essential pro- and anticoagulant co-factor of this pathway. *F5* variants have been associated with breast cancer and cancer associated thrombosis. We recently demonstrated that *F5* expression was enriched in breast tumors compared to normal tissue, and that *F5* expression was linked to tumor aggressiveness and patient survival. The ectopic expression of *F5* in breast tumors suggests a yet undiscovered role for FV in cancer pathogenesis.
Aims: To explore the possible biological roles of FV in breast cancer.
Methods: The Gene expression based Outcome for Breast cancer Online (GOBO) was used for gene correlation analyses. Results were validated in a Scandinavian breast cancer cohort* and a cancer genome atlas (TCGA) dataset. DAVID and REVIGO were used for gene ontology analyses. Hematoxylin-eosin staining and CIBERSORT was used to enumerate tumor infiltrating lymphocytes (TILs) and immune cell proportions, respectively. A transwell assay was used to evaluate the effect of exogenous FV on T-cell migration. *All patients have given their written informed consent to participate in the study.
Results: *F5* breast tumor expression was significantly correlated with an immune response gene module ($\rho>0.3$, $P<1.0x10^{-50}$). Gene ontology analyses of the most correlated genes indicated an association with activation/differentiation of lymphocytes. Accordingly, tumors with high *F5* expression were more infiltrated with lymphocytes and CIBERSORT analysis indicated that high *F5* expression was associated with tumor infiltration of multiple T cell lineages, including gamma delta T cells, activated CD4 memory T cells, and naïve CD4 T cells. However, exogenous FV had no direct effect on the *in vitro* migration capacity of Jurkat T cells, proposing that presence of additional factors are needed to induce T cell infiltration in tumors. Migration studies of different types of T cells are in progress, as well as T cell activation studies.
Summary/Conclusion: This study suggests that FV plays a role in breast cancer via immune stimulatory effects. FV seems to be involved in lymphocyte recruitment to the tumor site, but further studies are needed to identify the potential FV mediator(s) contributing to lymphocyte infiltration. FV emerge as an interesting candidate with potential therapeutic relevance for the cancer-inflammation-thrombosis circuit.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Safety of rivaroxaban in the diagnostic work-up of deep vein thrombosis

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Background: Deep vein thrombosis (DVT) causes significant morbidity in the general population. Guidelines recommend administration of low-molecular weight heparin (LMWH) if the diagnostic work-up is expected to be delayed. Rivaroxaban has the advantages over LMWH of oral administration and standard dosing, but the safety and feasibility of administering prophylactic rivaroxaban in the pre-diagnostic phase of DVT remain unexplored. Furthermore, we believe the management of DVT patients in the Emergency Department could be improved by simplifying the diagnostic work-up, as well as save time for patients and resources for clinicians whilst preserving safety considerations.
Aims: The Ri-Schedule study (NCT02486445) is a prospective management outcome study which aims to assess the safety and feasibility of a new scheduled work-up strategy incorporating rivaroxaban with on-hour diagnostic work-up according to predefined criteria.
Methods: We included consecutive outpatients with suspected DVT referred to Østfold Hospital Norway between 2015 and 2018. We administered 15 mg x 2 rivaroxaban over 24 hours to eligible patients according to predefined criteria. The primary endpoint was major bleeding or death related to bleeding within 48 hours after the last tablet had been ingested in patients where DVT was excluded, or until anticoagulation was initiated in patients where DVT was confirmed. Major bleeding was defined as bleeding which led to decreased hemoglobin concentration of ≥2 g/dl, transfusion of ≥2 units of red blood cells, occurred in a critical site or contributed to death. Based on documented bleeding rates for LMWH, an accepted bleeding rate for major bleedings was defined a priori at 0.2% (p0= 0.002), with a one-sided 95% CI of <0.8% (p1=<0.008). The secondary endpoint was proportion of patients eligible for scheduled work-up.
Results: Of the 1653 patients referred for suspected DVT and evaluated for eligibility, 624 (37.7%) fulfilled criteria for scheduled work-up and received rivaroxaban. Median age was 65, and 342 patients were female (55%). There were no major bleedings, resulting in a 48-hour major bleeding rate of 0% (one-sided 95% CI <0.4). There were 65 bleeding incidents in 59 of the patients receiving rivaroxaban (9.9%, 95% CI 7.8-12.5%). Of these, three bleedings were adjudicated as non-major clinically significant. There were no deaths. No patients (95% CI 0.0-0.61) experienced worsening of pre-existing symptoms or developed symptoms of pulmonary embolism between inclusion until VTE diagnosis was confirmed.
Summary/Conclusion: We found that rivaroxaban administered to patients in the pre-diagnostic phase of DVT is a safe and feasible alternative to LMWH when given according to predefined criteria. With its oral administration route and standard dosing, it may simplify the diagnostic work-up of DVT without compromising safety.

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Tissue factor is constrained by interaction with the PDZ-1 domain of magi-1 protein

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Background: Membrane associated guanylate kinase inverted (MAGI) are a family of three scaffolding proteins which are involved in tight-junction assembly and cell-attachment. Tissue factor (TF) contains a putative PDZ-binding domain within the cytoplasmic domain of TF (ENSPL; residues 256-260) may interacts with PDZ domains. MAGI proteins each contain 6 confirmed PDZ domains which modulate the activity of membrane proteins and can restrict proteins to the intercellular regions
Aims: To identify the MAGI protein that is capable of interacting with TF, and to characterise the responsible PDZ domain
Methods: The interaction of TF with each of the three MAGI proteins was examined using the proximity ligation assay (PLA) and by co-immunoprecipitation using mouse antibodies to TF (HTF1), and rabbit polyclonal antibodies to each of MAGI1-3. In addition, the five PDZ domains from the MAGI-1 were cloned into the FLAG-HA-pcDNA3.1 plasmid. The hybrid proteins were expressed in MDA-MB-231 cell line and assessed for their ability to associate with the endogenous TF. Finally, two DNA regions encompassing the N-terminal of MAGI-1, including or excluding PDZ-1, were also cloned and expressed in MDA-MB-231 cells. The interactions of TF with the recombinant proteins were then examined using PLA and immunoprecipitations, carried out using a mouse-anti TF antibody (HTF1) and a rabbit anti-HA tag (C2954)
Results: PLA studies showed significant association between TF and MAGI1 *in situ* and the interaction was confirmed by co-immunoprecipitation. PLA analysis indicated a high level of association between TF and recombinant PDZ-1 domain of MAGI1, with lower levels detectable with PDZ-4 and PDZ-5 domains. Finally, the examination of cells by PLA indicated a significantly higher association of the N-terminal protein with TF, on inclusion of PDZ-1 compared to the same protein but devoid of PDZ-1. This interaction was further confirmed by the increased co-immunoprecipitation of TF on expression of the N-terminal protein containing PDZ-1
Summary/Conclusion: We have demonstrated the ability of TF to interact with the PDZ-1 domain of MAGI-1. By localising TF to the intercellular gaps MAGI-1 may be vital in preventing the contact between cell-surface TF and the surrounding fluids. This in turn may regulate the activation of both coagulation and signalling mechanisms

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Kidney injury can be a predictor of intrahospital mortality and fatal bleeding in patients with acute pulmonary embolism

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Background: Pulmonary embolism due to haemodynamic disturbances may lead to multi organ damage including an acute renal dysfunction. On the other side, acute renal dysfunction in hospital settings are associated with adverse events and long-term mortality rate.
Aims: The aim of our study was an investigation of the predictive value of renal dysfunction in intrahospital environment regarding the mortality risk in pulmonary embolism patients hospitalized in intensive care units of six university hospitals.
Methods: The prospective cohort study, comprised 665 consecutive patients with APE confirmed using MDCT-PA. All patients underwent echocardiography examination on admission and blood samples were collected for Tni, BNP and creatinine assays.
Results: Based on estimated glomerular filtration rate (GFR), patients were divided into three groups: first with the GFR < 30ml/min, second with GFR 30-60 ml/min, and third with GFR >60 ml/min. There was a statistically significant distribution among groups regarding risk of pulmonary embolism ($p<0.0001$). During hospitalization in the first group the overall incidence of death was recorded in 28 (45.9%), in the second in 42 (18.9%), and in the third in 30 (7.9%) ($p<0.0001$). Pulmonary embolism as cause of death was recorded in the first group in 18 (29.5%), in the second in 25 (11.3%) and in third in 17(4.5%) patients ($p<0.0001$). Fatal bleeding was recorded in the first group in 1(1.6%), in the second in 1 (0.5%) and in third group in 3 (0.8%) patients ($p<0.05$). There were no significant differences regarding major bleeding among the groups (p 0.126). A multivariate analysis showed that age and comormidities, hemodynamic status, TnI and GFR strongly influenced overall death as well as death due to pulmonary embolism, while anticoagulation therapy influenced the fatal bleeding rate. After controlling for age, we found that admission GFR had a significant effect on in-hospital survival. Patients in the medium tertile of GFRc values (30-60 mL/min) had more than twice the risk of mortality (relative risk, OR 2.17 (CI 1.301-3.625) ; P = 0.001) of the patients in the highest tertile (> 60 mL/min), and the patients in the lowest tertile of GFR (<30m/min) had six times the higher risk of mortality compared to patients in the highest tertile [OR 6.006 (CI 3.487–6.006). Regarding discriminative value for prediction of intrahospital death, as assessed by AUC, GFR showed a potential diagnostic value for the identification of patients in the risk of death because the area under the corresponding ROC curve (Figure) was significantly [AUC= 0.725, (95% confidence interval 0.68-0.78), $p < 0.001$] higher than the threshold of diagnostic indifference (50%). When data were analyzed by the "best cut-off" derived from the ROC curve (59.12/min), the sensitivity was 64%, and the specificity was 70 %. Similarly, the analysis of the usefulness of GFR in the prediction of death of from APE yielded an area under the ROC curve of 0.73 (95% confidence interval [CI] 0.66-0.97; P<.001)
Summary/Conclusion: Renal dysfunction on admission in acute pulmonary embolism settings is strongly connected with overall pulmonary embolism mortality and fatal bleeding during in-hospital follow up. The estimation of GFR is very important not only for prediction of adverse events but also for prevention of bleeding complications regarding optimal dosage of anticoagulation regimen.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Plasma levels of leptin and future risk of incident venous thromboembolism

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Background: Leptin is a hormone produced mainly by adipocytes, and plasma levels of leptin are elevated in obesity. It has been shown to upregulate the expression of tissue factor and plasminogen activator inhibitor-1 *in vitro*. Leptin could therefore serve as a mediator for the risk of venous thromboembolism (VTE) in obese subjects. However, whether leptin is associated with VTE risk remains unknown.
Aims: To investigate the association between plasma levels of leptin and future risk of incident VTE in a nested case-control study.
Methods: A nested case-control study consisting of 416 subjects with incident VTE and 848 age- and sex-matched controls was derived from a population-based cohort (the fourth survey of the Tromsø study). Baseline information was collected by physical examination and blood samples in 1994-95, and participants were followed up to September 1, 2007. Logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE across quartiles of leptin levels determined in the control group. Given the sex-difference in leptin levels, analyses were performed separately in men and women based on sex-specific quartile cut-offs of plasma leptin. Ethical approval and informed written consent were obtained.
Results: Among control subjects (47% men), the median leptin level was almost 3 times higher in women (25.5 ng/mL, interquartile range [IQR] 15.8-36.7) than in men (9.2 ng/mL, IQR 5.6-13.4). Moreover, body mass index (BMI) and leptin levels showed a moderate to strong positive correlation (Spearman's rho of 0.63 in men and 0.72 in women). The risk of VTE increased across quartiles of leptin levels, particularly in men. In the age-adjusted model, male participants with leptin in the highest quartile had an OR for VTE of 1.70 (95% CI 1.04-2.79) compared to those with leptin in the lowest quartile. However, the association disappeared after further adjustment for BMI (OR 1.03, 95% CI 0.55-1.93). Among women, the OR for VTE was 1.35 (95% CI 0.85-2.15) for participants with leptin in the highest versus the lowest quartile, but again, ORs were substantially attenuated after adjusting for BMI (OR 0.81, 95% CI 0.45-1.47). Similar results were obtained in subgroup analyses (i.e., deep vein thrombosis, pulmonary embolism, and provoked and unprovoked events) in men and women.
Summary/Conclusion: In this study, plasma levels of leptin were not associated with future risk of VTE. BMI, a measure that reflects total body fat, appeared to fully explain the observed relationship between leptin and VTE. Our findings suggest that the effect of leptin on hemostatic factors reported *in vitro* is not clinically relevant for the mediation of VTE risk in obese subjects.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Pregnancy-related venous thromboembolism risk in women from families with thrombophilia: results from the MARseilles FAmily Study on venous Thrombosis (MARFAST)

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Background: The thrombophilia testing in relatives from families with known thrombophilia has not demonstrated its efficacy in assessing individual risk of venous thromboembolism (VTE). However the identification of at risk individuals is of major importance in order to handle at risk situations among which pregnancy is particularly challenging.
Aims: To identify risk factors for pregnancy-related VTE in women from families with known thrombophilia and to assess the management of pregnancies in those women.
Methods: A questionnaire on history/management of pregnancies and history of VTE was sent to all women over 18 years of age included in the Marseilles FAmily Study on venous Thrombosis (MARFAST); women who did not reply were contacted by phone. MARFAST includes families with at least 2 members with the following characteristics: one individual with a personal history of VTE and a positive thrombophilia testing (propositus) and a relative harboring the same defect. A complete thrombophilia testing was systematically performed and patients were classified into 3 groups: no defect, mild thrombophilia (Factor V Leiden (FVL) heterozygous (HTZ) or prothrombin mutation (PTM) HTZ) and severe thrombophilia (antithrombin, protein C, protein S deficiencies, FVL homozygous (HMZ), PTM HMZ, combined defects). ABO blood group was determined. Body Mass Index (BMI), degree of kinship, age at the time of VTE in propositi and the number of 1stdegree relatives with VTE were collected. Propositi were excluded for the statistical analysis.
Results: 1272 women have been contacted. Follow-up information were obtained for 665 patients (response rate= 52%; mail= 365, n= 297), including 488 relatives. 71 relatives (14.5%) had a personal history of VTE, among whom 20 episodes occurred during pregnancy. At the time of follow-up, relatives with a pregnancy-related VTE history were older than women with a history of pregnancy without VTE (mean age= 56.8 vs 47.1, p= 0.006). Besides, a first degree family history of VTE was more frequently reported in relatives with pregnancy-related VTE (95.0 vs 73.1%, p= 0.03). Eventually, non O blood groups were more prevalent in relatives with VTE (77.5 vs 62.8%, p= 0.04). In particular, AB blood group was highly prevalent in relatives with VTE (15.0% vs 4.0%, p=0.06). Of note, the prevalence of mild and severe thrombophilia was comparable between the 2 groups.
179 relatives with no history of VTE experienced at least 1 pregnancy before the thrombophilia testing; only 1 had received prophylactic anticoagulation during pregnancy and/or post-partum (PPP). 166 relatives with no history of VTE experienced their 1stpregnancy after being tested for thrombophilia; 60 had no defect, 75 had mild thrombophilia and 31 had severe thrombophilia; 9 (15%), 62 (83%) and 29 (94%) respectively received prophylactic anticoagulation during PPP. Of note, anticoagulation was initiated during the first trimester of pregnancy in 1 (11%), 24 (39%) and 10 (35%) in no defect, mild and severe thrombophilia respectively.
Summary/Conclusion: In families with thrombophilia, the thrombophilia screening did not allow the identification of women at increased risk of VTE during pregnancy. Other genetic factors appeared to be associated with VTE risk: ABO blood group and the family history as an indirect marker of the genetic risk. However, a positive thrombophilia testing in relatives strongly modified the management of pregnancies in those families, leading to a prophylactic anticoagulation in 86% of cases.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Transfusion requirements in adult patients with paroxysmal nocturnal hemoglobinuria naive to complement inhibitors receiving ravulizumab and eculizumab: results from a phase 3 non-inferiority study

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by hemolytic anemia and can occur concomitantly with bone marrow disorders such as aplastic anemia (AA). Accordingly, patients with PNH may require red blood cell (RBC) transfusion to treat anemia due to hemolysis or bone marrow hypoplasia. Ravulizumab, a novel long-acting C5 inhibitor (administered every 8 weeks), has been shown to be non-inferior to eculizumab (administered every 2 weeks) for treating adult patients with PNH. A phase 3 multicenter, randomized, active-controlled, open-label study (ALXN1210-PNH-301) in complement-inhibitor-naïve patients with PNH showed that 74% vs 66% of patients treated with ravulizumab and eculizumab, respectively, avoided transfusion over 26 weeks. Approximately one-third of enrolled patients had a history of AA, which provides an excellent opportunity to evaluate the efficacy of ravulizumab in patients with and without bone marrow failure.
Aims: To evaluate the efficacy of ravulizumab in patients with and without bone marrow failure. A subgroup analysis was performed to ascertain transfusion requirements based on history of AA and baseline reticulocyte count as a marker of bone marrow function.
Methods: Transfusion data from the 6-month primary evaluation period in study ALXN1210-PNH-301 were analyzed. The analysis included patients who provided informed consent and were randomized to receive ravulizumab or eculizumab. Patients were transfused according to protocol-specified transfusion guidelines. The number of patients receiving RBC transfusion, number of transfusions, and total number of units received during treatment were analyzed. Results for treatment groups were presented by presence or absence of AA.
Results: 246 patients were randomized to receive ravulizumab (n=125) or eculizumab (n=121). Approximately 75% of patients in each group had received a transfusion within 6 months prior to the first dose, with a total of 400 vs 337 transfusions (533 vs 492 units) in the ravulizumab and eculizumab groups, respectively. History of AA was seen in 33% (n=41) of patients in the ravulizumab arm and 31% (n=38) in the eculizumab arm. AA rates were representative of those expected in the general PNH population. Predictably, reticulocyte counts were higher at baseline for patients with no history of AA vs those with a history across both arms; these remained stable throughout treatment. After 6 months, 26% (n=32) of patients treated with ravulizumab received 107 transfusions (155 units) vs 33% (n=40) of those treated with eculizumab (144 transfusions totaling 222 units). In patients with a history of AA, 24% in the ravulizumab arm and 40% in the eculizumab arm received transfusion while on treatment (difference of -15.1% [95% CI, -36.3, 7.1]).
Summary/Conclusion: A similar proportion of patients treated with ravulizumab and eculizumab required transfusion; however, numerically fewer total transfusions and total units were received in the ravulizumab vs the eculizumab group. Fewer patients with a history of AA received transfusions on ravulizumab vs those on eculizumab, suggesting that in AA patients the benefit of more consistent C5 blockade by ravulizumab may be more apparent. Additional studies are needed to confirm these differences. These findings support use of ravulizumab in treatment-naive patients with PNH, with or without history of AA.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Prevalence and incidence of venous thromboembolism in Norway 2010 – 2017

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Background: Risk factors for VTE development are well characterised and there are many different options for both primary and secondary prophylactic treatment. Despite this, prior studies have shown an increasing incidence of VTE, driven by an increase in PE diagnoses. However, more recent data on trends in VTE from the Scandinavian setting are not available.
Aims: The study had two aims: to calculate the incidence and prevalence of VTE in Norway in 2017, and to assess whether the incidence of VTE had changed over time.
Methods: Patients in the nationwide Norwegian patient registry (NPR) aged >18 were included in the study if they had an inpatient or outpatient diagnosis of either deep vein thrombosis (DVT) or pulmonary embolism (PE; with or without DVT). Data was available from 2009. An incident event was considered a primary diagnosis of either PE or DVT with no history of either event; when comparing trends over time a fixed one year look back window was applied to prevent the introduction of bias. We calculated prevalence and incidence using the size of the Norwegian national population extracted from the Statistical Central Bureau (statistisk sentralbyrå) in Norway as the denominator, assuming patients were followed for an entire calendar year. All estimates were stratified by type of VTE, age-group, gender and calendar year, and, when assessing changes over time, age and sex standardised to the Norwegian population in 2010.
Results: We identified 63,274 individuals who met the inclusion and exclusion criteria. The prevalence of VTE in 2017 was 0.26% (95% CI = 0.25 - 0.26; 0.14% PE and 0.13% DVT), and the incidence 1.38 events per 1,000 person-years of follow-up (PYFU; 95%CI = 1.34 – 1.41; 0.67 events/1,000 PYFU PE and 0.71 events/1,000 PYFU DVT). The incidence of both PE and DVT increased with increasing age (PE: 0.12 [95%CI = 0.10 – 0.15] to 3.21 [95%CI = 2.72 – 3.78] and DVT: 0.70 [95%CI = 0.66 – 0.73] to 3.45 [95%CI = 2.95 – 4.04] events per 1,000 PYFU). The age and sex standardised incidence of PE increased by 11% between 2010 and 2017, whereas the age and sex standardised incidence of DVT decreased slightly by 6%. The standardised incidence of VTE was stable over the time period.
Summary/Conclusion: Our results indicate that previously reported increases in the incidence of VTE in Norway have stabilised; with a modest increase in PE incidence offset by a decrease in DVT incidence. Further research is needed to establish the cause of the changes in PE and DVT incidence over time.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Efficacy and safety of the long-acting complement C5 inhibitor ravulizumab in adult patients with atypical haemolytic uraemic syndrome (aHUS)

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Background: Ravulizumab is a long-acting C5 inhibitor designed by altering 4-amino-acids from eculizumab, leading to increased neonatal receptor recycling and increased elimination half-life, thus extending the maintenance dosing interval from every 2 to every 8 weeks.
Aims: The current analysis evaluated the efficacy and safety of ravulizumab in adults with atypical haemolytic uremic syndrome (aHUS).
Methods: This study (NCT02949128) was a global, phase 3, single arm study in complement inhibitor-naïve adults with aHUS. Patients ≥18 years of age who fulfilled diagnostic criteria for aHUS and active thrombotic microangiopathy (TMA) were included and received ravulizumab. No plasma exchange or infusion was allowed during the study. The primary endpoint was complete TMA response during the 26-week initial evaluation period, as evidenced by normalization of platelet count and lactate dehydrogenase and ≥25% improvement in serum creatinine from baseline. Patients had to meet all complete TMA response criteria at 2 separate assessments obtained at least 28 days apart, and any measurement in between. Secondary endpoints included change in estimated glomerular filtration rate (eGFR) and chronic kidney disease (CKD) stage improvement from baseline.
Results: The efficacy analysis set comprised 56 patients with a median (range) age of 40 (20–77) years, 19 (34%) of whom were male. A complete TMA response was achieved in 30 patients (54%; Table). Selected secondary endpoints are shown in the Table. More than 99% complete and sustained suppression of free C5 was observed throughout the dosing interval. The most frequent serious adverse events (SAEs) were hypertension and pneumonia, which occurred in 3 patients (5%) each; 4 deaths were reported (three occurring within 1 month of study initiation). No deaths were considered treatment-related. There were no identified meningococcal infections (all patients were vaccinated prior to treatment).
Summary/Conclusion: Treatment with ravulizumab at 8-weekly dosing intervals resulted in improved haematological and renal endpoints with no unexpected safety concerns.

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Hidden antithrombin variant detected by whole genome sequencing that modulates the N-glycosylation efficacy. Clinical implications and basic mechanisms.

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Background: Venous Thrombosis (VT) is a complex common disease where genetic alterations play crucial roles. However, they are difficult to detect, particularly if no intermediate phenotype is found. N-glycosylation is a postranslational modification which decorates the peptide chains with oligosaccharides at N-X-S/T sequon. It is very relevant on the correct folding, secretion, function and half-life of glycoproteins. Antithrombin (AT), the main endogenous anticoagulant, has 4 N-glycosylation sequons. One of them, at N167 position, is inefficiently glycosylated and is responsible for the beta glycoform (3N-glycans) with higher heparin affinity. The implication of the correct N-glycosylation on the thrombotic risk is sustained on the clinical data of patients with congenital disorder of glycosylation, a recessive disorder that cause AT deficiency. However, no N-glycosylation defect specific to AT associated to thrombosis has been described yet.
Aims: To identify the genetic variants and the associated mechanism causing a strong history of VT in a family with normal thrombophilia screening.
Methods: Thrombin generation was studied. Whole genome sequencing (WGS) was performed in symptomatic relatives. Recombinant antithrombins were produced by directed mutagenesis and expressed in HEK-EBNA cells. Anticoagulant activity (anti-FXa and anti-FIIa) and electrophoretic characteristics of plasma and recombinant antithrombin purified by FPLC was assessed by chromogenic and wester-blot assays.
Results: The proband was a 44y male who presented idiopathic portal thrombosis. He had two daughters with early VT. Classical thrombophilia tests were negative, but they all had higher endogenous potential of thrombin generation. WGS identified a new variant in heterozygous state p.E227K in *SERPINC1*, the gene encoding antithrombin. Functional and antigenic levels of AT were in the normal range in different time points except in two patients, who showed low functional antithrombin during the acute phase, while under curative dose of heparin. However, longer incubation times of plasma with the target proteases revealed an impaired anticoagulant activity in carriers. Western blot analysis of plasma AT showed the presence of an abnormal variant protein in carriers. Recombinant expression of the variant in HEK-EBNA cells demonstrated that: i) the mutation did not significantly impair secretion of the variant protein, but it has impaired anticoagulant activity; ii) the mutation causes the loss of N-glycosylation efficacy at N224. The presence of lysines flanking this position but also N167 lead us think the possibility that this electropositive residue could rest efficacy on the N-glycosylation. This hypothesis was confirmed by directed mutagenesis introducing or eliminating lysines close to consensus sequons at N167, N224 and N187. The results confirmed that the Lysine at +1, +3, +4, -2 and -4 positions significantly impaired the efficacy of the N-glycosylation.
Summary/Conclusion: This study identified a new variant of *SERPINC1* hidden by functional methods but that reduced the anticoagulant capacity of antithrombin, and affects the efficacy of N224-glycosylation. This is the first evidence of a higher risk of thrombosis associated to an N-glycosylation defect specific to antithrombin and show for the first time the role of lysines in the modulation of the N-glycosylation efficacy.
PI18/00598(ISCIII&FEDER); 19873/GERM/15(Fundación Séneca); GENMED(ANR-10-LABX-0013); «EPIDEMIOM-VTE» University of Bordeaux

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Recurrence risk of transient inflammation-associated venous thromboembolism: similar to provoked, unprovoked or in-between?

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Background: Inflammation increases the risk of venous thromboembolism (VTE). After resolution of inflammation, the coagulation system is no longer activated by this trigger. Inflammation might therefore behave as an reversible risk factor, and be associated with a decreased risk of recurrence.
Aims: To explore the role of transient inflammation in the risk of recurrent VTE
Methods: We performed a retrospective cohort study on subjects with a first VTE who were included in the BEAST case-control study between 2008 and 2010. In the BEAST study patients suspected of pulmonary embolism (PE) and/or deep venous thrombosis (DVT) were asked whether they had experienced transient inflammatory signs (i.e. upper or lower respiratory infection, gastro-enteritis, fever or malaise) in the four weeks prior to presentation at the emergency department. The aim of the BEAST study was to determine the association between those transient inflammatory signs and the risk of DVT and/or PE.
The primary endpoint for the current study was recurrent VTE confirmed by radiology reports and use of therapeutic anticoagulation for at least three months. Subjects were asked informed consent for retrieval of information from medical reports. Rates of recurrent VTE were compared between inflammation- and non-inflammation-associated VTE and analyses were stratified by unprovoked vs. unprovoked index-VTE. Follow-up started at cessation of anticoagulation for the index event. Cumulative incidences were determined with a cumulative incidence function accounting for death as competing risk. In addition, Cox proportional hazards regression was used to estimate hazard ratios (HR) adjusted for sex.
Results: In total, 109 eligible subjects contributed 798 person-years follow-up (PY FU) (median 8.3 years); median age was 52 years (interquartile range 38 – 66) and 63 (57.8%) were men. There were 48 unprovoked index-VTE of which 20 were inflammation-associated. Of the 61 provoked index-VTE, 24 were inflammation-associated.
In stratum concerning unprovoked index-VTE, recurrence rate of inflammation-associated index-VTE was lower than of non-inflammation-associated index-VTE (4.9/100 person years (PY) vs. 7.5/100 PY). Cumulative incidences for recurrence were 34.3% (95% CI 13.3% - 56.7%) and 48.9% (95% CI 28.2% - 66.7%) respectively, at 8 years of follow-up.
In stratum concerning provoked index-VTE, recurrence rates were similar (inflammation-associated (3.0/100 PY vs. non-inflammation-associated 2.7/100 PY). Cumulative incidences for recurrence were 23.4% (95% CI 10.7% - 38.8%) for inflammation-associated index-VTE and 21.1% (95% CI 7.4% - 39.3%) non-inflammation-associated index-VTE at 8 years of follow-up.
Sex-adjusted HRs for inflammation-associated vs. not inflammation-associated index-VTE were 0.70 (95% CI 0.27 – 1.77) and 0.97 (95% CI 0.31 – 2.97) for unprovoked and provoked respectively.
Summary/Conclusion: Transient inflammation might lower the recurrence risk after an otherwise unprovoked index-VTE. Transient inflammation could therefore be considered as a minor provoking factor.

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Patients with high post-endovascular treatment plasma levels of neutrophil activation markers are at higher risk of poor functional outcome, HT, and mortality.

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Background: Endovascular therapy (EVT) dramatically improved the outcome of patients with ischemic strokes secondary to large vessel occlusions. But 57% of EVT-treated patients still shares a poor functional outcome.
Aims: We investigated possible relationships between plasma levels of neutrophil activation markers and clinical characteristics and outcome in EVT-treated stroke patients.
Methods: Neutrophil activation markers were measured in plasma samples from 72 acute ischemic stroke patients. For each patient, samples were drawn before EVT (baseline), 1 hour after the end of EVT, and 24 hours after EVT.
Results: Baseline levels of neutrophil activation markers were not associated with in hospitalmortality,outcome, or hemorrhagic transformation (HT). At 1 hour after EVT, there was a statistically significant increase in plasma myeloperoxidase (MPO) and citrullinated histones (H3c) as compared to baseline. Although intravenous t-PA treatment prior to EVT did not affect baseline MPO plasma levels, it lowered the increase in plasma MPO observed at 1 hour post-EVT. In hospital-mortality was associated with higher plasma levels of H3c at 1 hour post-EVT, and with higher plasma levels of MPO, MMP9, and neutrophil elastase at 24 hours post-EVT. Patients with a favorable outcome (mRS≤2) had lower MPO, MMP9, and H3c plasma levels at 1 hour post-EVT, and lower MPO, neutrophil elastase, and H3c plasma levels at 24 hour post-EVT, as compared to patients with a mRS>2. Patients evolving towards HT had higher MPO and H3c plasma levels at 1 hour post-EVT, and higher MPO and neutrophil elastase plasma levels at 24 hour post-EVT, as compared to patients without HT.
Summary/Conclusion: In conclusion, patients with high post-EVT plasma levels of neutrophil activation markers are at higher risk of poor functional outcome, HT, and mortality.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Plasma levels of complement component C5 are associated with future risk of incident venous thromboembolism

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Background: Despite a well-known crosstalk between the complement and hemostatic systems, it remains largely uncertain to what extent the complement system is involved in the pathogenesis of VTE. Recently, C5 deficient mice were shown to have lower incidence of venous thrombosis and smaller thrombi than wild-type mice. We recently reported that increased plasma levels of the terminal soluble C5b-9 complement complex (TCC), which is the final activation product of complement, was associated with future risk of incident VTE.
Aims: To investigate (i) whether the plasma levels of the main parent molecule of TCC, C5 in its native form, is associated with future VTE, and (ii) to explore whether the ratio between TCC and C5 would improve the prediction of VTE risk.
Methods: A nested case-control study consisting of 415 subjects with incident VTE, and 848 age- and sex-matched controls was derived from a population-based cohort (the fourth survey of the Tromsø study). Plasma levels of complement C5 and TCC were measured at the time of inclusion. The odds ratios (ORs) with 95% confidence intervals (CI95%) for VTE across quartiles of plasma levels of C5 and the TCC/C5 ratio compared to controls were calculated using logistic regression models.
Results: The risk of VTE increased linearly across quartiles of plasma C5 levels (*p* for trend <0.001). Participants with C5 levels in the highest quartile (>65 µg/mL) had 73% higher OR for VTE (OR 1.73, CI95% 1.23-2.43) compared with those in the lowest quartile (≤49 µg/mL) in analyses adjusted for age and sex. Further adjustment for body mass index (BMI) and C-reactive protein (CRP) lowered the OR for VTE slightly (OR 1.57, CI95% 1.10-2.24). A substantially higher OR for VTE in the highest quartile of C5 was observed in samples collected shortly before VTE event. The TCC/C5 ratio yielded lower ORs for VTE than the C5 levels alone.
Summary/Conclusion: Our findings show that plasma C5 levels are associated with future risk of VTE. The ORs for VTE by C5 levels were even higher for blood samples collected shortly before the VTE event. The association between plasma C5 levels and VTE risk was not explained by chronic inflammation alone, as adjustment for CRP only marginally affected the risk estimates. The TCC/C5 ratio did not strengthen the association with VTE risk compared to the C5 levels alone.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Detection of pulmonary embolism in exhaled breath condensate - a porcine model

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Background: The search for biomarkers for pulmonary embolism has mainly been focused on plasma biomarkers. Exhaled breath condensate (EBC) can be collected non-invasively and consists mostly of water vapor, but small amounts of endogenous proteins are also present. Biomarkers specific for chronic lung diseases and cancer have been identified in the EBC, yet the potential of EBC for diagnosis of pulmonary embolism (PE) has not been investigated.
Aims: We hypothesized that EBC is a possible source of quantifiable proteins in a porcine model of intermediate-high-risk PE.
Methods: Fourteen pigs were anaesthetized and mechanically ventilated through a tracheal tube. EBC was collected by condensation of the exhaled air via the ventilator system one hour after intubation. The EBC collection time was 2 x 15 minutes, the initial condensing temperature was -80 ºC. Sixty milliliters of venous blood was drawn from each of the pigs and distributed to 4.75" uncoated extra corporal tubes and left for coagulation at room temperature for approximately three hours. Serum was discharged before the autologous emboli were induced through a 26 French sheath inserted in the right external jugular vein. Transesophageal echocardiography and biventricular heart catheterizations evaluated the hemodynamic responses to the infused emboli. Biochemical signs of PE were evaluated by consecutive arterial blood gas analysis. EBC samples were collected again 30 minutes and 2.5 hours after PE. Label free quantitative nano liquid chromatography - tandem mass spectrometry (MS) was used to identify and quantify proteins of the EBC. The raw MS data files were searched against Uniprot databases, *Sus scrofa* and *Homo sapiens* downloaded using MaxQuant (v1.5.5.1) for label free quantification. Perseus (v1.5.8.5) was used for further filtering and statistical analysis. Proteins identified as potential contaminants, only identified by site or by the reverse part of the database were removed. Paired t-tests were used to calculate the differences and associated p-values for proteins in the EBC samples obtained before PE compared with the EBC samples collected after PE. The study was approved by the Danish Animal Research Inspectorate (licence number: 2016-15-0201-00840).
Results: The 14 pigs suffered intermediate-high-risk PE with hemodynamic signs of right ventricular strain but preserved cardiac output and unaltered systolic blood pressure. The arterial blood gas analysis showed lower partial pressure of oxygen after PE while the lactate concentration was unaltered. The mean volume of obtained EBC was 1.4 ml per 15 minutes of collection. The mean protein concentration in the EBC was 2.8 ± 0.1 µg/ml corresponding to a mean concentration of proteins in the exhaled breath of 4.3 µg/100 l exhaled breath ± 0.3. In total 1027 proteins were identified in the EBC samples. After filtering in Perseus, 45 proteins were only identified by site, 9 were identified in the reverse database and 76 were potential contaminants. The mean number of identified proteins in the EBC samples was 249. In paired analysis, 94 proteins were present at markedly altered amounts in the EBC samples collected after PE compared with the EBS samples collected before PE.
Summary/Conclusion: We identified many hundred proteins in the ECB collected from pigs with intermediate-high-risk PE. EBC is obtained non-invasively and may be a new source for biomarkers for PE.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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P-059

The pharmacokinetics and pharmacodynamics of nadroparin in children with venous thromboembolism

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Background: Low-molecular-weight heparins (LMWH) are commonly used in pediatric venous thromboembolic disease (VTE). Since pediatric VTE is a rare disease, data on therapeutic LMWH dosages and their efficacy and safety in children is limited, in particular for nadroparin.
Aims: This retrospective study evaluated the therapeutic dosages of nadroparin required to reach therapeutic target ranges (TTR) (anti-Xa level between 0.5-1.0 IU/mL), and, the safety and efficacy of nadroparin in terms of mortality, bleeding complications, development of recurrent VTE and cloth resolution.
Methods: Medical charts were reviewed for all children with VTE, aged 0 – 18 years old, treated with therapeutic dosages of nadroparin in Erasmus MC Sophia Children's Hospital in Rotterdam between 1 January 2007 and 31 December 2018.
Results: A total of 144 children were included (men: women= 1:1.1). Of those children, 69% (n = 100) reached TTR. The median (±min/max, number) dose to reach TTR was 394.0 IU/kg/day (195.8 – 660.6, n = 34) in neonates, 296.0 IU/kg/day (220.7 – 518.9, n =12) in infants, 261.1 IU/kg/day (200.0 – 387.8, n = 14) in children 2-6 years old, 288.1 IU/kg/day (175.4 – 436.2, n = 10) in children from 6-12 years old and 207.3 IU/kg/day (131.0 – 315.0, n = 30) in adolescents. ($p<0.0001$). Younger children needed significantly more dose adjustments to reach TTR than older children. The median time to reach TTR did not differ significantly between age groups. None died due to VTE. Major and clinically relevant non-major bleedings were observed in 3/144 (2%) and 8/144 (5.5%) patients, respectively. Nineteen children (13%), including 10 neonates, suffered from severe subcutaneous catheter complications. Recurrent VTE occurred in 8/138 (5.8%) patients during nadroparin treatment. In 87.5% of those patients, anti-Xa levels were below 0.5 IU/mL. Clot resolution was achieved in 87.2% (n = 109/125) of patients after treatment.
Summary/Conclusion: Younger children needed higher nadroparin doses and more dose adjustments to reach TTR. Nadroparin therapy in pediatric VTE seems to be effective and safe. Use of subcutaneous catheters should be discouraged.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Comparison of coagulation properties of microparticles derived from platelets, erythrocytes, leukocytes and endothelial cells

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Background: Membrane microparticles (MPs) released by activated or apoptotic cells possess coagulation activity since they express phosphatidylserine and some of them tissue factor (TF).
Aims: Direct comparison of coagulation properties of MPs produced *in vitro* by platelets, erythrocytes, leukocytes (monocytes and granulocytes) and endothelial cells (ECs).
Methods: Platelets, erythrocytes, monocytes and granulocytes were isolated from the blood of healthy volunteers. ECs from human umbilical vein and monocytic THP-1 cells were cultured under standard conditions. Platelets were activated by thrombin receptor activating peptide, erythrocytes by calcium ionophore A23187, monocytes, granulocytes and ECs by bacterial lipopolysaccharide. MPs were sedimented from the culture media and cell supernatants at 20000 g for 30 min. MPs were counted by flow cytometry and their size was evaluated by dynamic light scattering. Coagulation activity of MPs was examined using modified plasma recalcification assay and a new "thrombodynamics" assay which allows visualizing the process of clot formation in plasma [Dashkevich NM, et al. Thromb. Res. 2014; 133: 472-6]. TF activity was measured by its ability to activate factor X.
Results: All MPs significantly accelerated plasma coagulation in a recalcification assay. The shortest lag times were detected for MPs from monocytes, longer for MPs from THP-1 cells and ECs, and the longest for MPs from platelets, erythrocytes and granulocytes. Coagulation activity of all MPs was completely inhibited by a phosphatidylserine blocker lactadherin. In a "thrombodynamics" assay MPs were able to induce formation of small clots within the plasma volume. Minimal concentrations inducing formation of such clots were detected for MPs from monocytes, slightly higher for MPs from THP-1 cells, higher for MPs from ECs, and the highest for MPs from platelets and erythrocytes (MPs from granulocytes were not tested by this method). Average diameters of MPs of different cellular origin ranged within 200-600 nm with no direct correlation with their coagulation activity. The largest MPs were produced by ECs and granulocytes (550-600 nm), slightly smaller by monocytes and THP-1 cells (400-500 nm), smaller by platelets (300-350 nm) and the smallest by erythrocytes (200-250 nm). The highest TF activity was detected in MPs from monocytes, slightly lower activity in MPs from ECs and THP-1 cells and no activity in MPs from platelets, erythrocytes and granulocytes. Anti-TF blocking antibody prolonged coagulation lag times in a recalcification assay for MPs from monocytes, ECs and THP-1 cells and equalized them with those for MPs from platelets, erythrocytes and granulocytes. Inactivated factor VII (also serving as a TF blocker) inhibited clot formation in a "thrombodynamics" assay induced by MPs from monocytes, THP-1 cells and ECs, but not by MPs from platelets and erythrocytes.
Summary/Conclusion: Significant variations of coagulation activity were revealed between MPs of different cellular origin. Higher activity of MPs from monocytes, THP-1 cells and ECs in comparison with MPs from platelets, erythrocytes and granulocytes was mainly determined by the presence of active TF.
This work was supported by the Russian Foundation for Basic Research (grant # 17-04-00347).

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Inhibitor effects on clot growth: different profiles of inhibition

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Background: A new pharmacodynamic test system for coagulation has been developed in which clot growth rate and size can be documented with a video-microscopic system: Thrombodynamics. An additional read out is the thrombin generation on a fluorogenic substrate during clot formation at several distance from the surface (defined by the stationary amplitude: Ast). Thrombin in the clot is related to fibre structure and lysability.
Aims: To test effects of variations in clotting factor and inhibitor concentrations.
Methods: Video-microscopic recording of clot growth and fluorimetric thrombin generation at several distances from the activating surface.
Results: Increase in factors VIII, IX and X results in dose dependent increase in both clot growth and Ast.
Decrease in endogenous inhibitors, antithrombin, Heparin Cofactor II, protein S, protein C and TFPI, have only minor effects on clot growth but significant dose dependent effects on Ast. The lower the inhibitor the higher the Ast Ast increases at 50% inhibitor level for protein C to 125%; for protein S to 165%, for HCoFII to 190%, for TFPI to 255% and for antithrombin to 770%. In Factor V Leiden heterozygotes Ast is high and variable (mean 462% , CV 62%, n=5).
Heparinoids reduce both growth and Ast; high levels result in a full stop of growth and thrombin formation.
DOACs showed dose dependent partial inhibition of clot growth to a plateau at about 50% of normal growth. Also Ast reduced dose-dependently to a plateau. Thrombin Ast plateau was 40% (Dabigatran), 45% (Argatroban) and 65% (Rivaroxaban), 70% (Apixaban) and 65% (Edoxaban) of plasma without DOAC.
Summary/Conclusion: It is concluded that DOACs and heparinoids inhibit adequately both growth and Ast. This is rational for treatment in case of high levels of factor VIII, IX and XI, which we showed to increase both growth and Ast. However, when used in a situation with reduced endogenous inhibitors, DOACs overcompensate the deficiency by also reducing normal clot growth. The smaller clots resulting from slower growth may be related to a more pronounced risk of bleeding.

ECTH 2019
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Alpha2-plasmin inhibitor and its C-terminal proteolytic variation in venous thrombosis

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Background: Alpha2-plasmin inhibitor (A2-PI) is the main inhibitor of plasmin. During circulation in plasma A2-PI undergoes both N- and C-terminal cleavages. The N-terminal cleavage is mediated by antiplasmin cleaving enzyme (APCE) resulting in a 12 amino acids shorter Asn1-A2-PI, which is cross-linked to fibrin more efficiently by activated factor XIII. The binding of plasmin(ogen) kringle domains 1-3 to the Lys-binding sites on the C-terminal part of A2-PI is the first and important step for the formation of stable plasmin-antiplasmin complex. The C-terminally cleaved form of A2-PI lacks the plasminogen-binding site, which is located in the C-terminal 26 amino acids of the molecule. The C-terminally cleaved form remains an active plasmin inhibitor, but reacts more slowly with plasmin.
Aims: In this case-control study we investigated whether total A2-PI antigen level and A2-PI C-terminal cleavage are associated with venous thrombosis.
Methods: Citrated plasma samples were obtained from 308 healthy controls (HC) and from 300 patients with venous thromboembolism (VTE) at least three month after the acute event. Total and C-terminally intact A2-PI antigen levels were measured by sandwich ELISA methods using two monoclonal capture antibodies, one is reacting with all form of A2-PI and the other reacting with the plasminogen binding part of non-truncated A2-PI, respectively and a polyclonal anti-A2-PI antibody for detection.
Results: The female/male ratio was higher in the control (189/119) than in the patient group (146/154). Patients were older than controls (median(IQR) year: 38(26-49) vs. 58(44-70), and had higher body mass index (BMI) (median(IQR): 29.0(25.9-32.9 vs. 25.1(21.6-28.1). Only BMI showed independent association with total and C-terminally truncated A2-PI antigen levels. Both total and C-terminally truncated A2-PI antigen levels adjusted for BMI were significantly elevated in the VTE group comparing to controls (adjusted mean (95% CI) 71.5 (70.4-72.5) mg/L vs. 64.9 (63.9-66.0) mg/L; $p<0.001$ and 30.0 (29.1-30.9) mg/L vs. 22.2 (21.3-23.1) mg/L; $p<0.001$, respectively). The ratio of C-terminally truncated A2-PI in patients was higher than in controls (41.6±6.3% vs. 33.6±9.1%). Total A2-PI in the upper quartile (>72 mg/L) significantly increased the risk of VTE (OR (95% CI): 3.835 (2.72-5.41); $p<0.001$). C-terminally truncated A2-PI in the upper quartile (>28.1 mg/L) also significantly increased the risk of VTE (OR (95% CI): 6.09 (4.28-8.67); $p<0.001$) and the association remained significant after adjustment for BMI, age, gender, fibrinogen and high total A2-PI level (OR (95% PI): 5.33 (3.00-9.46; $p<0.001$).
Summary/Conclusion: The increase of C-terminally truncated A2-PI concentration present a higher risk of VTE than the elevation of total A2-PI concentration alone.

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Rosuvastatin use reduces the procoagulant phospholipid (PPL) activity in patients with venous thromboembolism: a randomized controlled trial

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Background: Growing evidence support that statin therapy protects against incident and recurrent venous thromboembolism (VTE). However, the underlying mechanism(s) for the protective effect of statins on VTE risk is not known. Plasma levels of procoagulant phospholipids (PPL) are mainly reflecting plasma levels of extracellular vesicles expressing negatively charged phospholipids (i.e. phosphatidyl serine) on their external surface
Aims: To investigate the impact of rosuvastatin treatment on plasma PPL activity in subjects with a previous history of VTE in a randomized controlled trial.
Methods: STAtins Reduce Thrombophilia, or the START trial (NCT01613794), is a multicenter, randomized, controlled, open label clinical trial aimed to investigate the impact of rosuvastatin treatment on the coagulation profile of participants with a previous history of VTE. Patients were recruited from three Dutch anticoagulation clinics (Leiden, Hoofddorp, and Rotterdam). After anticoagulation withdrawal, patients were randomized to rosuvastatin 20 mg day^{-1} (n=126) or no intervention (n=121) for 4 weeks. Plasma samples were collected before and after the intervention. A slightly modified factor Xa-dependent PPL clotting assay (in-house) was used to measure plasma levels of PPL in citrated platelet free plasma. Plasma PPL clotting times were converted to PPL activity in mU/ml by the using a standardized UPTT calibrator (BioData Corporation, Horsham, Pennsylvania, USA).
Results: Plasma PPL activity decreased from baseline to study end for rosuvastatin users (mean change, -0.48 mU/ml; 95% CI -0.81 to -0.15), while a minor increase was observed for non-users (mean change, 0.17 mU/ml; 95% CI -0.18 to 0.53) for VTE, yielding a statin treatment effect of 22% reduction in PPL activity. For pulmonary embolism (PE), the statin treatment effect was particularly strong, and accounted for a 37% reduction in PPL activity. The observed decrease in plasma PPL activity was not associated with the concomitant changes in total cholesterol levels.
Summary/Conclusion: Rosuvastatin treatment for 4 weeks substantially decreased plasma PPL activity in patients with a prior VTE, and particularly in patients with a prior PE. Our findings suggest that statin treatment may be beneficial adjuvant therapy against recurrent VTE.

ECTH 2019
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Replacement of pt-inr monitoring of warfarin with FIIX-NR reduces thromboembolism, anticoagulation variability, testing frequency and dose adjustment need without raising time in range

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Background: The new Fiix prothrombin time (Fiix-PT, Fiix-test) is only affected by reduced FII and FX. In the double-blind randomized clinical Fiix-trial, monitoring of warfarin with Fiix-PT (Fiix-NR) was compared to high time-in-range PT-INR monitoring. Fiix-NR monitoring increased time-in-range (TTR), reduced normalized ratio variability and testing frequency, reduced dose-adjustment need and reduced thromboembolism (TE) without increasing major bleeding (MB). Consequently, in 2016, we replaced the PT-INR with Fiix-NR in our warfarin monitoring practice.
Aims: We now report anticoagulation outcome before and after replacement of the PT-INR with Fiix-NR in our warfarin population.
Methods: All patients managed at our anticoagulation management service in Reykjavik were initially included irrespective of target range but the analysis includes only patients on maintainance phase anticoagulation (after the first 60 days of warfarin treatment). All were dosed by specialized staff using the DAWN anticoagulation software®. The incidence of TE and MB (by ISTH criteria) before and after replacing the monitoring test was compared by extracting ICD codes from electronic hospital chart systems. Relative risks of adverse events were calculated. Other anticoagulation indicators included testing frequency and intervals, anticoagulation (normalized PT ratio) variability (i.e. variance growth rate; VGR), dose adjustments and time in range (TTR, Rosendaal method). Clinical data is shown as relative risk plots with annual incidence and p-values (Chi square with Yates correction). Anticoagulation indicators are shown as medians with interquartile ranges with p-values calculated using the Mann-Whitney test.
Results: After excluding the warfarin naïve period in 309 patients monitored with PT-INR and 211 patients monitored with Fiix-NR (38 and 27 observation years, respectively), the analysis is based on maintainance phase data during 1959 and 1966 observation years. Indications and target ranges did not differ during the two periods. The point estimates of the relative risk calculations consistently suggested a 30-40% reduced TE during Fiix-NR monitoring but MB was similar before and after. Fiix-NR monitoring associated with a reduction in total TE (excluding TIA) from 2.55% annually (per patient year) to 1.27% (RR 0.66 (IQR 0.48-0.91); p<0.005), total arterial TE (excl TIA) from 2.19% to 1.12% (RR 0.67 (0.48-0.95); p<0.02), cerebral infarction (excl TIA) from 1.07% to 0.46% (RR 0.60 (0.35-1.03); p<0.05). TIA´s were not reduced by Fiix-NR monitoring (0.61% vs 0.51%, respectively; p = 0.82). The MB incidence was 2.91% vs 2.64%, respectively (n.s.). The intracerebral bleeding incidence was low or 0.25% with both monitoring methods. Less variability of anticoagulation was evident in the Fiix-NR monitoring group by a lower VGR (0.13 vs 0.19; p<0.0001), 18% fewer tests per patient year (p<0.0001), a 24% prolongation of the testing interval (from 21 to 26 days;p<0.0001) and 24% fewer dose adjustments ppy (from 4.9 (1.1-11.1) to 3.7 (0.8-8.1); p<0.0001). The median TTR was 77% both before and after.
Summary/Conclusion: These clinical practice results confirm the blinded Fiix-trial results, namely that Fiix-PT monitoring during VKA monitoring appears safe and leads to about 30-40% reduction in thromboembolism without increasing bleeding compared to standard monitoring. The main explanation appears to be reduced anticoagulation variability as the TTR was identical.

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The Value of Neuroimaging and Implications for Long-term Neurological Sequelae of Thrombotic Thrombocytopenic Purpura: A Tertiary Centre Experience

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening disease caused by severe deficiency of the von Willebrand cleaving protease, ADAMTS13. The formation of platelet-rich thrombi in the microvasculature results in ischaemic organ damage, which can involve the central nervous system.
Aims: Despite neurological manifestations clinically, abnormalities on neuroimaging may not be evident; we aim to explore if detectable changes correlate with longer term outcomes.
Methods: A retrospective review of patients attending a single UK TTP tertiary referral centre from 1998 to 2018 was performed. Only patients with an ADAMTS13 activity <10% at presentation were included. Data collection included patient demographics, presenting neurological symptoms, neuroimaging modality, results and long-term neurological complications.
Results: A total of 59 patients (42 female and 17 male) were studied comprising 86 acute patient episodes. 43/59 patients had a single episode of TTP vs. 16/59 having multiple relapses. 42/59 patients had neurological symptoms at 1st presentation with the most common presenting symptoms being reduced GCS, 24/59 and headache, 14/59.
Neuroimaging data was available in 47/59 patients at 1st presentation; 29/47 patients had neuroimaging performed, all due to neurological symptoms. Of these, 15 had CT imaging, 10 had MRI and 4 had a combination of both. In total, 36 individual scans were performed within 4 weeks of presentation and reports were available on 32 of these. 14/19 CT scans and 8/13 MRI scans were reported as normal. Abnormalities were reported on 10 scans (5 CT and 5 MRI scans), with the most commonly reported abnormalities being cerebral infarcts and small vessel ischaemia.
16/59 patients experienced >1 acute episode, comprising 24 acute relapse episodes. 9/24 presented with neurological symptoms of which 5 had neuroimaging. In the 5 relapse episodes where neuroimaging was performed, there were 3 CT scans, 2 MRI scans and 1 MR angiogram. 4 scans were reported as normal; 1 CT scan and 1 MRI were reported as abnormal showing areas of hypoattenuation.
Surviving patients (54) were assessed for long-term neurological complications; 23/54 were reported persistent long-term symptoms at their last clinic review (follow-up range 1-30 years). The most frequent long-term complications were minor cognitive impairment/reduced concentration, followed by recurrent headache.
Summary/Conclusion: This study confirms that neurological symptoms ranging from headache to reduced GCS are common presenting symptoms in acute TTP, affecting around 70%. Symptoms are less common at relapse likely due to earlier presentation. Around 60% of our patients underwent neuroimaging with CT scanning being the most common investigation. Around 60% of scans performed were reported normal. Cerebral infarcts and small vessel ischaemia consistent with underlying pathophysiology of TTP was the most frequent abnormality found. Around 40% of patients reported long-term neurological complications with a higher incidence in those demonstrating neurological symptoms and abnormal imaging in their 1st acute presentation.
This study suggests that abnormal neuroimaging in patients with neurological signs in their first TTP presentation may predict those likely to experience long-term neuropsychological complications. The study highlights the growing importance of careful long-term follow-up and support for patients with acute and long term neurological disease resulting from TTP.

ECTH 2019
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Limitations of the Villalta scale in diagnosing the post-thrombotic syndrome

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Background: The Villalta scale is the recommended tool for diagnosing post-thrombotic syndrome (PTS) in clinical trials. The Villalta scale does not consider possible comorbidities, reflect the chronology of deep venous thrombosis (DVT) and leg problems, and might not capture limitations in physical performance, e.g., venous claudication as a PTS-symptom. Hence, both sensitivity and specificity of the scale could be low.
Aims: To evaluate the diagnostic accuracy for diagnosing PTS by the Villalta scale compared to a predefined clinical reference standard.
Methods: During the years 2006-09, the Catheter-directed Venous Thrombolysis (CaVenT) study randomized 209 patients with a high proximal DVT to conventional therapy alone or to additional catheter-directed thrombolysis. In 2017, we invited the 170 CaVenT participants who were still alive to participate in a new cross-sectional follow-up study of the long-term complications after a DVT. In the absence of a gold standard test, we diagnosed PTS by the following predefined clinical criteria: 1. previous objectively verified DVT; 2. development of chronic complaints in the DVT leg; 3. complaints had appeared or worsened following the DVT; and 4. an alternative diagnosis to the patient's complaints not likely. All four criteria had to be present to qualify as PTS and are in line with our clinical practice. We obtained informed consent from all participants prior to the study.
Results: We included 88/170 patients (52%). With our clinical criteria as reference, we found a 75% sensitivity and a 66% specificity of the Villalta scale. The 15 patients, who were diagnosed with PTS by the Villalta scale only, more often reported comorbidities as possible explanations of their leg problems, and more often assessed their discomforts as pain. The 11 patients who were diagnosed with PTS by our clinical criteria only, reported less comorbidity, less pain, but more fluctuating heaviness and edema.
Summary/Conclusion: Our findings indicate that the diagnostic accuracy of the Villalta scale has limitations. Incorporating chronicity, the chronology of DVT and the leg problems, fluctuations of heaviness and edema, and comorbidity could contribute to improved diagnostic accuracy in PTS assessment.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Characterisation of a novel genetically modified FXIII-A L34V model shows altered thromboembolism dynamics

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Background: Coagulation factor XIII (FXIII) is a key enzyme that stabilises blood clots by crosslinking fibrin molecules together, as well as crosslinking fibrinolysis inhibitors into fibrin. The human FXIII-A V34L sequence variant leads to increased activation rates, forming clots with thinner fibrin fibres and smaller pores, and is protective against thrombotic disease. However, the mechanism(s) by which V34L protects against thrombosis is unclear.
Aims: To establish a murine FXIII-A V34L model and study its role in mechanisms of thromboembolic disease.
Methods: FXIII-A 34V and FXIII-A 34L (wild-type) mice were weighed weekly for 12 weeks to compare growth. Plasma FXIII activation was assessed by biotin incorporation assay, and antigen levels by western-blotting. Whole blood clot formation, strength and lysis was measured by ROTEM (EXTEM, EXTEM+tPA, FIBTEM), providing clotting time (CT), maximum clot firmness (MCF) and lysis time (LT). Whole blood clot contraction and erythrocytes extrusion (haemoglobin) were measured for 2h, before the final clot weight was quantified. For pulmonary and cerebral embolism models, mice were anaesthetised and injected with AlexaFluor^{647}-fibrinogen before injuring the inferior vena cava and carotid artery, respectively, with $FeCl_3$ for 3 minutes. After 57 minutes, the mice were perfused-fixed and FITC-albumin (in gelatin) was injected in the circulation. Lungs or brains were harvested, dehydrated, optically cleared and imaged with a light-sheet microscope visualising both FITC-fluorescing vasculature and AlexaFluor^{647}-fluorescing emboli. Image stacks were used to reproduce a 3D image of an organ and emboli larger than 50 μm^3 were quantified using IMARIS software.
Results: No significant difference in animals' growth was observed between the groups. FXIII-A 34L plasma showed a 40.4% increase in activation rates compared to 34V, similar to comparison with human variants. No difference in plasma FXIII-A and fibrinogen antigen levels was observed between the groups. ROTEM studies showed no difference between the groups for CT, MCF and LT, whether in the presence (EXTEM) or absence (FIBTEM) of functional platelets. The clot volumes and serum haemoglobin levels were not significantly different at each time-point between the groups, and the final clot weight was similar. However, 34L mice showed a 45.8% decrease in cerebral emboli count compared to 34V mice, while there was no significant difference in pulmonary embolism data between the two mouse strains.
Summary/Conclusion: The murine FXIII-A 34L variant increased activation rates over the 34V variant comparable to that of the human variant (+68%), despite similar circulating levels. No differences were observed between the variants in *ex-vivo* studies of whole blood clotting, however *in-vivo* studies point to decreased cerebral embolism profiles for mice homozygous for the FXIII-A 34L variant. These results indicate that by altering embolism dynamics FXIII-A 34L variant could possess protective capabilities in the cerebral circulation.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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The risk of deep vein thrombosis and pulmonary embolism attributed to recognized prothrombotic genotypes

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Background: Studies have shown that 45-60% of all venous thromboembolism (VTE) events in the population can be attributed to heritability. The factor V Leiden mutation (FVL) is a single nucleotide polymorphism (SNP) that has consistently shown a stronger association with deep vein thrombosis (DVT) than with pulmonary embolism (PE). This phenomenon is often referred to as the "*FVL paradox*". Whether the FVL paradox applies to other prothrombotic genotypes, and whether the proportion of events in the population that can be attributed to prothrombotic genotypes is larger for DVT than for PE, has been scarcely investigated.
Aims: To estimate the population attributable fraction (PAF) of recognized prothrombotic genotypes in DVT and PE, respectively, using a population-based case-cohort.
Methods: Cases with incident VTE (n=1,480) and a randomly sampled sub-cohort (n=12,897) were derived from the Tromsø Study (1994-2012) and the Nord-Trøndelag Health (HUNT) Study (1995-2008). DNA-samples were genotyped for 17 SNPs associated with VTE. For each SNP of interest, hetero- and homozygous individuals were defined as risk allele carriers. Hazard ratios were estimated in Cox regression models, and PAFs were estimated for SNPs significantly associated with VTE. A cumulative model was constructed by adding SNPs one-by-one in order of the individual PAFs.
Results: Six SNPs were significantly associated with overall VTE. For DVT, the PAFs according to the individual SNPs were 0.4% for rs1799963 (Prothrombin G20210A), 7.6% for rs2066865 (FGG), 11.2% for rs6025 (FVL), 12.3% for rs2289252 (F11), 13.6% for rs2036914 (F11) and 21.2% for rs8176719 (ABO). For PE, the corresponding PAFs were 1.2% for rs1799963 (Prothrombin G20210A), 5.6% for rs2066865 (FGG), 3.3% for rs6025 (FVL), 14.3% for rs2289252 (F11), 15.5% for rs2036914 (F11) and 15.6% for rs8176719 (ABO). The overall PAFs for the six-SNP model was 52.5% for DVT and 33.3% for PE.
Summary/Conclusion: Our findings suggest that the proportion of events that can be attributed to prothrombotic genotypes is larger for DVT (53%) than for PE (33%).

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Anticoagulant treatment duration and risk of recurrent venous thromboembolism: an instrumental variable analysis from the MEGA follow-up study

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Background: Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE) recurs frequently. A pooled analysis of randomized trials showed that the minimum duration of OAC in VTE should be 3 months (Boutitie *et al*, BMJ 2011).
Aims: Although observational studies generally cannot look into the association between duration of OAC and venous recurrence due to confounding (by indication), the MEGA study was conducted in 6 separate regions where policy differed as to how long anticoagulation should be prescribed.
Methods: A population-based study from the Netherlands (MEGA) including patients who had a first VTE between 1999-2004. Patients were followed for recurrence until 2010. Patients who had recurrence during OAC (n=66) or who had active cancer/missing cancer status (n=456) were excluded. First, potential determinants of OAC treatment duration such as thrombophilia, cardiovascular- and VTE risk factors were studied. Next, we classified different subgroups and durations of OAC, divided in 0-4 months, 4-7 months and >7 months. Finally, MEGA was conducted in 6 geographical areas where duration of treatment policies were different: in the Leiden area VTE patients were treated for a median of 142 days, while in the other areas VTE patients were treated for a median of 187 days. Since patients from all these regions are likely similar in terms of underlying illnesses and confounding factors (except for treatment duration), we had the opportunity to analyze if VTE recurred differently in the Leiden- versus non-Leiden area. In such an instrumental variable (IV) analysis, region serves for treatment duration without introducing confounding by indication. Differences in treatment duration were calculated by back-transformation of the natural logarithm of treatment duration. Follow-up started at time of OAC withdrawal. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated between groups and adjusted for neighborhood socioeconomic status and type of thrombotic event (DVT/PE).
Results: There were 4209 patients included in this study with a median follow-up of 5.4 years. The type of thrombotic event (PE) and the patients from the non-Leiden area were on average treated 1.49 (95%CI, 1.44-1.55) fold longer and 1.27 (95%CI, 1.21-1.34) fold longer as compared with DVT patients and patients from the Leiden area, respectively. Other above-mentioned risk factors were not associated with increased treatment time, which is as expected, according to the guidelines at the time. In this observational study we did not know the indications for the durations of treatment, therefore we expected confounding by indication. Indeed, we observed that the longer patients received OAC, the higher the risk was of recurrence (adjusted HR 1.29 (95%CI, 0.99-1.68, in >7 months versus 0-4 months). In our IV analysis, which method adjusts for confounding by indication, we observed that the adjusted HR was 1.14 (95% 0.92-1.41) in the Leiden area versus the non-Leiden area.
Summary/Conclusion: The result from our IV analysis confirms that patients with a first VTE should be prescribed OAC at least 3 months and given the slightly increased risk of recurrence in the Leiden area versus the non-Leiden area possibly even longer than 3 months.

ECTH 2019
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Unravelling the molecular mechanism of tick protein BSAP1, a putative extrinsic coagulation pathway inhibitor

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Background: Ticks are a group of hematophagous parasites counting circa 900 species among 3 families. Although ticks are mostly considered as adverse parasitesthattransmit tick-borne diseases and thereby cause economical losses withinanimal husbandry, they could be used as a valuable source of new leads for drugs and biopharmaceuticals development. Due to their parasitic nature, ticks have to stay unnoticed by a host during feeding time exceeding several weeks for particular species. For that reason, tick saliva acquired in the course of evolution numerous bioactive compound to evade host's immune responses and battle hemostasis. One of front line defense mechanism with which parasites have to deal is the extrinsic coagulation pathway. This pathway is activated byblood vessel damage and excretion of tissue factor (TF) by endothelial cells. Two putative TF binding cysteine-rich proteins have been identified and isolated from the African soft tick *Ornithodoros savignyi* - barium sulfate adsorbing protein 1 and 2 (BSAP1 and BSAP2, respectively).
Aims: In the present study, we attempt to unravel molecular mechanism of BSAP1 anticoagulant activity from structural point of view.
Methods: Samples of native BSAP1 firstly is studied by MALDI-TOF mass spectrometry. BSAP1 gene in the pET23a vector is transfected to BL21 Star *E.Coli* strain and expressed in LB media. After refolding and purification BSAP1 is studied by solution NMR spectroscopy.
Results: Tick proteins are often subjected to post-translation modifications (PMTs) which could drastically affect their activity. To address this matter, BSAP1 obtained from tick saliva were studied by MALDI-TOF mass-spectrometry. Although, N- and C-termini of BSAP1 are proteolitically cleaved loosing up to 3 amino acid residues, observed mass peaks indicate absence of any other PTMs along the sequence. Absence of PMTs allowed us to express BSAP1 in a standard *E.coli* expression system and refolded under oxidative conditions. BSAP1 is expressed at relatively high yield of 1.5-2 mg/L of bacterial culture and preserving N-terminal methionine (met-BSAP1). Metabolically ^{15}N- and ^{13}C-labelled met-BSAP1 were further used for investigation by NMR spectroscopy.
BSAP1 ^{15}N and ^{13}C HSQC spectra were well resolved and contained almost all atom resonances which allowed structure calculation. One of the main challenges for structure elucidation of cysteine rich proteins by NMR spectroscopy is unraveling of the cysteine linkages. To address this issue, we used the in-house developed technique called selenocysteine (Sec) scanning in which NMR spectra of a native protein are compared with similar spectra of its counterpart possessing a single cysteine/selenocysteine mutation. Selenocysteine was introduced in the sequence using expression in the bacterial strain with expanded genetic code. Using C18U and C22U mutants the final C6–C22, C18–C49, C39–C54 disulfide linkage was determined. Determined disulfide was included to structure calculation of BSAP1 along with experimentally observed NOE restraints and TALOS predicted dihedral angles. The calculated structure revealed that BSAP1 has the flexible highly charged C-terminus and the structured core region. The protein core is arranged in the two loops connected by disulfides.
Summary/Conclusion: The structure of BSAP1 was successfully solved by NMR spectroscopy. This method along with other biophysical techniques will be further used to study interaction of BSAP1 with TF.

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Additive effect of oxygen saturation and chronic obstructive pulmonary disease on the risk of incident venous thromboembolism

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Background: Chronic obstructive pulmonary disease (COPD) is a moderate risk factor for venous thromboembolism (VTE). It remains unknown whether lowered oxygen saturation (SpO_2), alone and in combination with COPD, affects the risk of VTE.
Aims: To investigate whether oxygen saturation, alone and combined with COPD was associated with an increased risk of VTE.
Methods: Exposure information (spirometry and SpO_2) was collected in 8686 participants from the fifth (2001/02) and sixth (2007/08) surveys of the Tromsø Study, a population-based cohort. Incident VTE-events were registered from the date of inclusion to December 31, 2016. Cox-regression models with exposures and confounders as time varying co-variates were used to estimate hazard ratios (HR) with 95% confidence intervals (CI) for VTE. The study was approved by the Regional committee of medical research ethics, and all subjects gave informed written consent.
Results: During a median follow-up of 9.1 years, 330 participants developed incident VTE. Subjects with SpO2 ≤96% (lowest 20th percentile) had a 1.5-fold higher risk of VTE (adjusted HR 1.48, 95% CI: 1.13-1.93) compared with those with SpO2 ≥98%. Overall, COPD was associated with an 18% increased risk of VTE (HR 1.18, 95% CI: 0.92-1.50) compared to those with normal airflow in a multivariable adjusted model. The VTE risk increased with the severity of COPD. Subjects with COPD and lowered oxygen saturation (≤ 96%) had 1.7-fold increased risk of VTE (multivariable adjusted HR 1.72, 95% CI 1.17-2.54) compared to those with normal airflow and oxygen saturation (≥ 98%).
Summary/Conclusion: Lowered oxygen saturation was associated with increased VTE risk. COPD combined with lowered oxygen saturation had an additive effect on VTE risk, and may suggest a need for particular attention on VTE preventive strategies in COPD patients with lowered oxygen saturation.

ECTH 2019
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The LumiraDx Platform INR test: Performance and ease of use in an anticoagulation clinic setting

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Background: Vitamin K antagonists (VKAs), such as warfarin, have a narrow therapeutic window; patients on these therapies would therefore require regular international normalised ratio (INR) monitoring to maintain optimal coagulation. The LumiraDx Platform is a new, point-of-care (POC) *in vitro* diagnostic system that can be used for INR testing using fingerstick blood samples.
Aims: This study aimed to evaluate the accuracy and precision of the LumiraDx Platform INR test compared with a laboratory reference method, the IL ACL Elite Pro (Instrumentation Laboratory), in a POC setting.
Methods: The observational, cross-sectional study was conducted at 11 anticoagulation clinics across the Glasgow and Clyde, and Lanarkshire regions. Written informed consent for POC and laboratory testing was obtained from all participants. Precision of the LumiraDx INR test was assessed by correlating duplicate measurements of samples (n=366 subjects) that were either directly applied to the test strips or applied using transfer pipettes. Three test strip lots were used to complete the study. Accuracy was determined by comparing capillary whole blood INR, ascertained by the LumiraDx INR test, with paired venous plasma INR, measured by a laboratory reference method. Furthermore, the accuracy of the LumiraDx INR test was assessed across a range of haematocrit levels (25–55%), measured by the HemoCue Hb 201+ System (HemoCue), using venous whole blood. A questionnaire was completed by the healthcare professionals who were involved in testing to assess the ease of use of the LumiraDx INR test.
Results: Precision (% coefficient of variation) was <4 when samples were applied by direct application (n=284, mean INR 2.54, mean %CV 3.46) or via a capillary transfer pipette (n=291, mean INR 2.53, mean %CV 3.73), as well as between test strip lots. There was a strong correlation of INR results of directly applied samples (n=596; r=0.965; 95% CI: 0.959, 0.970) and when using capillary transfer pipettes (n=598; r=0.958; 95% CI: 0.950, 0.964) when compared with the reference method across the INR range 0.8–7.5. Results demonstrated that accuracy was maintained across the haematocrit range of 25–55%. Feedback from the users indicated that the instructions of the LumiraDx INR test were easy to follow, the results displayed were clear and easy to read, and the system was easy to prepare and clean.
Summary/Conclusion: INR results, tested in multiple clinical settings, showed that the LumiraDx INR test correlated well with laboratory testing, as well as between the different application methods and test strip lots. Overall, based on the tests performed, the new, portable, LumiraDx Platform INR test provides reliable INR monitoring of patients on VKA therapy when used by healthcare professionals at the point of care.
This study was sponsored by LumiraDx UK Ltd, Dumyat Business Park, Alloa, UK (UK Company Number 09206123) (NCT03682419).

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Thrombin generation in patients with lower-leg injury

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Background: Background: The thrombin generation assay (TGA) is a global coagulation test of which previous studies have shown that it can identify patients at risk of venous thrombosis (VT), for example, hospitalized non-surgical patients. Although we know that lower-leg injury leads to a procoagulant state, it is currently unknown whether thrombin generation is also increased in patients with lower-leg injury and whether it can be used to identify patients at risk of VT.
Aims: (1) To determine whether TGA-parameters are increased in patients with lower-leg injury. (2) To determine whether TGA-parameters are increased in patients with VT after lower-leg injury as compared with such patients without VT.
Methods: Blood samples were collected from the POT-CAST (Prevention Of Thrombosis after Lower Leg Plaster Cast; sampling shortly after trauma) and POT-KAST (Prevention Of Thrombosis after Knee Arthroscopy; sampling before procedure) trials. All patients with lower-leg injury who developed VT (23 POT-CAST patients) and a random group of controls who did not (46 POT-CAST and 16 POT-KAST patients) were included for current analyses. Thrombin generation was measured by performing a CAT-assay (Calibrated Automated Thrombogram®; Diagnostica Stago, France). Thrombin generation was initiated using assay reagents according to the manufacturer's specifications (tissue factor and phospholipids) and tested in duplicate. For aim 1) we compared control subjects with lower-leg injury with those who were scheduled for arthroscopy. For aim 2) we compared TGA-parameters between patients with and without VT. TGA-parameters were expressed as mean differences (aim 1) and odds ratios (aim 2) with 95% confidence intervals. ORs were calculated by categorizing TGA-parameters in the controls by the following cut-offs: 25th percentile, 25-75th percentile, and 75th percentile. Either <25th or >75th percentile, dependent on which TGA-parameter was involved, was used as reference. All outcomes were adjusted for age, sex, BMI, hospital of inclusion, and date of inclusion.
Results: (Aim 1) All TGA-parameters, except for ETP, showed a prothrombotic tendency in patients with lower-leg injury compared to controls (mean differences: ETP -7.94 nM*min (95%CI -173.83 to 157.94 nM*min), thrombin peak 94.83 nM (95%CI 43.10 to 146.56 nM), lag time -1.29 min (95%CI -1.99 to -0.59 min), time to peak -2.67 min (95%CI -3.84 to -1.50 min), velocity index 77.34 (95%CI 36.55 to 118.12)). The lower ETP is explained by a stronger decline of the thrombin generation curve in the attenuation phase in the injury patients versus the controls, which implies a faster inhibition of thrombin formation. (Aim 2) No association was found between TGA-parameters and risk of VT for patients with lower-leg injury, with OR ranging between 0.98 (95%CI 0.21 to 4.53) and 2.21 (95%CI 0.41 to 11.83) for the most prothrombotic quartile for lag time and velocity index, respectively.
Summary/Conclusion: Lower-leg injury leads to increased thrombin generation, but also to increased inhibition of thrombin formation. None of the TGA-parameters is useful to identify patients who will develop VT after lower-leg trauma.

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Identification of a circulating lipid profile associated with venous thromboembolism

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Background: The cell membrane plays an essential role in the different reactions that occur during coagulation, highlighting the presence of phospholipids, lipids and glycolipids, with pro- and anticoagulant actions. In addition, an association between certain proteins related to lipid metabolism and thrombotic risk has been described.
Aims: The aim of our study was to identify, through an advanced lipidomics analysis, the main lipid groups associated with venous thromboembolic disease (VTE), allowing us the knowledge of new factors that could interact with those already known, identifying new routes associated with this patology.
Methods: Plasma samples of 30 VTE patients, without any known thrombotic risk factor, and of 30 healthy volunteers, paired by age sex with patients, were selected. We analyzed the lipids present in the plasma samples by non-directed liquid-chromatography mass spectrometry (LC-MS). The annotation of the lipid groups was carried out using the specific package of *R*: Lipid MS (*v 0.1.0*), which allows us to process the corresponding data and identify the class of lipids to which they belong. Results will be analyzed by logistic regression analysis, identifying the lipid classes associated with VTE. We also analyzed the correlation degree between the different lipid groups and clinical parameters, the thrombin generation test (TGT) and coagulation modulators.
Results: Combining positive and negative ionization modes, a total of 379 lipids were identified in our plasma samples after analysis of LC-MS. These lipids were grouped according to the lipid class to which they belonged, identifying a total of 18 classes of circulating lipids in plasma, of which 11 had a different lipid profile. We obtained decreased levels of CEs (P = 0.041), and increased levels of carnitines (P = 0.010), ceramides (P <0.001), DGs (P = 0.003), FAs (P = 0.001), LPGs (P = 0.002), MG (P = 0.001), PCs (P = 0.006), PEs (P = 0.001), PGs (P = 0.007) and SMs (P <0.001) in patients with VTE compared with healthy controls. We included these 11 variables in a predictive model and we obtained an area under the ROC curve of 0.950 (95% CI: 0.896-1), higher than the estimated area for each lipid class separately. Finally, we observed a correlation between some of the lipid classes detected and the levels of free circulating DNA in plasma (P <0.05).
Summary/Conclusion: We have identified 11 plasma lipid classes associated with VTE. These results suggest the existence of new molecules and routes of action involved in the coagulation system. It would be necessary to carry out further studies to identify the specific lipids for each class responsible for the effect on thrombotic risk, which would allow us to better understand its pathophysiological mechanism in this disease.
ISCIII (PI14/00512, FI14/00269, CPII15/00002, PI17/00495), FEDER, Generalitat Valenciana (ACIF/2017/138), IIS La Fe (2014/0718, 2016/0820_VLC-BIOMED).

ECTH 2019
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The role of bacterial proteases in prothrombin activation

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Background: Clinical evidences accumulated in the last decades indicate that severe infectious diseases are positively related to remarkably increased thrombotic risk of thrombosis, up to Disseminated Intravascular Coagulation (DIC). Furthermore, other physio-pathological conditions such as Ageing and Inflammatory Bowel Diseases (IBDs), both associated with increased gut permeability, have been also recognized as risk factors for TDs (*Buford Microbiome2017; Vogt et al, SciRep2017; Novacek et al, Gastroenterology2010*). In our earlier work we have discovered that subtilisin from *Bacillus subtilis* is able to clot human blood by cleaving ProT at physiological (i.e. R320-I321, R271-T272) and non-physiological (A470-N471) sites to form active thrombin species (*Pontarollo et al, JBC2017*).
Aims: The general aim of this work is to deepen and extend our previous work on subtilisin to other proteases from both non-pathogenic (*B. subtilis*) and pathogenic (*Staphylococcus aureus*) bacteria to address novel molecular mechanisms of thrombotic diseases.
Methods: We have combined different biochemical techniques (i.e. limited proteolysis, high resolution mass spectrometry, enzymatic chromogenic assay), coagulation assays (i.e. fibrin generation and platelet aggregation), and intestinal permeability test.
Results: *Bacillus subtilis* is a non-pathogenic Gram+ bacterium present as a commensal in the human gut microbiota. It secretes two proteases, i.e. subtilisin and neutral protease (NP), where subtilisin is a serine protease while NP is a zinc-protease. We found that NP cleaves ProT at N162-L163, Ala470-N471 in the γ-loop, and R320-I321 in the activation loop. At variance with subtilisin (which is able to clot fibrinogen both in solution and in human plasma), NP can efficiently generate fibrin only with purified fibrinogen. Importantly, addition of *B. subtilis* cell culture medium to human plasma or whole blood samples can efficiently induce clot formation. A central issue to evaluate the role that gut microbiota proteases might play in thrombosis is the assessment of the gut mucosa permeability to proteases. To this aim, we have devised an *in vitro* cell system based on human epithelial colorectal adenocarcinoma cells (Caco-2) grown in a Transwell apparatus, simulating the intestinal epithelium. Subtilisin and NP were first labelled with fluorescein and then the passage of intact fluorescienated proteases was assessed, yielding translocation rate of 25-35% after 7hrs.
Staphylococcus aureus is a Gram+ bacterium and a major human pathogen responsible for both mild and systemic infections. Here we report that an extracellular serine protease (EpiP) from *S. aureus* is able to induce blood clotting by cleaving ProT at the very same cleavage sites as factor Xa (i.e. R155-S156, R271-T272, R320-I321), generating mature aT.
Summary/Conclusion: These results highlight a novel biochemical mechanism whereby proteases secreted from non-pathogenic bacteria in the gut microbiota (*B. subtilis*) or from a major human pathogens (*S. aureus*) can trigger coagulation. This mechanism can explain increased thrombotic risk observed under physio-pathological conditions associated to increased gut permeability (e.g. Aging and IBDs), allowing translocation of bacterial proteases from the intestinal lumen to the bloodstream, as well as thrombotic complications in infectious diseases. Our results pave the way to the development of novel therapeutic strategies based on the bacterial proteases inhibition.

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Role of factor VIII and von Willebrand factor in the relation between risk of venous thrombosis and their risk factors

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Background: We recently observed that individuals with increased levels of procoagulant factors I-IX have an increased risk of venous thrombosis, with Factor (F)VIII/von Willebrand factor (vWF) being the strongest. We also observed an increased risk of VT in individuals with a decreased kidney function and individuals affected by major illnesses.
Aims: The aim of this study was to further disentangle how FVIII and vWF increase the risk of venous thrombosis and which underlying variables impact the association. We therefore set out to determine whether an association exists between FVIII/vWF and age, self-reported major illness, overweight/obesity and C-reactive protein. Next we studied whether the risks for venous thrombosis for aforementioned risk factors were attenuated by adjusting for FVIII/vWF.
Methods: This study included 2126 venous thrombosis patients and 2834 controls from a case-control study (MEGA study). We first calculated mean differences by means of linear regression in FVIII between controls with and without the aforementioned risk factors, adjusted for age, sex and estrogen use where applicable. Then we calculated with logistic regression the odds ratios (ORs) for venous thrombosis for all the considered risk factors and adjusted these for age, sex and estrogen use. Finally, we performed a mediation analysis where FVIII was added to the regression models.
Results: Higher FVIII levels were found for all the risk factors, except overweight. Age category 60-70 years versus 18-30 years and raised C-reactive protein (CRP>5mgL^{-1} versus CRP<5mgL^{-1}) provided the highest mean differences: 30 (95%CI 23-37) IUdL^{-1} and 12 (95%CI 8-16) IUdL^{-1}, respectively. Venous thrombosis risk attenuated for almost all the risk factors after being adjusted for FVIII, most notably obesity (defined as BMI>30kgm^{-2}), OR from 2.27 (95%CI 1.90-2.72) to 1.98 (95%CI 1.64-2.40) and raised C-reactive protein (>5mgL^{-1}), OR from 1.58 (95%CI 1.35-1.85) to 1.20 (95%CI 1.01 to 1.42). These results were consistent for deep vein thrombosis, pulmonary embolism, provoked and unprovoked venous thrombosis. Results for vWF were very similar as compared with FVIII.
Summary/Conclusion: We found an association between FVIII/vWF and risk factors for venous thrombosis, and the associations between these risk factors and VT were attenuated when corrected for FVIII/vWF.

ECTH 2019
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MicroRNA-494 upregulates TFPI-2 expression in MCF-7 breast cancer cells

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Background: Tissue factor (TF) pathway inhibitor (TFPI)-2 is a Kunitz-type serine proteinase inhibitor that inhibits factor (F)VIIa-TF, FXa, kallikrein, trypsin and plasmin, and that has been shown to be involved in breast cancer pathogenesis. TFPI-2 is also a tumor suppressor inhibiting extracellular matrix degradation by targeting the enzymatic activity of trypsin and plasmin, thereby reducing tumor invasion and metastasis. Cancer is associated with an increased risk of thrombosis and besides metastasis thrombosis is the leading cause of death among cancer patients. MicroRNA (miR)s are important in the regulation of gene expression in various diseases, including cancer, and miR-494 has been shown to regulate the expression of coagulation proteins.
Aims: To investigate whether miR-494 is involved in the regulation of TFPI-2 in MCF-7 breast cancer cells.
Methods: miR-494 mimic was transiently transfected into MCF-7 breast cancer cells. AHR, ELF-1 and TFPI-2 mRNA levels were measured using qRT-PCR. Prediction of transcription factor binding sites in the *TFPI2* 5´flanking region was identified by *in silico* analyses using the PROMO database. Potential binding sites for miR-494 in the 3´-untranslated region (UTR) were identified using the miRNA prediction programs miRSVR and Target Scan. For transient knock-down of ELF-1 or AHR, MCF-7 cells were transfected with the respective siRNAs. Luciferase reporter constructs containing elements of the *TFPI2* 5´-flanking region comprising the wild type or mutated ELF-1 or AHR binding sites were cloned into pGL3-Promoter vector. Site directed mutagenesis were used to generate constructs with mutated binding sites. The constructs were transfected into MCF-7 cells with or without miR-494 mimic. Pearson correlation analysis of *TFPI2* and miR-494 expression was performed in tumors from 358 breast cancer patients (written informed consent was obtained).
Results: The TFPI-2 mRNA level was significantly increased 48-72 h after transfection with miR-494 mimic. No specific binding sites for mir-494 in the 3´-UTR of *TFPI2* were identified, however, mir-494 was predicted to bind the 3´-UTR of the transcription factors AHR and ELF-1, which have potential binding sites in the *TFPI2* promoter. Accordingly, ELF-1 mRNA levels were significantly downregulated whereas AHR mRNA levels were significantly upregulated after transfection with miR-494 mimic. Knock-down of ELF-1 and AHR significantly increased and reduced TFPI-2 mRNA levels, respectively, indicating an indirect regulation of *TFPI2* by miR-494 through these factors. A significant increase in luciferase activity was obtained when the constructs containing the potential AHR or ELF-1 binding sites were co-transfected with miR-494 mimic. No effects on luciferase activity were seen when the constructs with mutated AHR or ELF-1 sites were transfected with miR-494 mimic. TFPI-2 mRNA and miR-494 levels in tumors from breast cancer patients were positively correlated ($r = 0.16$, $P = 0.002$).
Summary/Conclusion: TFPI-2 mRNA levels were upregulated by miR-494 in the breast cancer cell line MCF-7 most likely by an indirect association where miR-494 targeted the transcription factors AHR and ELF-1. The association between miR-494 and TFPI-2 was also supported in a breast cancer cohort. This indicates that miR-494 may be involved in breast tumor suppression and, potentially, reduced cancer associated thrombosis, through regulating TFPI-2 expression.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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P-080

Hospital-based Diagnoses of Low Back Pain and Risk of Myocardial Infarction, Stroke, and Venous Thromboembolism

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Background: Sedentary occupation, obesity, underlying diseases, or immobilization may increase the risk of cardiovascular disease in patients with low back pain. In addition, patients with low back pain may be at increased risk of cardiovascular outcomes due to treatment with non-steroidal anti-inflammatory drugs, oral glucocorticoids, or opioids.
Aims: We examined whether risks of myocardial infarction (MI), stroke, and venous thromboembolism (VTE) were higher in patients with a hospital-based inpatient or outpatient diagnosis of low-back pain than in a general population comparison cohort.
Methods: Using nationwide population-based Danish medical registries, we identified a cohort of patients (>18 years old) who were diagnosed with low back pain during 2004-2012 and had no prior diagnoses of MI, stroke, or VTE. Each patient was matched to 10 individuals from the general population by age, sex, and calendar year. The date the patient was diagnosed with low back pain was used as the index date in both cohorts. Individuals in the general population cohort could not have a preceding diagnosis of low back pain, MI, stroke, or VTE. If they were diagnosed with low back pain after the index date, they were transferred to the low back pain cohort. All study subjects were followed from the index date until the occurrence of MI, stroke, VTE, death, emigration, or 30 November 2013 (ensuring at least eleven months of follow-up), whichever came first. We computed 10-year cumulative incidence of the study outcomes, accounting for death as a competing risk. Using Cox regression analysis we computed hazard ratios (HRs) for cardiovascular outcomes , adjusting for comorbidities, medication use, education, and employment status.
Results: Among 121,367 patients with low back pain (median age = 46 years, female = 51%), 4,856 (4%) experienced a cardiovascular event during 10 years of follow-up. A total of 1405 patients developed MI, 1925 developed ischemic stroke, 292 developed hemorrhagic stroke, and 1234 developed VTE over median follow-up time of 4.8 years. Compared with the matched general population cohort, the 10-year cumulative incidence of cardiovascular disease per 1000 persons was higher among patients with low back pain for MI (20 *vs.* 16), ischemic stroke (30 *vs.* 22), and VTE (18 *vs.* 14), but not for hemorrhagic stroke (4 *vs.* 5). Correspondingly, the 0-10 year adjusted HR was increased for MI (adjusted HR: 1.27 [95% CI: 1.20–1.35]), ischemic stroke (adjusted HR 1.30 [95% CI: 1.24–1.37]), and VTE (adjusted HR: 1.39 [95% CI: 1.30–1.47]), but not for hemorrhagic stroke (adjusted HR: 1.01 [95% CI: 0.90–1.15]). During the first 30 days of follow-up, the risk among low back pain patients was even higher for MI (adjusted HR: 1.84 [95% CI: 1.21–2.80]), ischemic stroke (adjusted HR: 2.49 [95% CI: 1.86–3.34]), hemorrhagic stroke (adjusted HR: 3.63 [95% CI: 1.84–7.17]), and VTE (adjusted HR: 4.43 [95% CI: 3.17–6.20]).
Summary/Conclusion: A hospital-based diagnosis of low back pain was associated with increased short-term risks of MI, ischemic stroke, hemorrhagic stroke, and VTE. The risks persisted over the long term for MI, ischemic stroke, and VTE. However, the absolute risks were low.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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P-081

Dabigatran therapy provides better inhibition against intracardiac activation of hemostasis as compared to vitamin K antagonists during cryoballoon catheter ablation of atrial fibrillation

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Background: Transcatheter ablation is a well-established treatment in symptomatic atrial fibrillation (AF) patients. Cerebral thromboembolism (stroke or TIA) is rare but feared complication of the procedure. In the past few years periprocedural anticoagulation strategies evolved from interrupted vitamin K antagonists (VKAs) to uninterrupted VKAs or dabigatran therapy.
Aims: Here we aimed to test which periprocedural anticoagulation strategy results in less activation of hemostasis during the ablation procedure by measuring markers of coagulation activation from intracardiac (left atrium) blood samples drawn before and immediately after the procedure.
Methods: In this observational study, 54 paroxysmal/persistent AF patients undergoing cryoballon ablation were included (age: 54.3±11.8 years; male: 34 [65%]). Patients were grouped according to their periprocedural anticoagulation strategy: interrupted VKAs (n=24), uninterrupted VKAs (n=11), uninterrupted dabigatran (n=17). Blood was drawn from the left atrium before and immediately after the ablation procedure. Cryoablations were performed according to standard protocols, during which patients received i.v. 150 IU/kg unfractionated heparin. Pre-ablation blood samples were taken from the left atrium through the Mullins sheath immediately after transseptal puncture and before the administration of heparin. Post-ablation left atrium blood samples were collected through the left atrium sheath after removal of the ablation catheter once the ablation procedure was finished. Heparin-insensitive markers of hemostasis activation and endothelial damage were tested from all samples: D-dimer, quantitative fibrin mononer (FM), plasmin-antiplasmin complex (PAP), FVIII activity, von Willebrand factor (VWF) antigen. All patients provided written informed consent.
Results: Baseline clinical and procedural characteristics did not differ among groups. INR of patients on interrupted and uninterrupted VKAs were 0.96±0.05 and 2.33±0.32, respectively, average peak level of dabigatran was 176.5±77.0 ng/ml; all patients on anticoagulants were in the therapeutic range. D-dimer levels increased in all 3 groups post-ablation, but significantly lower levels were observed in the dabigatran group as compared to the other two groups (median [IQR]: 0.30 [0.16-0.53] vs. 1.09 [0.7-1.9] and 0.72 [0.56-0.83] mgFEU/L for dabigatran vs. interrupted and uninterrupted VKAs, p<0.001). PAP levels were parallel to this observation. Post-ablation FM levels were elevated in interrupted and uninterrupted VKA groups (26.4 [16.5-46.6] and 10.1 [4.2-20.3] mg/L), but strikingly, FM levels remained below cut-off in all patients on dabigatran (3.9 [2.9-4.9] mg/L, p<0.001). VWF antigen and FVIII activity showed a similar increase in all groups post-ablation as compared to pre-ablation levels, suggesting comparable procedure-related endothelial damage.
Summary/Conclusion: Dabigatran provides better inhibition against intracardiac activation of hemostasis as compared to VKAs during cryoballoon catheter ablation. Our study provides laboratory evidence to previous clinical observations suggesting the superiority of dabigatran to VKAs in stroke prevention in patients with atrial fibrillation.
Funding: GINOP-2.3.2-15-2016-00043, NKFI-FK128582

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

P-082

Early mortality risk is associated with prolonged clot lysis time in acute pulmonary embolism: contribution of NETosis

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Background: Acute pulmonary embolism (PE) is poorly characterized in terms of fibrin properties. Our group showed that formation of dense clots with impaired susceptibility to lysis, which has a prognostic value in predicting recurrent episodes, is observed in patients following PE (Zabczyk M et al. *Arterioscler Thromb Vasc Biol* 2017;37:365-373, Cieslik J et al. *Blood* 2018;131:797-807). Given evidence for the role of neutrophil extracellular traps formation (NETosis) in acute thromboembolism we hypothesized that in acute PE unfavorable clot properties are associated with increased NETosis markers and they might predict mortality.
Aims: To investigate plasma clot properties in acute pulmonary embolism (PE) and their association with PE severity.
Methods: We studied 126 noncancer normotensive patients (aged 58±14 years, 52.4% males) on admission for acute symptomatic PE. Patients with hypotension or shock, known cancer or pregnant women were ineligible. Plasma fibrin clot permeability (K_s), efficiency of fibrinolysis using clot lysis time (CLT), endogenous thrombin potential (ETP), proteins involved in fibrinolysis, and citrullinated histone H3 (citH3), a specific marker of NETs formation, were evaluated. The early mortality risk score and simplified PE severity index (sPESI) were calculated. The Jagiellonian University Ethical Committee approved the study, and participants provided informed written consent.
Results: Intermediate-high risk PE (n=40, 31.7%) was associated with impaired fibrinolysis as evidenced by 26.4% prolonged CLT, while in patients with intermediate-low mortality risk (n=53, 42.1%) CLT was 16.5% prolonged compared to those with low-risk PE (n=33, 26.2%; $p<0.05$). Similar differences were noted for plasminogen activator inhibitor-1 (PAI-1) antigen and ETP (all $p<0.05$), but not for K_s. Subjects with sPESI score ≥2 (n=65, 51.6%) and 1 (n=41, 32.5%) had 35.3% and 16.5% longer CLT compared with low-risk patients with sPESI=0 (n=20, 15.9%, both $p<0.01$), respectively. K_s was lower (-19.2%) in subjects with sPESI≥2 compared to sPESI=0 ($p<0.05$). Increased levels of fibrinogen, C-reactive protein (CRP), PAI-1, and citH3 along with higher ETP were observed in patients with sPESI≥2 or 1 compared to low risk patients (all $p<0.05$), with no differences in antiplasmin, plasminogen, thrombin activatable fibrinolysis inhibitor (TAFI) activity, P-selectin, platelet factor 4, and factor VIII. After adjustment for potential confounders, including age, sex, body-mass index, and fibrinogen, PAI-1 and citH3 levels (odds ratio [OR]=1.09, 95% confidence interval [CI] 1.05-1.15 and OR=1.80, 95%CI 1.26-2.70, respectively) were the independent predictors of prolonged CLT >121.5 min (top quartile, n=32), while low K_s ≤5.46 x$10^{-9}$$cm^2$ (lower quartile, n=32) was predicted by C-reactive protein (OR=1.49, 95%CI 1.16-2.10 per 10 units increase).
Summary/Conclusion: Impaired clot lysis driven by enhanced NETs formation is associated with increasing early mortality risk in acute PE patients, suggesting a prognostic value of hypolysability in this disease. This work was supported by the Polish National Science Centre (UMO-2015/B/NZ5/02215 to A.U.).

ECTH 2019
European Congress on Thrombosis and Haemostasis
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In-depth epitope mapping of anti-ADAMTS13 autoantibodies in immune-mediated thrombotic thrombocytopenic purpura patients using a large library of ADAMTS13 fragments

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Background: In immune mediated thrombotic thrombocytopenic purpura (iTTP), patients develop an immune response against the multidomain enzyme ADAMTS13. ADAMTS13 consists of a metalloprotease (M) and disintegrin-like (D) domain, eight thrombospondin type 1 repeats (T1T8) and a cysteine-rich (C), spacer (S), and 2 CUB domains (CUB12). Epitope mapping using relatively large fragments of ADAMTS13 (e.g. MDTCS, MDT, T2T8 and CUB12) revealed that almost all iTTP patients have anti-CS antibodies while up to 60% of the patients also have autoantibodies against other ADAMTS13 domains which are, however, not well characterized.
Aims: We aimed to determine the epitope of anti-ADAMTS13 autoantibodies in a large cohort of iTTP patients using an extensive library of ADAMTS13 fragments.
Methods: A library of 15 ADAMTS13 fragments (M, MDT, MDTC, DTC, DT, CS, S, T2T5, T6T8, T2T8, CUB2, CUB12, MDTCS, T2C2, and ADAMTS13) expressed as a fusion protein with albumin domain 1 (AD1) was used for epitope mapping of 231 plasma samples either from remission, or acute phase from 93 iTTP patients using ELISA.
Results: From the 231 samples, 135 had high enough antibody titers to determine the anti-ADAMTS13 autoantibody profile. While 79.3% of the samples (107/135) contained anti-MDTCS autoantibodies, 65.9% (89/135) had autoantibodies against T2C2. The anti-MDTCS autoantibodies could bind to M in 8.4% (9/107), DT in 17.8% (19/107) and CS in 65.4% (70/107) of the samples. The anti-T2C2 autoantibodies targeted T2T5 in 44.9% (40/89), T6T8 in 36% (32/89) and CUB12 in 61.8% (55/89) of the samples. Limited changes in the autoantibody profile could be observed throughout acute and remission phases.
Summary/Conclusion: In-depth epitope mapping was performed in an extensive cohort of iTTP patients in acute and remission phases using a library of 15 ADAMTS13 fragments which enables a better insight in the immune response in iTTP patients.

ECTH 2019
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High plasma levels of mannose-binding lectin are associated with non-O blood type and increased risk of venous thromboembolism

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Background: Recent studies have implicated a role for the complement system in the pathogenesis of venous thromboembolism (VTE), but the exact mechanisms remain elusive. The lectin pathway of complement, activated by mannose-binding lectin (MBL), ficolins and collectins, is a potentially procoagulant pathway.
Aims: To investigate whether plasma MBL levels alone or in combination with any genetic variants associated with high plasma MBL levels are associated with risk of VTE.
Methods: A nested case-control study with 417 VTE patients and 849 age- and sex-matched controls was conducted from a population-based parent cohort (The Tromsø Study). Plasma MBL levels were measured at inclusion using ELISA. Protein quantitative trait loci (pQTL) analysis was performed on whole exome sequencing data conducted in 709 subjects (355 VTE patients and 354 control subjects). Logistic regression models were used to estimate odds ratios (OR) with 95% confidence intervals (CI) for VTE and subgroups across groups of MBL or groups combining MBL levels and blood type. Mann-Whitney Wilcoxon test was used for comparison of groups.
Results: Subjects with plasma MBL levels in the highest quartile (≥2423 ng/mL) had increased OR for VTE (OR 1.21; 95%CI: 0.92-1.59) and deep vein thrombosis (DVT) (OR 1.32; 95%CI: 0.96-1.81) compared to those with MBL levels in the combined lower three quartiles after multivariable adjustment. In the pQTL analysis, rs8176719 SNP of the *ABO* gene, known to differentiate O from non-O blood groups, displayed genome-wide significant (p=1.5x10^{-9}) association to high plasma MBL levels. Controls with non-O blood type had 42% higher median plasma MBL levels than controls with type O blood (1634 [IQR: 439-1917] ng/mL vs 1147 [IQR: 418-3076] ng/mL, p=0.003). Subjects with high plasma MBL levels displayed increased ORs for VTE (OR 1.78, 95% CI 0.87-3.63), DVT (OR 1.44, 95% CI 0.62-3.36), and pulmonary embolism (PE) (OR 2.37, 95% CI 0.96-5.85) compared to those with low plasma MBL levels among subjects with O blood group, whereas high MBL levels had no additional impact on VTE risk in subjects with non-O blood group.
Summary/Conclusion: Plasma MBL levels are partly determined by ABO blood group, reflected by a significantly higher plasma MBL level in those with non-O blood group. Subjects with high plasma MBL levels and blood group O had increased VTE risk and displayed similar ORs to individuals with non-O blood group.

ECTH 2019
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Exploring a novel interaction between the intrinsic pathway of coagulation and complement

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Background: Complement-coagulation crosstalk is now a widely accepted concept, however many interactions remain unexplored. The complement and coagulation cascades are implicated during systemic inflammation leading to detrimental effects if allowed to activate uncontrollably, as observed in disseminated intravascular coagulation (DIC). Investigating the interactions between coagulation factors and the complement system may reveal therapeutic targets for the treatment of thrombo-inflammatory complications as seen in DIC. In this study we investigated interactions between the intrinsic pathway of coagulation and components of the alternative pathway of complement.
Aims: To understand the role of the alternative pathway regulator, properdin, in the mechanisms of contact activation within the intrinsic pathway.
Methods: Surface plasmon resonance (SPR) was initially used to assess the binding of coagulation factors to complement proteins in a purified system. SPR was performed by separately immobilising properdin and complement component (C)3b to the sensor chip surface, and employing factor (F)XI and activated FXI (FXIa) as analytes. Chromogenic assays were developed to determine the effect of properdin on the autoactivation of FXI by 500 kDa dextran sulphate (DXS_{500kDa}), and the effect of properdin and DXS_{500kDa} on FXIa catalytic activity. These chromogenic assays were optimised to determine the effect of properdin on FXI activation by FXIIa, with phospholipids, polyphosphates (100mers) and high-molecular weight kininogen (HK). SDS-PAGE was performed to examine FXIa cleavage of properdin and was analysed using mass spectrometry (MS). SDS-PAGE also examined FXIa-induced cleavage of FIX in the presence of properdin and DXS_{500kDa}.
Results: FXI and FXIa both bound to properdin, with FXI displaying a lower affinity (nM) than FXIa (pM). FXI and FXIa both bound to C3b, with FXI displaying a higher affinity (pM) than FXIa (nM). FXI autoactivation by DXS_{500kDa} was reduced with properdin. The presence of DXS_{500kDa} reduced V_{max} of FXIa, and this effect was partially reversed by properdin. SDS-PAGE revealed cleavage products when FXIa, DXS_{500kDa} and properdin were incubated together; and MS determined that properdin appears to be a substrate for FXIa, only in the presence of DXS_{500kDa}. The presence of properdin does not appear to modulate FXIa cleavage of FIX in the presence of DXS_{500kDa} as shown by SDS-PAGE. A chromogenic assay exploring FXIIa activation of FXI, revealed that properdin caused an overall decrease in measured FXIa activity.
Summary/Conclusion: These findings suggest a novel mechanism that may potentially regulate contact activation of coagulation by the involvement of a complement regulatory protein. Other physiological surfaces are currently being explored to investigate further functional mechanisms involving the intrinsic pathway and complement activation.

ECTH 2019
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Plasmatic protein C levels are determined by genetic variants in the promoter and the intron 2 of the protein C gene

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Background: Plasma levels of protein C (PC) are associated with different genetic variants in the PC gene (PROC). *In vitro* studies have suggested that polymorphisms (SNP) in the promoter region and in the intron 2 of PROC alter transcriptional regulation, enhancing or decreasing the expression of PC, although the results are not conclusive.
Aims: Our objective was to analyze 9 of the main SNPs in the promoter and in the intron 2 of PROC and determine their association with plasmatic levels of PC in our population.
Methods: We selected a group of 60 healthy individuals with increased antigenic PC levels, between 85-186% (119.9 ± 26.3%). We genotyped by direct sequencing the following SNPs: rs2069904G>A; rs1799808C>T; rs1799809G>A; rs1799810A>T in the promoter region, and rs2069912T>C; rs2069913C>G; rs2069914G>A; rs2069915G>A; rs2069916C>T in the intron 2 of PROC. We analyzed the results using the statistical package *SNPassoc* from *R*, performing the calculation of the distribution of each SNP, the equilibrium of *Hardy-Weinberg* and the association of the SNPs with PC levels by means of a logistic regression model, adjusted for age and sex.
Results: : Frequency of the minor allele of each SNP was: 30.33%, 36.07%, 40.16%, 40.16%, 25.40%, 22.95%, 28.69%, 45.08 %, and 35.25%, respectively. There are no significant differences neither in the genotypic frequencies nor in the allelic ones. We did not observe any haplotype between groups. However, a significant association between 3 of the 9 analyzed SNPs and PC levels was observed, being for rs1799809G> A and rs1799810A>T (P = 0.0097 in both cases) of dominance, and for rs1799808C>T (P = 0.0454) recessive. In addition, we observed differences in the distribution of antigenic PC levels according to the genotype for rs1799809G> A and rs1799810A>T (P = 0.0370 in both cases).
Summary/Conclusion: Previous studies have described the association between rs1799808C> T and rs1799809G> A, PC levels and thrombotic risk, finding that the T-A haplotype has increased PC levels and lower thrombotic risk. In addition, *in vitro* studies have shown that the presence of the allele A for rs1799809 increases the expression of PROC, possibly by splicing. Our results corroborate how SNPs in the promoter region of PROC can contribute to the regulation of its transcription, giving rise to the modulation of PC plasmatic levels.
ISCIII-FEDER (PI14/00512, PI14/00079, FI14/00269, CPII15/00002, PI17/00495), Generalitat Valenciana (PROMETEOII/2015/017, ACIF/2017/138).

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Plasma P-selectin as Predictive Biomarker for Incident Venous Thromboembolism in Women

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Background: P-selectin (PSEL) is a transmembrane ligand receptor expressed on activated platelets and endothelium that serves a function in initial immune response by facilitating leukocyte adhesion and extravasation. In venous thromboembolism (VTE), PSEL is implicated as a mediator of synergistic mechanisms between inflammation and hypercoagulability. Clinical studies have reported elevated plasma PSEL levels for incident VTE in HIV-infected individuals, acute deep vein thrombosis (DVT), cancer-related VTE, and recurrent VTE, as well as attenuated plasma levels following anticoagulant therapy. Plasma PSEL has also been proposed as a potential biomarker to complement D-dimer in DVT diagnostics. Notwithstanding, the potential of plasma PSEL as a predictive biomarker for incident VTE in the general population remains elusive.
Aims: Here, we aimed to investigate the association between plasma PSEL levels and incident VTE risk.
Methods: In a nested case-control study, 415 subjects who experienced an incident VTE and 843 age- and sex-matched controls, were selected from a population-based cohort (the fourth survey of the Tromsø study). Plasma specimens were obtained at study inclusion, and baseline PSEL (ng/mL) values quantified by an enzyme-linked immunosorbent assay. Logistic regression models were employed to calculate odds ratio (OR) with 95% confidence interval (CI 95%) for VTE per standard deviation (SD) increase of PSEL. To assess potential regression dilution bias, analyses were restricted by years from specimen collection to VTE event (YBE), which ranged from 0.1 to 12.8 years.
Results: Results showed for the overall population, no significant association between VTE and plasma PSEL levels (range: 9.3-124.1 ng/mL) (OR per SD: 1.00, CI 95%: 0.89- 1.13) after adjustment for age, sex and body mass index. However, data evinced substantial regression dilution bias. YBE revealed increased risk for incident VTE in women (OR per SD: 1.5, p <0.05, YBE: <3), particularly driven by sub-cohorts 'Unprovoked VTE' (OR per SD: 2.3, p <0.05, YBE: <3) and 'Pulmonary embolism (PE)' (OR per SD: 1.8, p <0.05, YBE: <5). No association was found for YBE restrictions above 6 years.
Summary/Conclusion: To our knowledge, this is the first nested case-control study to discover an association between plasma PSEL levels measured at study inclusion and incident VTE risk based on sex. The augmented risk for unprovoked VTE and PE in women at a short-term perspective preceding VTE event (<6 years), may imply latent pathophysiological mechanisms invoking elevated PSEL levels. Although further studies are required to elucidate the true potential for PSEL as predictive biomarker for incident VTE, our results not only confirm YBE restriction as factor that needs accounting for, but also that sex stratification is warranted, in the present study design.

ECTH 2019
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Decadal results from a single centre cohort: Maternal and fetal outcomes in women with antiphospholipid antibodies

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Background: Antiphospholipid syndrome (APS) is defined by the presence of antiphospholipid antibodies (aPL) with thrombosis and/or obstetric morbidity. The obstetric morbidity includes recurrent first trimester loss, stillbirth, intrauterine death, pre-eclampsia (PET), premature birth and foetal growth restriction. We developed a treatment protocol for these patients and with the current rigid treatment regimen we aim to prevent obstetric morbidity and maternal thrombosis.
Aims: To report the results on maternal and fetal outcomes from a single centre cohort on 375 women and 522 pregnancies.
Methods: This is a retrospective observational study. Data was retrospectively collected from the Lupus Unit/Department of Thrombosis and Haemostasis at St. Thomas' Hospital in London, UK. Patients were identified from clinic lists (Jan 2010 to December 2017). Women persistently positive for antiphospholipid antibodies were included if complete pregnancy outcome data was available.
Results: Five-hundred-and-twenty-two pregnancies in 375 women were included in the study. The overall live birth rate was 80%. Fetal outcomes were as follows: Pregnancy loss =/< 12 weeks gestation and pregnancy loss between 12-24 weeks gestation was 16% and 5%, respectively. The rate of intrauterine death > 24 weeks was 1%.
Preterm birth at < 34 weeks gestation due to PET and preterm birth < 34 weeks of baby < 10thpercentile due to PET was 2% and 0.2%, respectively. Preterm birth at < 34 weeks without PET and a birthweight > 10thpercentile was 0.7%.
Maternal outcomes were as follows: 3% developed pre-eclampsia after 34 weeks and 42% had a caesarean section.
Summary/Conclusion: These results show that adhering to our current treatment protocol, approximately 80% of women with antiphospholipid antibodies will have a successful pregnancy outcome.

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Novel thermostable inhibitor of contact activation TICA effectively blocks contact activation in low tissue factor thrombin generation

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Background: Thrombin generation and other clotting assays suffer from a wide variation of pre-analytical variables. One of those pre-analytical variables is contact activation through blood withdrawal methods, different syringes, differences in blood coagulation tubes, blood transport and sample handling. It has been shown that the addition of contact activation inhibitors in low tissue factor activated thrombin generation leads to a correction of the increase in thrombin generation due to contact activation. We compare the novel 'thermostable inhibitor of contact activation' (TICA) to the current standard 'corn trypsin inhibitor' (CTI).
Aims: Comparing the effectiveness of novel contact activation inhibitor TICA to the current standard CTI in low tissue factor-induced thrombin generation and recalcification in sodium citrate anticoagulated platelet poor plasma (PPP) and platelet rich plasma (PRP).
Methods: We compared TICA, CTI and plasma without any contact activation inhibitors in low tissue factor PPP thrombin generation and in PRP recalcification thrombin generation, the latter the most sensitive condition for contact activation. In addition, we compared low tissue factor activated thrombin generation in plasma from severe hemophilia A patients with and without TICA during and after blood drawing. Thermostability – as a measure of shelf life - was measured and compared to CTI.
Results: TICA is able to fully block contact activation in PRP recalcification experiments and is comparable to CTI. TICA significantly lowers low tissue factor induced thrombin generation by blocking contact activation through FXIIa inhibition. Pre-loading vacuum blood collection tubes with contact activation inhibitors is superior in inhibiting contact activation compared to addition of the inhibitor during the thrombin generation assay itself. TICA did not alter coagulation activity when added to FXIIa deficient plasma in thrombin generation. In contrast to CTI TICA is heat stable which will be of benefit to shelf life of pre-loaded blood drawing tubes.
Summary/Conclusion: TICA is able to fully block contact activation and has several advantages over CTI.

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Does a meal affect thrombin generation? New insights from the NEO study

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Background: Obesity is a well-known risk factor for venous thrombosis (VT). Most of the clotting determinants, including the thrombin generation potential, are measured in a fasting state. However, information regarding the effect of a postprandial state on the thrombin generation potential is lacking.
Aims: We aimed to study the effect of a standard meal on different parameters of the thrombin generation potential in the general population and to study the difference between lean and individuals with overweight or obesity.
Methods: We report the results of a study on 91 participants from the Netherlands Epidemiology of Obesity (NEO) study, a population-based cohort study. Thrombin generation was determined using the Calibrated Automated Thrombogram ® (Diagnostica Stago, Asinères, France), using assay reagents according to the manufacturer's specifications (low tissue factor) and tested in duplicate. Thrombin generation assays were performed in every participant after an overnight fast, 30 minutes, and 150 minutes after a standard liquid mixed meal (400 mL, 600 kCal). Endogenous thrombin potential (ETP), lag time, and peak height were selected as the main determinants to describe the differences in thrombin generation. Paired samples t-test was used to analyse differences between thrombin generation parameters at different time points, in the total population of the study and stratified by low and high BMI, with 25 kg/m2 as cut-off. Associations between the difference in thrombin generation parameters between the three time points and BMI were examined using linear regression analyses while adjusting for confounding factors, age and sex.
Results: Twenty-five participants with BMI below 25 kg/m2 and sixty-six with BMI above 25 kg/m2 were included. The two groups were similar regarding sex (64% and 63.6% women) and age distribution (mean age 53 and 56 years), but differed in terms of diabetes and impaired glucose metabolism [100% of the lean subjects had normal glucose metabolism (glucose<6.1 mmol/L at fasting state), versus 78.8% of those with overweight]. The means of the main TGA parameters at the three time points (ETP, Lag time and Peak) did not differ in the lean group, but showed a clear shift towards a prothrombotic state comparing fasting state with 30 and 150 min postprandial in subjects with overweight (mean difference between 0 and 150 min postprandial: ETP: 109 nM·min; 95% CI: 59 to 158 nM·min; Lag time: -0.7 s; 95% CI: -1.0 to -0.4 s; Peak: 13.3 nM; 95% CI: 22.7 to 3.7 nM). The difference between the two BMI groups remained after adjustment for age and sex. Analyses were also performed taking the median of the BMI distribution in the total study population as cut-off (27 kg/m2), which obtained similar results.
Summary/Conclusion: The thrombin generation potential, as a result of the balance between all pro- and anticoagulant factors, appears to be increased after consumption of a standard mixed meal only in the overweight and obese population.

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Neutrophil extracellular traps accelerate clotting and produce a denser clot structure in plasma

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Background: The neutrophil is a type of leukocyte that is involved in innate inflammatory processes. Neutrophils are capable of extruding their DNA and granular proteins as traps for microbes, called Neutrophil Extracellular Traps or NETs. Neutrophils and NETs have been implicated in both arterial and venous thrombosis. NETs have been suggested to provide a "scaffold" for thrombosis and to increase the resistance of clots to fibrinolysis and thrombolysis. However, the exact interactions between neutrophils or NETs and fibrin(ogen), and the mechanisms behind this interaction are not yet fully understood.

Aims: This study aims to investigate the role of NETs in blood coagulation, fibrin formation, clot stability and clot mechanical properties, in order to decipher how NETs interact with the fibrin network, and to ultimately elucidate novel mechanisms that contribute to thrombosis.

Methods: Turbidity measurements were used to measure the kinetics of clots formation. Confocal microscopy and permeation analysis were used to investigate the effects of NETs on overall clot network structure. Magnetic tweezers are being used to investigate effects on clot stiffness and viscous properties.
Results: Neutrophils themselves did not significantly promote clot formation. NETs had no significant influence on clot formation with purified fibrinogen. However, NETs increased the speed of clot formation in pooled normal plasma both with and without thrombin. Blocking tissue factor and factor XII did not significantly influence this effect. Our confocal results suggested that clots with NETs were denser than those without NETs.
Summary/Conclusion: NETs may interact with other components of the plasma, resulting in accelerated clotting and the formation of a denser clot architecture. These effects are not mediated through tissue factor or factor XII but could involve other components present in the plasma.

ECTH 2019
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Impaired right ventricular function in patients with chronic thromboembolic disease

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Background: Chronic thromboembolic disease (CTED) is defined as radiological evidence of residual perfusion defects after pulmonary embolism (PE) but without significant pulmonary hypertension (mean pulmonary arterial pressure ≥25 mmHg by right heart catheterization at rest). These patients suffer from functional limitation and have a higher risk of venous thromboembolism recurrence.
Aims: In this study we wanted to explore if CTED patients had signs of increased PA pressure and right heart burden by echocardiography
Methods: Inclusion criteria were history of PE, age 18 - 75 years and PE diagnosed 6 – 72 months prior to inclusion. Patients with left ventricular systolic or diastolic heart failure, valvular disease, chronic pulmonary disease and chronic thromboembolic pulmonary hypertension were excluded. All patients underwent echocardiography with standard and novel methods and ventilation/perfusion (VQ)-scan. The person performing and analyzing the echocardiographic examinations was blinded to the result of the VQ-scan. VQ-scans were analyzed according to the European Association of Nuclear Medicine-criteria, and deemed either positive or negative. Data are presented as mean ±SD or median ±IQR as appropriate. Multiple linear regression was used for the statistical analysis, with adjustment for age, BMI and systolic blood pressure. Informed consent was obtained for all participants.
Results: 78 patients were included, of whom 20 patients (26 %) had radiological signs of CTED. For the statistical analysis, we divided the patients into two categories according to the result of the VQ-scan, i.e. positive or negative. The median age in the VQ-negative group was 59 ±8 years compared to 67 ±6,5 years in the VQ-positive, although not statistically significant, p = 0,71. Time since PE-event was not statistically different in VQ-negative versus VQ-positive; 37 ±19 months versus 33 ±17 months respectively. We found no differences in left ventricular systolic or diastolic function using standard echocardiographic measurements such as left ventricular ejection fraction, EA-ratio (ratio between transmitral pulsed doppler early (E) diastolic and atrial (A) velocity) and E/e´(ratio between E and early velocity (e´) by tissue velocity). Several parameters of right ventricular function and hemodynamics were recorded and analyzed; TAPSE (tricuspid annular plane systolic excursion), RV S`(tricuspid lateral annular systolic velocity), right ventricular strain (speckle tracking imaging technique) and right ventricular ejection fraction (using dedicated three dimensional software) revealed no significant difference between the groups. Pulmonary valve acceleration time was reduced in the VQ-positive group; 120 ±26 ms versus 145 ±22 ms, p<0,001. Pulmonary artery diameter was increased in the VQ-positive group; 25 ±3,6 mm versus 21 ±3,3mm, p=0,002. Right ventricular isovolumetric relaxation time and right ventricular myocardial performance index (Tei-index) were both significantly increased in the VQ-positive group; 50 ±22 ms versus 33 ±23 ms ,p= 0,004, and 0,44 ±0,15 versus 0,36 ±0,10, p<0,01 respectively. Tricuspid regurgitation maximum velocity (TR V max) was increased in the VQ-positive group; 2,7 ±0,4 cm/s versus 2,3 ±0,3 cm/s, p<0,001.
Summary/Conclusion: Patients with CTED have increased PA pressure and impaired RV systolic and diastolic function compared to post-PE patients without residual perfusion defects. These findings indicate that CTED patients should be more thoroughly followed up

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The ratio of factor VIIa:tissue factor content within microvesicles determines the differential influence on endothelial cells

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Background: We have previously shown that tissue factor (TF)-rich microvesicles (MV) can induce cellular apoptosis or proliferation in endothelial cells. The induction of either apoptosis or proliferation appears to be dependent on the ration of fVIIa:TF, and is variable on incubation with MV derived from different cell lines.
Aims: To study the effect of fVIIa:TF ratio on endothelial cell.
Methods: Microvesicles were isolated from HepG2, BxPC-3, 786-O, MDA- MB-231 and MCF-7 cell lines, by ultracentrifugation. The density of the MV was determined using Zymuphen assay, and the associated fVIIa and TF antigen and activity measured by ELISA and thrombin-generation assay, respectively. Human coronary artery endothelial cells (HCAEC) were incubated with these MVs, or with combinations of fVIIa- recombinant TF, and cell proliferation and cellular apoptosis assessed by crystal violet and TUNEL assay, correspondingly. Finally, the activation of PAR2 receptors on HCAECs were blocked using the SAM11 anti-PAR-2 antibody (20 µg/ml), or the activity of the TF-fVIIa complex in MV was blocked using HTF1 anti-TF antibody (20 µg/ml) or anti-factor VII antibody (10 µg/ml) prior to addition of MV to cells. HCAECs were treated with the pre-incubated MVs and the rate of cell proliferation and apoptosis measured by crystal violet or TUNEL assay.
Results: The purified microvesicles exhibited a range of fVIIa:TF ratios with HepG2 and 786-O cells having the highest (54:1) and lowest (10:1) ratios respectively. The reversal from pro- apoptotic to proliferative was estimated to occur at a fVIIa:TF molar ratio of 15:1, but HCAEC could not be rescued at higher TF concentrations. The purified microvesicles induced HCAEC proliferation or apoptosis according to this ruling. Blocking PAR2 activation on HCAEC,or inhibiting fVIIa or TF-procoagulant function on microvesicles prevented the influence on HCAEC.
Summary/Conclusion: The induction of cell proliferation or apoptosis by TF-associated MV is dependent on the ratio of fVIIa:TF and involves the activation of PAR2. At lower TF concentrations, fVIIa can counteract the pro-apoptotic stimulus and induce proliferation.

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Pregnancy outcomes in patients with atypical haemolytic uraemic syndrome

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Background: Pregnancy outcomes in patients with atypical haemolytic uraemic syndrome (aHUS) are not well reported.
Aims: Here, we present characteristics and outcomes for patients with aHUS who became pregnant while enrolled in the Global aHUS Registry.
Methods: The Global aHUS Registry (NCT01522183), initiated in April 2012, collects demographics, disease history, treatment and outcomes data for patients with aHUS. The current analysis includes patients from the Registry with evaluable pregnancy data supplemented with pharmacovigilance information; numbers of pregnancies, outcomes and treatment status were evaluated.
Results: As of December 2018, 39 pregnancies were recorded in 36 patients. In 3 cases, no outcomes were reported; two pregnancies were ongoing. Outcome data were available for 34 pregnancies: 24 with eculizumab exposure and 10 without (Table). Of eculizumab-exposed pregnancies, 17 patients were dosed according to recommendations in the prescribing information. There was a higher proportion of live births in non-exposed vs eculizumab-exposed patients (80% vs 58%, respectively) and a more elective terminations in eculizumab-exposed patients (33% vs 10%, respectively; reasons cited in Table footnote). Relapse of aHUS occurred in 9% of patients (1 exposed, 2 non-exposed) with known pregnancy outcomes. Five patients received dialysis during pregnancy, all of whom were eculizumab-exposed (including 1 patient with an aHUS relapse). No malformations or anomalies were reported at or within 3 months of birth. There was a higher proportion of complement mutations in eculizumab-exposed patients with known outcomes (63% vs 33%, respectively).

Table. Pregnancy outcomes in patients with aHUS per eculizumab exposure

Outcome	Pregnancy with exposure to eculizumab (n=24)	Pregnancy without exposure to eculizumab (n=10)
Live birth	14 (58)	8 (80)
Elective termination[a]	8 (33)	1 (10)
Miscarriage	2 (8)	0
Late foetal death	0	1 (10)
aHUS relapse	1 (4)[b]	2 (20)[c]
Genetic mutation identified/tested[d,e]	12/19	6/13
Genetic mutations listed (n)	C3 (1); C3, CFH (1); C3, CFH, MCP, CFB (1); CFH (3); CFH, MCP (3); MCP (2); THBD (1)	CFH (3); MCP (2); THBD (1)

Data presented as n (%) unless otherwise stated. [a]Reasons for elective termination were given for 5 patients citing the following reasons: health concerns of the mother; medical advice due to eculizumab treatment; severe maternal medical condition; high-risk medical reason; maternal pathology and risk of exacerbation affecting prognosis. [b]Relapse occurred during first trimester. [c]Relapse occurred during second trimester. [d]Two patients had one pregnancy each with eculizumab exposure and one without eculizumab exposure; genetic data are included in both groups. [e]Number of patients tested includes those without known outcome. C3, complement component 3; CFB, complement factor B; CFH, complement factor H; CFI, complement factor I; MCP, membrane cofactor protein; THBD, thrombomodulin.
Summary/Conclusion: Real world data from the Global aHUS Registry showed no increase in poor outcomes in pregnant patients exposed to eculizumab with the proportion of miscarriages consistent with the general population (up to 20% of pregnancies[1]). Further evaluation of this aHUS subpopulation is ongoing.

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Characterisation of a novel F5 gene mutation (Ala2086Asp, Factor V Besançon) associated with factor V deficiency and recurrent venous thrombosis

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Background: Coagulation factor V (FV), present in plasma and platelets, is a multi-domain protein (A1-A2-B-A3-C1-C2) that acts as a pivotal regulator of coagulation. Single-chain FV serves as a carrier of tissue factor pathway inhibitor (TFPIa) in plasma and expresses anticoagulant activity as a cofactor of both TFPIa and activated protein C (APC). However, after cleavage by factor Xa (FXa) or thrombin, FV is converted into a procoagulant cofactor (FVa) that potently enhances prothrombin activation by FXa. FVa activity is regulated *via* proteolytic inactivation by APC and its cofactor protein S. FV deficiency is usually associated with a mild-to-severe bleeding diathesis.
Aims: We investigated a 37-year-old male patient with moderate FV deficiency (FV:C 3%, FV:Ag 4%) and recurrent venous thrombosis (11 events).
Methods: Following informed consent, a blood sample was obtained from the patient. Thrombin generation was measured in platelet-rich plasma (PRP) and platelet-poor plasma (PPP) by Calibrated Automated Thrombography. Moreover, thrombophilia screening and *F5* gene sequencing were performed. The structures of the wild-type and mutant FV C2 domain were analysed by molecular dynamics (MD) simulation. The time-courses of FV activation by thrombin and of FVa inactivation by APC ± protein S on synthetic phospholipid vesicles were followed in 1/500 diluted plasma, using a prothrombinase-based assay to quantify FVa activity in timed subsamples. The APC-cofactor activity of FV was probed with the Immunochrom assay.
Results: Thrombin generation in the patient's PPP was lower than in control PPP, in line with the patient's FV deficiency. However, thrombin generation in the patient's PRP was higher than in control PRP, indicating a hypercoagulable state. The response to APC (Protac) was impaired in both PPP and PRP, possibly due to the complete lack of APC-cofactor activity of FV (Immunochrom APCsr 1.44 *vs.* 2.04 in normal plasma) and to the low full-length TFPIa level (24%) accompanying the patient's FV deficiency. Genetic analysis revealed heterozygosity for the thrombophilic prothrombin 20210G>A mutation and homozygosity for a novel missense mutation in the C2 domain of FV (Ala2086Asp, FV Besançon), affecting a conserved residue and likely responsible for the patient's FV deficiency. The MD simulation indicated that FV Besançon favours a more "closed conformation" of the FV C2 domain, predicting impaired binding to phosphatidylserine-containing membranes. FV Besançon was activated by thrombin ~1.5 faster than normal FV. While the rates of APC-catalysed inactivation in the absence of protein S were similar for FVa Besançon and normal FVa, FVa Besançon inactivation was less stimulated by protein S.
Summary/Conclusion: We have identified and characterised a *F5* gene mutation (FV Besançon) associated with FV deficiency and recurrent venous thrombosis. FV Besançon contributes to a hypercoagulable state by: a) markedly decreasing the FV level and hence the anticoagulant activity of FV without compromising its procoagulant activity; b) indirectly reducing the full-length TFPIa level; and c) increasing the rate of FV activation and decreasing the rate of FVa inactivation in the presence of protein S, possibly *via* impaired binding to phospholipids. A specific role of platelet FV Besançon cannot be excluded and deserves further investigation.

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Design of a "catch and release assay" for circulating FXIa

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Background: Reduced *in vivo* levels of FXI have been associated with a lower thrombosis risk while at the same time do not lead to an increased bleeding risk. For this reason, a shift in anticoagulant therapy toward FXIa inhibition is currently explored. However, it is not known if increased FXIa levels lead to a higher risk on thrombosis. Previous studies focused on the indirect measurement of FXIa by quantification of FXIa-inhibitor complexes via sandwich ELISA's but the detected levels of FXIa by this assay were influenced by contact activation during the process of blood drawing.
Aims: To develop an assay to directly measure the amount of FXIa present in blood at the time of blood drawing by isolation of circulating FXIa homodimers through complexation with a multimeric form of the natural FXIa inhibitor Fasxiator in the presence of a contact activation inhibitor. Upon isolation, the multimeric complex is disrupted resulting in a decrease in affinity of the inhibitor and release of FXIa. Finally, FXIa could be quantified using chromogenic substrate specific for the FXIa enzyme.
Methods: Fasxiator (*Bugarus Fasciatus*: 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 desthiobiotin tag to enable tetramer formation of Fasxiator with Avidin. In a proof of concept study desthiobiotin-Fasxiator was coupled to avidin and subsequently used to inhibit and isolate FXIa from plasma. To release FXIa, the multivalent enzyme-inhibitor complex was disrupted by incubation with biotin. Lineweaver Burk plots were made and Biacore assays were performed to study the kinetic parameters of FXIa inhibition.
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. Disruption of the tetrameric Fasxiator-FXIa complex lead to maximal dissociation of FXIa and regain of its catalytic activity. By making use of this "catch and release" system we were able to detect FXIa down to 50 pM in blood plasma.
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 a tetrameric Fasxiator a suitable candidate for FXIa isolation. Upon disruption of the tetrameric Fasxiator complex, active FXIa was regained and activity could be assessed. The so designed "catch and release" FXIa assay is a promising approach to isolate and measure FXIa in circulation.

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Opium as a risk factor for coronary artery disease in young Iranians: results from the premature CAD Milano-Iran (MIran) study

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Background: The spreading of opium addiction, especially in Asian countries, pose new health related concerns. Despite this, in some areas its use is still believed to protect from cardiovascular disorders, such as coronary artery disease (CAD). However, whether opium use has an association with CAD is still unclear.
Aims: To investigate the association between opium use and risk of CAD.
Methods: We set up a case-control analysis, i.e., the premature CAD Milano-Iran (MIran) study. We enrolled young patients who underwent a coronary angiography at the Heart Centre of Tehran, between 2009 and 2012. Incident cases with critical CAD (stenosis >50%) were contrasted with subjects with no history of CAD (controls) for habitual opium use. Relative risks were calculated in terms of odds ratios (ORs) by logistic regression models adjusted for age, sex, cigarette smoking, body mass index, hypertension, hyperlipidaemia and diabetes, and stratified by sex. Interaction analyses have been performed between opium use and hypertension, hyperlipidaemia and diabetes.
Results: 1011 patients with CAD (65% female) and 2002 controls (57% female) were included in the study. Mean age was 43.6 years for cases and 54.3 for controls. Habitual opium users had a 4.3-fold increased risk of CAD (95% CI 3.0 - 6.2) compared with non-users. The risk remained high even when all main cardiovascular risk factors were taken into account (fully adjusted OR 3.8, 95% CI 2.4 - 6.2). The association was stronger for men, with a fully adjusted OR of 5.5 (95% CI 3.0 - 9.9), than for women (fully adjusted OR 2.6, 95% CI 0.5 - 12.6). No interaction was observed for the combination of opium addiction and hypertension (OR for the combination 9.0, 95% CI 3.7 - 21.7; expected OR 10). On the contrary, a small excess in risk was found in opium users with hyperlipidaemia (OR 16.8, 95% CI 8.9 - 31.7, expected OR 12.2), suggesting supra-additive interaction. Finally, opium users with diabetes had a lower risk of CAD than the sum of the two separate risk factors (OR 1.8, 95% CI 0.5 - 5.9, expected OR 8.8).
Summary/Conclusion: Despite common beliefs, our study showed that opium addiction is associated with an increased risk of CAD, even when other major cardiovascular risk factors are taken into account. The increased risk is consistent in both sexes and it is particularly high for subjects with hyperlipidaemia. Our results should help implementing prevention and educational programs against the non-medical use and spread of opium consumption worldwide, especially in Asian countries.

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A validated metabolomic plasma profile associated with venous thromboembolism

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Background: Recent studies show the existence of new modulators of blood coagulation, which include contact phase proteins (quinine-kallikrein system), products released by activated and necrotic cells (polyphosphates, histones and nucleic acids), and inflammatory mediators.
Aims: The application of advanced analytical techniques in the study of venous thromboembolism.
Methods: In a previous untargeted metabolomics analysis in the plasma of 40 patients with VTE and 32 healthy volunteers, we were able to identify 15 metabolic variables capable of distinguishing this pathology. Next, we selected plasma samples from 60 patients with VTE and 60 healthy volunteers to validate these metabolic variables in a targeted analysis by UPLC-MS / MS. The identification and quantification of these variables was done through the use of chemical standards and calibration lines. We analyzed the association between the concentration of the plasmatic metabolites quantified and the risk of VTE by a binomial regression analysis called ELASTIC NET (R v3.5.0). We also analyzed the correlation degree between quantified metabolites and clinical parameters, the thrombin generation test (TGT) and coagulation modulators.
Results: 4 of the 15 metabolic variables identified in the untageted study were successfully identified with their chemical standard and quantified in plasma samples. We observed the differences in the levels of these 4 metabolites (M1, M2, M3 and M4) between patients and controls (P <0.001), with a fold-change of -1.2 (M1); 1.6 (M2); 2.4 (M3) and 2.1 (M4). Next, we performed a binomial regression analysis with the four quantified metabolites and we obtained a predictive model of VTE with an area under the ROC curve of 0.98 [95% CI = 0.97-1]. Odds-ratio values for each variable were: 0.92 (P = 0.173) (M1); 1.03 (P <0.001) (M2); 1.12 (P = 0.173) (M3) and 23.98 (P = 0.001) (M4). Finally, we observed an association between the quantified metabolites and the levels of LDL, calprotectin and myeloperoxidase (P <0.05).
Summary/Conclusion: We have identified and validated in a retrospective case-control study a group of plasma metabolites with a high predictive capacity for VTE.
Recent studies have shown that one of these metabolites (M1) inhibits the activity of FXa, and we have also observed an association between these metabolites and biomarkers of lipid metabolism, cell activation, inflammation and NETosis. All this suggests the existence of new molecules and routes of action involved in the coagulation system.
ISCIII (PI14/00512, PI14/00079, FI14/00269, CPII15/00002, PI17/00495), FEDER, Generalitat Valenciana (PROMETEOII/2015/017, ACIF/2017/138), IIS La Fe (2014/0718, 2016/0820_VLC-BIOMED).

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The impact of age on the pharmacological activity of factor X inhibitors, rivaroxaban and apixaban

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Background: According to both trial and clinical data on novel oral anticoagulants (NOACs) elderly patients are at greatest risk of bleeding. It is unclear whether age per se affects anticoagulation response.
Aims: In order to investigate the age-related increase in sensitivity to NOACs, the pharmacological activity of the direct factor Xa inhibitors, rivaroxaban and apixaban, was compared between young and elderly subjects ex-vivo.
Methods: Two groups of study subjects were recruited; one group of fit elderly people and one group of fit young people. After signing informed consent, all eligible subjects that fullfilled inclusion criteria provided a blood sample and demographic data. Plasma samples were separately incubated with rivaroxaban (100-500ng/ml) and with apixaban (75 and 150ng/ml). Clotting parameters, clotting factor activity and thrombin generation were measured.
Results: 36 fit elderly and 30 fit young subjects [median(IQR) age: 83(75-87) v 30(26-38) y] were recruited for rivaroxaban and provided a blood sample. A subset of 17 elderly and all 30 young subjects were available for analysis with apixaban. Rivaroxaban produced a greater prolongation of both PT ($p<0.05$) and modified PT ($p<0.001$) across the concentration range in the elderly compared to young subjects. Apixaban produced a greater prolongation of PT ($p<0.001$) and mPT ($p<0.001$) at the highest concentration (150ng/ml) in the elderly compared to young subjects. Factor IIa and Xa activity levels were significantly lower in elderly than young subjects after exposure to both rivaroxaban and apixaban. Rivaroxaban prolonged time-based parameters of thrombin generation and suppressed the rate and amount of thrombin generation to a significantly greater extent in the elderly compared to young subjects (%change from baseline for ETP; rivaroxaban:-35.0±4.4 v -29.8±7.4 nM*min; $p=0.004$, apixaban:-37.5±2.4 v -25.5±2.0 nM*min;$p< 0.01$).
Summary/Conclusion: The impending validated assays for use with NOACs will be of considerable benefit for bleeding patients and those requiring urgent invasive procedures. Their more routine use might also benefit some elderly patients who, because of their increased sensitivity to rivaroxaban and apixaban, may require lower doses of the drug for therapeutic anticoagulation.

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The effects of a peripheral venous catheter compared to repeated venepunctures on markers of coagulation, inflammation, and endothelial function

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Background: Standard venepuncture is the recommended blood sampling technique for coagulation testing. Frequent venepunctures can, however, cause pain, skin damage, and bruising at the local site of blood sampling. Therefore, peripheral venous (PV) catheters are often used for serial blood sampling, but studies suggest that PV catheters increase markers of coagulation activation and inflammation. Whether the increase is caused by irritation of the vessel wall or diurnal variation is unknown
Aims: The study aim was to compare the effects of a PV catheter and repeated venepunctures in healthy volunteers on selected markers of coagulation, inflammation, and endothelial function in serial blood samples
Methods: The study included 10 healthy non-fasting volunteers after informed consent and was conducted according to the Declaration of Helsinki. A PV catheter was inserted at 07:45 in a hand vein in each subject, and blood samples were collected at 8:00, 10:00, 12:00, and 14:00. In the contralateral arm, blood was simultaneously obtained by venepunctures. Markers of coagulation, i.e. prothrombin fragment 1+2 (F1+2) and thrombin-antithrombin (TAT), inflammation, i.e. interleukin 6 (IL-6), and endothelial function, i.e. plasminogen activator inhibitor 1 (PAI-1), tissue plasminogen activator (tPA), von Willebrand factor (vWF), and tissue factor (TF), were measured in plasma
Results: The concentrations of TAT and F1+2 were significantly increased (10:00; $p<0.01$, 12:00; $p<0.05$, and 14:00; $p<0.01$) in PV catheter samples compared with venepuncture samples. There was no increase in concentrations of PAI-1, tPA, vWF, or TF, and no differences in these markers between sampling methods at 10.00, 12.00, and 14.00. In most subjects, IL-6 concentrations increased in both PV catheter samples and venepuncture samples, but this increase was only significant in PV catheter samples ($p=0.001$). Also, there were no significant differences in IL-6 between sampling methods at 10.00, 12.00, and 14.00. The size of the response was, however, characterized by a large inter-individual variation
Summary/Conclusion: Blood collection through a PV catheter induces coagulation activation by increasing concentrations of TAT and F1+2. A PV catheter does not affect the plasma concentrations of the endothelial derived proteins PAI-1, tPA, vWF, and TF. The effects of blood sampling techniques on IL-6 are complex and need further investigation. Our results indicate that future studies should use venepuncture as blood drawing technique for investigation of coagulation activation markers in serial blood samples

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Discrepant interpretation of HIT screening results on the same sample - data from a UK NEQAS for Blood Coagulation exercise

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Background: Heparin Induced Thrombocytopenia (HIT) is an immune complication of treatment with heparin, in some cases resulting in life-threatening thrombosis. An accurate diagnosis is required to ensure appropriate clinical decisions are made. Several assays are now available to diagnose HIT, and several studies have demonstrated varying sensitivity and specificity of different tests.
Aims: We describe here an external quality assessment (EQA) exercise in which laboratories employing different HIT assays were asked to test and interpret results for a sample from a donor thought to have HIT.
Methods: Plasma was obtained with informed consent from a patient identified as having HIT antibodies by an ELISA assay. Plasma was buffered and lyophilised in aliquots and sent to participants in the UK NEQAS for Blood Coagulation (UK NEQAS BC) HIT programme. In the same exercise a sample was included which was expected to give normal results. For each sample, median, coefficient of variation (CV) and range of results were determined for each method and reagent group. Participants were also asked to make an interpretation (positive, equivocal or negative) for each sample.
Results: Sample S18:01 was a sample previously identified as having raised HIT antibody levels by an ELISA assay. 10 centres using the Lifecodes/Immucor PF4 IgG kit reported a positive HIT screen, along with 1 centre using a PIFA Heparin/PF4 rapid assay kit, 1 using the Stago STic Expert kit, and 1 failing to state their method. Two centres using the Lifecodes/Immucor PF4 IgG kit reported a negative HIT screen, one of which noted the presence of non-neutralising antibodies in the plasma, having performed the test with additional heparin; this was noted by another centre reporting a positive screen. One centre using the HemosIL HIT-ab PF4-H kit reported an "inconclusive" result. Sample S18:02, expected to give a negative screen, was described as negative by all centres. Heparin induced platelet activation and serotonin release assays, considered reference standard methods for diagnosis of HIT, were not used by any of the participants in this exercise
Summary/Conclusion: This exercise demonstrated the variability in interpretations that can arise for a sample with HIT antibodies. Some assays may be sensitive to non-pathogenic antibodies, and it is important that laboratories and clinicians understand the clinical implications of reported HIT assay results.

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Molecular genetic investigations in antithrombin deficient children; investigation of genotype-phenotype relationship

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Background: Antithrombin (AT) is the most important circulating inhibitor of blood coagulation proteases. Hereditary AT deficiency is classified as type I and type II. In type II deficiency, the defect may involve the reactive site (IIRS), the heparin-binding site (IIHBS) or it may exert a pleiotropic effect. Individuals with inherited AT deficiency have a highly increased thrombotic risk. The mutation profile of *SERPINC1* is heterogeneous, the most prevalent mutations are AT Cambridge II, AT Budapest 3 (ATBp3) and AT Basel. The ATBp3 mutation underlies the vast majority of AT deficiencies in the Hungarian population and there is evidence that its elevated frequency may be due to a founder effect. Clinical management of AT deficiency is sometimes a challenge, especially in childhood. No widely accepted recommendations are available, at least partly due to low amount of clinical and laboratory data.
Aims: Our aims were to establish a database of AT deficient children, to investigate the genetic background of the disease and to collect data on clinical symptoms and treatment modalities.
Methods: Genetic analysis was performed in AT deficient children recruited in our diagnostic center. The inclusion criterion was AT deficiency diagnosed by functional heparin cofactor FXa-based assay. Clinical data, as venous thromboembolism or arterial thrombosis diagnosed by objective methods before the age of 18 years and data on therapeutic modalities were collected and the causative mutations in SERPINC1 were detected by Sanger sequencing. The mutation detection rate, the mutation spectrum and the genotype-phenotype correlations were determined.
Results: Among the n=55 children with laboratory phenotype of AT deficiency the mutation detection rate was 100%. The vast majority of children carried the ATBp3 mutation (n=24 homozygous and n=19 heterozygous). Type IIHBS AT deficiency was detected in two additional cases (AT Basel). Type I deficiency was diagnosed in n=10 children. Among the identified mutations two novel were found (p.Leu205Pro and p.Ser84Valfs*21). The age interval at the time of thrombotic complications was rather wide (between 2 days and 17 years). The clinical symptoms were also heterogeneous and not only venous but also arterial thrombosis was registered (ischemic stroke). Provoking factors of thrombosis were found only in sporadic cases (2 pregnancies and 1 surgical procedure) in the background. Four out of the 24 ATBp3 homozygous children had congenital vascular anomaly. Therapeutic and preventive strategies were heterogeneous.
Summary/Conclusion: AT deficiency is a rare disease, however it should be screened in children with unprovoked thrombosis. Based on our experience ATBp3 deficiency is not as benign as it is suggested since the majority of children carrying this mutation suffered their first thrombotic event before the age of 18. By performing adequate laboratory methods for the diagnosis of AT deficiency the causative mutation is detected in all cases. Clinical management of AT deficient children needs harmonization.

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New fibrinolytic technology based on serine protease subtilisin

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Background: Pharmacological fibrinolysis with tPA depletes plasminogen resource that able to decrease the effect of their administration over time. Formed plasmin cleaves the Lys-Lys and Lys-Arg bonds contained as in fibrin as well as in fibrinogen and in clotting factors V, VIII, X. In turn plasmin-mediated fibrinolysis is inhibited by fibrin/fibrinogen degradation products in high concentrations.
Aims: We are reporting about new fibrinolytic technology using serine protease subtilisin that allows to fibrinolysis bypassing plasmin.
Methods: Subtilisin's substance was produced from Bacillus subtilis, gram+ and catalase-positive bacteria, and then immobilyzed at polymer by original technology with a stream of accelerated electrons (axis-technology). Due to that enzyme's globule has covering with polymer-clad shell while active enzyme's site has remained accessible.
Fibrinolytic properties of subtilisin's substance were examined in vitro and then in animal models of tail veins thrombosis. Basing on obtained results it was developed and studied new fibrinolytic medication.
Results: After i.v. injection of new fibrinolytic medication the mass of fresh (within 2-hours formed) thrombi has decreased their mass in 8-fold during 90 minutes, and 'old' thrombi (with lifetime over 24h) were lost up to 85-87% of their weight in 2 hours as well. After per os using of that, total thrombosis resolution was observed within 36 hours. The study has shown high enteral absorption and bioavailability up to 18%.
One result else was that repeated administration of this agent were not accompanied the loss of fibrinolytic activity due to there was not a phenomenon of plasmin inhibition by cumulated fibrin/fibrinogen degradation products.
Primary clinical study was involved 154 patients with acute deep venous thrombosis of lower limb. All patients were similar by age, disease duration, therapy and other parameters that allowed to test 3 doses of new fibrinolytic agent each-to-other and in comparing to placebo as well. The bloodstream recovery and the lysis of clot floating segment were checked by Doppler before and after 10 days of fibrinolytic therapy. The study has revealed dose-depended effect of new fibrinolytic medication increased therapeutic effectiveness up to 75% in compare with placebo. That is important the lysis of clot floating segment was observed almost in 96%. Any bleedings, sides effects, and drug-drug interactions were not found till now.
The new study is ongoing in patients with cerebral venous sinus thrombosis. Primary results seems encouraging.
Summary/Conclusion: Subtilisin's-based technology looks like high attractive because it performs non-plasmin path of fibrinolysis. New fibrinolytic technology together with developed medication 'Trombovazim' was patented in Russia and in USA. We assume this fibrinolytic technology able to be a success in different cases of at least venous thrombosis.

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The association of antithrombin activity with the risk assessment of acute pulmonary embolism and predictive significance for 30-day mortality

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Background: Antithrombin activity is often decreased in patients with acute pulmonary embolism (PE). Its significance in the risk assessment of early mortality in such patients is still unknown.
Aims: To investigate the association of AT activity with the risk stratification and its predictive significance of 30-day mortality in acute PE-patients.
Methods: Consecutive patients with acute PE admitted to single-center were included in the study. All patients were treated according to the current guideline for the management and treatment of acute PE. The morning after admission a blood sample was taken from the antecubital vein for the measurement of AT activity. According to current European guideline for the management of acute PE, patients were stratified into the four risk groups for 30-day mortality: high-risk, intermediate-high, intermediate-low and low-risk group. Quartiles of AT activity values were compared between the risk groups. A Kaplan-Meier curve was used to depict the 30-day mortality difference between the first and other quartiles of AT activity and prediction value for 30-day mortality was calculated by Cox's proportional hazard model.
Results: This study included 292 consecutive PE patients. The incidence of 30-day mortality was 9.2%. Across the risk groups, 61.1% of patients with high-risk PE were within the first quartile of AT activity as compared with 23.8%, 14.1% and 14.3% of patients who had intermediate-high, intermediate-low and low-risk, respectively ($p<0.001$). Patients who were within the first quartile of AT activity had significantly increased 30-day mortality than patients in other quartiles (log rank $p<0.001$). When sex and age were included in the Cox's proportional hazard model, PE patients within the first quartile of AT activity had a higher probability for 30-day death than patients within other quartiles (HR=4.79; 95%CI 2.16 – 10.65; $p<0.001$).
Summary/Conclusion: Patients with high-risk PE have significantly more often the lower activity of AT then patients with intermediate-risk and low-risk PE. Those within the first quartile of AT activity have a higher probability of 30-day death than patients within other quartiles.

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Plasma clot formation, lysis, fibrin fiber density and mechanics from patients with acute PE before and after initial treatment with low molecular weight heparin

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Background: Pulmonary embolism (PE) is a major cause of morbidity and mortality in patients with venous thrombosis. Treatment, both initial and long term, is aimed at preventing recurrent episodes. Determining differences in clot properties may facilitate understanding of mechanisms underlying the disease, as well as guide treatment, e.g. with heparin, in PE patients. Furthermore, it may have the potential to indicate when recurrence may occur.
Aims: To determine plasma clot formation, lysis, fibrin fiber density and mechanics from patients with acute phase PE and 24 hours after treatment with low molecular weight heparin (LMWH).
Methods: Blood samples were taken from 47 patients aged 23-86, who presented with acute phase PE, and again from the same patients 24 hours after initial treatment with LMWH. Plasma clot formation and lysis was determined by turbidity. Mechanical properties were measured using an in-house magnetic microrheology apparatus which allowed for determination of storage (G') and loss (G'') modulus. Clot permeability (Ks) was measured and images of each clot were taken using laser scanning confocal microscopy.
Results: Plasma clots from acute PE patients formed quicker (3.96 ± 2.43min vs 11.18 ± 15.8min) and had a shorter time to max OD (97.33 ± 37.33min vs 126.67 ± 42.17min) than plasma clots formed 24 hours after initial treatment with LMWH. There were no differences in lysis parameters, including time to half lysis (33.50 ± 30.16min vs 31.33 ± 32.33min, acute PE vs LMWH treatment) or average rate of lysis (2.20 ± 1.53 ΔOD/s vs 2.06 ± 1.35 ΔOD/s). Storage modulus (G') was higher for acute PE samples compared to those 24 hours after LMWH treatment (2.02 ± 2.14 Pa vs 1.30 ± 1.40 Pa). Similarly, loss modulus (G'') was higher for acute PE samples compared to samples 24 hours after LMWH treatment (1.05 ± 0.60 Pa vs 0.92 ± 0.39 Pa). Clot permeability was similar for each group (5.13 ± 2.47 X 10^{-9} cm^2 vs 5.35 ± 2.35 X 10^{-9} cm^2, acute PE vs LMWH treatment respectively).
Summary/Conclusion: LMWH treatment for acute PE patients slowed both initial and final clot formation, while not changing how these plasma clots are broken down. Additionally, treatment with LMWH lowered clot stiffness and viscosity when compared to initial acute PE presentation. These data indicate that clot mechanical properties may help to predict recurrent episodes. Further studies, with larger sample sizes, are needed to determine how both initial and long term treatment with LMWH and warfarin effect clot structure and mechanics for the prevention of thromboembolic disease.

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Examination of the interacting domains between the extracellular domains of tissue factor and β1-intergin and their influence on cell proliferation

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Background: Tissue Factor (TF) has been shown to interact with β1 integrin which can induce signalling and influence cell proliferation
Aims: This study aimed to identify the domains within TF and β1 integrin which are involved in the interaction of these two proteins.
Methods: MDA-MB-231 or HBDEC cells (10^4) were seeded in glass bottomed dishes and transfected with FLAG-HA-pcDNA3.1 plasmids encoding DNA-fragments to express peptides corresponding to the first (residue 1-110), second (residue 106-211) or combined fibronectin-like domains of TF, or a peptide corresponding to the EGF-4-βTD domains of β1 integrin (residue 572-706). The ability of the expressed peptides to associate with the native β1 integrin or alternatively, the ability of EGF-4-βTD to associate with endogenous TF, were assessed using the proximity ligation assay (PLA). In addition, MDA-MB-231 or HBDEC cells (10^5) were seeded in 12-well plates and transfected as stated above. The cells were incubated for 48 h after which the rate of cell proliferation was assessed using the crystal violet staining procedure.
Results: The expression of the fragment corresponding to the second fibronectin-like domains of TF (residue 106-211) reduced the rate of cell proliferation in MDA-MB-231 cells. In contrast, the expression of the first (residue 1-110) or combined (residue 1-211) fibronectin-like domains had no significant influence. However, all three fragments were shown to be capable of associating with β1 integrin on the surface of MDA-MB-231 and HBDEC. Furthermore, the fragment corresponding to the EGF-4-βTD domains within β1 integrin was capable of associating with the endogenous TF on the surface of MDA-MB-231 cells, but had no detectable influence on the rate of cell proliferation.
Summary/Conclusion: This study is the first to identify an association between the second fibronectin-like domain of TF, and β1 integrin protein *in situ*, and further elucidates the mechanism of TF-β1 integrin signalling in the enhancement of cellular proliferation.

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Soluble fibroblast activation protein (sFAP) and alpha2-plasmin inhibitor p.Arg6Trp polymorphism in venous thrombosis

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Background: Alpha2-plasmin inhibitor (A2-PI) is the main inhibitor of plasmin. During circulation in plasma, A2-PI suffers from cleavages, that significantly influence its activity. The secreted full length A2-PI (Met-A2-PI) is shortened by 12 amino acids resulting in a version with Asn on the N-terminus (Asn-A2-PI). The cleaving enzyme was identified and named as antiplasmin cleaving enzyme (APCE), which is identical to the soluble form of fibroblast activation protein (sFAP), a prolil-specific serine protease. Significant correlation was shown between sFAP plasma levels and the percentage of A2-PI N-terminal cleavage. The Asn-A2-PI form is more efficiently cross-linked by activated FXIII (FXIIIa) to fibrin A-chains. Only the cross-linked A2-PI can effectively inhibit fibrinolysis, therefore the ratio of N-terminal forms may affect the resistance of the clot to fibrinolysis. Soluble FAP cleaves Met-A2-PI(Arg6) 8-fold faster than Met-A2-PI(Trp6), thus the p.Arg6Trp polymorphism of A2-PI may have an effect on the risk of thrombotic disorders.
Aims: In this case-control study we investigated the association of A2-PI p.Arg6Trp polymorphism and plasma sFAP antigen levels with the risk of venous thromboembolism (VTE).
Methods: 300 VTE patients and 308 healthy controls (HC) were enrolled in the study. sFAP, and fibrinogen levels were measured in citrated plasma samples using FAP DuoSet ELISA kit (R&D System) and Clauss assay, respectively; A2-PI Arg6Trp genotype was determined with real-time PCR (LightCycler 480).
Results: The minor allele frequency did not differ significantly between HC and VTE groups (0.21 and 0.19, respectively). Possession of the Trp allele did not decrease significantly the risk for VTE (OR (95% CI): 0.825 (0.591-1.150). There was no statistically significant difference in the median sFAP levels of HC and VTE groups (median (IQR): 76.8 (65.7-90.1) mg/L and 80.2 (66.9-95.5) mg/L respectively, p=0.105). In a multiple linear regression model only BMI showed independent correlation with sFAP levels (b: 0.224; p=0.002). Elevated sFAP levels (>90.5 mg/L) had a moderate increasing effect on the risk of VTE (OR: 1.47 (1.034-2.104), p=0.032), but after correction for BMI, the effect was not statistically significant any more (OR: 1.225 (0.828-1.814), p=0.113).
Summary/Conclusion: In our study neither sFAP levels nor A2-PI Arg6Trp polymorphism influenced the risk of VTE significantly.

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Analysis of the differential expression of miRNAs present in plasma and contained within microparticles in severe forms of pulmonary hypertension

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Background: * J Oto and O Tura-Ceide have contributed equally to this work.
Pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH) are serious forms of precapillary pulmonary hypertension (PH) with reduced prognosis, despite recent advances in their treatment. Epigenetic mechanisms might be involved in the pathogenesis of these diseases.
Aims: To study the epigenetic mechanisms in severe precapillary PH through the identification of dysregulated microRNAs (miRs) contained in circulating plasma microparticles (MPs) and their differential expression with respect to paired total plasma.
Methods: Thirty patients with PH and 10 healthy subjects were prospectively recruited. Patients with PH were classified in: PAH, surgical CTEPH and non-surgical CTEPH (n=10/each). Plasma samples were collected and MPs were isolated from all subjects. Total RNA was isolated from both plasma and MPs with the *miRNeasy Mini kit* (Qiagen) and the expression of 179 miRs was quantified using the Human Serum/Plasma Focus, miRCURY LNA miRNA Focus PCR *V5* (Qiagen). Expression levels were normalized with the endogenous and stable miR-425-5p selected using *BestRef*. Statistical analysis of differentially expressed miRs (in plasma and MPs) among all groups was performed using R (v3.5.0).
Results: Fourteen miRs (miR-301a-3p, miR-339-5p, miR-652-3p, miR-331-3p, miR-484, miR-18a-5p, miR-127-3p, miR-133a-3p, miR-629-5p, miR-361-5p, miR-200c-3p, miR-144-5p, miR-34a-5p and miR-10b-5p) contained in MPs and 4 plasma miRs (miR-133a-3p, miR-28-5p, miR-26a-5p and miR103a-3p) were differentially expressed among the different groups. Among them, miR-133a-3p was found to be dysregulated in both MPs and plasma.
Summary/Conclusion: Patients with precapillary PH show a differentiated profile of miRs in both plasma and circulating MPs. miR-133a-3p might play a prominent role in PH as it is downregulated in both plasma and MPs in patients with PAH or CTEPH.
Funding sources: Instituto de Salud Carlos III (PI15/01085, PI15/00582, CPII15/00002 and CP17/00114), SETH and SEPAR 164/2016.

ECTH 2019
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The effects of acute and chronic exercise on Factor VIII and von Willebrand antigen plasma levels in well trained athletes

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Background: Physical exercise induces a range of physiological changes including the release of hemostasis proteins such as von Willebrand factor (vWf) and factor VIII (FVIII) to blood. The magnitude and permanence of these changes have been related to the volume and the intensity of exercise as well as the physical fitness of the athlete.
Aims: The aim of this study was to evaluate the effects of acute intensive and chronic moderate physical exercise on plasma concentration of vWf and FVIII in well-trained athletes.
Methods: Two professional male soccer teams(team 1 and team 2) participated in this study. Team 1 (N=18, mean age 20±2 years, BMI: 22.5±1.9 kg/m2, Fat: 13.8±3.4%) performed one high-intensity interval training to exhaustion. Von Willebrand factor antigen (vWf: Ag), FVIII plasma levels, platelet count (PLT) and high sensitivity C-reactive protein (hs-CRP) were measured before and immediately after the end of the specific training. The same blood tests were performed at the beginning and at the end of a 7 weeks period of moderate intensity training for team 2 (N=15, mean age: 22.2±4.3 years, BMI: 23.1±1.3 kg/m2, Fat: 7.8±2.6%).
Results: No differences were found in hs-CRP levels at the end of the study, for both teams. In team 1, PLT, FVIII, and vWf: Ag were found significantly increased at the end of the study ($p<0.002$, 95%CI). Particularly, PLT were increased by 14.8% FVIII by 102.3%, and vWf: Ag by 79.8%, at the end of the specific training. Contrariwise, in team 2, mean vWf: Ag plasma levels were significantly decreased by 7.7% at the end of the experimental period ($P=0.018$). No significant differences in PLT and FVIII were observed in team 2 at the and of the 7 weeks period.
Summary/Conclusion: 1. Acute intermittent training to exhaustion leads to a significant increase of PLTs, FVIII, and vWf Ag levels probably due to shear stress-induced endothelial activation. These findings are in accordance with previously published data supporting that intense exercise can be beneficial on specific types of bleeding disorders.
2. Chronic moderate exercise reduces vWf: Ag levels indicating a possibly beneficial effect on the endothelium of these players.

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Empowering cancer patients for non-pharmacological primary prevention and early recognition of cancer-associated venous thromboembolism (VTE): the EMPATIC-CP survey

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Background: Venous thromboembolism (VTE) is a leading cause of death and morbidity in patients with cancer.
Aims: The aim of the present study was to explore the current practices regarding non-pharmacological primary VTE prevention and education for early recognition of potential VTE-related symptoms in cancer patients.
Methods: A specific electronic questionnaire was designed to assess the current practices in a sample of Medical Oncology Departments from different healthcare institutions in Spain.
Results: The preliminary data from 30 out of 52 centres selected were analysed. Education specifically addressed to recognizing alarm symptoms for cancer-associated emergencies was routinely performed: never (0%), always (48%) or only for specific situations (52%).
The rates regarding specific patient education programmes for primary prevention and early recognition of VTE were: never (18%), always (10%), only for patients with central venous catheter (CVC) (8%), only for patients with CVC and/or other particular conditions (52%), and only for specific cases (12%).
Patient education (multiple-choice questions) was performed by: specialist physicians (75%), medical residents (55%), outpatient clinic nurses (38%), daycare hospital nurses (58%), and pharmacists (14%). Education aimed at recognizing VTE recurrence and/or bleeding complications in cancer patients with VTE was routinely performed in 62% of the participating centres.
Summary/Conclusion: Patient education for recognizing potentially life-threatening symptoms related to cancer, anticancer therapies and cancer-associated complications such as VTE poorly covered in our setting.
The implementation of strategic educational programmes aimed at increasing patient awareness about cancer-associated emergencies and VTE is an area requiring further research and development.

ECTH 2019
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The glycan structure of factor XIII B subunit

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Background: Coagulation factor XIII (FXIII) is essential for maintaining hemostasis. It stabilizes the fibrin clot by cross-linking fibrin γ-, and α-chains through ε(γglutamyl)lysyl isopeptide bonds. Cross-linked fibrin becomes more resistant against the shear stress of circulating blood and against fibrinolityc degradation. FXIII is of tetrameric structure; it consists of two potentially active A subunits (FXIII-A) and two protective/inhibitory B subunits (FXIII-B). The latter prolongs the half-life of FXIII-A in the circulation and prevents its spontaneous activation in plasmatic conditions. FXIII-B is in excess, 50 % of it circulates as free non-complexed dimer. FXIII-B is a glycoprotein containing 8.5% carbohydrate. It has two N-glycosylation sites at Asn142 and Asn525 in the 3rd and 9th sushi domains. Neither the structure nor the functional role of the glycans on FXIII-B has been explored.
Aims: The aim of the study was to reveal the glycan structures present on FXIII-B and to de-glycosylate the native protein making it available for further functional studies.
Methods: FXIII-B was purified from human plasma as described by Lorand et al. Methods Enzymol 1981;80(Pt C):333-41). The protein was denatured in a solution containing SDS, dithiotreitol and Nonidet P-40. The glycan structures were released from the denatured protein by PNGase-F endoglycosidase. After labeling with 8-aminopyrene-1,3,6-trisulfonic acid, the released sugars were purified by magnetic bead technique and analyzed by capillary electrophoresis using laser induced fluorescence detection (CE-LIF). In separate experiments sialic acid was removed by sialidase enzyme. In the case of native (non-denatured) FXIII-B de-glycosylation was carried out by the combination of F1-F2-F3 enzymes or by PNGase-F endoglycosidase in a concentration 10-fold higher than used with denatured FXIII-B. De-glycosylation was controlled by CE-LIF and by periodic acid-Schiff (PAS) staining of SDS polyacrylamide gels.
Results: The total glycan profile included 9 individual structures. The core structure contained two N-acetyl glucoseamines and three mannoses. In four core structures the first N-acetyl glucoseamines were fucosylated. Each structure contained at least one sialic acid and. PNGase-F in the usual concentration was able to remove the glycans completely from the denatured protein but not the native one. Using the combination of F1-F2-F3 enzymes the native FXIII-B could be de-glycosylated with the exception of the first core N-acetyl glucoseamines. Complete de-glycosylation was achieved by PNGase-F when used in elevated concentration. The completeness of de-glycosylation was confirmed by the lack of carbohydrates using CE-LIF or PAS staining. Sialic acid moieties could be removed from both the denatured and the native protein.
Summary/Conclusion: The composition and the structural arrangement of the glycan moieties attached to FXIII-B was revealed for the first time, what helps in understanding their contribution to the protein structure. Complete de-glycosylation and de-sialysation of the native protein was achieved. Their production makes is possible to perform functional studies for exploring the role of glycan structure in the function of FXIII-B.

ECTH 2019
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Dietary zinc modulates several haemostatic parameters

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Background: Zinc is a vital cofactor in many biological reactions in the human body. Zinc is suggested to be endocytosed from plasma into the cytoplasm and a-granules of platelets during their maturation from precursors. The cation is subsequently released during platelet activation. Several studies have shown that *ex vivo* manipulation of zinc has an impact on platelet aggregation and other haemostatic parameters, including fibrinolysis. The bioavailability of zinc in the body entirely depends on dietary intake. Despite this the direct impact of zinc deficiency on haemostatic parameters in human subjects has not been studied.
Aims: To investigate if the bioavailability of dietary zinc in human subjects impacts on haemostatic parameters.
Methods: Thirty-six healthy volunteers aged over 18 years were recruited and informed written consent obtained. Dietary intake of zinc was restricted for 2 weeks (1 mg/day). Blood was collected from volunteers on habitual diet and at the end of the depletion period in 3.2% Sodium Citrate tubes. Platelet rich plasma (PRP) was isolated and haemostatic parameters were measured as detailed below.
Results: Lagtime to platelet aggregation was prolonged by approximately 3 min upon dietary depletion of zinc compared to habitual samples. Maximal platelet aggregation in response to collagen was significantly decreased during the depletion phase (45.2 ± 10.2 %) compared to habitual phase (95.6 ± 23.0 %). Time to thrombin generation was significantly delayed in depletion samples (18.2 ± 4 min) compared to habitual samples (13.2 ± 4.5 min). Maximal thrombin generation was also significantly lower (152.2 ± 4 nM) compared to the habitual phase (226.1 ± 6.3 nM). Visualisation of real-time clot formation and lysis were observed using a Hemacore Thrombodynamics Analyzer. Rate of clot growth was significantly slower in the depletion period (33.4 ± 12.2 µm/min) compared to the habitual period (49.8 ± 11.1 µm/min). Fibrinolysis of PRP clots by tissue plasminogen activator (tPA) was delayed in the depletion phase (48.2 ± 3.1 min) compared to the habitual phase (37.4 ± 4.5 min). A similar attenuation of tPA-mediated PRP clot lysis was observed in an absorbance-based assay of fibrinolysis (100.6 ± 28.2 min in depletion versus 83.6 ± 24.4 min in habitual phase). Clots formed from PRP during dietary zinc restriction exhibited defective clot retraction (final clot weight 481.4 ± 81.2 mg) compared to the habitual period (382.2 ± 41.3 mg).
Summary/Conclusion: These data show that manipulation of dietary zinc has an impact on its bioavailability and uptake by platelets. Restricted levels of zinc in the diet attenuate platelet aggregation, clot formation and fibrinolysis thus directly impacting clot formation and stability. These data show for the first time that dietary zinc has a direct impact on haemostasis in humans.

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A phase 3 study of ravulizumab (ALXN1210) vs eculizumab in adults with paroxysmal nocturnal hemoglobinuria currently treated with eculizumab: subgroup analyses by transfusion history and demographics

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, potentially life-threatening hematologic disorder caused by uncontrolled activation of the terminal complement pathway. PNH may lead to intravascular hemolysis, which is a major contributor to morbidities and premature mortality associated with the disease. Effective treatment options for PNH are aimed at inhibiting complement activation, thereby preventing the risk of life-threatening thrombosis, while reducing symptoms associated with free hemoglobin and alleviating the need for transfusions to treat anemia. Ravulizumab, a novel C5 inhibitor that is administered every 8 weeks, has been shown to be non-inferior to eculizumab (administered every 2 weeks) in two phase 3 trials. Due to the rarity of PNH, there has been a paucity of data evaluating treatment in unique patient populations.
Aims: The aim of these protocol-specified analyses was to examine the safety and efficacy of ravulizumab and eculizumab in specific patient subgroups on the basis of observed transfusion history (received a transfusion of packed red blood cells within 12 months prior to Day 1) and demographics.
Methods: ALXN1210-PNH-302 was a phase 3, open-label, randomized, active-controlled, multicenter study designed to evaluate whether patients with PNH could safely and effectively switch from eculizumab to ravulizumab. The study enrolled adult patients who were previously treated with eculizumab for ≥6 months and had lactate dehydrogenase (LDH) levels ≤1.5 times the upper limit of normal prior to baseline; all patients provided informed consent. Patients were randomly assigned to switch to ravulizumab or continue with eculizumab. The primary efficacy endpoint was percent change in LDH from baseline to Day 183. In the primary study, baseline characteristics between treatment groups were similar. In the current subgroup analyses, we examined the impact of observed transfusion history, sex, age at first study treatment exposure, race, and geographic region on percentage change in LDH.
Results: Overall, a total of 195 patients received treatment with either ravulizumab (n=97) or eculizumab (n=98). The subgroup analyses demonstrated that there were no apparent sensitive subgroups. Point estimates for percent change in LDH from baseline to Day 183 favored ravulizumab overall and across all patient subgroups including observed transfusion history, sex, age at first study drug exposure, race, and geographic region.
Summary/Conclusion: These prespecified subgroup analyses showed point estimates favoring ravulizumab with respect to normalizing LDH levels in patients with PNH at Day 183. The findings confirm the robustness of the primary results of the phase 3 study and support the use of ravulizumab in patients who were previously treated with eculizumab, regardless of transfusion history, sex, age at first study treatment exposure, race, or geographic region.

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Prediction and measurement of human PEG plasma levels after administration of PEGylated biologics and their value for safety assessment

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Background: PEGylation technology has proven to be a valuable tool for prolonging the half-life of proteins through the covalent binding of one or more large polyethylene glycol (PEG) molecules. There are at least 12 PEGylated biopharmaceuticals currently approved in Europe and the United States in several indications including hemophilia A and B. The pharmacokinetic (PK) properties of PEGylated proteins are initially driven by the two major parts of the molecule: the protein itself and its conjugated PEG. An understanding of the PK, metabolism and biodistribution of PEG in PEGylated proteins is essential in order to understand the long-term safety of these compounds. As excretion processes have been demonstrated in animal studies, including for large PEGs (up to 60 kDa), once a steady state is reached, there are no further increases in blood or tissue concentrations.
Aims: Predictions of human steady-state levels of PEG and their value for human long-term safety will be discussed.
Methods: Plasma PEG-60 concentration-time profiles after one to two times per week administration of BAY 94-9027 therapeutic dose in humans (weekly dose 60–120 IU/kg) were simulated, based on single-dose administration of a human 30-year equivalent dose of PEG-60 in rats. For comparison, long-term plasma PEG measurements were available for 120 adults/adolescents from PROTECT VIII (age at enrollment, 12–62 years) and 59 children (age 2–11 years) from PROTECT VIII Kids. In an ongoing extension, up to the cut-off date, plasma PEG levels were measured at baseline, after 4–5 months, 8–9 months, then every 6 months up to 6 years during the extension study, and in children at baseline and after ≥50 exposure days (EDs), followed by measurement every 6 months for up to 4.6 years. PEG levels were determined using size exclusion chromatography and mass spectrometry with a detection limit (lower limit of quantification, LLOQ) of 0.1 mg/L.
Results: Median (range) total time in PROTECT VIII for adults/adolescents (August 2018 cut-off) was 3.9 (0.8–6.0) years and 223 (23–605) EDs; median (range) total time for children in PROTECT VIII Kids (January 2018 cut-off) was 4.0 (1.0–4.6) years and 313 (98–447) EDs. Nearly all PEG measurements were below LLOQ. Borderline positive results during the PROTECT VIII extension were observed in 6 patients (maximum level, 0.119 mg/L); all patients had negative follow-up measurements. These data were compared with published data of other PEGylated products to understand the relationships between overall PEG dose and PEG plasma levels in humans. The simulation of human steady state concentrations, which were based on rat data, resulted in a C_{trough} value of about 0.11 mg/L and a C_{max} value of about 0.15 mg/L. These simulated values were in the range of the highest measured concentrations in human patients from PROTECT VIII.
Summary/Conclusion: It is important to evaluate whether PEG levels at steady state are associated with any possible adverse effects. In patients receiving therapeutic doses of PEGylated biologics, the time to reach steady state was different based on PEG size, and when using different PEG loads/doses there was a clear relationship between PEG load/dose and plasma levels, suggesting that simulations based on preclinical data can accurately predict results in humans.

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Global hemostatic assays (OHP and ETP) have no additional value compared to D-dimer in the diagnosis of acute venous thrombosis

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Background: An alternative biomarker to D-dimer would be of great clinical use in the diagnosis of acute venous thrombosis (VTE). Global hemostatic assays (GHA) have been shown to differ between normal controls and prothrombotic patients and could theoretically be superior to analysis of specific proteins for measurement of hemostatic imbalances that affect several coagulation factors or inhibitors. Overall Hemostasis Potential (OHP) is a global fibrin generation assay and the Endogenous Thrombin Potential (ETP) is an automated global thrombin generation assay. A few studies have evaluated the use of ETP (1,2) and OHP (3) as a complement to D-dimer in the exclusion of VTE. Even though GHA have been studied for several decades, their role has yet to be established as routine clinical markers.
Aims: A prospective clinical evaluation of the parameters of the OHP and ETP assays in Swedish out-patients with suspected VTE.
Methods: Patients with clinically suspected PE or DVT in the lower limb were recruited from the medical emergency department of Karolinska University Hospital. VTE was verified by imaging techniques (ultrasonography, computed tomography or ventilation/perfusion lung scintigraphy, as appropriate). VTE was ruled out by the imaging techniques or absence of VTE in a three month follow up of medical records. OHP, ETP, D-dimer and fibrinogen were analyzed in 64 patients with VTE and 104 without. OHP and fibrinogen was also performed in 42 healthy controls. A database of clinical data was collected from medical records.
Results: The evaluated parameters where OHP, OCP, OFP from the OHP assay and AUC, C_{max}, Tl_{ag} and T_{max} from the ETP assay. Most of the evaluated parameters showed no difference between emergency department patients with VTE and non-VTE. However, the ETP AUC showed a statistically significant difference between the medians between VTE (105%) and non-VTE (100%) (p=0.001, Mann Whitney U test). There was also a statistically significant correlation between VTE and ETP AUC as well as ETP C_{max} (Pearsons' partial correlation), which was independent of adjustment for clinical factors by multivariable analysis. The OHP parameters OCP and OHP as well as fibrinogen were increased in the emergency department patients compared to the healthy controls. In the comparisons of ROC-curves for the different parameters, ETP AUC had the highest area under the ROC-curve (0.65).
Summary/Conclusion: Neither the ETP nor the OHP assay are clinically useful as biomarkers of acute venous thrombosis. The results of the OHP assay indicates that the emergency patients that were used as cases and controls were in a prothrombotic state, seemingly in part due to an increased fibrinogen level. Presumably these assays are sensitive to the acute phase effect and comorbidities that are unavoidable in out-patients at the emergency department. This would indicate that the global hemostatic assays do not contribute further to the existing diagnostic arsenal.

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Obtaining renal function results before empiric rivaroxaban in the diagnostic work-up of deep vein thrombosis

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Background: The Ri-Schedule study (NCT02486445) is a prospective management outcome trial to determine the safety and feasibility of administering rivaroxaban in the pre-diagnostic phase of deep vein thrombosis (DVT) work-up. Rivaroxaban is contraindicated in patients with severe renal failure. We analyzed serum creatinine and GFR using a point-of-care device (i-STAT blood analyzer) for quick assessment of renal function before the patient received rivaroxaban in the Ri-Schedule study.
Aims: Our aim is to evaluate the need for obtaining renal function results prior to the use of rivaroxaban in the pre-diagnostic phase of DVT work-up.
Methods: We included 1653 consecutive outpatients referred to the Emergency Department of Østfold Hospital, Norway, for suspected lower-extremity DVT between 2015 and 2018. Patients with no contraindications were eligible for scheduled diagnostic work-up and received rivaroxaban 15 mg every 12 h until completion of diagnostic work-up, which did not exceed 24 hours. Patients with GFR < 45 mL/min/1.73 m^2 were excluded from scheduled work-up. Patients with a positive D-dimer were scheduled for ultrasonography the next morning, whereas patients with negative D-dimer were not.
Results: Six hundred twenty-four of the 1653 patients (37.7%) received rivaroxaban. Renal function was assessed with the point-of-care device in 388 patients. For the remaining patients, laboratory renal function results were either available, or the physician attending preferred to wait for these due to the patient's clinical condition. Of the 388 patients, 35 patients had GFR < 60 ml/min. Of these, 27 patients had known renal function impairment before the analysis was performed, whereas eight patients had normal creatinine on their last available results in the hospital laboratory system. All eight patients had GFR > 45 mL/min/1.73 m^2 and received rivaroxaban. Of the 1653 patients included in the study, 67 (4.1 %) had GFR < 45 mL/min/1.73 m^2. Twenty-two patients (1.3 %) had GFR <45 mL/min/1.73 m^2 as their only contraindication to receiving rivaroxaban.
Summary/Conclusion: Our results suggest that there is no need to determine renal function prior to the use of up to two tablets of rivaroxaban in the pre-diagnostic phase of DVT in patients with no known impairment of renal function.

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The effect of DOAC-Remove on activated protein C resistance testing in venous thromboembolism patients receiving direct oral anticoagulants

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Background: Direct oral anticoagulants (DOACs) may falsely increase the activated protein C resistance (APC-R) ratio. The DOAC-Remove is a new reagent designed for the *in vitro* use for eliminating interference of DOACs on routine coagulation assays. To our knowledge, there have been no reports on the APC-R testing in VTE patients following the use of DOAC-Remove.
Aims: We sought to investigate whether DOAC-Remove is helpful in determining APC-R in real-life patients treated with DOACs.
Methods: We assessed 74 patients with venous thromboembolism (VTE) aged 47.2 ±14.3 years including 25 patients receiving rivaroxaban (mean blood concentration 112 [59-315] ng/ml), 25 subjects on apixaban (98 [6-138] ng/ml) (both measured by the anti-factor Xa chromogenic assay, Biophen DiXaI; Hyphen Biomed, France) and 24 patients taking dabigatran (52 [36-79.3] ng/ml) (measured using the Hemoclot thrombin inhibitor assay; Hyphen Biomed, France). APC-R was determined using the Russell viper venom time (RVVT)-based functional clotting test (ProC Ac R assay, Siemens, Germany). APC-R and DOAC concentrations were tested at baseline and following treatment with DOAC-Remove (5-Diagnostics, Switzerland) performed according to the manufacturer's instruction. All patients had indication for thrombophilia screening and the presence of factor V Leiden (FVL) mutation was confirmed using Real-Time PCR and Taq Man genetic probes (Life Technologies, USA). The study was conducted in accordance with the Declaration of Helsinki and all patients provided written informed consent.
Results: FVL mutation was found in 20 (27%) patients, including 1 *homozygous man*. The APC-R ratio at baseline was measurable in 43 patients (58.1%), including 20 (80%) on rivaroxaban, 19 (76%) on apixaban and 4 (16.7%) on dabigatran. In VTE patients with measurable APC-R at baseline, the ratio higher than 2.9 (non-APC-R patients) was found in 23 patients (53.5%). In 16 (37.2%; 9 on rivaroxaban and 7 on apixaban) subjects APC-R ratio was below 1.8 suggesting FVL mutation which was genetically confirmed. Moreover, 4 (9.3%) FVL carriers, all on dabigatran, showed either APC-R negative (n=1, ratio >2.9) or equivocal (n=3, ratio 1.8-2.9) results. At baseline there were no correlations between plasma DOACs concentrations and APC-R ratios, regardless of FVL. However, in 11 patients taking rivaroxaban (n=5) or apixaban (n=6), in whom blood was collected 2-4.5 hours since the last dose, APC-R was unmeasurable (drug concentration >126 ng/ml and >128 ng/ml, respectively). DOAC-Remove eliminated rivaroxaban and dabigatran completely (0 ng/ml) and apixaban by 96.2% (median, 3 [range 0-14] ng/ml). After administration of DOAC-Remove all APC-R ratios were measurable (n=54, APC-R ratio >2.9 and n=20, ratio <1.8). In 4 patients treated with dabigatran (drug concentrations: 47, 50, 55 and 106 ng/ml) with false negative APC-R who were carriers of FVL, DOAC-Remove resulted in APC-R ratios <1.8, consistent with the presence of this mutation.
Summary/Conclusion: To our knowledge, this report is the first to show that addition of DOAC-Remove to plasma samples of patients taking DOACs, who are tested for FVL, effectively reduces drug concentrations and allows to diagnose FVL mutation using APC-R in all VTE patients. This work was supported by a grant from the Jagiellonian University Medical College (K/ZDS/007717 to A.U.).

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Clinical outcomes of international normalized ratios measured every 8 weeks versus 12 weeks in venous thromboembolism patients with stable warfarin dose: prospective cohort study

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Background: Assessment of warfarin dosing every 6 to 8 weeks using international normalized ratio (INR) is a routine practice in our Anticoagulation Clinic for stable patients, while extended period up to 12 weeks was not assessed in term of clinical outcomes.
Aims: To compare the clinical outcomes of warfarin dosing every 12 weeks versus every 8 weeks. The primary outcomes were to assess the development of major bleeding and venous thromboembolic events, and the secondary outcomes were the time in therapeutic range (TTR) and number of patients need dose changes.
Methods: Prospective cohort study was performed at Venous Thromboembolism Anticoagulant Clinic, King Abdulaziz Medical City, Riyadh in Saudi Arabia. Patients aged ≥18 years who were prescribed warfarin every 12 weeks or 8 weeks treated for venous thromboembolism or cerebrovascular events were eligible to participate in this study. All stable participants were recruited starting from January 2016 and followed-up for 24 months with last follow-up ending in January 2019.
Results: A total of 140 patents were included in the 8-week group and 110 patients were included in the 12-week group. The mean age of participant in both groups was not different, i.e., 62.8±9.5 years and 61.4±10.2 years in 8-week and 12-week group, respectively. Proportion of male patients was similar between the two groups (91 (65%) versus 73 (66%), p=.834). the median follow-up duration was similar between the two groups (23 months versus 22 months). The proportion of patients who had dose changes was not different between the two groups, 18 (12.9%) versus 16 (14.5%) in 8 and 12-week group, respectively, p=.736. The percentage of TTR was similar in both 8 and 12-week group, 95.4% versus 93.8%, p=.856. Two in the 8-week group (1.4%) versus one (0.9%) of the patients in the 12-week group had a new venous thromboembolism. The proportion of Major bleeding events in both groups was also statistical similar, 6 (4.3%) versus 4 (3.6%) in the 8 and 12-week group, respectively, (p=.803).
Summary/Conclusion: Our study suggests that VTE patients on stable warfarin dose may extend their INR measurement and periodic visit to the VTE Anticoagulation Clinic up to 12 weeks.

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Plasma lipopolysaccharide-binding protein is a biomarker for future deep vein thrombosis in women; results from untargeted discovery and validation studies

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Background: Venous thromboembolism (VTE) is a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE). Although DVT and PE are often considered together, certain VTE risk factors predispose differentially for DVT and PE. The VTE incidence 1-2 cases per 1000 individuals annually and slightly increasing. Reduced VTE incidence may be achieved by targeted prevention actions in high-risk individuals, but established risk factors inadequately predict incident VTE. The identification of plasma biomarkers with predictive potential for VTE is warranted. VTE biomarker identification may be obscured by merging of PE and DVT, and long follow-up in prospective studies may cause regression dilution bias.
Aims:
(i)To use untargeted proteomics data to identify plasma biomarker candidates for future DVT attenuated by an opposing effect size for PE and by regression dilution bias.
(ii)To perform targeted candidate validation.
Methods: A VTE-case-control discovery study (control=86, DVT=55, PE=25) and a nested case-control validation study (control=670, DVT=213, PE=119) were derived from the general population surveyed by the Tromsø study in 1994-95. EDTA-anticoagulated plasma was obtained at study enrolment, and VTE events were registered until 2007. For the discovery study, untargeted mass spectrometry (MS)-based proteomics was used to profile plasma. For the validation study, a two-step targeted proteomics was performed. First, suspension bead array technology (SBA) was used for targeted candidate validation and included the HPA001508 antibody against LPS-binding protein (LBP). Second, absolute LBP plasma levels were quantification by ELISA. Protein measurements were used as continuous variables in logistic regression models to estimate odds ratios (ORs) with 95% confidence intervals (CI). Regression dilution bias was assessed by limiting the maximum follow-up time. The study was approved by the regional research ethics committee, and all subjects gave informed written consent.
Results: LBP was identified as a candidate biomarker for DVT with a disparate effect size for PE in the discovery study. The increased risk of DVT was driven by 11 women with less than three years between blood sampling and DVT (OR: 2.26, 95% CI 1.08-4.73, per SD increase in LBP). In the validation study, SBA data confirmed the association between LBP and DVT in women at limited follow-up, and ELISA was used for absolute quantification. Based on ELISA measurements, the OR for DVT was 2.16 (95% CI 1.56-2.98) per SD increase in LBP level for women with less than 3 years between blood sampling and DVT (n=24). For sensitivity analyses, we restricted the cohort to persons who were not part of the discovery cohort. Here, 13 women got a DVT within three years, giving an OR of 2.01 (95% CI 1.08-3.89) per SD increase in LBP. Adjustment for age, BMI, and C-reactive protein level only marginally attenuated the results. The Pearson correlations between the measurements of LBP across the three techniques ranged from 0.52-0.72.
Summary/Conclusion: LBP was discovered as a predictive biomarker for DVT in women by an untargeted MS-approach. Two complementary techniques, orthogonal to MS, were used for validation in a larger cohort that included validation in a sub-cohort that was independent of the discovery cohort. For women, the OR for DVT was 2.12 (95% CI 1.57-2.93) per SD increase in LBP when the time between blood sampling and DVT was restricted to 3 years. For longer follow-up, we observed strong regression attenuation.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Antiphospholipid antibodies and thrombosis in none BCR-ABL myeloproliferative neoplasms

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Background: None *BCR-ABL* myeloproliferative neoplasia (MPN) are a group of haematologic diseases that develop from the myelopoietic precursor. The main complications of these diseases are thrombosis which pathogeneis is multifactorial. In this study, we examined if the appearance of thrombosis may be affected by antiphospholipid antibodies in MPN patients.
Aims: To evaluate the antiphospholipid antibodies including *lupus* anticoagulant (LA) in relationship with the risk of thrombosis in patients with none *BCR-ABL* MPN´s.
Methods: The study was conducted at the Lithuanian University of Health Sciences. It included 108 patients with *BCR-ABL* negative MPN. Data of previous venous and arterial thrombosis and thrombosis risk factors was collected. There were two different selective and one confirmatory test for the LA determination. Investigations were performed by the automatic analyzer STA-R Max (Diagnostica STAGO). The detection of antibodies against β2-glycoprotein-1 (β2GP1) was performed using a chemiluminescent method. The detection of antibodies against cardiolipin (aCL) was performed by ELISA using commercial Aeskulisa Cardiolipin-GM (AESKU.DIAGNOSTICS, Germany).
Results: LA was present in nine (8.3%) MPN patients. Solid phase aPL were detected in 18 (16.7%) MPN patients. Patients with positive aPL were older than those without aPL (72.50 and 63.0 years, p=0.017). PV patients were more likely to have aPL than ET or PMF patients (66.7%, 27.8% and 5.6%, p=0.6). aPL were more frequently found in MPN patients with thrombosis than in patients without thrombosis (25.4% and 6.1%; p=0.007). In subjects with thrombotic complications IgG aCL were most frequent (13.6%) MPN patients with arterial thrombotic complications were significant more frequently positive for aPL or LA compared to those without arterial thrombosis (38.8% and 11.9%; p=0.001).
Summary/Conclusion: Antiphospholipid antibodies increase the risk of thrombosis development in patients with none *BCR-ABL* MPNs.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

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Why do we not investigate suspected deep vein thrombosis according to current guidelines?

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Background: Deep vein thrombosis (DVT) is a common diagnosis in patients with a swollen lower limb and the suggested method of patients review with pre-test probability including Wells score and D-dimer is well established.
Aims: We have noted that many patients are not investigated in accordance with current recommendations and have therefore conducted a retrospective descriptive directory study to examine the current procedure of DVT-investigation at the emergency department (ED) of Södersjukhuset in Stockholm.
Methods: Our study has been conducted though chart reviews of 1000 patients who presented at the ED and was triaged into the category "swelling or pain in extremity" during 2016. Exclusion-criteria were symptoms from upper extremity, diagnosed DVT, previous visit to the ED for the same symptom, < 18 years of age, locked case record and incorrect triage. Data was gathered on demographic composition, whether Wells score and D-dimer had been documented, symptoms persisting over one week, duration of stay at the ED, conducted imaging and final diagnosis. A comparison between the groups who had and had not been investigated in accordance with current recommendations were done with regard to missed DVT and the usage of imaging.
The study has ethical approval from the regional ethical review board in Stockholm.
Results: Out of 1000 visits to the ED due to swelling or pain in extremity a total of 398 patients were investigated for suspected DVT. Out of 602 patients not investigated no patients were diagnosed with a DVT within 3 months. Out of 398 patients 74 DVT were verified. More than half of the patients review were not conducted according to current guidelines. These investigations did not miss any thromboses but did use significantly more imaging (55,8 % vs 78,4 %, $p < 0,001$). Out of 157 referred patients 28 was not investigated for DVT and 24 were assessed not to have a DVT by Wells score and D-dimer, none of these 52 patients were diagnosed with a DVT within 3 months. The prevalence of DVT was 1,6 times higher in patients not referred than those referred (20,1 % vs 12,6 %).
Summary/Conclusion: Our study is mainly applicable in a major city hospital with high patient load and long waiting hours. The study suggests that if the ED-physician assesses that DVT-investigation is not warranted, the risk of a treatment-demanding DVT is very low. The adherence to current guidelines must be considered poor. The fact that the current recommendations has been unchanged for a long period of time makes lack of knowledge a less likely cause, perhaps a lack of faith in one's own clinical ability or a timesaving approach straight to imaging is the explanation. Regardless does this contribute to unnecessary imaging, increased number of medical contacts and often unnecessary treatment while waiting for imaging.
In Sweden this accounts for about 2500 imaging studies that could have been avoided without loss in patient safety. ED-visits could also be avoided for approximately one out of three referred patients via stricter diagnostics conducted by the referring colleague.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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An unusual case of abdominal pain

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired, life-threatening hematological disease that has fascinated clinicians for more than a century because of its diverse manifestations and intricate pathophysiology. Pathogenesis involves hematopoietic stem cells and, in most cases, somatic mutations in the PIGA gene are present. In patients with PNH a disrupted GPI anchor leads to dysregulated complement activation. In addition to other well-known hematological manifestations (e.g. Coombs negative hemolytic anemia), PNH encompasses a broad spectrum of sign and symptoms, such as abdominal pain, thrombosis, fatigue, red urine and erectile dysfunction, that makes it a challenging diagnosis.
Aims: The association between PNH and Budd-Chiari syndrome is well known, though in most reported cases patients had other pro-thrombotic conditions such as pregnancy or cirrhosis, or thrombosis in the hepatic veins had been heralded by thrombotic events in other districts. Here, we report the case of Budd-Chiari syndrome caused by PNH in a young man with no personal or family history of thrombophilia who presented with abdominal pain and B symptoms.
Methods: A 30-year-old Caucasian male presented to the emergency department for abdominal pain accompanied by vomiting and diarrhea of one week of duration. In the preceding three weeks the patient had had fever, profuse sweating, pruritus and unintentional weight loss of 10 kg. His past medical history was notable for hypertriglyceridaemia, isolated thrombocytopenia of undetermined origin and remote EBV infection. At physical examination abdominal palpation revealed enlargement of liver and spleen. Complete blood count showed slight anemia, mild thrombocytopenia, elevated LDH levels, normal coagulation tests and a negative HIV serology. An abdominal CT scan with contrast agent demonstrated hepatosplenomegaly, no deep lymphadenopathies and the absence of contrast agent diffusion in the hepatic veins. A color doppler examination confirmed the diagnosis of hepatic vein thrombosis (Budd-Chiari syndrome); thus, anticoagulation with enoxaparin was started. A thrombophilia screening including LAC, Factor II, Factor VIII, Protein S and C, Factor V Leiden turned negative, as negative were the results of antinuclear antibody testing and the search for JAK2 mutations. Further exams showed a Coombs negative hemolytic anemia, while flow cytometry revealed a deficient expression of CD55 and CD59, consistent with PNH.
Results: Treatment with eculizumab, a humanized monoclonal antibody targeting the complement component C5 and preventing its cleavage by convertases, was started. At the last follow-up visit the patient reported that anemia and fatigue had improved, with no recurrence of abdominal pain or B-symptoms.
Summary/Conclusion: In the present case, an unspecific diffuse abdominal pain was investigated with a multidisciplinary approach. An accurate differential diagnosis ruled out leukemia, solid tumors, infectious diseases. Following a hematological consult, further investigations were planned. Even if the patient did not have any evidence of hemoglobinuria, the presence of thrombosis, weight loss, abdominal pain, fatigue and fever, were suggestive for an hematological disease. This case highlights the importance for clinicians of suspecting PNH, including this disease in the differential diagnosis when confronted with an unprovoked thrombosis in an unusual site, in a young man without personal or family history of thrombophilia.

ECTH 2019
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The effects of Edoxaban on routine coagulation tests

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Background: Edoxaban is a direct oral factor Xa inhibitor licenced in some regions for a number of indications in relation to thrombosis treatment and prophylaxis. Due to fixed dosage regimes and predictable pharmacokinetics, routine laboratory monitoring is not required . The British Committee for Standards in Haematology states that a specifically calibrated chromogenic anti-Xa assay should be used if measurement is required and recommends against the use of PT and APTT for monitoring. It does however, recommend that laboratories should know the sensitivity of their PT and APTT assays to the presence of direct anti-FXa inhibitors and offer advice on the interpretation of their assays in the presence of DOACs.
Aims: This study aims to compare sensitivity of multiple prothombin time (PT) and activated partial thromboplastin time (APTT) reagents across two platforms to the presence of edoxaban
Methods: 10 normal volunteer samples were spiked with Edoxaban (provided by Daiichi Sankyo UK Ltd.) to provide 8 concentrations including potential trough, peak and accumulation levels (0-1500ng/ml). The PT was measured using Innovin and thromboreLS on the Ssmex CS5100 and PT-Fibrinogen HS Plus and RecombiPlasTin 2G on the ACL TOP700. The APTT was measured using ActinFS and SynthaSiL on the Sysmex CS5100 and APTT-SP and SynthaSil on the ACL TOP700.
Results: At 250ng/ml edoxaban (close to the average peak level for 60mg daily dosing) there is a significant difference between results with different PT reagents, ranging from a 1.2x increase over baseline clotting time with Innovin, the least sensitive reagent, up to 1.9x increase with the most sensitive reagent PT Fib HS+.Less variation is seen between APTT reagents across both platforms at 250ng/ml with SynthaSil on the CS5100 being the least sensitive at 1.3x increase in clotting time from baseline and APTT-SP on the ACL top being the most sensitive at 1.4x increase at in clotting time from baseline. However, all the tested PT and APTT reagents gave clotting times exceeding our laboratories reference range at 125ng/ml .
Summary/Conclusion: This data highlights the need for laboratories to be aware of the impact of edoxaban on their clotting screen reagents and the importance of relevant clinical details accompanying clotting screen requests.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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The role of red blood cells surface proteins in hemostasis and thrombosis

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Background: Venous thromboembolism is the third most common vascular disease after myocardial infarction and stroke. Endothelial cells, platelets and coagulation factors are all active players in hemostasis whilst red blood cells (RBCs) are considered passively trapped in the growing fibrin network. However, most circulating blood cells are RBCs and evolving data suggest a more intricate role for RBCs when incorporated into clots. Factor XIII (FXIII)-deficient humans suffer from bleeding tendency and impaired wound healing. FXIII-/- mice have less dense fibrin networks with fewer RBCs inside the thrombi, producing smaller and paler clots compared to their littermate counterparts with normal FXIII status. FXIII is a transglutaminase in plasma and its active form, FXIIIa, catalyzes formation of ε-N(γ-glutamyl)-lysyl bonds, first making crosslinks between fibrin γ-chains and at later stages of clot formation, polymerization of its α-chains and this stabilizes the clot which becomes more resistant to fibrinolysis. Understanding the molecular mechanisms underlying thrombus formation in general, and the contribution of RBCs in this process in particular, may be of importance for development of alternative therapeutic approaches to counter thromboembolic disease.
Aims: To investigate if RBC surface proteins are attached by FXIIIa-dependent crosslinking to protein partners in the fibrin mesh.
Methods: In vitro generated clots, prepared in tubes with a mixture of human thrombin 1U/ml (SIGMA-Aldrich) and FXIII 13E/ml (Cluvot, CSL Behringer) in a buffer containing 1.25mM Tris-HCl, 2.5 mM CaCl2 and PBS. An 0.08% suspension of RBC was added and the mixture incubated 15 min at 37°C to activate FXIII. In some experiments 5mM N-(biotinyl)Cadaverine (Zedira) was added for competitive binding to lysine moieties. Finally, clots formed following addition of 3U/ml fibrinogen during slow-movement rotation. Hemoglobin from trapped RBCs was removed by excessive washing in cold RBC lysis buffer, resulting in white membrane "ghosts". Correlative light electron microscopy (CLEM, Delphi) analysis combined EM images with fluorescence images of clots incubated with primary antibody anti-N epsilon gamma glutamyl lysine antibody [81D4] and Alexa fluor 488 (both from Abcam) using overlay precision. Mass spectrometry (MS) was performed to identify cross-linked proteins. Clots were mechanically disrupted by sonication and boiling to allow protein separation on SDS-PAGE gels (TGX, Biorad). Preparation of protein-containing gel slices was performed including digestion with trypsin or chymotrypsin prior to analysis on a Fusion Orbitrap (Thermo Fisher).
Results: Using a reductionistic approach, we made clots in vitro by mixing fibrinogen, thrombin and FXIII in the presence of calcium ions and RBCs. Fluorescently labelled ε-N(γ-glutamyl)-lysyl bonds were found juxtaposed with RBCs in CLEM analysis. By MS, we identified specific lysine positions on the fibrin(ogen) α chain, which engaged N-(biotinyl)Cadaverine to bind. By combining the exact weight of these fibrin fragments with potential binding partners in our database of erythroid-specific peptides, we have started to identify candidate RBC proteins targets.
Summary/Conclusion: We are currently working with identification of erythro-specific proteins which may potentially facilitate a more active incorporation of RBCs in clots made in vitro. Clearly, these preliminary data require further investigation but if substantiated could establish a more active role for RBCs in hemostasis

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The FibWave: a new rapid and relevant tool for the evaluation of anticoagulant drugs

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Background: Anticoagulants like DOACs, are known to impact several coagulation assays and parameters. Although a lot of assays are available for the assessment of anticoagulant drugs, many limitations have been highlighted, like the lack of sensitivity of routine assays or the need of specific calibrators and controls for specific ones. More global coagulation assays like the thrombin generation test (TGT) showed good sensitivity towards anticoagulants considering also the inter-individual coagulation potential. However, TGT assesses thrombin which is not the final endpoint of the coagulation process and the use of a slow-reacting fluorogenic substrate distances the test from the physiological conditions. The FibWave® (FW) is a new assay based on the evaluation of the kinetic of the clot formation which circumvents the limitation of the TGT without altering its capacity to assess the coagulation potential at the individual level.
Aims: To assess the impact of DOACs on the kinetic of fibrin formation using the FibWave method and compare the results with the calibrated automated thrombogram (CAT).
Methods: DOACs were spiked at final concentrations ranging from 0 to 500ng/mL in normal pool plasma. The clotting conditions were the same for FibWave and CAT. Briefly, the coagulation was initiated by the intrinsic or extrinsic pathway using either ellagic acid plus phospholipid or relipidated tissue factor as activator, respectively. Coagulation was then triggered by the addition calcium chloride or FluCa for FibWave and CAT, respectively.
Results: All CAT parameters were significantly impacted by anticoagulant treatments. On FibWave, the FW-delta parameter (i.e. the amount of fibrin generated) was impacted by dabigatran dose-dependently. A curvilinear concentration-dependent correlation was observed for FW-time-to-peak (i.e. time to maximal fibrin formation velocity) for all DOACs. Dabigatran showed a dose-dependent curvilinear decrease for FW-max1 (i.e. maximal fibrin formation velocity) and FW-max2 (i.e. maximal fibrin formation acceleration) parameters.
Summary/Conclusion: The FibWave is as a new global, rapid and relevant pharmacological tool for the assessment of DOACs. Further works are needed with other anticoagulant agents as well as in other clinical settings.

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Incidence of prothrombotic risk factors in perinatal stroke onset

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Background: Perinatal stroke is heterogeneous disorder occurring between 22 weeks of a pregnancy and the first month of postnatal life. It refers to the blockage (ischemic stroke-IS) or breakage (hemorrhagic stroke-HS) of a brain blood vessels. IS is 17 times more common in perinatal period compared to later childhood (incidence of 2.5/100000 per year), while the incidence of HS is 0.17/1000 live-births. Outcome of perinatal stroke is poor with many lifetime sequels. Significance of prothrombotic risk factors in perinatal stroke is still the subject of discussion but the tendency of a body to form clots can theoretically be the predisposing factor for childhood stroke onset.
Aims: Aim of this study was to investigate the incidence of prothrombotic risk factors and their association with perinatal IS and HS.
Methods: Between 2011. and 2019. year among 60 pediatric patients with stroke, 17 neonates (1-16 days of age) were identified: 7 with IS 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 on the DNA samples extracted from peripheral blood lymphocytes. Protein C, protein S, antithrombin, plasminogen, factor VIII and lupus anticoagulant antibodies (LA) were measured by automated hemostasis analyzers. Previously published data from healthy control group from Serbia were used for comparison with our results.
Results: In HS group we identified three (30%) heterozygous carriers for FV Leiden (R506Q), one (10%) heterozygous carrier for prothrombin 20210G>A mutation, three heterozygous carriers (30%) and two homozygous (20%) for MTHFR 677C>T variant. Two patients (20%) had elevated level of factor VIII and one (10%) had elevated plasminogen levels. All HS patients had at least 1 analyzed risk factor while two patients (20%) had 2 risk factors.
In IS group, four patients (57.14%) were heterozygous carriers for MTHFR 677C>T variant. One of them (14.29%) had elevated level of factor VIII. 4/7 (57.14%) IS patients had at least one prothrombotic risk factor, while one patient (14.29%) had two risk factors. In three IS patients none of the analyzed factors were identified.
Summary/Conclusion: Results of this study showed that all patients with HS and 57.14% IS patients had at least one prothrombotic risk factor. Variants FV Leiden (R506Q) and prothrombin 20210G>A had higher prevalence in a group of patients with HS. In group with IS 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 tests in the clinical setting of pediatric stroke.

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Evaluation of the new QXpert Hemostasis Analyzer for routine coagulation tests

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Background: The QXpert (Grifols, Barcelona, Spain) is a fully automated random-access multiparameter hemostasis analyzer that is equipped with a cap-piercing system. It is designed to perform coagulation, chromogenic and immunologic assays.
Aims: We evaluated its performances for routine clotting assays i.e. prothrombin time (PT) activated partial thromboplastin time (aPTT), and fibrinogen using liquid ready-to-use reagents from Grifols.
Methods: PT (in sec. and INR) was measured using the DG-PT RecombiLIQ reagent, a liquid recombinant human thromboplastin. APTT (in sec.) was measured using the DG-APTT Synth reagent that contains ellagic acid and synthetic phospholipids. Fibrinogen (in g/L) was measured according to Clauss using the DG-FIB L Human reagent, a liquid human thrombin solution. Normal and abnormal lyophilized plasma samples (DG-C1 and DG-C2, Grifols) were evaluated to assess the preliminary within-run and total precision for the three evaluated parameters. In addition, we evaluated 200 plasma samples obtained from the laboratory routine workload, including patients treated with either vitamin K antagonist (n=95) or unfractionated heparin (UFH, n=20) as well as patients with liver failure (n=10), disseminated intravascular coagulation (n=6), single factor deficiency (n=9), lupus anticoagulant (n=7) and inflammatory state (n=11), and test results were compared to those obtained on the ACL TOP 700 analyzer (Instrumentation Laboratory, IL) using IL reagents.
Results: Preliminary and total precisions, evaluated as the coefficients of variation (CVs), were below 5.4% for all three tested parameters both in the normal and the pathological ranges. No carryover was detected in alternating aPTT measurement of heparinized (>1.0 IU/mL UFH) and normal plasma samples. PT, aPTT and fibrinogen test results obtained on the QXpert analyzer were well correlated with those obtained on the ACL TOP 700 analyzer using IL reagents, with r in the range from 0.857 to 0.973. Even though analytical comparison of test results obtained on the two analyzers were significantly different, concordance as whether test results obtained on the two analyzers were normal or not, or within the therapeutic range or not (for INR), was excellent or good for the three tested parameters.
Summary/Conclusion: These results suggest that measurement of PT, aPTT and fibrinogen can be performed on the QXpert Hemostasis Analyzer using the evaluated liquid reagents with satisfactory precision.

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Course of fatigue symptoms following initiation of rivaroxaban for the treatment of venous thromboembolism

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Background: Rivaroxaban, a direct oral Factor Xa inhibitor, was the first new oral anticoagulant to be approved for treatment of venous thromboembolism (VTE). Clinical trials have shown that rivaroxaban is non-inferior to conventional anticoagulation for VTE in efficacy and safety. Increased level of fatigue after the initiation of rivaroxaban has been observed in clinical practice, but data on this potential side-effect is currently lacking.
Aims: The study aimed at evaluating fatigue prospectively in consecutive patients diagnosed with deep vein thrombosis (DVT) or pulmonary embolism (PE) treated with rivaroxaban or other anticoagulant agents using the Chalder Fatigue Score (CFS) questionnaire.
Methods: Patients were recruited after a diagnosis of VTE. Fatigue scores assessed by the CFS were obtained at baseline, after three weeks of treatment, and one month after the discontinuation of treatment, or at 6 months if treatment was continued beyond this time. Patients were split into two groups; one receiving rivaroxaban, and the other receiving either apixaban, enoxaparin, warfarin or dibigatran. Data on the fatigue scores were analysed by a linear mixed model using time and type of treatment as explanatory variables. An interaction term allowed for different trends over time for the two types of treatment. The model was reduced by the likelihood ratio test and checked by QQ- and residual plots.
Results: Data were collected from 126 patients. Mean age was 59 years; 77 males. Fifty-seven (45%) were diagnosed with DVT, 48 (38%) with PE and 21 (17%) had both DVT and PE. Eighty-eight patients (70%) were treated with rivaroxaban, and 38 patients were treated with other anticoagulants (30%). Our analyses found a significant decrease in the mean fatigue score from baseline to the last measurement for both groups indicating an overall decline in the levels of fatigue over time. No difference was detected between the rivaroxaban and the other-anticoagulants group after 3 weeks or after 4-6 month of treatment. However, increased levels of fatigue were observed in some patients in both groups.
Summary/Conclusion: An overall decline in total fatigue was observed after 4-6 months as compared to baseline, both in patients receiving rivaroxaban as well as in patients receiving other anticoagulants. Some patients treated with rivaroxaban did experience a substantial increase in fatigue scores after 3 weeks of treatment, which is in agreement with observations from clinical practice, but this increase was also observed in patients receiving other anticoagulants. In conclusion, we found no indication that rivaroxaban increases fatigue compared to other anticoagulants.

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Impact of duration of anticoagulant therapy on survival and bleeding or thrombotic complications in patients with cancer-associated thrombosis: data from a retrospective cohort study

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Background: Cancer-associated venous thromboembolism (VTE) represents a major cause of mortality and morbidity among cancer patients. The optimal duration and doses of anticoagulant therapy are not yet been established, since thombosis in cancer is associated with both high thrombotic recurrence rates and high haemorragic risk. Few studies have assessed haemorragic and thrombotic outcomes beyond the first six months of therapy since now.
Aims: The aim of our study was to determine differences in terms of overall survival, thrombotic recurrence and haemorragic events between patients with cancer associated thrombosis undergoing six versus more than six months of anticoagulant therapy.
Methods: in our retrospective study 360 patients with active cancer and associated venous thomboembolism were enrolled from Cardiovascular Medicine database consecutively from August 2016 to August 2018. The primary composite outcome was defined by thrombotic recurrences, major bleedings and all-cause mortality among patients who stopped therapy within the first six month of follow-up and patients who continued therapy for more than a six-month period.
Results: A total of 360 patients (median age 66 ± 13 years; 192 males) with cancer and VTE were enrolled. The most common cancer types included genitourinary cancer (17.2%), breast cancer (13.3%), colorectal cancer (12.2%), lung cancer (11.4%) and lymphomas (11.7%). Within the first six month of therapy 67 patients died and 8 patients were lost to follow-up. Of the remaining 285 patients, 139 stopped therapy within the first six months and 146 patients continued therapy beyond six months (for a maximum of 24 months of follow-up). Cancer represented the first cause of death (78% of patients), followed by sepsis (10%) and pulmonary embolism (5%). Patients who stopped therapy within the sixth month of follow-up (due to thrombocytopenia or other causes) experienced more thrombotic recurrences. Moreover, all fatal thrombotic recurrences occured during the first six months of follow-up, irrespective of type and lenght of the anticoagulant therapy. Overall survival, recurrent thrombotic events and bleeding rates didn't significantly differ among patients who stopped and patients who continued anticoagulant therapy after the first six months of follow-up.
Summary/Conclusion: Among newly diagnosed cancer patients with VTE, anticoagulant therapy lasting more than six months was not associated with a clear clinical benefit or reduction of mortality. Progression of neoplastic disease represented the most important cause of death in patients with cancer-associated thomboembolism. The management of the first six months of follow-up from the diagnosis of venous thromboembolism in cancer patients remains crucial beacause of high mortality and high risk of fatal thrombotic recurrences even in potentially curable cancer types.

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Validation of a model with coaguation-related markers for the diagnosis of periprosthetic joint infection

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Background: Bacterial infection activates coagulation through immunothrombosis. Neutrophils are the first line of defense against bacterial infection, but its mechanism of action in periprosthetic joint infections (PJI) is unknown. Possibly it is through the release of neutrophil extracellular traps (NETs), since they have been found in the bacterial biofilm of PJIs. Currently there are no diagnostic tests of PJI with sensitivity / specificity of 100% and often the diagnosis of PJI is uncertain due to lack of compliance with some major or minor criteria, hindering the management of these patients. In a previous study we obtained a model to estimate the pre-surgical risk of PJI using markers of NETs and parameters of the thrombin generation test (TGT).
Aims: The aim of the present study was to validate the diagnostic capacity of PJI of our model, which includes markers related to coagulation.
Methods: We obtained citrated plasma, in the preoperative period of prosthetic surgery, from 32 patients prospectively recruited. The PJI was diagnosed in 9 of these patients, following the analytical and microbiological criteria of the International Consensus on Musculoskeletal Infections (ICMI). The diagnosis of 9 patients was uncertain, which is relatively frequent. We validate our predictive model of PJI that includes markers of NETs (DNA and calprotectin) and TGT variables (lagtime and starttail), with a multivariable logistic regression model with R (v3.5.0).
Results: All parameters of NETs and TGT were significantly increased in patients with PJI and with uncertain diagnosis compared with patients undergoing prosthetic surgery due to non-septic causes. The area under the ROC curve (AUC) of our predictive model was 0.79 (P = 0.019, 95% CI [0.58, 1]). With our model, we calculated the presurgical probability of PJI for each patient and found that this probability was higher in patients with a confirmed diagnosis of PJI than without PJI (P = 0.009).
Summary/Conclusion: The markers of NETs, DNA and calprotectin, and TGT, lagtime and starttail, seem to have diagnostic utility of PJI before surgery. Our diagnostic model could reinforce the clinical criteria currently available in order to reduce the number of uncertain diagnoses and thus be able to make an early and effective diagnosis and treatment to minimize side effects of the PJI such as tissue damage, bone degradation and replacement surgery. ISCIII-FEDER (PI14/00079, PI14/00512, FI14/00269, CPII15/00002, PI17/00495), Generalitat Valenciana (ACIF/2017/138) y Sociedad Española de Trombosis y Hemostasia.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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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.
Aims: Analyze laboratory parameters before and after plasma exchange in patients with code thrombotic thrombocytopenic purpura (TTP) and examine the effect derived therapeutic plasma exchange (TIP) on the clinical condition of the patient.
Methods: We studied a retrospective analysis of the five-year period at the Department of Clinical Transfusion Institute for Blood Transfusion 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, 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-a 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 TIP in 80% of patients had complete remission, while in 3 (6.66%) patients occurred exacerbation and repeated TIP.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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D-dimer as a stand-alone test to rule out deep vein thrombosis

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Background: Current guidelines recommend the use of clinical decision rules, such as Wells score, in combination with D-dimer to assess the need for objective imaging to rule out deep vein thrombosis (DVT). However, the clinical decision rule has limitations, and use of D-dimer as a stand-alone test has been suggested.
Aims: To assess the safety and efficiency of D-dimer as a stand-alone test to rule out DVT in outpatients referred with suspected DVT.
Methods: We collected data from consecutive outpatients referred with suspected DVT in 2008-2018. D-dimer levels were analyzed using STA® Liatest® D-Di assay. D-dimer as a stand-alone test was theoretically applied in retrospect, and the number of misclassified events were estimated as if such an approach had been initially used. All patients were followed for three months.
Results: Of 1765 included patients, 293 (16.6%) were diagnosed with DVT. A total of 491 patients (27.8%) had a negative D-dimer (<500 ng/mL). Of these, nine were diagnosed with DVT, yielding a failure rate for D-dimer as a stand-alone test of 1.8% (95% CI 0.8%>3.5%). The majority of the misdiagnosed patients had a distal DVT. In analyses restricted to proximal DVTs, the failure rate was 0.6% (95% CI 0.1%>1.8%). D-dimer as a stand-alone approach reduced the proportion of required ultrasounds from 81.8% to 72.2%.
Summary/Conclusion: D-dimer as a stand-alone test may be safe for excluding proximal DVT among outpatients. The strategy has the potential to simplify and increase the efficiency of the diagnostic work-up in patients with suspected DVT.

ECTH 2019
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Less is more: Reduced anticoagulation therapy in patients after mechanical aortic On-X valve replacement in real life. Experience of a Spanish single centre

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Background: The On-X valve is a bileaflet mechanical heart valve designed to function with less anticoagulation than previously recommended. Recently, Puskas *et al* reported the final results of the PROACT (Prospective Randomized On-X Anticoagulation Clinical Trial). In this study, patients with one or more risk factors for thromboembolic complications randomized, after mechanical aortic valve replacement (MAVR) to low-dose warfarin plus aspirin experienced significantly fewer bleeding events than those treated with standard warfarin plus aspirin, while the incidence rates of thrombotic and all-cause mortality were not different.
Aims: To evaluate the incidence of thromboembolic and bleeding complications among patients after MAVR in our centre in "real life" conditions.
Methods: We conducted a retrospective analysis of patients undergoing to MAVR with the On-X valve from March 2014 through March 2019 in our centre in Toledo (Spain). All of them received acenocoumarol (target INR 2.0 to 3.0 for the first 3 post-operative months, and then 1.5 to 2.0) and 100 mg aspirin daily. Those without one of the following conditions were considered in the low-risk group: chronic atrial fibrillation, left ventricular ejection fraction <30%, left atrial dimension >50 mm, spontaneous echocardiographic contrast in the left atrium, significant vascular disease, history of neurological events within 1 year, hypercoagulability, left or right ventricular aneurysm, and women receiving estrogen replacement therapy. We collected patients characteristics, risk stratification, anticoagulation data, and thrombotic and bleeding events.
Results: A total of 38 patients (25 men and 13 women) were included. The mean age was 63 years (34-83). 14 patients (36.8%) had a high risk profile. Three patients (7.9%) had atrial fibrillation at baseline. The median follow-up time was 30 months (8-58). 27 patients (71.1%) had a TRT Rosendaal <65%. Only 15.1% of the INR determinations were less than 1.5. The mean INR was 1.92. During follow-up, 5 patients suffered hemorrhagic events: 5 minor bleeding (4 patients) and 1 major bleeding. One patient had a thrombus in aortic tube and another one, mild chronic hemolysis. There were neither valvular thrombotic events nor deaths.
Summary/Conclusion: In our experience, reduced anticoagulation (INR 1.5-2) plus low doses of antiaggregation in patients undergoing aortic valve replacement with the On-X valve prosthesis has a low rate of bleeding and thrombotic complications, confirming in real life the outcomes of the PROACT study. More studies, with more patients and longer follow-up, are needed to confirm these results.

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Plasma level of complement factor B is associated with risk of future venous thromboembolism

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Background: Deep vein thrombosis (DVT) and pulmonary embolism (PE) are collectively referred to as venous thromboembolism (VTE). The complement system plays a central role in intravascular immunity and immunothrombosis, and growing evidence support a key role for the complement system in the multifactorial etiology of VTE. A large proportion of complement activation results from amplification by the alternative pathway (AP). Complement factor B (FB) is the key catalytic enzyme and substrate in the AP. It is not known whether plasma levels of FB are associated with future risk of incident VTE.
Aims: To investigate the association between blood plasma levels of FB and future risk of VTE.
Methods: A nested case-control study consisting of 332 VTE cases and 673 age and sex-matched controls was derived from the population-based Tromsø study. Blood samples were collected at inclusion in 1994-95. Relative levels of FB in plasma from EDTA-anticoagulated blood were determined by suspension bead array using the anti-FB antibody HPA001817. Mean fluorescence intensities were recorded and normalized for inter plate variations and used as a continuous or discretized categorical variable based on quartiles of the control population. Logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CI). The study was approved by the regional research ethics committee, and all subjects gave informed written consent.
Results: The VTE risk increased linearly with plasma levels of FB (OR 1.23, 95% CI 1.08-1.42, per 1 SD increase in FB). ORs for both unprovoked (OR 1.30, 95% CI 1.07-1.59) and provoked (OR 1.19, 95% CI 1.02-1.40) VTE were elevated. The risk of DVT increased across quartiles of plasma FB, and subjects with plasma FB levels in the highest quartile had an OR for DVT of 1.70 (95% CI 1.10-2.65) compared to those in the lowest quartile. The DVT risk was mainly driven by unprovoked events where subjects in the highest quartile had an OR for unprovoked DVT of 2.32 (95% CI 1.19-4.75). The OR for PE was elevated 1.4 to 1.9-fold in subjects with FB levels above the reference (lowest quartile) with similar estimates for provoked and unprovoked events.
Summary/Conclusion: High plasma levels of FB are associated with future risk of VTE. The increased OR of DVT was mainly driven by unprovoked events and displayed a dose-response relationship. Plasma FB levels above the lowest quartile showed a moderately increased OR for PE. Our findings support the concept that the potential for complement amplification via the AP is important for the risk of VTE.

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Plasma Von Willebrand Factor (VWF) and ADAMTS13 profiles in patients with Antiphospholipid syndrome (APS) and Systemic lupus erythematosus (SLE)

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Background: Thromboembolism is a major cause of morbidity in both antiphospholipid syndrome (APS) and systemic lupus erythematosus (SLE) patients; however, the precise thrombogenic mechanisms are not fully elucidated. Von Willebrand Factor (VWF), a large multimeric glycoprotein crucial for haemostasis and thrombus formation, is physiologically regulated through proteolytic cleavage by the plasma metalloprotease ADAMTS13, with the VWF:ADAMTS13 axis playing a critical role in the development of thrombosis. Whilst the VWF:ADAMTS13 axis has been proposed to be dysregulated in both patients with APS and SLE, it remains to be established whether there are any differences in the mechanisms between APS and SLE leading to this dysfunction.
Aims: This ongoing cross-sectional study seeks to establish plasma VWF and ADAMTS13 antigen levels in patients with APS and patients with SLE, identify possible differences between the two conditions, and assess for associations between antigen levels, antiphospholipid antibody status, and thrombotic history. A further aim is to determine the presence of anti-ADAMTS13 autoantibodies in both cohorts and establish associations with ADAMTS13 activity and clinical phenotype.
Methods: Plasma VWF and ADAMTS13 antigen levels were measured by ELISA in 93 APS, 48 SLE (antiphospholipid antibody negative), 28 thrombotic controls (TC) and 66 healthy controls (HC). Samples with low ADAMTS13 antigen (< 100 ng/mL) were further analysed for anti-ADAMTS13 autoantibodies using an ELISA based method.
Results: VWF antigen levels were significantly higher in APS (median 1.3 IU/ml; 95% CI 1.1-1.5; $p<0.0001$), SLE (1.8 IU/ml; 1.6-2.0; $p<0.0001$) and TC (1.4 IU/ml; CI 1.1-1.8; $p<0.0001$) compared to HC (mean: 0.8 IU/ml; 0.7-1.0). VWF levels were significantly higher in APS compared to SLE patients ($p = 0.001$). ADAMTS13 antigen levels were significantly lower in APS (138.9; 123.7-157.1; $p<0.0001$) and SLE (175.3; 154.2-205.3; $p=0.01$) patients compared to HC (234.8; 197.7-283.3), with ADAMTS13 levels significantly lower in APS compared to SLE patients ($p=0.0002$). The ADAMTS13:VWF ratio in APS (11.2; 5.9-32.9) and SLE (10.0; 8.4-11.9) patients was lower compared to TC (11.7; 5.9-32.9; $p<0.0001$) and HC (31.4; 27.4-38.3; $p<0.0001$). Low ADAMTS13 antigen levels (< 100 ng/mL) were detected in 32% (32/93) of APS and only 2% (1/48) of SLE patients. IgM anti-ADAMST13 antibodies were detected in 58% (11/19) of APS patients with < 100 ng/mL ADAMTS13 antigen levels that have been tested to date.
Summary/Conclusion: In summary, both APS and SLE patients exhibited elevated VWF and reduced ADAMTS13 antigen levels, more marked in the APS group, with anti-ADAMTS13 autoantibodies detected in a subset of APS patients. These findings suggest dysregulation of the VWF:ADAMTS13 axis. The mechanisms underlying this dysregulation appear to differ between APS and SLE patients, suggesting possible differential underlying pathogenic mechanisms between the two conditions.

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Even if new scores enhance D-dimer testing relevance, D-dimer testing will still be limited if not combined with an appropriate clinical score

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Background: Plasma D-Dimer levels (D-Di) can be used as a reliable biomarker for coagulation activation and fibrinolysis. D-Di are determined to rule out venous thromboembolism events (VTE) in patients with low-medium clinical probability, and in many others clinicals situations with more or less validated score. However, for daily practice several studies have pointed out that D-Di testing is often misused. Although it is well known that D-Di testing cannot be used as a stand-alone test and should be combined with a clinical score to improve both the diagnostic specificity and accuracy, in practice clinical scores are poorly used.
Aims: Focusing on the 2923 D-Di tests performed at the hospital Henri Mondor during 2018, the aim of the study was to evaluate the use of D-Di testing according to clinical scores published in the literature.
Methods: We established a list of patients that were tested for D-Di from January 1st, 2018 till December 31st, 2018. For every patient the following characteristics were determined: the hospital department, age, anticoagulation treatment status, D-Di levels, clinical status leading to D-Di testing, such as Deep Venous Thrombosis (DVT), Pulmonary Embolism (PE), Disseminated Intravascular coagulation (DIC)... Determining these characteristics allowed us to calculate an retrospective clinical probability using Wells score, Geneva revised score ADJUST PE score, IMPROVEDD score, HERDOO2 score, DASH score, DIC evaluation, and ADD-RS score. In false positive patients, 30 other conditions that increase D-Di were determined 6 months before and after D-Di testing.
Results: 51% of the patients were female (mean age 55.7 years) and 49% were male (mean age 56.4 years) . Less than 1% of the patients recieved anticoagulants. Values of D-Di under 500 ng/mL, considered as low or negative, were found in 1276 patients. 537 patients had D-Di between 500 and 1000 ng/mL, 689 patients between 1000 and 5000 ng/mL and 139 patients over 5000 ng/mL. 99% of the D-Di were performed to exclude venous thrombo-embolism (VTE). 87% of the tests were executed at the emergency department. Here, 82.6% of the D-Di were performed because of a suspicion for PE, 8.7% for DVT, 2% for both, and 5.6% for vaso-occlusive crisis in sickle cells patients as the hospital is a reference center. When PE was suspected, diagnosis for PE was 3%. 67% of the cases where DVT+PE was suspected were diagnosed for DVT+PE. 12% of the cases were diagnosed for DVT when DVT was suspected. Of note in the DVT+PE, D-Di should not be tested following current guidelines. For DVT diagnosis, the age-adjusted rule was applied when patients were not already validated for the disease. Moreover, D-Di were used as a stand-alone rule out exam and compressive ultrasonography was not systematically prescribed for diagnosis. For PE diagnosis, since D-Di were erroneously prescribed, patients were rarely associated with VTE. If for emergency patients D-Di are tested to exclude VTE, the particularity in patients is that D-Di should be used to consolidate the hypothesis of VTE. In hospitalized patients, D-Di levels were also determined for other reason than VTE like DIC and sometimes in nonvalidated conditions. At least in 15% of false positive patient with inexplicated high D-Di level developped a cancer within the year.
Summary/Conclusion: The current study demonstrates the limit of using D-Di levels in real practice. D-dimer testing should be combined with a relevant clinical score, adapted to the clinical situation.

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Plasma circulating free DNA levels and DNase I activity are associated to an increased risk of venous thromboembolism

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Background: Plasma circulating free DNA (cfDNA) comes from cellular death or *neutrophil extracellular traps* (NETs). Plasma cfDNA levels are elevated in venous thromboembolism (VTE) patients and increase VTE risk.
Aims: Our aim was to compare plasma cfDNA levels and DNase I activity (cfDNA inhibitor) in VTE patients and healthy controls.
Methods: One hundred and seven VTE patients and 53 healthy volunteers (controls) were recruited. Plasma samples were obtained between 6 and 24 month after the thrombotic event in VTE patients and at recruitment in controls. In every plasma sample, cfDNA was quantified with PicoGreen (Life Technologies) and DNAse I activity was measured with DNase I assay Kit (Abcam) after optimization. Statistical analysis was performed with R software (v 3.5.0).
Results: cfDNA levels were slightly higher in VTE patients (1771.15 ± 223.81 ng/ml) than in controls (1724.69 ± 226.55 ng/ml) (P=0.15). DNase I activity was significantly increased in VTE patients (6.17 ± 1.61 µU/ml) vs controls (5.5 ± 1.25 µU/ml) (P<0.001). In a bayesian regression logistic model an interaction between cfDNA levels and DNase I activity in plasma was detected (OR=1.32, IC 95% [0.96, 1.97]). Moreover a synergistic effect was observed and simultaneous increasing of both, cfDNA levels and DNAseI activity, were associated with higher VTE risk. Thus, patients and controls were distributed in tertiles according to DNase activity and VTE risk was calculated according cfDNA levels. We observed than VTE risk in subjects in the lowest tertile of DNase I activity (4.42 µU/ml) ranged from 50 to 62%, in the medium tertile (5.95 µU/ml) ranged from 65to 75%, and in the highest tertile (7.48 µU/ml) ranged from 76 to 95%.
Summary/Conclusion: Elevated levels of cfDNA and DNase I activity are associated with an increased risk of VTE with a synergistic effect. Thus, subjects with elevated levels of both parameters show the highest risk of VTE. Elevated DNase I activity in plasma does not seem to be a VTE risk factor by itself, however, we speculate that DNase I activity is elevated in plasma as a response to high level of cfDNA which is known to be a VTE risk factor.
ISCIII-FEDER (PI14/00079, PI14/00512, FI14/00269, CPII15/00002, PI17/00495), Generalitat Valenciana (ACIF/2017/138) y Sociedad Española de Trombosis y Hemostasia.

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Profile of thrombin generation in children using low tissue factor picomolar concentration with and without thrombomodulin

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Background: The appearance of benchtop fully automated analyzers for thrombin generation testing (TGT) has lead to an increased presence of TGT in routine laboratories. ST Genesia (Stago) uses a calibration performed in plasma with a known amount of human thrombin and has some commercial kits with different tissue factor concentrations. The use of a low tissue factor picomolar concentration with and without thrombomodulin (STG - ThromboScreen, TS) reveals the effect of thrombomodulin on endogenous thrombin potential (ETP).
Aims: This study aimed at assessing the performance of the STG-Thromboscreen application on the ST Genesia analyzer on pediatric platelet poor plasma samples and defining a reference normal range.
Methods: 55 healthy children were enrolled. Blood samples were analyzed for hemogram, TG using STG-Thromboscreen, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, proteins C and S, activated protein C resistance test, antithrombin III, factor VIII, von Willebrand factor antigen and activity, lupus anticoagulant test, FV G1691A and FII G20210A polymorphisms. Parental informed consent was obtained before collecting the sample. Samples for TGT testing were processed by a double centrifugation tube method (10 minutes / 2500g), and stored at -80°C.
Results: The median age was 11 years (range 1-17 years), 60% males. Hemogram and basic coagulation parameters were strictly normal in all cases (median PT ratio 1.00, range 0.88- 1.16; median APTT ratio 1.00, range 0.83-1.21; median fibrinogen 2.9 mg/dL, range 1.8-4.5 mg/dL), as well as thrombophilia profile and any of them had polymorphisms. Median FVIII percentage was 108% (range 57%>133%) and median von willebrand antigen and activity were 108% and 96% respectively. Median protein S value was 84% (range 64%>133%), median protein C value was 83% (range 41%>136%), and median antithrombin III value was 111% (range 65%>131%). All lupus anticoagulants tests were negative. All the samples were processed in 6-8 weeks from collection.
Median ETP was 66.25 % (95%CI 63.57%>68.93%), and median peak height was 69.16% (95%CI 64.54%>73.78%). These values are slightly different from those reported on healthy adult population. Adding TM markedly reduced ETP, with a median ETP inhibition of 61.26 % (95%CI 58.29%>64.22%). Coefficient of variations were between 14.19% and 25.75% in the different analyzed parameters.
Summary/Conclusion: This analyzer allows the study of TGT on pediatric samples. Our results suggest that thrombin generation profile in children could be slightly different from that of adults. Larger studies are required to stablish reference ranges on children and to elucidate the origin of the coefficients of variation observed.

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European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

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Current approach to antithrombotic therapy in patient with acute myocardial infarction in pregnancy

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Background: Acute myocardial infarction (AMI) in pregnancy is relatively infrequent but life-threatening event accounting for more than 20% of all maternal cardiac deaths. Antithrombotic therapy adminstration, including antiplatelet and anticoagulant therapy, poses a major clinical challenge.
Aims: To present current approaches to antiplatelet and anticoagulant therapy administration in patients with AMI in pregnancy based on our specific clinical experience.
Methods: A comprehensive PubMed and MEDLINE literature search along with clinical examination, multiplate aggregometry and standard coagulometer and biochemical assays.
Results: Determining optimal antithrombotic therapy requires understanding specific oethiopathogenesis of AMI in pregnancy encompassing numerous nonatherosclerotic causes such as spontaneus coronary artery dissections responsible for 15-43% of cases, myocardial infarction with normal coronary arteries, paradoxical embolism, hypercoagulablity, vasospasm, Kawasaki disease and coronary thrombosis (about 17% of cases) always keeping in mind pharmacokinetic changes as well as modifications in a hyperdynamic circulation, the coagulation and vascular milieu, hemodynamic parameters.
In patients with STEMI (ST elevation myocardial infarction) percutaneous coronary interventions (PCI) with stent implantation are the treatment of choice, while in selected patients a coronary artery bypass graft (CABG) is a possible a treatment option in pregnant women with acute coronary syndrome. Dual antiplatelet therapy and anticoagulation therapy administration is the *sine qua non* in patients treated with PCI. Apart from low dosage aspirin, there is limited experience with the use of P2Y12 adenosine diphosphate receptor antagonists (clopidogrel, prasugrel, ticagrelor and cangrelor), glycoprotein IIb/IIIa inhibitors in pregnancy. Considering specific coagulation changes in pregnancy we suggest using aggregometry examination of degree of platetelet inhibitions induced by certain antiplatet agents.
We present the case of a pregnant woman aged 32 with extensive acute myocardial infarction of anterolateral wall complicated with heart failure and ventricular fibrillation and succesful Ceserean section one day after primary percutaeous coronary intervention with drug eluting stent implantation. Aggregometry assays having confirmed a suboptimal response to ticagrelor and a moderate response to aspirin, it was decided to perform Ceserean section. Intensive local haemostatic measures being taken, the patient bled no more than any other woman having a Ceserean section. A healthy male child was born with Apgar score of 8. Upon intensive therapy, the patient's condition was stabilised, her aggregometry assays showing an optimal response to aspirin and ticagrelor in the further course of treatment. A thorough search of professional literature revealed that this is the first case of delivery by a Ceserean section in a patient on dual antiplatelet therapy with aspirin and ticagrelor.
Summary/Conclusion: Relying on specific aggregometry and coagulation assays and local haemostatic measures it has been shown that it is possible to successfully perform a Ceserean section delivery in a patient undergoing *recent PCI* treated with dual antiplatelet therapy with aspirin and ticagrelor. Further research and experience is needed to reach more reliable conclusions on this comlex issue.

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The role of thrombotic risk factors in the aspect of pregnant women anemia correction

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Background: In 2017, 43.4% of pregnant women in Russia had anemia. Traditionally, anemia is considered as a pathological condition increasing the risk of premature birth, pre-eclampsia and fetal hypoxia. However, in a number of modern studies, a decrease in the frequency of stillbirth and pre-eclampsia has been demonstrated in women with mild anemia. According to the Russian valid order 572n, all cases of pregnant women hemoglobin decrease below 110 g/l are identified as anemia of pregnant women (O99.0 by ICD-10) and are treated with iron supplements. However, moderate physiological hemodilution can be a factor that partially compensated thrombotic readiness of women with thrombotic risk. Besides the prescription of iron supplements in the absence of true iron deficiency can cause undesirable side effects, including the activation of opportunistic pathogens. The literature describes urinary tract infections as the most frequent manifestation of excess exogenous iron.
Aims: Study the effect of varying severity anemia on the pregnancy course and outcome depending on the presence of thrombotic risk factors.
Methods: We'd performed a case-control study that included 454 pregnancy and delivery histories. Parameters of peripheral blood in each trimester, pregnancy outcomes and presence of urinary tract infections were studied. Patients were divided into groups: risk group - with history of pregnancy loss and/or pregnancy complications and/or thrombotic risk factors (n=322) and low-risk group (n=132).
Results: The risk group patients had higher hemoglobin values (p=0.019) and erythrocyte counts (p=0.001) in the 1st trimester than low-risk group patients.
Risk group patients with mild anemia during pregnancy had significantly less such outcomes as "fetal loss" (p=0.000) and "placental insufficiency" (p=0.032) than patients without anemia.
Anemia in the 1st trimester is assotiated with premature birth only in the low-risk group patients (p=0.008 for mild anemia and p=0.003 for severe). Critical hemoglobin level in the 1st trimester, which was assotiated with preterm labor in the low-risk group, was 115 g/l (p=0,04).
Moderate and severe anemia (hemoglobin level less than 90 g/l) in the 1st trimester is assotiated with the placental insufficiency in the risk group (p=0.021) and in the general group (p=0.015).
Hemoglobin decrease in the 3rd trimester less than 7.9% of the initial level in the 1st trimester is assotiated with preterm labor only in the risk group (p=0.041).
In the low-risk group, patients with urinary tract infections during pregnancy significantly more often had mild anemia (p=0.012). For severe anemia and for risk group patients this relation was not noted.
Summary/Conclusion: The presence of severe anemia from the 1st trimester is certainly unfavorable for all patient groups. But for patients with history of pregnancy loss or obstetric complications or thrombotic risk factors, mild anemia can be a factor that prevents pregnancy loss and placental insufficiency. Even 1st trimester mild anemia is not assotiated with pregnancy complications in this group. Perhaps, for women with risk factors, it is advisable to establish a lower limit values for normal hemoglobin during pregnancy.
Iron preparations were prescribed to all studied patients with anemia. But in mild anemia treatment of women without risk factors, a differentiated approach is appropriate depending on the presence of true iron deficiency, since in undifferentiated treatment they have urinary tract infections more often.

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The mechanism of coagulating action of positively charged polyamidoamine dendrimers of low generation

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Background: Nano-sized, monodisperse polyamidoamine (PAMAM) dendrimers with internal cavities and surface functional groups (-NH_2, -OH, or -COOH) are used for drug delivery in systemic administrations. Only large PAMAM-NH_2 dendrimers (G4 – G7) induce aggregation of platelets and blood coagulation. We observed that small cationic PAMAM dendrimers (G1-G3) in high concentrations accelerate the coagulation of fibrinogen by thrombin.
Aims: The aim of this work was to find out the mechanism of their coagulation action.
Methods: Overall plasma hemostatic potential, thrombin generation, prothrombin time, thrombin activity, fluorescence, zeta potential, and polymerization of fibrinogen were studied in the presence of various concentrations of PAMAM dendrimers (G< 4).
Results: PAMAM dendrimers (G1 –G3) to a concentration of 0.6 mM practically did not cause RBC hemolysis in plasma. The studied cationic dendrimers inhibited the overall hemostatic potential in plasma, increased the prothrombin time, strongly suppressed the generation of endogenous thrombin, and slightly increased thrombin activity. All effects were enhanced with increasing generation and concentration of the dendrimers. During fibrinogen coagulation by thrombin, the lag phase decreased and completely eliminated, the fibrin formation rate accelerated, and the plateau height enhanced with increasing concentration of G2 and G3 dendrimers. The effect of cationic dendrimers on fibrinogen fluorescence excited at 280 nm showed that the fluorescence peak at 347 decreases uniformly in intensity, but does not shift with increasing dendrimers concentration. This indicates the formation of fibrinogen-dendrimer complexes, resulting in changes in the microenvironment of Trps residues and protein conformation. For example, for G3 dendrimer values of quenching constants obtained from the linear parts of the Stern-Volmer plot were: K_{SV}' = 1.23 ± 0.15*10^5 M^{-1} and K_{SV}'' = 2.0 ±0.2.*10^4 M^{-1}. The zeta potential of fibrinogen (- 17.65 MV) gradually changed towards positive values with increasing concentration of G3 dendrimer, reaching a maximum of -0.5 MV with a dendrimer:protein ratio of 10:1, Consequently, at least 10 molecules of G3 PAMAM-NH_2 can bind to one fibrinogen molecule. The strong interaction should be attributed to the electrostatic attraction of a positively charged dendrimer with a negatively charged fibrinogen. Analysis of changes in the fluorescence spectrum, zeta potential and fibrinogen polymerization by thrombin showed that cationic dendrimers are strongly bind with negatively charged fibrinogen, change its conformation and coagulability.
Summary/Conclusion: Our results show that PAMAM-NH_2 dendrimers of low generation (G < 4) are not cytotoxic, do not inhibit the thrombin activity, but inhibit the external pathway of the coagulation system activation. However, they increase fibrinogen coagulation by thrombin which is caused by a change in its conformation as a result of a strong electrostatic interaction between cationic dendrimers and negatively charged fibrinogen.

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Lot Specific differences in POC INR testing: UK NEQAS BC investigation

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Background: UK National External Quality Assessment Scheme for Blood Coagulation (NEQAS BC) has been providing External Quality Assessment(EQA) for 20+ years for Point of Care (POC) INR testing. Currently we provide EQA for Roche XS series , Abbott i-STAT, Siemens Xprecia Stride and Werfens Hemochron .As part of our process of analysis, lot related medians are compared and lots which are >10% different from the overall median are investigated.
Aims: In this time only 3 lot related issues have required investigation and we report here on the latest instance.
Methods: NEQAS BC distributes two lyophilised plasma samples in 4 surveys per year for each device type with distribution staggered over the year. For the device concerned surveys are distributed in Jan, April, June and October. In an exercise sent in April 2018 one lot of test strips(code chip 312) for one device was noted to be giving higher INR results compared to the overall medians for that device in one of our two samples- lot medians were 2.3 and 3.5 compared to overall medians of 2.1 and 3 (between 10 and 15% increase).
Results: We contacted the company concerned and were informed that a change in calibration had occurred: newer batches of test strips being calibrated against rTF/16 whereas older batches had been calibrated against rTF/09. Our results showed the newer batches (calibrated with rTF/16) were reading higher than the older batches of test strips with code chip 312 giving the highest readings.
The June 2018 survey comprised of a low INR sample (median =1.5) which did not show any differences and a higher INR sample which showed lot differences. The lots calibrated against rTF/09 had median INR of 4.2 and the lots calibrated against rTF/16 had a median INR of 5.6.
Information from our two surveys was reported to the company and also to the UK Medicines and Healthcare products Regulatory Agency (MHRA). In September 2018 a Field Safety notice was issued indicating that users of test strip lots calibrated against rTF 16 should any POC INR results of 4.5 or above check with a venous sample. The October 2018 and January 2019 NEQAS BC surveys did not show any differences between lots but all four INR samples were low with medians of 2.7 and 1.6 for survey in October and 1.8 and 1.5 for January.
The company has now moved back to calibrating test strips against rTF/09. The April 2019 NEQAS BC survey showed lots from three distinct groups 1) early batches (calibrated against rTF/09) users , 2)discrepant batches (calibrated against rTF/16) users and 3) latest batches (calibrated against rTF/09)users. Onelot (code chip 312) from the rTF/16 batch users showed results >10% different from the overall medians.
Summary/Conclusion: UK NEQAS BC continues to monitor batch medians for EQA samples for all the POC INR testing as part of our EQA process.

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Altered coagulation status in the pathogenesis of Buruli ulcer

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Background: Buruli ulcer (BU) skin lesions, caused by the *Mycobacterium ulcerans* exotoxin mycolactone, display restricted infiltration of immune cells and coagulative necrosis. Mycolactone blocks expression of secreted and cell surface proteins by inhibition of the Sec61 translocon. We have shown that endothelial cells are particularly sensitive to the actions of mycolactone, leading to the hypothesis that endothelial cell dysfunction may contribute to the development of BU lesions. In this line, we found exposure to the toxin rapidly depletes expression of thrombomodulin (TM) and this may have an impact on anti-coagulation at the endothelial cell surface.
Aims: To study whether mycolactone-triggered endothelial cell dysfunction and local coagulopathy in BU skin lesions may contribute to BU pathogenesis.
Methods: Quantitative proteomic analysis of membrane fractions: Human dermal microvascular endothelial cells were incubated for 24 hours with DMSO or mycolactone in triplicate. After hypotonic lysis, membranes were isolated by differential centrifugation. Acetone precipitated proteins were reduced, alkylated and digested then labelled with tandem mass tags and detected by LC-MS/MS.
Sequential staining strategy in serial tissue sections of BU patient skin biopsies (n = 8): Non-necrotic regions were identified by Haematoxylin and eosin staining. Vessels were tracked and analysed using smooth muscle actin and the endothelial marker CD31. The haemostatic markers fibrin, TM, platelet glycoprotein CD61, tissue factor and von Willebrand factor (vWF) were examined by immunohistochemical staining.
Results: Gene ontology analysis of the proteomic data set showed proteins associated with blood coagulation and the plasminogen activating cascade were enriched in the downregulated fraction. This was validated by Western blot which confirmed reduced expression of tissue factor pathway inhibitor and vWF in mycolactone-treated primary endothelial cells. In BU patient skin punch biopsies, fibrin staining around TM-negative vessels was significantly higher than TM-positive ones. In addition, tissue factor was seen in endothelial cells, as intracellular puncta in macrophages and other unidentified cells as well as in tissues adjacent to fibrin-positive wounds and outer vessel walls in a TM-independent fashion. Notably, tissue factor seen outside of the tunica externa correlated with abnormal fibrin deposition in these areas. Interestingly, CD61 was barely detectable in the analysed vessels in BU skin biopsies.
Summary/Conclusion: Our findings demonstrate widespread disruption of coagulation pathways in BU lesions that may facilitate pathogenic fibrin deposition and impact on tissue repair.

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Cerebral venous sinus thrombosis as first manifestation of behçet' s disease

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Background: Cerebral venous sinus thrombosis (CVST) is a rare entity and its clinical presentation is variable, even life-threatening. The most frequent symptoms and signs are headache, focal seizures, unilateral or bilateral paresis and papilledema. Transverse sinus and superior sagittal sinus are the most frequently involved sites of CVST. Patients with hypercoagulable states, adjacent infections, low cerebral blood flow, on oral contraceptives, pregnancy and specific autoimmune disorders are at high risk of CVST.
Aims: Aim of the present is to highlight an interesting case of CVST with diplopia and headache,who was concurrently diagnosed with Behcet's disease (BD). The incidence of CVST in BD patients is estimated 3.1 per 1000 person-years and 15.1 per 1000 person-years among the BD patients and BD patients with neurologic involvement, respectively.
Methods: A 26-year old Caucasian man, with no previous medical history, was admitted to our clinic due to sudden onset of horizontal diplopia and fronto-temporal headache for the last week. No history of fever, facial/sinus infection or head trauma was documented. The patient had never experienced similar symptoms neither was there any family history of similar conditions noted. He was afebrile and physical examination revealed painful aphthous stomatitis, chronic skin rash and pseudofolliculitis with acne form nodules on both upper arms and forearms.
Results: Visual acuity of both eyes and cranial nerves examination were normal, while fundus examination showed bilateral papilledema. Cerebrospinal fluid analysis revealed mildly increased intracranial pressure and aseptic CSF profiles. His brain MRI showed presence of cerebral edema and no space occupying lesion, MRA showed no aneurysms, while MR Venography revealed right transverse sinus and superior sagittal sinus thrombosis. His coagulation profile and blood testing for thrombophilia and connective tissue disorders were normal apart from a positive HLA-B51 testing. His echocardiogram revealed a small atrial septal aneurysm and blood chemistry showed moderately elevated inflammation markers. The patient was treated with low molecular weight heparin SC for 1 week followed by warfarin administration in the absence of any concomitant aneurysms. Due to highly suspected underlying BD, he was given 1 gr methylprednisolone IV for three days followed by *per os* corticosteroids and methotrexate IM treatment. Diplopia and headache had gradually resolved and repeated MRV one month later showed resolved CVST.
Summary/Conclusion: CVST is a rare potentially lethal entity. BD is a systemic inflammatory vascular disease with variable clinical manifestations and masquerades. CVST is one of its major neurological manifestations, whose early recognition and treatment bear a good prognosis.

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Patient Preferences for the Treatment of Paroxysmal Nocturnal Hemoglobinuria: Results of a Patient Survey of Ravulizumab (ALXN1210) and Eculizumab in the UK

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Background: Eculizumab administered every 2 weeks (q2w) has been the standard of care for paroxysmal nocturnal hemoglobinuria (PNH) since its approval in 2007. However, its q2w dosing frequency may negatively impact patients' quality of lives. Ravulizumab, a novel C5 inhibitor, administered every 8 weeks (q8w) showed noninferiority to eculizumab in two phase 3 trials on all primary and key secondary efficacy measures. In global substudy ALXN1210-PNH-302s, a significantly higher proportion of patients (pts) were shown to prefer ravulizumab over eculizumab. In regions where two treatment options are available for PNH, understanding patient preferences for ravulizumab or eculizumab could help guide future treatment decision-making.
Aims: To evaluate patient preference for ravulizumab or eculizumab treatment in clinical trial substudy ALXN1210-PNH-302s from the UK population.
Methods: In the phase 3 open-label study (ALXN1210-PNH-302), adult PNH pts stable on eculizumab for ≥6 months received weight-based ravulizumab or eculizumab in accordance with labeling for 26 weeks (randomization period). Pts were then enrolled in an extension period, all receiving ravulizumab. This substudy enrolled PNH pts who participated in the extension period and had received a minimum of 2 maintenance doses of ravulizumab during the extension phase. In the substudy, patient treatment preference was evaluated at one time point using an 11-item PNH Patient Preference Questionnaire (PNH-PPQ©). Differences in preference between ravulizumab and eculizumab were examined with an exact binomial test (Question [Q] 1), frequency distributions (Q2-3), and paired *t* tests (Q4-11), with $P<0.05$ considered statistically significant. Standardized effect sizes (*d*) were calculated for differences between evaluations of ravulizumab and eculizumab on Q4-11.
Results: Of 98 pts enrolled, 95 pts from 8 countries completed Q1 of the PNH-PPQ, including 35 pts from the UK. The mean age of UK participants was 53 years; 54.3% were female. Mean time since diagnosis was 13.7 years and mean number of days between the last randomized study treatment and completion of PNH-PPQ was 278.1. Overall, 91.4% of pts (n=32) preferred ravulizumab vs 8.6% (n=3; $P<0.001$) who had no preference (n=2) or preferred eculizumab (n=1). For specific aspects of treatment, ravulizumab was predominantly preferred on frequency of infusions (100%), ability to plan activities (97%), convenience of receiving treatment (97%), overall quality of life (88%), and effectiveness of medication until the next infusion (76%). Frequency of infusions was selected by the most participants (n=15; 43%) as the most important factor determining treatment preference, followed by being able to plan activities (n=5; 14%) and overall quality of life (n=5; 14%). Moderate to large effect sizes were observed for factors favoring ravulizumab over eculizumab, including the frequency of infusions disrupting everyday life ($d= –2.14$, $P<0.001$), feeling fatigued after infusions ($d= –0.71$, $P<0.001$), and being able to enjoy life while receiving treatment ($d= 0.95$, $P<0.001$).
Summary/Conclusion: In this clinical substudy of eculizumab-experienced PNH pts, a majority of UK pts preferred ravulizumab compared to eculizumab because of reduced infusion frequency (q8w vs q2w), better ability to plan activities, more convenient treatment, improved overall quality of life, and effectiveness of medication until the next infusion.

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Patient's preferences for decision-making in anticoagulation: Beyond the clinical criteria

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Background: According with the European Heart Rhythm Association, "consider patient preference" is one of the cornerstones for choosing anticoagulant therapy as it could be related to patient's adherence, physician confidence and, at the end, with clinical effectiveness. Preference for Direct Oral AntiCoagulants (DOAC) has been widely evidenced, with no differences among the available DOACs.
Aims: To assess the main value drivers and preferences of the patients being treated with DOAC, according to routine clinical practice in Spain.
Methods: Observational, multicentric (26 hospitals), cross-sectional study, based on patients and caregivers' interview, was conducted in Spain. Patients (≥18y-o) treated with a DOAC for ≥6 months were included in the study (after provided written informed consent). Patient's caregiver was included when possible. The study questionnaire was ad-hoc designed for the study purpose. Importance and preference questions were scored according to a 10-points Likert scale. Patients were distributed in three study groups according to their self-reported preference regarding DOAC posology: (A) Once daily, with water; (B) once daily, with food; (C) twice daily.
Results: A total of 335 patients and 55 caregivers were included, being considered as valid 332 patients and 55 caregivers. Once daily administration was preferred by 274 patients (82.5%) [60.8% (A); 21.7% (B)] and 47 caregivers (85.5%) [58.2% (A); 27.3% (B)]. Included patients aged a mean (SD) of 73.7 (10.7) years, and 51.5% were women. Comorbidities were frequent in these patients (80.7%), as well as polymedication (6.6 (3.3) daily drugs). Patients were treated with DOAC a mean of 23.8 (18.1) months and 44.0% were naïve to oral anticoagulation. Caregivers aged 58,7 (13,9) years and 69.1% were women. Most caregivers were informal (84.7%), combining caregiving tasks with labor activities (52.7%). Caregiving was conducted a mean of 39,1 (44,7) weekly hours. No statistically significant differences were found among preference study groups in terms of socio-demographic and main clinical data. Both, patients and caregivers were highly satisfied with the received DOAC (9.0 and 9.1 points, respectively). However, only 41.0% of the patients were treated according to their preferences [23.8% (A); 44.4% (B); 96.6% (C)]. DOAC users are mainly concerned by hemorrhagic risk (major and mild; 7.3 and 5.6 points, respectively), followed by drug administration frequency (5.6 points). Caregivers showed similar priority profiles, although posology was ranked in fourth position: major hemorrhagic risk (8.0), mild hemorrhagic risk (6.6); drug interactions (5.6) and administration frequency (5.2).
Summary/Conclusion: After efficacy and safety, administration frequency has been identified as a main priority for both, patients and caregivers. Most patients prefer once daily medication (82.5%), and, when possible, once daily, with water (60.8%). However, only 41.0% are treated according to their preferences (23.8% when considering the main option, "with water"). Clinical criteria must be the main driver for decision-making, even though closely followed by patients' preferences or those of the caregiver, when needed. Specific guidelines or tools should be developed for helping in the balancing of clinical criteria and preferences for DOAC choice in routine clinical practice.
The study has been sponsored by Daiichi Sankyo Spain, and coordinated by IQVIA (including medical writing).

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Evaluation of the challenges in the diagnostic methods for the detection of lupus anticoagulant in a third level hospital

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Background: The lupus anticoagulant (LA) detection requires four mandatory steps including mixing studies, screening and confirmation tests. These tests have shown a variable sensitivity and specificity. Mixing LA test improves the discrimination of LA in presence of an inhibitor and the deficiencies of coagulation factor, such as those that occur in patients receiving antagonists of the vitamin K (VKAs).
Aims: The objective is this study is to review the experience and the challenges over the LA tests in a third level hospital.
Methods: A Cross-sectional study was performed from January 2018 to April 2019. On 92 patients with an INR above 1.4 in whom a study for detection of lupus anticoagulant was required.
Results: Out of 92 patients (43 men and 48 women), 53 patients were diagnosed with venous thrombosis (57%) and 12 patients with arterial thrombosis (11%). The remaining 28 patients had other non-thrombotic conditions (factor coagulation deficiencies, connective tissue diseases and liver disease). 74 patients were taking VKAs; 6 patients, direct oral anticoagulants (DOACs), and 11 patients did not take anticoagulants (two of them had factor V and X deficiency). 71 patients had an INR between 1.4 to 3 and 43 a positive silica clotting time (SCT). From these patients, performing a mixing LA test, 15 maintained positivity and 28 became negative. In the group of 71 patients, 65 presented a positive dRVVT, which subsequently, with the mixing LA test, remained positive 43 and in 28 the dRVVT test was negative (24 patients are taking VKAs and DOACs, and 4 had a connective tissue disease).
Among the 92 patients, 21 had an INR higher than 3. All of them were positive for both tests SCT and dRVVT. The mixture LA test negativized 12 and 4 SCT and dRVVT respectively. The positive mixing LA tests only coincided in 2 patients who had been diagnosed with APS. All of 21 patients are taking VKAs.
Finally, from the 92 patients, a total of 18 were diagnosed with APS, presenting huge variability in the SCT tests, but all cases had a positive dRVVT test. A high variability of anti-beta2GPI and anticardiolipin antibodies results was observed.
Summary/Conclusion: The combined use of dRVVT and SCT assays reports clearer and objective results of LA. In patients with discrepant results between LA tests should be mandatory to perform new LA tests beyond the period of treatment with anticoagulants. The performance of additional tests such anti-beta2GPI and anticardiolipin antibodies would be a helpful tool in most of the patients taking oral anticoagulants.

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Recurrence of VTE in the long-term period: a perspective study

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Background: Venous thromboembolism, including deep vein thrombosis (DVT), pulmonary embolism (PE) or both, is the third most frequent cardiovascular disease, accounting from 70 to 120 cases every 100.000 people/year. Most of recurrences occur in the first months after an acute event, then decrease progressively.
Aims: The risk of recurrence within five years after an acute event remains up to 5%; our primary endpoint was to assess the risk of recurrence in the long term (beyond 5 years) and whether other factors (presence/ discontinuation of therapy, sex, age) could be implicated.
Methods: We consecutively enrolled 270 patients referring to our Center and then followed in a period of ten years (from 2006 to 2016); exclusion criteria were age<18 years, provoked VTE, VTE at unusual sites, presence of known thrombophilia, end stage kidney or liver disease.
Results: 88 patients (32.6%) had a recurrence (15.2% within 5 years, 17.4% beyond 5 years; p=0.49). Among 182 patients with no recurrence, 37 were continuing anticoagulant drugs, while among the 88 patients with a new event, 26 were still under treatment (RR=0.73, p=0.09). With regards to the patients with recurrence under treatment, in 20 cases this occurred after 5 years (22.7%, p=0.003).
Summary/Conclusion: In our population we sought recurrence in one third of the cases, with no difference considering the timing of recurrence; moreover we observed that the presence of anticoagulant treatment was able to delay the onset of recurrence. In conclusion, we could not assess whether the anticoagulant therapy was able to modify the risk of recurrence. Further studies are needed to confirm this data.

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Argatroban for therapeutic anticoagulation in two patients with acute thrombosis and heparin resistance

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Background: Argatroban is a small molecule direct thrombin inhibitor, cleared by the liver with a 45-minute half-life, quick onset of action over 1-3 hours and is monitored using the APTT. Argatroban has been licensed for the treatment of heparin-induced thrombocytopenia (HIT) and its clinical use has been largely limited to such.
Aims: We present two cases where argatroban was successfully used off-license in patients with acute thrombosis requiring anticoagulant treatment where heparin resistance with unfractionated heparin had been encountered.
Methods: The first case was a woman with abdominal arterial thrombosis, of unknown aetiology, treated with therapeutic low-molecular-weight heparin who developed pulmonary embolism despite therapeutic anticoagulation (and had evidence of heparin resistance on anti-Xa monitoring). The second patient had provoked abdominal arterial thrombosis from sepsis and could not attain therapeutic anticoagulation with intravenous unfractionated heparin. Institutional ethical approval was not required for a case series and written consent was obtained from both patients.
Results: In both cases, therapeutic anticoagulation was achieved with the use of argatroban, as a temporising measure to treat the acute thrombotic event.
Summary/Conclusion: Conventionally argatroban has been described for use in heparin-induced thrombocytopenia (HIT). The use of argatroban is briefly discussed, especially in the context of heparin resistance where anticoagulation can be challenging. This report adds to the growing literature on argatroban usage outside of HIT.

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Reference intervals of routine coagulation tests in healthy term-pregnant Greek women

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Background: Since haemostatic reference intervals are generally based on samples obtained from non-pregnant women, those intervals should not be considered for the evaluation of the coagulation status of pregnant women. Pregnancy is an acquired thrombophilic condition and, therefore, it is important for a laboratory scientist to be cognizant of the ranges pertaining to the first-line coagulation test reference values during pregnancy. This knowledge would enable not only detection of an additional coagulation triggering but also prevention of a possible thrombotic episode.
Aims: The aim of this study was to establish the reference intervals of Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT), and Fibrinogen (FIB) in Greek women prior to labor.
Methods: Blood samples (citrated plasma) from 741 healthy term pregnant women were collected. PT (Ratio and %), APTT (Ratio) and fibrinogen (FIB) levels were measured in a coagulation analyzer. The participants were divided into two groups according to their age: A) <35 years and B) >35 years old. After subtracting 5% of the extreme values, a normality test was performed using SPSS 21 statistical software. Depending on the results of the previously mentioned test, the median, minimum, maximum values with or without means and Standard Deviations (SD) were calculated. Subsequently, the A and B study groups were statistically compared by means of Mann-Whitney test or t-student test.
Results: The median, minimum and maximum values of healthy pregnant women were as follows: 1) women of all ages: PT=110%, range: 97 - 122%; PT-Ratio=0.91, range: 0.82 - 1.03; APTT-Ratio=0.87, range: 0.72 - 1.09; and the mean value (±SD) of FIB was 521 ± 81.4mg/dl; 2) Group A: PT=110%, range: 97 - 120%; PT-Ratio=0.91, range: 0.83 - 1.03; APTT-Ratio=0.88, range: 0.72 - 1.1; and the mean value (±SD) of FIB was 525 ± 79.9mg/dl; 3) Group B: PT=111%, range: 96 - 122%; PT-Ratio=0.9, range: 0.82 - 1.04; APTT-Rati=0.84, range: 0.72 - 1; and the mean value (±SD) of FIB was 503 ± 92.28mg/dl. The PT and fibrinogen measurements were not significantly different between the groups A and B, whereas a statistically significant decrease of APTT in the group B was found (p=0.01).
Summary/Conclusion: The PT and APTT in healthy term-pregnant women of all ages are at the lower limit of normal reference values, whereas the fibrinogen level is elevated. In addition, the APTT is reduced in older pregnant women (i.e., >35 years old) while there is no difference in PT and fibrinogen relating to age.

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Evaluation of EQA material on Yumizen G 200 – coagulation analyser: UK NEQAS for Blood Coagulation study

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Background: The YumizenG200 device is a two channel analyser for in vitro diagnostics of patient citrated plasma samples. This semi–automated coagulometer performs tests using nephelometric, turbidimetric and chromogenic endpoint detection methods. The device can be used for performing coagulation screening tests, clotting factor assays, D – Dimer and Antithrombin III.
Aims: To determine the suitability of UK National External Quality Assessment Scheme for Blood Coagulation (NEQAS BC) lyophilised plasma samples for use with Yumizen G200 and to establish an external quality assessment (EQA) programme for this device.
Methods: NEQAS BC samples previously used in the laboratory EQA programme were selected. For each tested parameter (PT/INR, APTT, Fibrinogen, Thrombin Time and DDimer) 5 aliquots of each of three samples (low, normal, abnormally high) were tested on the Yumizen G200 using reagents provided by the manufacturer. Obtained results were compared to the test specific medians established previously in NEQAS BC EQA surveys.
Results: INR: Mean INR results on the YumizenG200 for the 3 samples were 1.6, 2.5 and 5.6 respectively. These were comparable to the median INR values from EQA surveys (1.34, 2.3 and 5.27 respectively) with coefficients of variation (CV %) < 5% (1.83 – 3.67).
PT: Mean PT ratios on the YumizenG200 and median results from UK NEQAS BC participants for samples were as follows: FV deficient plasma 1.5 vs 1.29; FX deficient plasma 1.6 vs 1.57; FVII deficient plasma 1.80 vs 1.84 with CVs% < 5% (1.32 – 2.44).
APTT: Mean APTT ratios on the YumizenG200 and median results NEQAS BC participants for samples were: normal plasma – 1.11 vs 0.98; FXI deficient plasma 1.48 vs 1.25; VWD type 3 plasma 2.19 vs 1.91. Two lupus anticoagulant positive EQA samples were also tested for APTT. Sample 1: NEQAS BC median DRVVT ratio 1.74 vs mean APTT on the YumizenG200 1.34. Sample 2: NEQAS BC median DRVVT ratio 2.84 vs mean APTT on the YumizenG200 1.85 with CVs% for APTT testing <5% (1.53 – 4.78).
Clauss fibrinogen: Mean results on the YumizenG200 for 3 samples were 2.02, 2.52 and 3.94g/l respectively. These were comparable to the median values from NEQAS BC EQA exercises (1.87, 2.4 and 3.9g/l respectively) with CVs% (5.53 – 7.24).
Thrombin time (TT): Mean TT ratios on the YumizenG200 and median results from NEQAS BC participants for samples were as follows: normal plasma 0.98 vs 1.01; dabigatran spiked plasma 6.4 vs 4.87; spiked unfractionated heparin 0.15IU.ml plasma 1.41 vs 1.45 with CVs% 1.16 and 2.98 respectively where results were obtained.
DDimer: Mean DDimer results on the YumizenG200 for 2 samples were 0.68 and 0.40 ug/ml FEU with corresponding CVs% 7.72 and 2.75. These were comparable to the median DDimer values for Siemens Innovance reagent group from NEQAS BC EQA exercises (0.63 and 0.39ug/ml FEU respectively).
Summary/Conclusion: The study has demonstrated the suitability of NEQAS BC EQA lyophilised plasma samples for testing on YumizenG200.

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Fibrinogen structure and function under oxidative stress condition

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Background: Oxidative stress is linked with many pathological conditions including cancer, neurodegenerative and cardiovascular diseases. Among plasma proteins, fibrinogen is the most sought-after target of oxidative stress reagents. Structural changes in fibrinogen molecule affect dynamic of thrombus formation as well as dissolution. The link between structural and physiological changes might clarify connections of modifications of the molecule to the disease progression.
Aims: The aim of the work was to observe the effect of pathological condition during disease onto structure and function of fibrinogen.
Methods: Patients samples were obtained in accordance with The Ethic Committees of participating institutions (The Institute of Hematology and Blood Transfusion, Prague and The Military University Hospital, Prague, Czech Republic). For the determination of the degree of fibrinogen modification carbonyl groups evaluation was used. Oxidative status was determined by malondialdehyde concentration in plasma. For identification of modified amino acids residue by mass spectrometry fibrinogen was purified from citrated plasma by precipitation with a 25% saturated ammonium sulfate. Fibrin net architecture was studied by scanning electron microscopy. Molecular dynamics simulations of fibrinogen were performed in Gromacs software with Gromos 54a7 force field. PTMs were introduced into the truncated crystal structures 3GHG and 2A45 by Vienna-PTM 2.0 server.
Results: MDA concentration and carbonyl content were significantly higher compared to controls. Oxidative changes affected profoundly the architecture of fibrin net. Density and fiber diameters differed in comparison with control samples. Modified amino acids residues were identified and selected structures of the modified chains were characterized by molecular dynamic simulations.
Summary/Conclusion: Observed changes in oxidative status, variations in clot architecture together with data from LC-MS/MS and molecular dynamics gave us a chance to better understanding to thrombotic/bleeding complications linked with oxidative stress in diseases.
This work was supported by the Ministry of Health, Czech Republic (nr. 00023736), by Grant from the Ministry of Health [NV18-08-00149], by Grant Agency of the Czech Republic (nr. P205/12/G118) and by ERDF OPPK (CZ.2.16/3.1.00/24001).

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In vitro determination of the amount of in vivo formed active thrombin in cohorts of patients with various diagnosis

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Background: D-dimer determination is a standard procedure in the treatment of thrombosis. Thrombin plays pivotal role in thrombosis and hemostasis. D-dimers half-life is in hours, thrombin has much shorter half-life since it is rapidly inhibited by naturally occurring inhibitors. Thrombin binds to fibrin where it is quite efficiently protected from inhibition by heparin-antithrombin. There is one high affinity thrombin non-substrate binding site on a variant fibrinogen γ' chain (a constitutive feature of fibrin as well as fibrinogen).
Aims: The aim of the study was to determine active thrombin bound to D-dimers in cohorts of patients with different diagnosis and to discuss the effect of pathological conditions during the disease on the measured values.
Methods: Patients (76) and control samples (131) were obtained in accordance with The Ethic Committees of participating institutions (The Institute of Hematology and Blood Transfusion, Prague and The Military University Hospital, Prague). The amount of D-dimers was determined using immunochemical methods. The amount of the active thrombin bound to D-dimers was determined by adding specific substrate for thrombin and measuring the increase of fluorescence caused by the enzymatic reaction.
Results: We found that active thrombin bound to isolated D-dimers is stable for many hours. The measurement of very low activities of thrombin is possible. The presence of active thrombin in samples of acute care patients was linked with thrombotic diagnosis (e.g. pulmonary embolism, myocardial infarction) in nearly 63 % of cases. Active thrombin was detected in only 7 % of control samples, on the other hand in 45 % of acute care patients. In 70 % cases of acute care patients without thrombosis as main diagnosis, presence of active thrombin was linked with complications or death. In an *in vitro* experiment we found that the activity of thrombin bound to D-dimers reflected its concentration at the time of thrombus formation.
Summary/Conclusion: Since the thrombin concentration in growing thrombus is one of the key factors determining thrombus mechanical stability and resistance to fibrinolysis the measurement of the residual activity of thrombin on D-dimers may contribute to improvement in diagnosis of thrombosis. Moreover, false elevations of D-dimers can be observed in patients with high levels of rheumatoid factor and several other conditions; the determination of thrombin activity is not affected by these conditions.
This work was supported by the Ministry of Health, Czech Republic (nr. 00023736), by Grant Agency of the Czech Republic (nr. P205/12/G118), by Grant from the Ministry of Health [NV18-08-00149] and by ERDF OPPK (CZ.2.16/3.1.00/24001).

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Plasma fractionation: conventional and chromatographic method for factor VIII purification

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Background: Plasma protein fractionation is by far the largest industry segment in therapeutic protein manufacture. Plasma of blood contains about 60÷80 g/L of protein, of which about 95.0 % are used for many therapeutic products. Currently therapeutically important proteins are serum albumin, immunoglobulin G (IgG), factor coagulations.
Improvement in therapy of hemophilia A requires a steady increase in the production of factor VIII (FVIII).
Aims: optimization of a process purification FVIII by the use different methods of fractionation.
Methods: pre-fractionation; ion-exchange and affinity chromatography.
Results: The use of silica-based chromatographic supports with immobilized dye-ligands can be used in many preparative and analytical applications. Dye-ligands have been considered as one of the important alternatives to natural counterparts for specific affinity chromatography.
Demonstrated that the process of purification of the FVIII by the method dye-ligand chromatography with Diasorb-Procion Gelb M4R allowed to achieve a maximum purification rate of 6.28 times.
The combinations of the pre-fractionation method (barium citrate, aluminum hydroxide (III), and PEG-4000) with the method of affinity chromatography on selected sorbent allows obtaining FVIII with a degree of purification of 93.35 times.
It has been demonstrated that the use of ion-exchange chromatography on DEAE-Sepharose FAST FLOW and affinity chromatography allows obtaining FVIII with a degree of purification of 166.52 times.
It has been found that the combination of pre-fractionation, ion-exchange and affinity chromatography stages provides a purification rate of FVIII 499.44 times.
Summary/Conclusion: The process purification FVIII have been developed in combination of methods of pre-fractionation of additional proteins, ion-exchange chromatography on DEAE-Sepharose and affinity sorption macrospores silica sorbents Diasorb-Procion Gelb M4R, which achieve a maximum degree of purification of 499.44 times.

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A case of slow resolution upper extremity DVT

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Background: Despite a lack of direct evidence proving safety and efficacy of anticoagulation for upper extremity deep vein thrombosis (DVT), it still remains the cornerstone of therapy, with the goal of relieving acute symptoms and preventing embolization. Direct oral anticoagulants (DOACs) are considered more suitable than vitamin K antagonists (VKA), as they have evidence of similar effects in non-cancer patients and a safer adverse events profile. Nevertheless, in case of malabsorption syndromes, there are a few data about pharmacokinetics of DOACs since plasmatic dosages have just been recently introduced. Moreover, it is difficult to relate them to drug efficacy. Some rare cases of resistance to VKA are described in literature.
Aims: Here by we present a case of a 36-year-old woman with a right upper arm deep vein thrombosis (DVT) developed in a context of a peripherally inserted central catheter (PICC) after a cholecystectomy for acute calcolous cholecystitis. The post-operative course was complicated by recidivant pancreatitis, leading to diarrhea, due to malabsorption of nutrients, and to a long-term hospitalization.
Methods: Medical history included Hashimoto thyroiditis and obesity. Her medications consisted in levothyroxine, lipase analogues, a proton-pump inhibitor and ursodehoxycholic acid. On US study large involvement of humeral, axillary, basilic and subclavian veins by DVT was detected, and CT chest scan excluded pulmonary embolism. At first low molecular weight heparin was started, then, after 7 days, an oral anticoagulation was introduced. We decided to use AVK in order to easily monitor drug efficacy, especially for expected drugs malabsorbtion.
Results: The patient seemed to be refractory to AVK, since it was demonstrated no improvement of DVT on repeated US studies in the following 2 months, even after PICC removal. Checking weekly INR, we always found levels in range but at lower limit (2.1), so we made a dosage titration until 10-12.5 mg daily. A thrombophilic screening was also evaluated, with all negative findings. At a second anamnestic evaluation, the patient admitted estroprogestinic (EP) intake, which she previously deliberately denied. Therefore, EP drug was obviously stopped and anticoagulation was continued without any other dose adjustments. Several US and a CT made in the following months demonstrated thrombosis complete resolution after one year-therapy.
Summary/Conclusion: Starting a VKA, instead of a DOAC, could be a better choice in order to evaluate its efficacy related to absorption in a context of pancreatic insufficiency. In this case a combined defect in absorption of VKA and fat-soluble vitamins, including vitamin K, was assumed. Therefore, we used high doses of VKA, although without reaching peaks described in literature. However, persistence of thrombosis without easy resolution, even after PICC removal, highlighted the presence of a hidden risk factor, which was in this case EP. At least, low INR levels and continuous EP therapy may have contributed to thrombus organization leading to a slow resolution of a thrombosis which could have been treated with administration of anticoagulant for at most 3 months.

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Management of the repeated thrombotic complications and antithrombin deficiency

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Background: Antithrombin is the inhibitor of thrombin and activated coagulation factors IX, X, XI, XII, kalikrein and plasmin. Antithrombin deficiency represents 3-7-fold increased risk of the venous thromboembolism (VTE) when compared with other thrombophilic states. Antithrombin deficiency may be inherited or acquired. Inherited antithrombin deficiency is rare thrombophilia inherited in autosomal dominant manner. In general, the activity is decreased below the reference range (below 70 % of normal values).
Aims: To present a study of a patient with complicated management of repeated thrombotic complications and antithrombin deficiency.
Methods: The authors present the case report of the patient with mild antithrombin deficiency. Despite this non-severe decrease in antithrombin activity, repeated thrombotic complications requiring the demanding management were developed. Thus, the authors discuss risk factors, diagnosis and treatment possibilities of this thrombophilia.
Results: During the last year, the antithrombotic management has been without further rethrombotic complications.
Summary/Conclusion: Antithrombin deficiency is a high-risk thrombophilic state and a rare clinical condition. Despite full anticoagulation, the rethrombosis may occur and could have a severe clinical consequneces. Thus, the close monitoring of the patient is inevitable.
Key words: antithrombin deficiency, venous thromboembolism, anticoagulant treatment
Acknowledgement: Authors thank the support of the projects of the Scientific Grant Agency (Vega) 1/0168/16 and Vega 1/0549/19. Informed consent of the patient was obtained.

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Risky pregnancy and anticoagulant prophylaxis of the thromboembolic events

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Background: Thrombophilic states aggravate the risk of placenta-related complications that can be prevented by the use of low molecular weight heparin (LMWH). Based on the results of the meta-analysis, LMWH provides the highest probability of live childbirth. Currently, the administration of LMWH in individuals with inherited thrombophilia and repeated pregnancy loss is not routinely suggested.
Aims: To evaluate results of the follow-up of selected changes in haemostatic parameters and modulate the dose of anticoagulant thromboprophylaxis.
Methods: Patients with risky pregnancy (past history of pregnancy complications and thrombophilic state) were included in this analysis. Selected parameters of special haemostasis (D-dimer levels, protein S, anti-Xa activity) were monitored in 4 intervals of blood sampling in the course of pregnancy and also after the postpartum period.
Results: We changed the dose of LMWH according to the actual results of the standard coagulation tests, anti-Xa activity and other specific haemostatic tests, and with regards to the current clinical state of the patient. No life-threatening thrombotic event was reported.
Summary/Conclusion: Thromboprophylaxis may prevent high-risk pregnant patients from throbmoembolic and pregnancy issues.
Key words: risky pregnancy, thrombophilia, pregnancy complications, anticoagulant thromboprophylaxis
Acknowledgement: Authors thank the support of the projects of the Scientific Grant Agency (Vega) 1/0168/16 and Vega 1/0549/19. Informed consent of the patient was obtained.

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Is CHA2DS2-VASC score useful in patients with low thromboembolic risk? A case report.

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Background: Atrial fibrillation (AF) is a disease characterized by an increased stroke and thromboembolic risk, which might vary significantly depending on the patients' characteristics and risk factors. Hence, various tools useful in predicting stroke risk were developed, being CHA_2DS_2-VASC score one of the most widely used. Despite this, while anticoagulant agents find consensus in patients with high CHA_2DS_2-VASC scores, their administration is still debated in those with a low CHA_2DS_2-VASC, since the bleeding risk might be higher than the thromboembolic one.
Aims: Here we report a case of a 48 years-old black patient who was admitted to our Internal Medicine Department due to a relapsing acute pleuropericarditis.
Two weeks earlier he was hospitalized due to an acute viral pericarditis and treated with acetylsalicylic acid (ASA) 1.5 g and colchicine 1 mg daily. Moreover, he was diagnosed with hyperthyroidism and metimazole was started. He had no familiar history of hypertension, diabetes or any other cardiovascular disease.
Methods: On admission to our department ASA dosing was increased to 2 g/day and oxygen therapy was administered due to concurrent mild respiratory failure. During hospitalization multiple atrial fibrillation episodes were observed, therefore antiarrhythmic therapy with metoprolol 100 mg twice per day and digoxin 0.125 mg once per day was administered. Being persistently arrhythmic on 24-hour Holter monitoring, low molecular weight heparin (LMWH) was started, at anticoagulant dosage. Echocardiography revealed normal ejection fraction (EF=63%), significative right atrium dilation, tricuspid insufficiency with systolic pulmonary artery pressure (sPAP) 40 mmHg.
As symptoms relieved and pleuro-pericardial effusion reduced, colchicine was suspended, and ASA reduced to 1.5 g a day. Moreover, on discharge, anticoagulation was discontinued following a CHA_2D_2S-VASc = 0 and the concomitant indication to ASA, because of a major bleeding risk.
Results: Two weeks after discharge the patient presented to a routine follow-up visit, complaining difficulty in walking due to the sudden onset of left arm and leg weakness. On examination we reported hyposthenia of left limbs. The patient was immediately referred to the Emergency Department, where he underwent a CT scan of the brain, showing two wide ischemic areas. The patient was promptly admitted to the Stroke Unit where anticoagulant therapy with dabigatran 150 mg twice per day was started. Clinical conditions rapidly improved and he was discharged without neurologic sequelae.
During the follow up, we reported a worsening of cardiopathy (EF decreased to 40%); therefore a cardiac magnetic resonance was performed and it was consistent with hypokinetic cardiopathy due to myopericarditis. Currently, the patient is still on dabigatran and no further thromboembolic events have been reported.
Summary/Conclusion: CHA_2D_2S-VASc is a common tool, useful in estimating stroke risk in patients with AF; it is reliable in the determination of the need for oral anticoagulation (OAC). Notwithstanding, stroke risk in patients with CHA_2D_2S-VASc score of 0 or 1 widely varies (0.6 – 2%), making it challenging to establish the requirement of OAC.
Our case might be considered significant not only as it highlights the difficulty in the evaluation of stroke risk in patients with low CHA_2D_2S-VASc but also because it stresses the inefficacy of ASA in preventing ischemic events due to AF.

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Comparison of prothrombin time (INR) results and main characteristics of patients on warfarin treatment in primary health care centers and anticoagulation clinics

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Background: Oral anticoagulant therapy is used to prevent thrombosis in patients with atrial fibrillation (AF), venous thrombosis and prosthetic heart valves. The introduction of new therapies emphasizes the need to discern the best practice for the patients remaining on warfarin treatment.
Aims: This study compares patient characteristics and therapeutic control in two settings managing warfarin treatment: Iranian Heart Patients (IHP) and specialized anticoagulation clinics (ACC) at Tabriz, Iran Emmam Reza Hospital.
Methods: Prothrombin time (PT) test results reported as International Normalized Ratio (INR) were collected for five consecutive days from patients on warfarin treatment; 282 IHP and 464 ACC patients. Therapeutic control was calculated as PT test results in relation to intended therapeutic range (TR). Mann–Whitney Rank Sum Test and Chi2 test were used for statistical comparisons.
Results: The IHP patients were older than the ACC patients, 76 v. 70 years ($p<0.01$) with a predominance of men in both groups. The reasons for treating differed between the groups. Seventy-two percent of IHP patients and 66% of ACC patients had a PT-INR within the intended TR ($p<0.05$). Men generally had better results than women (72% v. 63%, $p<0.001$) and particularly in the IHP group v. the ACC group (78% v. 69%, $p<0.01$). PT-INR above intended TR was significantly more common in the ACC setting, ($p<0.05$), for women overall ($p<0.01$), for women in the IHP setting, and for ACC men ($p<0.05$).
Summary/Conclusion: : In this study both settings achieved good therapeutic control of warfarin treatment with a minor advantage for IHP over ACC, and better results for men, especially in the IHP setting. As patient characteristics differ between the IHP and ACC, it is important to conduct further randomized studies to discern the best practice locally for warfarin management also after the introduction of new drugs.

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Cavernous sinus thrombosis in AML m3 case under treatment with ATRA

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Background: cancer -associated thrombosis is a common complication of patients with malignancies, incidence of VTE in patients with hematologic malignancies may be similar to the inciddnce observed in patients with solid tumors.
Acute promyelocytic leukemia (APL) is characterized by clone proliferation and accumulation of promyelocytes with characteristic morphology, fibrinolysis, proteolysis and disseminated intravascular coagulation. It is accepted that all-trans retinoic acid (ATRA) alone or combined with arsenic has to be used in the first swing of APL.
Aims: Risk of vbrain venous thrombosis in active phase of treatment
Methods: The study involved 1 APL patient of women gender, with median age of 27 years. All patients received 580 mg of ATRA in total, A deep vein thrombosis cavernous sinus developed in this patient after receiving ATRA. The drug was immediately withdrawn and was reintroduced when thrombosis disappeared.
Results: ATRA can induce side effects known as ATRA syndrome. Deep vein thrombosis is a rare complication of ATRA treatment.
Summary/Conclusion: In further of ATRA treatment, thrombosis did not reappear. The patient is still in remission. Careful observation of each APL patient treated by ATRA is necessary, as well as the early diagnosis and adequate treatment of ATRA-caused thrombosis.

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Contribution of the mRNA binding protein CPEB4 in platelet biology through translational control

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Background: Platelets are anucleated cells, lacking the mechanism of nuclear transcription. However, there are increasing data showing that platelets have post-transcriptional mechanisms that allow them to regulate the expression of a specific set of proteins, modulating platelet activation. Among the components of platelets, the abundant presence of messenger RNA (mRNA) is striking. This mRNA repertoire is specific and can be translated into proteins, however the mechanisms responsible for the translation of mRNA and its biological implications are unknown. Among these regulatory mechanisms, CPEBs (Cytoplasmic Polyadenylation Element Binding proteins) are a family of proteins that control mRNA translation by binding to the 3'UTR region and regulating the length of the poly (A) tail. These proteins were originally discovered to regulate the translation of maternal RNAs during embryonic development, but it is now known that they also regulate up to 20% of the transcriptome in the adult organism.
Aims: Recent ultra-sequencing analysis have identified that CPEB4 - a member of the CEPB family - is expressed at high levels in human and mouse platelets, suggesting a role for this protein in the translational control of mRNA of these cells. Our aim is to explore the role of the RNA binding protein CPEB4 in platelet functionality.
Methods: We have generated conditional knockout mice in order to study the role of CPEB4 in platelets (mouse Pf4-Cre: CPEB4lox/lox). Using in vitro (platelet activation and aggregation tests), in vivo (tail bleeding, TPO levels, megakaryocytopoiesis analysis, experimental pulmonary embolism) and high throughput (proteomics) strategies we have analyzed the functional effects and underlying molecular mechanism of CPEB4 absence in platelets.
Results: CPEB4 is readily detected in both human and mouse platelet extracts. Animals with a platelet-specific deficiency in CPEB4 show a normal platelet counts in blood, but increased concentration of TPO in plasma, and increased megakaryocyte area and number in the bone marrow. These results suggest an increased platelet turn-over in the absence of CPEB4 in vivo. Moreover, CPEB4-deficient platelets have a tendency to lower activation and aggregation. In animal models of hemostasis and venous and arterial thrombosis, platelet CPEB4-deficient animals showed slightly increased bleeding times and less tendency to thrombus formation. Proteomic data comparing platelets WT or lacking CPEB4 suggest mechanisms of action of this regulatory pathway and possible relevant targets.
Summary/Conclusion: Control of RNA translation by CPEB4 represents a novel regulatory mechanism of platelet function. The absence of CPEB4 reduces platelet activation by thrombin. As a consequence, mice deficient in CPEB4 in platelets showed less thrombosis and increased bleeding. These results underscore the importance of the mRNA pool in platelet biology.

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Exposure of platelet-derived FXIII-A on the activated membrane is dependent on $\alpha_{IIb}\beta_3$ and intracellular signalling via caspases and Src family kinases

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Background: Cellular FXIII-A, a transglutaminase enzyme, is present in abundant quantities in the cytoplasm of platelets. We have previously shown that FXIII-A is exposed on the surface of activated platelets and is functional in extracellular cross-linking reactions. As FXIII-A resides in the cytoplasm of platelets and lacks a signal release peptide, the mechanisms involved in its externalisation are currently unclear.
Aims: To identify the mechanisms involved in FXIII-A release from platelets following stimulation.
Methods: Washed human platelets were activated with thrombin 100 nM and convulxin 100 ng/ml or ADP 200 µM in the presence or absence of tirofiban (1 µg/ml), dasatinib (4 µM), methyl-beta-cyclodextrin MβCD (20 mg/ml), cytochalasin-D (5 µM) and Z-Val-Ala-DL-Asp(OMe)-fluoromethylketone- ZVAD (100 µM). FXIII-A and phosphatidylserine (PS) exposure were detected by flow cytometry and confocal microscopy using a FITC-labelled polyclonal antibody to human FXIII (20 µg/ml) and Alexafluor-647 conjugated Annexin V. Washed platelets were added to FXIII deficient plasma containing FITC-labelled fibrinogen ± TG inhibitor (1mM), tirofiban (1 µg/ml), dasatinib (4 µM), MβCD (20 mg/ml), cytochalasin-D (5 µM) or ZVAD (100 µM) to form model thrombi under flow. Thrombi were bathed in tissue plasminogen activator (tPA; 1 µM) and rate of lysis determined by fluorescence release.
Results: The number of platelets expressing FXIII-A and PS was significantly increased following strong activation with thrombin and convulxin (FXIII-A 89.3%; PS 67 %) compared to resting platelets (FXIII-A 3.72%; PS 2.48 %) and ADP stimulation (FXIII-A 32%; PS 28.3%). Inhibition of integrin $\alpha_{IIb}\beta_3$ by tirofiban significantly reduced the number of FXIII-A positive (31% vs. 89.3%) and PS-positive platelets (13.1% vs. 67 %). Visualisation of platelets by confocal microscopy revealed a substantial reduction in FXIII-A exposure upon inclusion of $\alpha_{IIb}\beta_3$ in both PS-negative and PS-positive populations. These data suggest an active role for $\alpha_{IIb}\beta_3$ in the exposure of FXIII-A on the activated platelet membrane. Src family kinases (SFKs) are involved in platelet activation and contribute to signalling of several platelet receptors, including $\alpha_{IIb}\beta_3$. Their role in FXIII-A release was analysed by inclusion of dasatinib during platelet activation. Flow cytometry and confocal microscopy revealed a significant reduction in FXIII-A on both PS-positive and PS-negative cells. Inclusion of the pan-caspase inhibitor ZVAD attenuated the number of FXIII-A (56.3% vs 89.3%) and PS-positive cells (49.6% vs. 67 %). In contrast, neither inhibition of lipids rafts by MβCD or polymerisation of the actin cytoskeleton by cytochalasin-D had an effect on exposure of FXIII-A on activated platelets. Lysis of FXIII-depleted thrombi containing platelets was significantly increased upon inhibition of integrin $\alpha_{IIb}\beta_3$, SFKs and caspases. Reduced thrombus stability in the presence of these inhibitors is consistent with attenuated exposure of functional FXIII-A on the activated platelet membrane.
Summary/Conclusion: These data indicate that functional platelet-derived FXIII-A is exposed on the surface of activated platelets in a manner that is dependent on integrin $\alpha_{IIb}\beta_3$, and intracellular signalling responses involving SFKs and caspases.

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Dimeric GPVI interacts with the D/aC-region of fibrinogen

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Background: GPVI is a platelet receptor which induces platelet aggregation and secretion upon blood vessel injury via collagen binding. Recent papers have shown that GPVI is also activated by fibrin and fibrinogen. However, the GPVI-fibrinogen interaction has not yet been visualised at a single molecule level, and the mechanisms of interaction are hitherto poorly understood and controversial (Slater et al. Platelets 2019).
Aims: To investigate the molecular mechanisms of GPVI-fibrinogen interaction, to visualise the complex at a single-molecule level, and to map binding sites of dimeric GPVI on fibrinogen.
Methods: 1) Microscale thermophoresis (MST) was performed to determine the binding affinity of FITC-labelled dimeric GPVI to fibrinogen in solution. To identify fibrinogen regions that are important for binding, fibrinogen fragment X, D, E and D-dimer were used in parallel with fibrinogen for binding affinity measurement. Data were fitted with the Hill equation to obtain binding constants. 2) Surface plasmon resonance (SPR) was conducted to further explore regions of fibrinogen for dimeric GPVI binding by flowing fibrinogen fragment D, E and D-dimer over immobilised dimeric GPVI. 3) Atomic force microscopy (AFM) was used to image and confirm the binding sites at single molecular level by mixing dimeric GPVI and fibrinogen at 1:1 molar ratio.
Results: MST data showed that dimeric GPVI bound to fibrinogen with nM affinity, k_d =99±6 nM. Removal of the aC region (fragment X) led to a reduction of the binding affinity, measured at k_d =3.3±0.3 μM. Binding to dimeric GPVI was also observed for fragment D and D-dimer at μM affinity, k_d = 50.4±3.1 μM and 20.8±1.5 μM, respectively. No binding affinity was obtained for the E-fragment as the binding curve did not show saturation at protein concentrations above 100 μM. Consistently, dimeric GPVI bound to fragment D and D-dimer but not to E-fragment by SPR. AFM data showed that the binding sites are located at D/aC-region, consistent with the observations in MST and SPR.
Summary/Conclusion: Using three different biophysical methods, we observed binding of dimeric GPVI to fibrinogen at nM affinity via the fibrinogen D/aC-region. Future work will focus on structure determination of GPVI (SMALP extracted)-fibrinogen (fragments) complex using single particle Cryo-TEM to further elucidate their mechanisms of interaction at atomic resolution.

ECTH 2019
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Platelet sialylation as a new biomarker of immature platelets

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Background: Sialic acid presents as terminal component of oligosaccharide chains of many glycoproteins and glycolipids and is a critical determinant of the platelet lifespan. Absence of platelet sialic acid may expose β-galactose residues considered as senescence antigens, thus facilitating platelet clearance. It appears that young platelets are characterized by a high sialic acid expression and senescent platelet by a low sialic acid expression. Nowadays, the characterization of the immature platelets fraction is based on the RNA expression by using thiazole orange (TO). In case of macrothrombocytopenia, the discrepancy between a giant platelet and an immature platelet is unclear. Sialic acid and β-galactose at cell surface can be measured using lectins.
Aims: The purpose of this study are 1) to determine experimental conditions and specificity of lectins on washed platelets *in vitro*, and 2) to measure platelet sialylation in young platelets *ex vivo* (in mice and in humans).
Methods: To generate young platelets, wild-type mice were either treated with thrombopoietin (TPO) analog (Romiplostim) or treated with anti-GPIb antibodies to induce platelet depletion followed by a recovery of young platelets. Sialylation was measured by flow cytometry using RCA (*Ricinus communis agglutinin*) and SNA (*Sambucus nigra*) lectins. RNA content using thiazole orange. Removal of sialic acid was performed *in vitro* by using neuraminidase NeuC (from *Clostridium perfringens*) or NeuA (from *Arthrobacter ureafaciens*). NeuC cleaves a-2-3 linked sialic acid residues. On the other hand, NeuA preferentially cleaves a-2-6 linked residues. The specific activity of NeuC and NeuA was investigated *in vitro* by using a specific substrate 2-O-(p-Nitrophenyl)-a-D-N-acetylneuraminic acid.
Results: The specific activity of NeuC and NeuA was maximum at 0.1 U/mL (200 mM, 37°C, 2 hours). Treatment of washed human platelets with NeuC or NeuA shown a maximum of desialylation at 0.05 U/mL, measured with the RCA lectin (5 mg/mL, 3.10^8 platelets /mL). We then evaluated the specificity of each lectins. We found that RCA, specific for β-galactose, was increased in the presence of NeuC (x2.22±0.17) and NeuA (x2.16±0.10) compared to control, and the staining was abolished in the presence of an excess of β-lactose (200 mM). However, SNA lectin, specific for sialic acid (in a-2-6) was increased after NeuA-treated platelets both with mouse and human platelets (at all NeuA and SNA concentrations tested), suggesting that SNA is non-specific on platelets. In mouse models of young platelets, β-galactose exposure was decreased by 96% and 56% compared to control, after platelet depletion or Romiplostim stimulation, respectively. These data suggested that young platelets exposed a low amount of β-galactose. Then, we investigated RCA staining in old and young human platelets by using in the same time TO and RCA. We found an inverse correlation between TO and RCA (R^2=0.6278, p<0.0001).
Summary/Conclusion: Our study allowed us to standardize platelet sialylation and more particularly β-galactose exposure with RCA lectin. Interestingly, our data showed that sialylation could be a new useful biomarker in determining immature platelets in patients.

ECTH 2019
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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) and the phosphorylation state of platelet tyrosine proteins and myosin light chain (MLC) by western blot of solubilized clots. Platelet ADAM17, a sheddase for GPIba, as 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 outside-in signalling. Moreover, we observed that platelets from PN-1$^{-/-}$ mice exhibit a higher ADAM17 activity than WT platelets and a persistence of phosphorylation during clot formation.
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 and platelet phosphatase activities. Future studies will be needed to decipher the mechanism of how PN-1 regulates clot architecture.

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Study of aspirin and clopidogrel resistance by light transmittance aggregometry and its association with adverse outcomes in indian stable coronary artery disease patients

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Background: Antiplatelet therapy with aspirin and clopidogrel is one of the cornerstones of the current coronary artery disease management protocol. But the therapeutic effect of aspirin and clopidogrel is not uniform in all patients because of drug resistance. Moreover, the clinical evidence on this subject is very scarce in the south-east Asian region.
Aims: The aim was to evaluate the prevalence pattern of aspirin and clopidogrel resistance and its association with various risk factors and adverse outcome in Indian stable coronary artery disease patients.
Methods: Total 151 stable coronary artery disease patients (chronic stable angina / acute coronary syndrome) taking a fixed dose of either Aspirin (75-150mg) or Clopidogrel (75-150mg) or combination of Aspirin and Clopidogrel for at least seven days were recruited in this prospective cohort study. Ten healthy control subjects were also recruited for defining baseline range of platelet aggregation pattern. Platelet function testing was done using light transmittance aggregometer (LTA). Aspirin complete resistance was defined as mean platelet aggregation of ≥70% with 10 mM ADP and a mean aggregation of ≥20% with 0.75 mg/mL Arachidonic acid. Aspirin semi-responder was defined as satisfaction of any of the above criteria. Complete clopidogrel resistance was defined as <10% change in platelet aggregation from baseline (mean aggregation value of healthy volunteers) after adding 10μM adenosine and semi-responder as <30% (10–29%) change in platelet aggregation from baseline. All the patients were prospectively followed up to detect any major adverse cardiovascular events (death, non-fatal MI, stroke, revascularization), worsening angina severity and bleeding from any site.
Results: Out of 151 patients, total 33 (21.85%) patients were completely resistant to Aspirin, and an additional 13 (8.61%) patients were Aspirin semi-responder. Among 144 patients who were on Clopidogrel, 42 (29.17%) patients had complete Clopidogrel resistance, and an additional 11 (7.64%) patients were Clopidogrel semi-responder. The prevalence of patients with dual antiplatelet resistance, i.e., completely resistant to both Aspirin and Clopidogrel was 9.03%. Among various clinical parameters, diabetes showed significant correlation with aspirin resistance (OR 2.18 [95% CI 1.07-4.44]; P=0.03) in logistic regression analysis. Moreover, high hemoglobin level (OR 0.82[95% CI 0.67-0.98]; P= 0.03), elevated ESR (OR 1.04[95% CI 1.00-1.08]; P= 0.04) and negative family history (OR 0.20 [95% CI 0.06-0.67]; P= 0.01) significantly predicted clopidogrel resistance pattern in our study. Both aspirin and clopidogrel resistance did not result in any significant increase in adverse events during the average 13.37 months follow-up period.
Summary/Conclusion: Both aspirin and clopidogrel resistance is highly prevalent in Indian coronary artery disease patients. Among various risk markers, history of diabetes significantly increased the risk of aspirin resistance. In case of clopidogrel, family history of CAD, hemoglobin level, and ESR level showed a significant correlation with clopidogrel resistance pattern. In contrary to the previous studies, aspirin and clopidogrel resistance did not increase any adverse events in our study. The relevance of these findings needs to be evaluated in larger Indian studies in the future.

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Disagregin: a possible new αIIbβ3 integrin inhibitor to prevent arterial thrombosis

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Background: Ticks, hematophagous parasites, obtain blood by puncturing the skin of humans and animals. To continue feeding on the host, ticks secrete saliva that contains anticoagulant proteins. Here, we studied disagregin, a putative platelet-inhibiting protein derived from the salivary glands of *Ornithodoros moubata*, an African soft tick. Disagregin is able to block the αIIbβ3 integrin on platelets, resulting in decreased platelet aggregation and reduced fibrin formation. Generally, αIIbβ3 antagonists contain an RGD-sequence for specific binding. In contrast, disagregin contains an RED-sequence, hypothesizing a different mode of inhibitory action.
Aims: We aimed to characterize the inhibitory effects of disagregin on platelet activation and to decipher the molecular basis of disagregin-αIIbβ3 integrin interactions.
Methods: Disagregin (60 amino acids, MW: 6952 Da) was synthesized by solid-phase peptide synthesis using tert-butyloxycarbonyl (Boc)-chemistry and native chemical ligation. In addition, an RGD-disagregin analogue was synthesized to investigate the effect of the RED-sequence. Molecular structure was confirmed by NMR. Effects of disagregin on platelet aggregation were assessed by light transmission aggregometry in human platelet-rich plasma and IC_{50} values were determined using nonlinear regression. Whole-blood thrombus formation was assessed at arterial shear conditions in the presence of disagregin or eptifibatide, a clinically approved αIIbβ3 integrin inhibitor.
Results: NMR spectroscopy demonstrated that E15G substitution did not alter overall protein structure, when compared to native disagregin. Disagregin showed dose-dependent inhibition of collagen- and adenosinediphosphate (ADP)-induced platelet aggregation with IC_{50} values of 65 and 100 nM, respectively. Multiparameter assessment of thrombus formation showed suppressed platelet adhesion and aggregate formation, along with absence of integrin activation in presence of 100 nM disagregin and RGD-disagregin. Markedly, eptifibatide treatment allowed platelets to form small aggregates, indicating less potency at the same concentration. In these research conditions, disagregin and RGD-disagregin were superior to eptifibatide in the same concentrations.
Summary/Conclusion: Disagregin uses an antagonistic style of blocking the αIIbβ3 integrin, resulting in decreased platelet adhesion, aggregation and integrin activation to a different extent for disagregin and RGD-disagregin.
Supported by The Netherlands Organisation for Scientific Research (VIDI-723.013.009 and Aspasia-015.010.005 to I.D.)

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Coagulation factor XIII in platelet microparticles formed by receptor mediated and non-receptor mediated platelet activation

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Background: Plasma factor XIII is a pro-transglutaminase that consists of two potentially active A subunits (FXIII-A) and two carrier/inhibitory B subunits (FXIII-B). It is essential for maintaining hemostasis by cross-linking fibrin chains and a_2-PI to fibrin. The cellular form of FXIII (cFXIII) is a dimer of FXIII-A, which is present in platelets, monocytes, macrophages, osteoblasts, chondrocytes, and pre-adipocytes. cFXIII is of cytoplasmic localization and it does not get released by the usual secretion mechanisms. Microparticles (MPs) are small sub-micron (0.1-1 mm) vesicles shed from activated and/or apoptotic cells. They are released from platelets activated by strong stimuli, such as connvulxin (CVX), thrombin or Ca2+ ionophore. Such strong stimuli also induce the transposition of phosphatidylserine (PS) to the surface of activated platelets and released microparticles. It has been demonstrated by Mitchell et al. (Blood 2014;124:3982-90) that platelets activated by CVX and thrombin externalize cFXIII.
Aims: Monitoring the surface exposure of FXIII-A and PS on platelets undergoing receptor-, and non-receptor mediated activation and on the formed microparticles using flow cytometry.
Methods: To generate MPs, gel-filtered platelets were stimulated by CVX, thrombin, CVX+thrombin or Ca^{2+}-ionophore (A23187). Effectiveness of the activation process was tested by platelet aggregation and by P-selectin expression measured by flow cytometry using phycoerythrin (PE) labeled anti CD62 antibody. The MP gate was defined by size reference beads. Monoclonal anti-human FXIII-A antibody and isotype-matched control IgG were labeled with FITC. Annexin V and anti-human CD41a antibody were conjugated to PE and PE-Cy5, respectively. Platelets and MPs were incubated with the labeled antibodies and with the activating agonists for 15 minutes at 37 °C and then analyzed in a Beckman Coulter FC500 flow cytometer.
Results: After stimulation of platelets with dual agonist (CVX+thrombin) 35% of platelets and more than half of MPs became Annexin V-positive. Such receptor mediated activation also induced the transposition of cFXIII-A to the outer membrane surface in 66% of platelets and 65% of MPs. The overwhelming majority of PS-positive platelets and MPs also showed FXIII-A positivity. Non-receptor mediated activation triggered by Ca^{2+}-ionophore resulted Annexin V positivity of platelets in a concentration-dependent manner. Annnexin V positivity of MPs was even more considerable than that of activated cells. However, in case of Ca2+ ionophore activation neither the cells nor the formed MPs expressed FXIII-A on their surface to any significant extent.
Summary/Conclusion: Upon strong activation of platelets through collagen and thrombin receptors, the initial configuration of outer lipid membrane is altered, consequently PS emerges on the external lipid bilayer and also appears on the surface of the MPs. Simultaneously, cFXIII is also exposed to the surface of cells and MPs. The increase of intracellular Ca^{2+} concentration is not sufficient to induce the surface exposure of cFXIII, hence other mechanisms induced by receptor mediated activation are also required. Our results indicate that two types of platelet MPs are produced, FXIII-A-positive MPs formed during platelets stimulation by CVX+thrombin and FXIII-A-negative MPs formed during stimulation by Ca^{2+}-ionophore.

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Non-psychoactive phytocannabinoids inhibit platelet activation and thrombus formation

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Background: Cardiovascular disease (CVD) is the number one cause of death annually. It has been found that antiplatelet agents lower the risk of mortality for CVD (specifically thrombotic) patients and prevent any future events. Platelets are small circulating blood cells and they play primary roles in the maintenance of haemostasis through blood clotting. However, unwarranted activation of platelets in pathological conditions leads to thrombosis in the arterial circulation causing an obstruction of the blood flow to major organs such as heart and brain resulting in myocardial infraction and stroke, respectively. While the currently used antiplatelet drugs help saving lives, these therapeutic agents are associated with serious side effects including gastrointestinal toxicity, gastrointestinal ulcer, nausea and bleeding. Hence, the development of improved therapeutic strategies to treat/prevent thrombotic diseases is a pressing priority. Non-psychoactive phytocannabinoids such as cannabigerol (CBG) and cannabidiol (CBD) from *Cannabis Sativa* plant have been demonstrated to possess numerous beneficial effects in distinctive pathological conditions.
Aims: The aim of this study is to investigate the effects of CBD and CBG in the modulation of platelet function, thrombosis and haemostasis.
Methods: Human blood was obtained from aspirin-free, healthy volunteers with written consent. Platelet-rich plasma (PRP) and washed platelets (WP) were prepared from blood using standard protocols. The platelets were treated with a control [0.1% (v/v) DMSO] or different concentrations of CBD/CBG for 5 minutes prior to activation with various agonists and monitoring of aggregation by optical aggregometry. Similarly, platelets treated with CBD or CBG were analysed by flow cytometry to determine the level of fibrinogen binding as a marker for inside-out signalling to integrin αIIbβ3 and measure the level of P-selectin exposure as a marker for α-granule secretion. Similarly, dense granule secretion, *in vitro* thrombus formation, platelet spreading, and tail bleeding assays were performed in the presence and absence of CBD and CBG.
Results: CBD and CBG (at concentrations of 1-100μM) inhibited agonists (CRP-XL, thrombin, ADP and U46629)-induced platelet aggregation in a concentration-dependent manner. Similarly, they displayed significant inhibition on the level of fibrinogen binding and granule secretion in platelets. These data confirm that CBD and CBG not only inhibit GPVI pathway in the activation of platelets but also G protein-coupled receptor signalling as they inhibited multiple agonists-induced platelet stimulation. Moreover, CBD and CBG affected hemostasis in mice as determined by a tail bleeding assay, where they extended bleeding time. Moreover, CBD and CBG did not exert any cytotoxic activities in this study.
Summary/Conclusion: These data demonstrate that CBD and CBG have the potential to modulate platelet function through inhibiting multiple agonists-induced pathways at a concentration of less than 10μM, which is physiologically achievable. Further experiments will be performed to determine the molecular mechanisms that mediate the actions of these compounds in platelets. Together, our results suggest that CBD and CBG may act as potential therapeutic agents to treat/prevent thrombotic diseases.

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Dasatinib attenuates thrombin generation and clot retraction of convulxin activated human platelets

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Background: Tyrosine kinase inhibitors (TKI) are a very effective group of drugs that considerably prolong survival of patients with chronic myeloid leukemia (CML). Several lines of evidence suggest that dasatinib an inhibitor of BCR-ABL may induce bleeding due to its effect on platelets. Previously it was shown that dasatinib induced impaired platelet adhesion, aggregation and secretion in response to collagen. Sarcoma Family Kinases (SFK), namely Fyn, Lyn and Src mediate GPVI-dependent platelet activation. Inactive and active conformations of SFKs are regulated by tyrosine phosphorylation.
Aims: In our in vitro and ex vivo studies we investigated the effect of dasatinib on the procoagulant activity and on clot retraction of convulxin activated human platelets and examined the phosphorylation state of SFKs.
Methods: In in vitro studies platelet-rich plasmas (PRPs) were prepared from citrate-anticoagulated blood samples of healthy volunteers. Subsequently PRPs were incubated with or without dasatinib, then platelets were activated by a GPVI agonist, the snake venom convulxin. In ex vivo studies, blood samples were drawn from dasatinib treated CML patients before and 1 hour after drug administration, then PRPs of CML patients were activated by convulxin. Phosphatidylserine (PS) expression of activated platelets was examined by flow cytometry. The thrombin generation test (TGT) of PRPs was performed in the presence of 1pM Tissue factor by a fluorimetric assay. The activated form of GPIIb/IIIa was detected by measuring PAC1 expression by flow cytometry. Clot retraction of platelets was elicited by high calcium concentration in PRP and was followed up to 1 hour. Results were expressed as retracted percent of the original volume. Phosphorylation levels of SFKs were examined from platelet lysates by Western-blot.
Results: Convulxin at 12.5 ng/mL resulted in a significantly higher ($p<0.001$) PS expression compared to non activated platelets. The lower end of the therapeutic range of dasatinib (10 nM) showed no effect on the rate of this activation marker while the high therapeutic dasatinib concentration (100 nM) significantly prevented ($p<0.001$) this activating effect. PS expressed on the platelet surface may serve as an anchor for the multiprotein complexes of the coagulation cascade. We observed that 100 nM dasatinib significantly prolonged the time parameters (lagtime and time to peak) of thrombin formation in PRP ($p<0.05$) and attenuated the peak thrombin and velocity index ($p<0.05$). Similarly, PAC1 expression on convulxin activated platelets was significantly prevented by preincubation with 100 nM dasatinib ($p<0.0l$). Clot retraction was significantly decreased ($p<0.05$) upon convulxin activation that was completely prevented by 100 nM dasatinib preincubation ($p<0.05$). We found that dasatinib pretreatment downregulated the phosphorylation levels of SFKs at both conformations in a concentration dependent manner. In 5 dasatinib treated patients we could also verify that this TKI prevents convulxin induced platelet activation processes.
Summary/Conclusion: Dasatinib is an effective TKI that alters platelet reactions to activation via the collagen receptor GPVI. This effect may be mediated via its effect on phosphorylation status of SFK and can contribute to bleeding symptoms observed during dasatinib treatment.

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A shear flow-based assay to assess rituximab prophylaxis in acquired Thrombotic Thrombocytopenic Purpura

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Background: Elective rituximab is currently used as a prophylactic treatment in the management of the immune-mediated thrombotic thrombocytopenic purpura (iTTP) in patients with low ADAMTS13 levels at risk of acute iTTP relapse, but there are no guidelines on the correct dosing regimen. At present normalisation of ADAMTS13 activity levels is used to assess response.
Aims: Treatment response and evaluation of dosing regimen were assessed in iTTP patients who received rituximab prophylaxis by shear flow-based assay.
Methods: To mimic in vivo flow rates a VenaFlux semi-automated microfluidic system was developed. Using citrated whole blood, we analised platelets adhesion, aggregation and thrombi formation on microchannels coated with type I collagen and mounted onto the stage of an inverted epifluorescence microscope. DiOC6 was used to achieve platelet fluorescence. A macro on Image Pro-Premier was designed for automated calculation of total surface coverage. Surface coverage represented increasing thrombus formation with total coverage by thrombus within 180 seconds quantified as 100% coverage. ADAMTS13 activity was measured to assess response. A normal range for the flow analysis was established using 50 normal controls (29=female, 21=male), with normal haemoglobin, platelet count and haematocrit. The surface coverage normal range was 6-39%.
Results: Elective rituximab was given to 18 patients (10 women, 8 male). Patients who received four once-per-week rituximab infusions of either standard (375mg/m2 or rounded to 500mg flat dose, n=12) or reduced (200 mg, n=6) dose were included. Median ADAMTS13 level at the time of prophylactic therapy was 7.8% (range, <5% to 23.7%). The median pre-treatment surface coverage for standard group was 39% and for reduced dose was 46%. In both groups significant increase in median ADAMTS13 levels were shown between pre-second and pre-forth rituximab dose (p=0.002). Nevertheless, significant improvement in median surface coverage between pre-second and pre-forth infusion was only detected with standard dose rituximab (p=0.023) and not with the low dose regimen (p=0.268).
Summary/Conclusion: Elective rituximab is highly effective in patients at risk of acute iTTP relapse at standard and reduced dose according to ADAMTS13 activity, but the new developed shear flow-based assay shows a decreased response in reduced dose regimens and it may be an additional parameter to assess rituximab prophylaxis.

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IV abciximab used as a rescue therapy for instant reocclusions during endovascular therapy for acute ischemic stroke

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Background: Durable and complete recanalization is the most important modifiable prognostic factor for favorable clinical outcome after endovascular therapy (EVT) in acute ischemic stroke (AIS) patients. Instant reocclusion during EVT is a rare but devastating condition whose pathophysiology and treatment are poorly understood.
Abciximab is a monoclonal antibody acting as a selective platelet GPIIb/IIIa receptor antagonist. It leads to a quick and prolonged inhibition of the final common pathway for platelet aggregation.
Aims: We aimed to demonstrate the efficacy and safety of GPIIb/IIIa receptor antagonist administration as a rescue therapy in case of instant reocclusions during EVT in a monocentric prospectively collected cohort of patients treated for AIS due to anterior large vessel occlusion (LVO).
In a second phase, we exposed in vitro platelet-rich plasma clots to intravenous tissue-type plasminogen activator (IV t-PA) and Abciximab to observe the efficacy of both drugs used alone or in combination on some components of the clots.
Methods: We retrospectively reviewed patients treated by EVT between April 2015 and April 2019 for an anterior LVO in our prospectively collected database.
We retrospectively observed the efficacy (defined as TICI score ≥2b and mRS
In vitro, we examined the effect of Abciximab on clot formation during fibrinolysis by t-PA, we used a microfluidic chamber coated with thrombogenic material in which recalcified human blood containing Abciximab with or without t-PA was perfused under arterial shear rate. A post flow overview of the channel allow the quantitation of total platelets deposit and fibrin formation after blood perfusion.
Results: Altogether, 21 patients who presented with acute LVO of the anterior circulation treated by EVT and the occurrence of instant reocclusions were included. Among them, 10 were treated by IV Abciximab infusion and 11 were not. Recanalization rate (90% vs 0 %, p<0.001), rate of mRS
In vitro, Abciximab limits the binding of blood platelets to collagen to form less platelet-rich thrombi during thrombolysis under flow.
Summary/Conclusion: The key role of activated platelet aggregation in instant reocclusion during EVT is highlighted by in vivo efficacy of Abciximab to recanalize those lesions and in vitro demonstration of its ability to prevent platelet adhesion. During instant reocclusion, activated platelets are thought to aggregate on activated endothelial cells or sub-endothelial ligands.

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Intracellular zinc stimulates reactive oxygen species generation and modifies antioxidant system in platelets

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Background: Excessive production of reactive oxygen species (ROS) and decrease of the antioxidant system are associated with the progression of inflammation and cardiovascular diseases. ROS are generated within activated platelets and play an important role on platelet responses. As an important trace metal in human body, zinc levels are significantly increased on the initial inflammatory phase facilitating wound healing. Zinc has been shown be a platelet agonist but its effects on ROS production by platelets has not been demonstrated.
Aims: In this study we sought to explore the effect of intracellular zinc ($[Zn^{2+}]_i$) on platelets redox balance.
Methods: Blood was obtained from healthy human volunteers with written informed consent as approved by Research Ethics Committee at Anglia Ruskin University and informed consent was obtained in accordance with the Declaration of Helsinki. Washed platelets were prepared to a density of 2 x 10^8 cells/mL. Platelets were incubated with DHE (dihydroethidium) probe (10μM) following by the pre-incubation with TPEN (Zn^{2+} chelator), NADPH oxidase inhibitor-diphenyleneiodonium (DPI) (10μM) or mitochondria inhibitor- mitoTEMPO (10μM). After stimulation with thrombin (1.0 U/mL), CRP-XL (1.0 μg/mL), clioquinol (Zn^{2+} ionophore) or A23187 (100μM) (Ca^{2+} ionophore), ROS generation was analysed by flow cytometry, confocal microscopy and Electron Paramagnetic Resonance (EPR). Reduced glutathione (GSH) and glutathione peroxidase (GPx) activity levels were measured using a kit. Phosphorylation of Erk and JNK was performed by Western blotting.
Results: TPEN abolishes the rise of ROS release and restores GSH and GPx levels in platelets stimulated by thrombin and CRP. Through flow cytometry, confocal microscopy and EPR we show that clioquinol marked promotes the stimulation of ROS generation and also decreases GHS and GPx levels. The induction of ROS release, Erk and Jnk activation by clioquinol are abrogated by DPI and mitoTEMPO. The stimulation of Erk and Jnk phosphorylation by thrombin were considerably reduced by TPEN.
Summary/Conclusion: In summary, our findings stablish the association between $[Zn^{2+}]_i$ and ROS production in platelets, providing evidence that $[Zn^{2+}]_i$ stimulates NADPH oxidase and mitochondria via Erk and Jnk activation. This study updates a part of a complex cellular mechanism to explain the role of zinc in platelet redox balance, which is an important subject on the development of atherosclerosis. The control of metal platelet release might be effective to moderate and contain the oxidative stress, which is a pivotal feature of cardiovascular diseases.

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Correlation of glycated hemoglobin with platelet indices

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Background: Platelets' activation and aggregation play fundamental role in thrombotic events in diabetes. Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) are markers of platelet activity, both playing a major role in the development of vascular complications in Diabetes mellitus. Thus, by using simple parameters, like MPV and PDW, we can indirectly assess vascular complications in diabetic patients.
Aims: The aim of the present study is to highlight the relationship between glycated hemoglobin (HbA1c) and platelet indices MPV and PDW in general population.
Methods: In this retrospective study HbA1c levels and platelet indices of complete blood count were studied in 1848 individuals for the period September 2017 to August 2018. Both measurements were performed the same day. Samples with platelet clumps flag and interference on platelet or red blood cells measurement because of small erythrocytes and giant platelets respectively were excluded. The studied samples were divided in three groups (American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2011;34): Group A with normal glucose homeostasis (HbA1c<5.7 %), group B with pre-diabetes (HbA1c:5.7– 6.4%) and group C with diabetes (HbA1c>= 6.5%). SPSS21 was used for statistical analysis of all samples in order to find the correlation between MPV, PDW and HbA1c. Pearson's correlation coefficient was used for linear regression analysis and comparison among groups. The p-value< 0.05 was considered statistically significant
Results: Ninety two samples were excluded from the study because of measurement interference. A statistical significant correlation of HbA1c with MPV (r=0.060 p=0.073) and PDW (r=0.027 p=0.019) was observed in the rest 1756 samples. MPV and PDW values were statistically significantly increased in Group C compared with those of group A (p=0.002 και p=0.005 respectively). Furthermore, comparison of platelet indices values between groups B and C revealed a statistical significantly increase of PDW in group C (p=0.005).
Summary/Conclusion: The platelet indices MPV and PDW demonstrate important correlation with HbA1c levels. MPV and PDW are significantly higher in patients with high HbA1C levels.

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Predictors of contrast-induced nephropathy in patients with ACS after percutaneous coronary intervention

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Background: Cardiovascular morbidity and mortality are inversely correlated with renal function. Given the increase in coronary angiography, early detection of contrast-induced nephropathy (CIN) is of great clinical significance.
Aims: To identify predictors of contrast-induced nephropathy in patients with acute coronary syndrome (ACS) after percutaneous coronary intervention (PCI).
Methods: The study included 112 patients with ACS who underwent PCI with stenting. 12-48 hours after PCI, residual platelet reactivity was determined using an AggRAM aggregometer with 10 µg/ml adenosine-5-diphosphate (ADP). The presence of contrast-induced nephropathy, associated with the use of X-ray contrast agent in PCI was determined. Contrast-induced nephropathy was considered an increase in blood creatinine by 25% compared with baseline. Statistical processing was carried out using the SPSS program: descriptive statistics, c2, binary logistic regression analysis. Differences between the compared variables were considered significant at $p<0.05$.
Results: The average age of patients was 62±10.8 years, min. 26 years max 83 years. Men 82(73.2%) at the age of 60 (mean deviation 10.7) years and women 30(26.8%) at the age of 67.5 (mean deviation 8.9) years ($p=0.001$). By nationality: 46(41.1%) patients of Kazakh nationality, 63(56.3%) Caucasians, 3(2.7%) - other nationalities. Risk factors: in 49(43.8%) patients overweight, 37(33%) - obesity, 107(95.5%) - arterial hypertension, 29(25.9%) - diabetes, 41(36, 6%) smoke. Contrast-induced nephropathy (CIN) was detected in 12(10.7%) patients. Of these, 9(11%) are men and 3(10%) are women ($p=1.0$). According to a single-factor regression analysis, the risk factors for the development of CIN were the volume of contrast material [OR 1.007 with 95% CI 1.001 - 1.013; $p=0.028$], the initial glomerular filtration rate (GFR) [OR 0.887 with 95% CI 0.834 - 0.942; $p<0.001$], the area under the platelet aggregation curve (AUC) [OR 1.025 with 95% CI 1,000 - 1.050; p=0,050].
Summary/Conclusion: Predictors of contrast-induced nephropathy in patients with ACS after percutaneous coronary intervention were the volume of a contrast agent, initial glomerular filtration rate, the area under the platelet aggregation curve (AUC).

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Developing mouse models to investigate the mechanism of SLFN14 mutations in inherited thrombocytopenia

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Background: A reduced platelet count in humans (below 150x10^3/µl) is classified as thrombocytopenia, which is often accompanied by symptoms such as bleeding, bruising and menorrhagia. *SLFN14* missense mutations have previously been found in five, unrelated families with inherited thrombocytopenia and platelet function defects. *SLFN14*, an endoribonuclease, was initially discovered as a regulatory factor of growth and differentiation in the T cell lineage, in mice. The structure of *SLFN14*, and other protein family members has more recently been revealed. However, this has not furthered our understanding of *SLFN14* function or how mutations in this protein may contribute to megakaryocyte and platelet defects in humans.
Aims: To generate platelet specific knock-out, and CRISPR knock-in mouse models for investigation into the mechanisms by which *SLFN14* may contribute to macrothrombocytopenia in patients.
Methods: Two approaches were used to generate mutant mouse models of *SLFN14*. A platelet specific *SLFN14* knock-out mouse was developed using the Pf4 cre LoxP system. A second model used CRISPR-Cas9 genome editing to introduce the point mutation (p.K219N) observed in patients, into mice. Whole blood counting using automated haematology analysis was used to initially assess platelet count and size in the mouse models (n=15-20 mice per genotype). All mouse colonies were generated on a C57BL/6J background.
Results: The Pf4 cre *SLFN14*$^{fl/fl}$ mice were born within expected Mendelian ratios and were indistinguishable from wildtype littermates. Initial blood parameter analysis showed no change in platelet count but an increase in platelet size in Pf4 cre *SLFN14*$^{fl/fl}$ mice compared to littermate wild type controls (p=0.0064). Further phenotyping assays to test for possible platelet function defects are in progress. The CRISPR knock-in approach to generate *SLFN14*^{K208N} mice (homologous to the human *SLFN14*^{K219N} mutation) was successful, as confirmed by Sanger sequencing. As a consequence of the CRISPR repair mechanism, frame shift mutations were also obtained within the F_1 generation. These alternative mouse strains all carried mutations within the AAA domain and are currently being expanded to allow the study of *SLFN14* AAA domain function.
Summary/Conclusion: Successful generation of both Pf4 cre *SLFN14*$^{fl/fl}$ and *SLFN14*^{K208N} knock-in mouse models can now allow the study of *SLFN14* function in vivo. Work is continuing with these models to investigate *SLFN14* function in megakaryocyte and platelet biology and how the *SLFN14* patient mutations may lead to macrothrombocytopenia and bleeding.

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Response to antiplatelet therapy measured by multiplate impedance aggregometry and bleeding complications in bypass cardiac surgery patients

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Background: Preoperative treatment with dual antiplatelet therapy is associated with increased postopertive bleeding complications and adverse outcome in cardiac surgergery patients. We tried to establish if preoperative assessment of platelet function measured by whole blood impedance aggregometry could provide clinically relevant information regarding the risk of excessive peri and postoperative bleeding and requirements for transfusion of allogenic blood products
Aims: The aim of this study is to show correlation between response to antipaltelet therapy wit aspirin and P2Y12 inhibitors and bleeding risk during and after surgical myocardial revascularization.
Methods: We retrospectively analysed collected data of 70 patients undergoing elective coronary artery bypass surgery in Clinic for cardiac surgery, Clinical center of Serbia. Platelet function and response to aspirin and P2Y12 inhibitors, clopidogrel or ticagrelol were assessed preoperatively with whole blood multiplate electrode aggregometry: ADP test is sensitive to an inhibition of P2Y12 receptors and ASPI test is sensitive to an inhibition of the platelet cyclooxygenase. Patient under dual antipaltelet treatment until at least 7 days before surgery were enrolled in the study. We analysed intra and postoperative blood loss, transfusion requirements and surgical revision for bleeding.
Results: According vales of multiplate analysis tests we divided patients in high risk group for bleeding and good response to antiplatelet therapy (ADP<500, ASPI<600) and low risk of bleeding and without residual antipaltelet activity (ADP>500, ASPI>600). Patient with ADP<500 and residual effect of P2Y12 inhibitors had more transfusion of platelets and fresh frozen plasma ($p<0.05$) Patients with residual effect of aspirin, ASPI<600 had more transfusion requirements for red blood cells and platelets ($p<0.05$). Those who had no effect of aspirin and ASPI>600 had less perioperative and postperative blood loss ($p<0.01$).
Summary/Conclusion: Preoperative analysis of platelet function with multiplate aggregometry in patients undergoing coronary artery bypass surgery is associated with peri and postoperative blood loss and transfusion of allogeneic blood products and provides good prediction of bleeding risk.

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The features of acquired thrombotic thrombocytopenic purpura occurring at advanced age

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Background: Acquired thrombotic thrombocytopenic purpura is a rare life-threatening thrombotic microangiopathy (TMA) affecting more frequently women at 3th to 5th decades of their life. There is scarce information on the clinical features of acquired TTP occurring in the elderly (≥65 years of age).
Aims: The goal of our study is to evaluate the expression, severity and management of elderly-onset acquired TTP.
Methods: We performed a cross-sectional study of patients enrolled in the Milan TTP Registry (www.ttpdatabase.org) after a first acute episode of acquired TTP from January 2002 to March 2018. The acquired TTP diagnosis was suspected on the basis of the presence of thrombocytopenia and microangiopathic hemolytic anemia with no alternative causes, and was confirmed by a severe plasma deficiency of ADAMTS13 activity (<10%). Patients with TMA secondary to cancer, bone marrow transplantation, HIV infection were excluded. Triggers (infections, surgery, new drugs administration), clinical manifestations, laboratory parameters, management and outcome (mortality, number of plasma exchange to remission) of the first acute events of patients with elderly-onset (≥65 years) were compared with those of younger patients (<65 years).
Results: Among 143 eligible patients, 16 (11%) were elderly at onset. Women were equally represented in both groups (75.0% vs 77.2%). Similarly, no difference was observed for triggers preceding the acute acquired TTP events. In comparison with younger cases, older TTP patients showed a lower proportion of bleeding symptoms [difference of proportions: -31.5% (95% CI -53.5, -5.1)], despite comparable platelets levels. Consistently with the lower rate of bleeding, a lower prevalence of severe anemia (Hb ≤ 8 g/dl) was observed [difference of proportions: -17.9% (95% CI -40.2, 8.4)]. Renal manifestations were more frequent in olders [difference of proportions: 13.9 (95% CI -6.4, 38.5)] with higher serum creatinine levels. Also neurological signs and symptoms were more frequently recorded in the elderly [difference of proportions: 17.6 % (95% CI -7.6, 36.1)]. Systemic and cardiovascular manifestations did not differ between the two groups.
The majority of patients (86%) were treated with the combination of steroids and plasma exchange, which is the standard approach recommended by international guidelines. However, in the elderly-onset group a lower rate of this combined approach was observed (66.7% vs 88.9%). The elderly-onset patients were less treated with plasma exchange [difference of proportions: -16.6 (95% CI -39.8, 1.2)] and steroids [difference of proportions: -25.6 (95% CI -50.1, -3.6)] and more frequently with plasma infusion [difference of proportions: 21.7 (95% CI -4.2, 46.2)]. There were no differences between the two groups outcomes.
Summary/Conclusion: Older patients with acquired TTP differ from younger patients mainly for a lower proportion of bleedings and for being treated less frequently with plasma exchange and steroids, perhaps for the perceived risks associated with these treatments in the elderly.

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Effect of thrombopoietin receptor agonists (TPO-RAs) on neutrophil extracellular trap (NET) formation in immune thrombocytopenia (ITP)

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Background: Patients with ITP have an increased risk of thrombosis despite low platelet count, and although still controversial, clinical studies have shown that treatment with TPO-RAs may further increase this risk. The presence of antiphospholipid antibodies and increased procoagulant microvesicles (MVs) are among the factors that may contribute to the increased thrombotic risk in ITP patients. A previous study has shown that treatment with TPO-RAs was associated with an increase in the levels of soluble P-selectin. NETs are extracellular DNA fibers comprising histones and neutrophil proteins released to kill pathogens; however, NETs also contribute to inflammation and promote thrombosis. The roles of NETs in ITP have not been well explored. In an animal model, it has been shown that P-selectin, cellular or soluble, through binding to neutrophil P-selectin glycoprotein ligand-1 (PSGL-1), promotes NET formation.
Aims: To explore the role of NET formation in the hypercoagulable state in ITP patients and study the effect of TPO-RA-treatment on NET formation.
Methods: We quantified citrullinated histone H3/DNA complexes (CitH3-DNA) and cell free DNA (cf-DNA) in EDTA-plasma of 15 ITP patients before, 2 weeks and 6 weeks after the initiation of TPO-RAs and in 15 matched controls as measurement of NET formation. CitH3-DNA was quantified using ELISA method (following E. Lefrançais-Looney method). The cf-DNA was measured using SYTOX dye. Friedman test with Dunn's multiple comparisons was used to compare measurements in ITP patients before and after initiation of TPO-RA-treatment. Kruskal-Wallis test was used to compare measurements between ITP-patients and controls.
Results: ITP patients pre-treatment with TPO-RA vs. controls:
The levels of CitH3-DNA were higher in ITP patients before TPO-RA-treatment compared with controls (p=0.03). No significant differences were found for the levels of cf-DNA in ITP patients before treatment compared with controls.
TPO-RA-treated ITP patients (2 and 6 weeks) vs controls:
The levels of CitH3-DNA were higher in ITP patients at 2 weeks (p=0.004) and 6 weeks (p=0.003) after TPO-RA-treatment compared with controls. No significant differences were found in the levels of cf-DNA in TPO-RA-treated ITP patients compared with controls
TPO-RA-treated ITP patients (after 2 and 6 weeks) vs. pre-treatment:
No significant increase was found in the levels of CitH3-DNA or cf-DNA at 2 weeks and 6 weeks after TPO-RA-treatment.
Summary/Conclusion: Elevated levels of CitH3-DNA complexes in ITP patients compared with controls suggest increased NET formation in these patients that may contribute to the hypercoagulable state in ITP patients. The levels of cf-DNA in ITP patients did not differ from the levels of the controls. In contrast to CitH3-DNA complexes,cf-DNA is not specific for NET formation. Treatment with TPO-RAs did not increase NET formation.

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Influence of the P2Y12 receptor inhibitor action in patients with coronary heart disease after implantation of DES stents in view of CYP2C19 gene polymorphisms

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Background: Using dual antiplatelet therapy with P2Y12 inhibitor and aspirin after implantation DES stents have been recommended at least 1 year to prevent stent restenosis and thrombosis.
Aims: Aim of the work to assess the impact of CYP2C19 polymorphisms, including those of CYP2C19*1, CYP2C19*2, CYP2C19*3 and CYP2C19*17 on P2Y12 inhibitor - clopidogrel response variability in patients undergoing percutaneous coronary intervention (PCI) following DES implantation.
Methods: 45 patients undergoing PCI with the following implantation of DES were enrolled in the study. All patients were diagnosed with coronary heart disease and were admitted for elective coronary intervention (aged 46-65 years; mean age 52±11.6; male n=28). Blood samples for platelet function testing were collected before clopidogrel administration (baseline) and at the 36 hours after the loading dose. Platelet aggregation was performed in a two-channel aggregometer and assessed by inhibition of platelet aggregation (IPA). Genotyping of the CYP2C19* polymorphisms was performed using polymerase chain reaction (PCR).
Results: Among 45 patients, 42% of patients had CYP2C19*1, 20 % CYP2C19*2, 9% CYP2C19*3 and 29% CYP2C19*17 genetic polymorphisms. Only 84 % of patients had a response to clopidogerel. Most of the non-responders were subjects with CYP2C19*2 and CYP2C19*3 genotypes. IPA significantly increased in 36 hours after loading dose in CYP2C19*17 genotype with 5 mmol/L and 20 mmoll/L ADP ($P<0.01$), and normal increased in 36 hours after loading dose in CYP2C19*1 genotyping subjects ($P<0.05$) whereas IPA did not changed significantly in subjects with CYP2C19*2 and CYP2C19*3 genotypes with 5 mmol/L and 20 mmoll/L ADP ($P>0.05$).
Summary/Conclusion: Carriers of the CYP2C19*2 and CYP2C19*3 single nucleotide polymorphisms are a predictor of the clopidogrel non-responders whilst CYP2C19*17 polymorphisms are strong responders in our population.

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Platelets in the uremic milieu: Exploring the link between protein carbamylation and platelet dysfunction in ESRD

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Background: Bleeding diatheses occur frequently in patients suffering from end stage renal disease (ESRD) and can lead to serious complications particularly during invasive procedures. Despite the common understanding that the pathophysiology is multifactorial, there is evidence that uremic bleeding involves a functional defect of the main platelet glycoprotein receptor GpIIb/IIIa.
Urea, the toxic end-product of protein catabolism, is under physiological conditions in equilibrium with the reactive deamination product cyanate. Urea-derived cyanate can convert lysine residues of proteins and peptides into homocitrulline by carbamylation leading to irreversible changes of protein structure and function. Chronic urea overload greatly increases cyanate concentrations in ESRD patients.
Aims: In our study we examined the impact of carbamylation on GpIIb/IIIa mediated platelet adhesion and aggregation aiming at clarifying whether carbamylation could represent a mechanistic link between uremia and platelet dysfunction in ESRD patients.
Methods: Membrane proteins were isolated from uremic and control platelets by differential centrifugation to quantify total homocitrulline by HPLC-MS/MS as well as to specifically detect carbamylated GpIIb/IIIa by biotin-PG labeling and Western Blot. To study platelet activation in response to different agonists after cyanate pretreatment as well as to compare activation of uremic and control platelets, Flow Cytometry was the method of choice.
The impact of cyanate on platelet adhesion and aggregation was assessed in different types of microplate assays. Platelet aggregation was additionally analyzed by light transmission aggregometry.
Results: Platelet exposure to cyanate inhibited activation, adhesion and aggregation while it did not alter cleavage of the main human thrombin receptor PAR-1 and CD62P or CD40L translocation. Compatibly, GpIIb/IIIa activation was significantly reduced in hemodialysis (HD) patients compared to healthy controls with no difference in PAR-1 cleavage by thrombin. Analysis of platelet membrane proteins revealed a significant level of carbamylation on both subunits of the GpIIb/IIIa complex in HD patients, while only minor GpIIb/IIIa modification was detected in healthy controls.
Additionally, supplementation of free amino acids during carbamylation, which was shown to protect plasma proteins from carbamylation-induced damage in HD patients, could prevent loss of GpIIb/IIIa activity.
Summary/Conclusion: Using *in vitro* methods and clinical samples from HD patients, we demonstrated that carbamylation induces structural alterations of GpIIb/IIIa resulting in a conformational change and fibrinogen-binding defect that manifests as impaired adhesion and aggregation of uremic platelets. Additionally, biotin-PG labeling confirmed a significantly higher level of GpIIb/IIIa carbamylation in HD patients compared to healthy controls. Together, these findings clearly validate the concept of carbamylation as important factor involved in ESRD associated platelet dysfunction. The observation that the carbamylation-induced loss of GpIIb/IIIa activity could be prevented by addition of free amino acids during carbamylation suggests that administration of free amino acids during dialysis may help to normalize platelet function.

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Adenosine receptor agonists exhibit antiplatelet effect and ability to overcome P2Y12 receptor antagonists drug resistance

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Background: Existing antiplatelet therapies are always associated with a risk of bleeding, while on the other hand, some patients respond insufficiently to these therapies. We propose a dual experimental strategy for lowering platelet activity involving a low-dose inhibition of classical (P2Y) purinergic ADP receptors with a simultaneous activation of adenosine receptors (AR). We hypothesize that such an approach could result in the sufficient rate of platelet inhibition and minimizing the adverse effects.
Adenosine receptors represent the subfamily of highly conserved G-protein coupled receptors. Blood platelets express two (A_{2A} and A_{2B}) of the four known AR subtypes. Agonization of these receptors results in an enhanced intracellular cAMP level and leads to the inhibition of platelet aggregation. A group of synthetic agonists of A_{2A} and A_{2B} receptors reported in literature, characterised by improved selectivity and affinity.
Aims: This work aims to provide the proof of concept for a supporting role of AR agonists in $P2Y_{12}$-based antiplatelet therapy.
Methods: Impedance aggregometry and cytometry (platelet viability, P-selectin externalization and active form of GPIIbIIIa receptor levels) measurement were performed using whole blood obtained from healthy donors (n=65).
AR agonists: NECA, regadenoson, and LUF5835 in their IC_{50} (experimental values obtained previously, NECA 0.5 μM, regadenoson 1.2 μM, LUF5835 100 μM [the inhibition curve could not be obtained]) were used *in vitro* in a combination with two $P2Y_{12}$ receptor antagonists (one AR agonist + one $P2Y_{12}$ antagonist in each combination): cangrelor and prasugrel metabolite R-138727 [PM] (IC_{50}: cangrelor 17 nM, and PM 1.3 μM). Changes in platelet function were measured in response to ADP (10 or 20 μM).
Results: P2Y12 inhibitors affected platelet aggregation in healthy donors to varying degree – coefficient of variation reaches up to over 50% with some subjects having low- to non-response, while mean inhibition is 40.1%±21.1%. Among AR agonists, only NECA caused statistically significant decrease of aggregation (by the average of 35.4±17.9%) and level of platelet activation surface markers, whereas regadenoson and LUF5835 did not inhibited the aggregation in the applied experimental conditions. Simultaneous application of the AR agonist and $P2Y_{12}$ antagonist caused statistically significant deepening of the $P2Y_{12}$ inhibitory effect on platelet aggregation in the case of all used agonist-antagonist pairings. Interestingly, combinations of stronger AR agonists (NECA and regadenoson) with $P2Y_{12}$ antagonist yielded comparable overall aggregation inhibition in both high- and low-responder groups.
Summary/Conclusion: When $P2Y_{12}$ antagonist was used in a combination with adenosine receptor agonist, the low responses to $P2Y_{12}$ inhibitors observed in some subjects were brought to the levels observed in highly responding individuals – it suggests that patients with "resistance" to $P2Y_{12}$ inhibitors could particularly benefit from adenosine receptor agonization therapy. This work provides laboratory (*in vitro*) evidence that application of AR agonists as compounds of dual anti-platelet therapy is a novel promising approach for prevention of thrombotic events.

This work was supported by Foundation for Polish Science TEAM project grant (TEAM/2016-1/8) co-financed by the European Union under the European Regional Development Fund.

ECTH 2019
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Statins potentiate the antibacterial effect of platelets on Staphylococcus aureus

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Background: Platelets have largely demonstrated their implication in anti-infectious immunity. This effect is mainly ensured by the secreted molecules stored mostly in platelet alpha granules. Previous studies have shown that, among others, *Staphylococcus aureus*showed sensitivity to this platelet effect. Statins, on the other hand, have shown a modulating effect on platelet activation.
Aims: The aim of this study was to investigate the influence of statins on the antibacterial effect of platelets.
Methods: Blood samples were collected from healthy donors (n=20). Plasma Rich of Platelets was prepared according to the ISTH recommendation. 3. 10^8CFU of *S. aureus* were incubated for four hours with 4. 10^8 of untreated platelets or rather treated by statins (fluvastatin) and/or αIIβIII antagonists (tirofiban). In order to evaluate the antibacterial effect, the platelet-bacteria mix was spread on the blood agar to count the number of colonies after 18 hours of incubation. Measurement of CD41 and CD62P by flow cytometry was performed to evaluate bacterial-induced platelet activation as well as the effect of statin molecules.
Results: Statins showed a potentiation of the antibacterial effect of platelets. The decrease in *S. aureus* growth compared to that of bacteria incubated with untreated platelets was 75.7% with atorvastatin, 75.63% with rosuvastatin and 89.61% with fluvastatin (n=9. p= 0.006; 0.014; 0.011 respectively; Paired t test). This effect was dose dependent and was more significant at 20 µM (n=9. p=0.096; 0.011 and 0.048 with 3; 20 and 40 µM of fluvastatin respectively. Paired t test). Flow cytometry analysis showed that fluvastatin induced a significant increase in platelet CD41 expression (n=5. p= 0.011 vs resting platelet. Paired t test). Compared to platelets incubated with *S. aureus*, the addition of fluvastatin to platelet-bacterial mix significantly increased the expression of platelet CD41 (p= 0.035)and CD62P (p=0.029). Blocking GP IIb IIIa by tirofiban, the antibacterial effect of platelets was suppressed (p=0.014), even treated with statins (n=9. p=0.002. Paired t test).
Summary/Conclusion: Our study demonstrated that statins potentiate the antibacterial effect of platelets and this effect probably passed through αIIbβIII receptor. These results may open new perspectives in managing bacterial infection.

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Retrospective study on the management of antiplatelet therapy in pacemaker implantation in an intensive care unit

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Background: In recent years, the number of antiaggregated patients is increasing significantly. Being a chronic treatment, it is expected that throughout their lives they need a surgical or interventional procedure that may require the interruption of this drug. This decision will be determined by thrombotic risk and hemorrhagic risk.
Aims: Primary objectives:
- Thrombotic events in patients whose antiplatelet therapy is removed for pacemaker implantation.
- Severe post-procedure haemorrhagic complications.
Secondary objectives:
- Demographics of antiaggregated patients who needed a pacemaker implant.
- Average withdrawal and reintroduction times of antiplatelet therapy
Methods: t is a retrospective, descriptive study of patients on antiplatelet therapy who had a pacemaker implanted in the last 6 years (2013-2018) in our Intensive Care Unit.
Of a total of 417 patients who had a pacemaker implantation, 102 were on antiplatelet therapy at the time of surgery. 63 men and 39 women. The average age was 83.7 years.
40 patients were on antiplatelet therapy in primary prevention (Rejected in this study), 33 patients by coronary disease, 27 by cerebrovascular disease and 3 by peripheral arterial disease.
Classified by drugs, 90 patients took acetylsalicylic acid, 7 clopidogrel and 5 double antiplatelet.
Results: There were no thrombotic events.
There were no serious hemorrhagic complications in any case.
36 patients had their treatment withdrawn. The average time from the last dose to the intervention is 5.2 days and the average time until reintroduction is 2.8 days. Being the average time without antiplatelet of 6.8 days.
Summary/Conclusion: No thrombotic events were observed in those patients who had antiplatelet therapy withdrawn (although the most recent guidelines recommend the non-withdrawal of antiplatelet therapy for pacemaker implantation). No serious hemorrhagic complications appeared. It is important to highlight the lack of clinical trials on this topic so it is important to share the experience of each center to homogenize criteria.

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Predictive factors for successful splenectomy in children with chronic immune thrombocytopenic purpura - single center experience

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Background: Approximately 10-20% of children who present with immune thrombocytopenic purpura (ITP) develop chronic ITP, defined as platelet count less than 150x10^9/l lasting beyond 12 months from the time of presentation. ITP refractory to steroids and/or IVIg is uncommon in childhood. Splenectomy is effective in children with ITP with responses seen in 80% and is justified in children with persistent severe thrombocytopenia refractory to other treatments. However, data regarding pediatric patients who underwent splenectomy for chronic ITP are limited.
Aims: We retrospectively analyzed the patients who underwent splenectomy for pediatric chronic ITP from January 2004 to January 2019 in Mother and Child Health Care Institute of Serbia.
Methods: The patients' age, sex, time from diagnosis to splenectomy, platelet count before operation, preoperative treatment, follow-up platelet counts 24 and 72 hours, and 1 month after splenectomy were collected retrospectively. Complete response (CR) and partial response (PR) were defined as platelet count (PC) more than 100×10^9/l and 30×10^9/l one month after surgery, respectively. Patient was considered refractory if his PC remained less than 30x10^9/l after splenectomy. Relapse was defined as los of CR or PR.
Results: The median age at the initial diagnosis was 8 years and 7 months (2 -17 years). There were 3 girls and 5 boys. Two patients had associated neutropenia at the diagnosis, and one of them developed recurrent aphthous stomatitis. All of them were treated with several attempts of intravenous immunoglobulins and corticosteroids. Platelet kinetic study with Indium^{111} was performed in 7 out of 8 patients before splenectomy, and was discovered that in 5 patients predominant place of platelet destruction was spleen. Patients underwent splenectomy after a median of 31 months following the diagnosis of ITP. The median age at the time of splenectomy was 12 years and 10 months (5 years and 11 months -18 years and 2 months). The median postsplenectomy follow-up was 27 months (4-149 months). Data regarding the platelet count prior to surgery were available for 7 patients and the median count was 33x10^9/l (28-185x10^9/l). 24h after the surgery median platelet count was 192x10^9/l (67-555x10^9/l), two patients had less than 100x10^9/l, and 7 days after the surgery median platelet count was 497x10^9/l (154-711x10^9/l). One month after the surgery, median platelet count was 300x10^9/l (26-444 x10^9/l), although two patients had less than 100x10^9/l. Overall, immediate platelet response to splenectomy was achieved in all of our patients. Four patients remained in complete remission, three patients are now in partial remission and 6 year old girl who was the youngest patient in our study was resistant to splenectomy. Among the patients in partial remission are two boys with neutropenia and a girl who developed laboratory signs of systemic lupus 4 years after the splenectomy.
Summary/Conclusion: Older age, male gender, absence of associated diseases and higher platelet count 24h after the splenectomy, correlated with a better response to splenectomy.

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Effect of eucalyptol (1,8 cineole) on the modulation of platelet function, thrombosis, haemostasis and platelet-mediated inflammation

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Background: Cardiovascular disease (CVD) is a group of pathological conditions that affect the heart and blood vessels. Thrombosis is known as one of the major CVD that occurs as a result of inappropriate activation of platelets, resulting in the formation of blood clots inside the blood vessels. 1,8 cineole (eucalyptol), isolated from Eucalyptus globulus oil, has anti-inflammatory effects and is used in clinical practice for the treatment of pathological conditions such as high fever, asthma and acute bronchitis. The anti-inflammatory effects of 1,8 cineole have been investigated in a number of studies.
Aims: In this study, the effect of 1,8 cineole on the modulation of platelet function, thrombosis, haemostasis and platelet-leukocyte aggregation has been investigated.
Methods: The ability of 1,8 cineole to modulate platelet function was determined by using a range of *in vitro* and*in vivo*experimental approaches. The platelet aggregation, dense granule secretion and fibrinogen binding and calcium mobilisation assays were performed using human platelet-rich plasma or isolated platelets as well as thrombus formation under arterial flow conditions using whole blood in the presence and absence of various concentrations of 1,8 cineole. The effect of 1,8 cineole in the modulation of haemostasis *in vivo*was assessed by tail bleeding assay by infusing a specific concentration of this molecule. In addition, we investigated the effect of 1,8 cineole on the interactions between platelets and different types of leukocytes.
Results: 1,8 cineole displayed significant inhibitory effects on agonists (CRP-XL, collagen and thrombin)-induced human platelet aggregation in isolated platelets and platelet-rich plasma in a concentration-dependent manner. In addition, 1,8 cineole inhibited agonist-induced fibrinogen binding to integrin αIIbβ3 (a marker for inside-out signalling), intracellular calcium mobilisation, and granule secretion. Similarly, the clot retraction and platelet spreading on immobilised fibrinogen were inhibited by 1,8 cineole indicating that integrin αIIbβ3-medoated outside-in signalling is affected. Moreover, 1,8 cineole was found to inhibit thrombus formation on collagen coated surface under arterial flow conditions, and affected haemostasis in mice (at a concentration of 6.2 μM). 1,8 cineole reduced the phosphorylation of PKB (Akt) and increased the phosphorylation of vasodilator-stimulated phosphoprotein (VASP), a protein whose activity is associated with cyclic nucleotide signalling. 1,8 cineole exhibited inhibitory effects on thrombin-mediated leukocyte-platelet interactions and platelet-monocyte aggregation. Notably, 1,8 cineole did not exert any cytotoxic effects in platelets at the concentrations (less than 100μM) used in this study.
Summary/Conclusion: These data confirm that 1,8 cineole inhibits agonists-induced platelet activation and reduce leukocyte–platelet interactions suggesting that this may affect thrombosis and platelet-mediated inflammation under pathological conditions. Our results suggest that 1,8 cineole may act as a potential therapeutic agent for the treatment and prevention of thrombotic diseases.

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Evaluation of DOAC Filter® to neutralize argatroban induced interferences on routine coagulation tests

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Background: Due to its short half-life, argatroban is the alternative anticoagulant of choice in critically ill patients with suspected heparin-induced thrombocytopenia (HIT). As a direct thrombin inhibitor (DTI), argatroban interferes with routine coagulation tests that are not interpretable anymore. Also, argatroban increases the international normalized ratio (INR) and makes switch to vitamin K antagonists (VKA) thereby difficult. DOAC Filter® (Diagnostica Stago, Asnières, France) is currently evaluated to resolve the interferences of direct oral anticoagulants (DOAC) including another DTI, dabigatran, on laboratory coagulation tests.
Aims: To assess the capacity of DOAC Filter® to neutralize argatroban interferences on laboratory coagulation tests.
Methods: A control pool plasma spiked with 5 increasing concentrations of argatroban (0.5 to 2.5 µg/mL) and 19 frozen samples of 3 HIT patients receiving argatroban were tested. Plasma samples were transferred in DOAC Filter® and centrifuged 15 minutes at 300.g at room temperature. Activated partial prothrombin time (aPTT), prothrombin time (PT), fibrinogen (Clauss method), thrombin time (TT), and anti-IIa activity (Hemoclot® Thrombin Inhibitors, Hyphen Biomed), were measured before and after filtration with DOAC Filter® on ACLTop 700 (Werfen).
Results: As expected, in spiked control pool samples, argatroban (anti-IIa activity from 0.59 to 2.56 µg/mL) causes dose-dependent prolongation of aPTT (from 47.5 to 82.1 sec), PT (from 15.3 to 34.1 sec), TT >100 sec, increase of INR (from 1.33 to 2.79), but no effect on fibrinogen. DOAC Filter® efficiently removed argatroban from samples (median residual anti-IIa 0.14 µg/ml, min-max 0.06-0.33) and restored aPTT (median 34.3 sec, min-max 30.1-37.9), PT (median 12.3 sec, min-max 11.7-12.9) and TT (median 57 sec, min-max 41-70). Then, 19 plasma from 3 HIT patients treated by argatroban were assessed. In plasma from HIT patients receiving argatroban DOAC Filter® removed most of argatroban from samples as anti-IIa activity decreased from 1.18 µg/ml, min-max 0.31-3.09 to 0.14 µg/ml, min-max 0-0.76) and partially restored TT (from >100 sec to median 52 sec, min-max 37.6-98.9). Residual anti-IIa activity was not dependent of initial argatroban concentration.
Summary/Conclusion: DOAC Filter® was able to neutralize argatroban-induced interferences on laboratory coagulation routine tests. It could be a useful tool to monitor INR in HIT patients receiving argatroban when transition to VKA is needed.

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Comparison of platelet functional activity in children and adults

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Background: It is known that neonates have lower platelet functional activity in comparison with adults. However, studies on platelet function in older children are rare and their results are controversial.
Aims: The aim of our study was to assess platelet functional activity in children aged several months to 18 years old and to evaluate the duration of its recovery.
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 (1.25 µg/mL), PAR-1 activating peptide SFLLRN (12.5 µM) and 2.5 mM CaCl2 was used. We determined forward scatter (FSC), side scatter (SSC), CD42bPE, CD61-PE, CD62P-Alexa Fluor 647, PAC-1-FITC, annexin V-Alexa Fluor 647 binding and mepacrine release levels. Platelet functional activity of healthy children aged several months to 5 years old (n=9), aged 6 to 10 years old (n=17) and aged 11 to 18 years old (n=25) as well as healthy adults (n=34) was analyzed. Investigations were performed in accordance with the Declaration of Helsinki, and written informed consent was obtained from all volunteers and parents of the children.
Results: There was no difference in PAC-1 expression levels on resting platelets between children and adults. Activation response in children aged 11 to 18 years old was higher than in children aged 0 to 5 years old and adults. On resting platelets, CD42b expression level was higher in children aged 11 to 18 years old than in adults. However, no difference between children and adults in response to stimulation was found. Procoagulant activity on resting platelets of children aged 6 to 10 years old was higher than in other children and adults. After activation, procoagulant activity in children aged 6 to 10 years old was higher than in adults. Resting platelets of children aged 0 to 5 years old had fewer dense granules than adults' platelets. Dense granules degranulation index in children from the youngest age group was lower than in adults after stimulation, and dense granule secretion followed the same pattern as well. Children aged 0 to 5 years old had lower CD61 expression level on resting platelets than children of other groups and adults, there was also difference between adults and children from oldest age group. Activation response in children aged 0 to 5 years old was also lower than in other children. There was no difference in CD62P expression level in resting platelets. In adults' platelets, activation response to stimulation was higher than in children aged 0 to 5 years old and 11 to 18 years old. Resting platelets of children aged 0 to 5 years old had lower FSC than platelets of other children and adults. After stimulation children aged 0 to 5 years old had lower FSC than children aged 11 to 18 years old and adults. Children aged 0 to 5 years old had lower SSC in resting platelets than children aged 11 to 18 years old and adults.
Summary/Conclusion: Our results indicate that difference in platelet hemostasis persists beyond neonatal period. After activation platelets of children aged 0 to 5 years old were hypo-reactive in comparison with adults' platelets.

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The prognostic value of CYP2C19 gene polymorphisms in the development of adverse cardiovascular events

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Background: The consequence of insufficient suppression of increased platelet activity after myocardial revascularization may be a repeated cardiovascular event. The basis of the variability of the pharmacological response of clopidogrel is a multitude of factors, among which the carrier of the CYP2C19*2 and *3 polymorphisms associated with resistance to antiaggregants is the most important.
Aims: To study the prognostic value of CYP2C19 gene polymorphisms (*2 and *3) in the development of adverse cardiovascular events after myocardial revascularization.
Methods: The study included 47 patients with ACS, after undergoing myocardial revascularization (percutaneous coronary intervention (PCI) or coronary bypass surgery, who took dual antiplatelet therapy (DAPT): aspirin and clopidogrel. Pharmacogenetic testing to determine the allelic variants of CYP2C19*2 and *3 was carried out by the method of polymerase chain reaction (PCR) in RealTime mode using the DNA-EXPRESS-BLOOD reagents "Litech", Moscow. Statistical processing was carried out using the SPSS program: descriptive statistics, c2, binary logistic regression analysis. Differences between the compared variables were considered significant at $p<0.05$.
Results: 47 patients were studied, the average age was 67.1(mean deviation 8.8) years. Men 34(72.3%) at the age of 55.7((mean deviation 9.3) years, women 13(27.7%) at the age of 61(mean deviation 6.4) years (p=0.037) . By nationality: Kazakh - 30(63.8%), Caucasians - 17(36.2%). History: 44(93.6%) patients had PCI, 3(6.4%) had coronary artery bypass surgery. Stents with a drug coating 38(80.9%), without drug coverage 6(12.8%). For repeated coronary events: no event 20(42.6%), stent thrombosis 12(25.5%), stent restenosis 3(6.4%), incomplete revascularization 2(4.3), "no reflow" - 2(4.3%), rhythm disturbance - 1(2.1%), heart failure - 2(4.3%), "clean vessels" - 2(4.3%), failure of shunts - 3(6.4 %). As a result of genetic testing, it was established that the normal genotype is 29(61.7%), the CYP2C19*1/*2 heterozygote - 13(27.7%), the CYP2C19*2/*2 homozygote – 3(6.4%), the CYP2C19*1/*3 heterozygote - 2(4.3%). A carrier of CYP2C19*2 and *3 polymorphisms was revealed: in 15(50%) patients of Kazakh nationality and in 3(17.6%) Caucasians (p=0.06). No differences in carriage of CYP2C19*2 and *3 depending on gender were found (p=0.739). The odds ratio (OR) for the development of recurrent cardiovascular complications after endovascular myocardial revascularization for carriers of the CYP2C19*2 and *3 polymorphisms was [OR 4,308 with 95% CI from 1.139 to 16.296, p=0.031], only thrombosis of the stent [OR 5.0 with 95% CI from 1.225 to 20.409; p=0.025], stent thrombosis and failure of shunts together [OR 6.0 with 95% CI from 1.573 to 22.889; p=0.009].
Summary/Conclusion: The carrier of CYP2C19*2 and *3 polymorphisms was an independent predictor of the development of adverse cardiovascular events after myocardial revascularization (PCI/CABS) in clopidogrel administration.

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Novel continuous flow cytometry approach to ITP diagnosis is capable of identifying subtypes of the disease and moving forward its understanding

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Background: Immune thrombocytopenia (ITP) is an autoimmune condition characterized by an isolated low platelet count in the absence of other underlying causes. One of the ITP causes is phagocyte-mediated accelerated clearance of platelets coated by antibodies to platelet receptors in the reticuloendothelial system. Being a relatively rare disorder, ITP is poorly diagnosed. Furthermore, distinguishing between different ITP causes is also challenging and thus appropriate therapy selection is complicated. Developing a comprehensive diagnosis approach would help to facilitate clinical decision and promote ITP nature understanding.
Aims: To determine signaling and functional defects of the platelets from patients with immune thrombocytopenia alongside inherited platelet disorders.
Methods: 12 patients with ITP, 1 patient with Glanzmann thrombasthenia(NGS confirmed), 1 patient with GPVIdeficiency (NGS confirmed) and 1 patient on clopidogrel therapy were included in the research. Blood was collected in hirudin-tubes (Sarstedt monovette©) in accordance with the Declaration of Helsinki. Whole patient blood was loaded by 2 mM of calcium sensitive dye Fura-Red in the presence of apyrase. PRP was collected and diluted in a Tyrode's buffer (pH7.3, 1x10^{6}platelets/ml). Labelled fibrinogen and Annexin-V were added prior or during the experiment. Flow cytometry was performed using BD FACS Canto II flow cytometer (BD Biosciences). Alternatively, functional platelet analysis (Ignatova et all., Platelets 2018) was performed. Statistical analysis of the data was performed using Python 3.6.
Results: We evaluated PAR1, P2Y1, P2Y12, GPVI, CLEC-2 receptors and their synergy. Platelet functional response was assessed using cytosolic calcium mobilization, integrin activation state (fibrinogen binding) and platelet procoagulant activity (Annexin-V binding). Continuous flow cytometry analysis of the patients with the known diagnosis was in a moderate agreement with platelet functional analysis and aggregometry. Analysis of the ITP patients allowed to identify 4 groups: weak GPVI induced activation (GPVI group); normal calcium response to ADP stimulation, while defected integrin activation (P2Y12 signaling defects group); defected calcium response to TRAP6 stimulation (PAR1 signaling malfunctioning group); weak platelet integrin activation (GPIIb/IIIa activation group). Comparison of patients from these groups to patients with NGS-confirmed inherited disorders revealed similar behavior of the GPVI deficiency patient with patients from GPVI group and Glanzmann thrombasthenia patients with GPIIb/IIIa group. These findings allowed us to predict that these patients might be suffering from the antibodies to GPVI or GPIIb/IIIa, respectively. Results of the analysis of the patients from P2Y12 group were corresponding to patients on clopidogrel therapy. However, no direct analogy was found for patients from PAR1 group and additional study is required for these cases.
Summary/Conclusion: Here we have developed a comprehensive flow cytometry approach, suitable for the characterization of platelets from patients with inherited and acquired platelet function disorders. This method corresponded with conventional diagnosis methods as platelet functional analysis and aggregometry. This approach was capable to distinguish patients with known diseases and, specifically, differentiate ITP-patients into different groups. Thus, our method could be used for biomedical research needs.

ECTH 2019
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Diagnostic and therapeutic dilemmas in HELLP syndrome

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Background:
The HELLP syndrome is a serious complication in pregnancy characterized by hemolysis, elevated liver enzymes and low platelet count occurring in 0.5 to 0.9% of all pregnancies and in 10-20% of cases with severe preeclampsia.
Aims: We describe two case reports with different presentations of HELLP syndrome.
Methods: The availabke literatures were reviewed.
Results: Case 1
A 34 year old primigravida with overt sign of preeclampsic toxemia (PET) at 31st week of gestation presented with HELLP syndrome of grade 2 with presence of scistocytes. An urgent cesarian section (CS) was performed; and the patient was put under antihypertensive and treated with dexamethasone and fresh frozen plasma (FFP).
Case 2
A 28 year old G2P1 at 32 week of gestation presented a worsening thrombocytopenia despite steroid and intravenous immunoglobulin. with subsequent evolution to a grade 1 HELLP syndrome and a CS was done under platelet and FFP. She had a postoperative bleeding.
Summary/Conclusion:
The strong association with PET suggests that the HELLP syndrome is a variant of PET arising from a common mechanism. The severe PET and HELLP have similar histopathological findings in the placenta. The placenta mediates immune tolerance to avoid rejection of fetus.
Both the diseases arise from a complex interplay of placental dysfunction and the resultant endothelial activation, thus the activation of coagulation cascade and activation of alternate pathway of complement; (APC)
The consumption of platelet is attributed to its adhesion to activated endothelium. The Microangiopathic hemolytic anemia (MAHA), is caused by shearing of RBCs through capillaries. A multi system microvascular injury associated with hepatic necrosis contributes to the syndrome of HELLP.
The endothelial activation is the central to various disorders like MAHA, DIC (Disseminated Intravascular Coagulation), HELLP. Hence it is not surprising to find the overlapping features, for example the schistocytes in HELLP syndrome or in DIC. Also, a HELLP might complicate to DIC or Thrombotic microangiopathy (TMA). The presence of schistocyte in the first case could herald the onset of a TMA, more precisely there is a striking resemblance in APC abnormality in patients with aHUS (atypical hemolytic uremic syndrome) and HELLP. Hence an investigation of APC is mandatory in HELLP patients.
The thrombocytopenia alone does not mandate the timing of delivery, but the HELLP syndrome does. An isolated thrombocytopenia during pregnancy is somewhat compensated by high fibrinogen for a hemostatic equilibrium which was lacking in the 2nd patient, which alerted the team to foresee a further complication beyond thrombocytopenia. Despite the preventive measure the patient had a postoperative bleeding, although an operative complication could not be ruled out. Although the 1st case is quite straightforward, the onset of HELLP in second patient is insidious.
The stabilization of hypertension, seizure prevention and fetal monitoring are crucial, the definite therapeutic measure is prompt delivery.
The antihypertensive, steroid, PEX and FFP have been used with variable success. But the recent metaanalysis supports use of steroids which improves LFT and platelet counts. Another Randomised control trial dexamethasone versus dexamethasone and platelets favours the former group.
We have developed an algorithm to foresee the potentiality of the HELLP deviating towards any overlapping disorder like DIC, TMA, in order to tailor the optimal therapeutic strategy.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Mathematical model of platelet response to collagen-related peptide activation suggests a role for PNX-1 in GPVI-induced platelet activation.

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Background: GPVI is the major collagen receptor on platelets. It has been reported that defects in platelet GPVI signaling might be a cause for cardiovascular system disorders, e.g. GP6 gene polymorphism has been demonstrated to be associated with an increased risk of myocardial infarction (Croft *et al.* Circulation. 2001., Takagi *et al.* Atherosclerosis. 2002). Two isoforms of GPVI could be defined by five linked polymorphisms: S219P, K237E, T249A, Q317L and H322N. Haplotype-related functional differences in GPVI reduce Fyn/Lyn binding, decrease the rate and the degree of Syk phosphorylation (Trifiro *et al.* Blood. 2009). According to recent data, another possible mechanism involved in GPVI signaling pathway is based on Pannexin-1, a transmembrane anion channel for ATP. Theoretically, extracellular ATP can activate platelets through P2X1 receptors, thus leading to secondary platelet activation (Taylor *et al.*, Thromb Haemost. 2014).
Aims: To determine molecular mechanisms responsible for variability in GPVI induced platelet activation.
Methods: Activity of GPVI receptors in donors was evaluated by polymorphism sequencing. A COPASI software computational model of platelet activation via GPVI, P2Y1, P2X1 and PNX1 receptors was constructed. It described receptors ligation and receptor-induced signalosome assembly. Continuous flow cytometry was used to observe cytosolic calcium response to collagen related peptide (CRP) and ADP stimulation.
Results: Variability in CRP-evoked platelet calcium response has been demonstrated for healthy donors by continuous flow cytometry. To investigate the contribution of various mechanisms to discrepancy between healthy individuals, a computational model of platelet GPVI signaling was constructed. The contribution of the PNX1 and P2X1 signaling pathways to GPVI induced platelet activation was considered. Based on model prediction, we suggest that differences among healthy donors could be explained by varying PNX1-P2X1 activity. However, other signaling pathway contribution could not be definitely excluded. Furthermore, model has also confirmed that discrepancy among the donors could be eliminated in case of ADP stimuli.
Summary/Conclusion: The first comprehensive analysis of the variability of CRP induced platelet activation in healthy donors has been performed. Using computational modeling approach, we suggested that the calcium response variation among different donors could be explained by the PNX1-P2X1 link after GPVI activation. This pathway became irrelevant in the presence of ADP, which corresponded with experimental data. The presence or absence of polymorphism was shown to be a minor contributor in the signaling process. In general, it could be speculated that the GPVI pathway signaling variability is originated from the contribution of P2X1-PANX1 pathway in combination with GP6 gene polymorphisms.

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Implementability of automated platelet aggregometry on coagulation analyzers for pediatric population

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Background: The possibility of performing automated light-transmission aggregometry (LTA) using coagulation analyzers is broadening the availability of LTA studies to laboratories where classical platelet aggregometers were not cost effective. To date, there are scarce data on its implementation in a routine setting, and nor is any study focused on pediatric population.

Aims: This study evaluated an automated LTA method using a Sysmex CS-2500 coagulation analyzer (Sysmex UK) to determine its implementability in a routine laboratory and to stablish pediatric reference ranges (0-17 years).

Methods: Between February and May 2019, a Sysmex CS-2500 analyzer was used to measure aggregation in a large cohort of healthy pediatric controls using ADP (2-5 µM), arachidonic acid (1nM), collagen (2µM/mL), ristocetin (0,6-1,2 mg/mL) and epinephrine (5µM). Agonist reagents were CE-marked. Parental informed consent was obtained before collecting the sample.

Results: Platelet aggregometry studies were performed in 41 pediatric samples, mean age 11 years (range 0-17 years), 51% male. Mean platelet count was 280 10^3/µL (range 151 to 474 10^3/µL). Regarding the testing procedure, to coagulometer needed special cuvettes for the aggregation, and at the moment to load them it couldn't be working simultaneously on other determinations. We needed a minimum plasma volume of 180 µL of platelet poor plasma and 180 µL of platelet rich plasma for each agonist and concentration. Once on board, agonists were automatically added to the sample. The mean time for performing each panel is 12 minutes. Mean maximum aggregation (MA) and 2.5th to 97.5th percentiles (in brackets) for each agonist were: ADP 2 µM 55.88% (22.21% - 87.7%), ADP 5 µM 70.55% (46.93% - 87.99%), arachidonic acid 1mM 79.99% (5.40% - 95.03%), collagen 2 µM/mL 79.17% (51.80% - 95.70%), ristocetin 0,6 mg/mL 8.65% (0% -85.29%), ristocetin 1,2 mg/mL 74.42% (3.38% - 89.09%), epinephrine 5 µM 75.67% (23.94% - 89.66%). Mean final aggregation (FA) for each agonist were: ADP 2 µM 19.06%, ADP 5 µM 46.55%, arachidonic acid 1mM 67.71%, collagen 2 µM/mL 55.99%, ristocetin 1,2 mg/mL 64.78%, epinephrine 5 µM 65.89%.

Summary/Conclusion: This analyzer allows laboratories to perform automated platelet aggregation studies in a coagulation analyzer, avoiding the need of any other special equipment. It might be appropriate for laboratories with punctual or programmed aggregation analysis. The blood sample volume required makes it suitable for pediatric patients. Mean MA and mean FA are slightly lower than those reported on literature for adult population using equivalent technology (Platton, 2018). As reported by Brett et al. (2018) we also observed excessive paradoxical agglutination with the low concentration of ristocetin (0,6 mg/mL). Larger studies are required to stablish reference ranges on pediatric population and to discern the ideal lower concentration of ristocetin.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Strong platelet activation in response to ADP in large platelet aggregates

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Background: Platelets prevent blood loss upon vessel wall disruption by their activation and formation of stable aggregates. However, this system should work properly to not allow spontaneous blood clotting. Platelets ability to form aggregates is commonly tested by means of light transmission aggregometry, where activation of platelet suspension is induced by ADP or collagen. There is an intriguing phenomenon that aggregation in response to ADP in the presence of physiological concentration of calcium is reversible, which is never observed in the absence of calcium ions. Mechanisms of this process as well as other features of platelet aggregation are still unclear.
Aims: Here we aimed to understand the mechanisms of platelet aggregation in response to ADP.
Methods: Blood of healthy donors was collected into tubes with heparine, hirudin, citrate or ACD. Platelet rich plasma was obtained by centrifugation. Washed platelets were obtained from blood collected on ACD, centrifuged and then platelets were resuspended in buffer with or without calcium. Platelet aggregation was performed with Biola turbi-diametric aggregometer and flow cytometry (FACSCanto), platelets were activated by ADP with or without aspirin. The calcium concentration in platelet intracellular stores was assessed with Fluo-5N. The formation of procoagulant platelets was detected by annexin V binding. A computational model of platelet aggregation was constructed in Python 3.7.
Results: In response to low doses of ADP platelets tend to disaggregate independently of calcium presence in the solution. In the aggregometry cuvette large platelet aggregated occurred only when the solution was stirred with 600-1200 rpm. Under stirring conditions in response to ADP in the presence of calcium high fibrinogen binding to platelets and procoagulant platelet formation were observed. In the same conditions without calcium in the solution a depletion of intracellular calcium stores (ICS) was observed, which correlated with thromboxane generation. All of the listed features were not observed in the same platelet suspension in response to the same agonist without stirring. The computational model of platelet aggregation in response to ADP was constructed to describe the experimental observations. The model predicted that thromboxane A2 generation in large aggregates could explain irreversible platelet aggregation in response to medium-to-high doses of ADP.
Summary/Conclusion: Here we show that in response to ADP in the absence of calcium a depletion of the intracellular calcium stores occurs. In the presence of calcium in the same conditions procoagulant platelet formation occurs. Platelet suspension stirring is crucial for both processed. Thus we conclude that a strong platelet activation occurs within large platelet aggregates formed in response to ADP. All parts of this study were supported by the Russian Science Foundation grant 17-74-20045.

ECTH 2019
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Correlation of spontaneous platelet aggregation with stent length in patients undergoing percutaneous coronary intervention

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Background: Light transmission aggregometry is the "gold standard" for platelet function assessment. Several studies in the past have shown that spontaneous platelet aggregation (SPA) measured in stirred platelet-rich plasma (PRP) is enhanced in patients with various disorders, including diabetes, acute coronary syndrome and myocardial infarction. It is also suggested that SPA can be used as a predictive marker of atherothrombotic events and systemic prothrombotic activity.
Aims: Our aim in this study was to evaluate SPA in blood from patients with stable coronary artery disease (CAD) undergoing coronary stent implantation.
Methods: Thirty patients with stable CAD (18 males, aged 63 ± 19 years) who were under dual antiplatelet therapy with clopidogrel 75 mg and acetylsalicylic acid 100 mg were included. All patients underwent single-vessel elective percutaneous coronary intervention (PCI) with drug-eluting stent implantation. Blood samples were obtained before and 48 hours after the procedure. Platelet-rich plasma (PRP) was obtained by centrifugation at 100 g for 10 minutes, and stirred in a light transmission aggregometer, at 37°C in the absence of chemical stimulants for 15 minutes.
Results: No statistically significant change in SPA was revealed before and after stent implantation (from 7.6 ± 6.9% to 9.8 ± 7.3%, p=NS). However, a significant correlation was found between changes in SPA (ΔSPA) and stent length (r = 0.52, p <0.001).
Summary/Conclusion: SPA following PCI and stent implantation increases with stent length. Stent length should be considered when deciding periprocedural antiplatelet prophylaxis. However, the long-term clinical impact of stent length on those patients' prothrombotic activation remains to be determined.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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A role for vitamin D in inter-individual variation in platelet function

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Background: Pathological platelet activation in intact blood vessels underlies thrombotic cardiovascular disease and is responsible for up to 33% of all deaths in the western world. Vitamin D deficiency has recently been suggested as an independent risk factor for cardiovascular disease. Vitamin D is a cholesterol-derived biomolecule that is synthesised in human skin only upon exposure to adequate sunlight. Due to reduced sunlight exposure, Vitamin D deficiency is common in northern latitudes and also in sections of the Middle-East where traditional dress often protects wearers from exposure to adequate sunlight.
Aims: The purpose of this study is to correlate platelet function with serum levels of Vitamin D in a normal healthy population.
Methods: 30 normal healthy volunteers were recruited with full ethical approval, and assessed for indices of platelet function. Agonists were prepared freshly by serial dilution of thrombin receptor activating peptide (TRAP; 2.9-33 uM), thromboxane A_2-mimmetic, (U46619; 0.1-15 uM) and collagen-related peptide (CRP; 0.1-2 ug/ml). Specifically, we examined the dose-response nature of each individual's responsiveness to platelet agonists in light transmission platelet aggregation and platelet ATP secretion assays. We assessed both the magnitude of the response (% maximal aggregation; fmoles ATP secreted per 10^6 platelets) and the potency of the agonists (threshold dose of agonist that induces platelet aggregation; EC_{50} value for ATP secretion). Following Vitamin D analysis of stored serum samples, the relationship between platelet function parameters and indices of platelet function are assessed through correlation analysis.
Results: We observed significant inter-individual differences in aggregation and secretion responses to TRAP, CRP and U46619. For example, the maximal amount of ATP secreted in response to U46619 is 9.53 ± 6.0 fmoles / 10^6 platelets; (mean ± SD; range 2.11 – 27.28), demonstrating a 10-fold range in secretion-capacity for this dense-granule component. A similar >10 fold difference between minimum and maximal values for ATP secretion was observed between individuals in response to TRAP and CRP. Serum Vitamin D levels varied from 4-80 nmol/IL in this healthy population. Values lower than 25nmol/L are indicative of a Vitamin D deficiency according to the UK Scientific Advisory Committee on Nutrition. Pearson's correlation analysis of our data demonstrate that low vitamin D levels are associated with a hypo responsiveness to U46619, and a high agonist-induced release of ADP (r=0.43).
Summary/Conclusion: The platelet function assays used in this study are unique, as they report on both the threshold of responsiveness for each agonist and the maximal capacity of the platelet to respond. Considerable inter-individual variation exists in normal healthy donors. In this small donor sample, we show a positive correlation between platelet responses and serum vitamin D.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Flow cytometry for platelet functional characterization in children with essential thrombocythemia

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Background: Childhood essential thrombocythemia (ET) is a rare chronic myeloproliferative disorder. Patients with ET may suffer from hemostatic complications.
Aims: To get insight into platelet (PLT) functional activity in this disorder, we investigated blood samples of children with ET.
Methods: 20 patients were examined. The median age was 12.5 years (range 1.5-17 years). The gender composition was 12 boys and 8 girls. In 10 patients, disease progressed without haemostasis-related clinical manifestations (group 1). 7 had ischemic symptoms: erythromelalgia, chest pain, headaches (group 2). 3 patients had hemorrhagic phenotype: ecchymosis and/or nosebleeds of different severity (group 3). We investigates PLT count and used flow cytometry-based platelet function (FC) in rest and after activation with collagen plus TRAP-6. Control groups (CG) for FC included 58 healthy children (median age was 10 years (range 2-17 years).
Results: The median number of PLT in groups 1, 2, and 3 were 1468 th/µl, 1562 th/µL and 780 th/µl, respectively. There were no differences in PLT between the patient groups. PLT size by forward side scattering (FSC) was significantly reduced in patients compared with CG, but not different between patient groups at rest. There were no significant differences in volume of dense granules at rest when compared with the CG and between the groups of patients. After activation, the volume of dense granule release was decreased compared to CG and in patients with hemorrhagic symptoms compared to those without them. PLT size after activation in patients with hemorrhagic symptoms was significantly increased compared to patients without it. PAC1 before and after activation was significantly decreased in the whole patient cohort compared with the CG.
Summary/Conclusion: Our data shows decrease of platelet size, the volume of dense granule release and PAC1 after activation in all patients compared to controls. In 3 children with hemorrhagic manifestations the volume of dense granule release after activation are reduced and PLT size increased in comparison with 17 patients without hemorrhages. Our data show that platelets in children with essential thrombocythemia are qualitatively abnormal.

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Sticky platelet syndrome in patients with thromboembolic complications

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Background: Sticky platelet syndrome (SPS) is a prothrombotic disorder of the platelet functiion that may have familial coincidence and is characterized by platelet hyperaggregability after the induction by low concentrations of epinephrine, adenosine diphosphate, or both substances. It affects relatively young people at the time of their first thrombotic complication, may be related to fertility and pregnancy, and has effective and easy management. However, the exact genetic background has still not been definitely determined.
Aims: To analyze the genetic markers of SPS.
Methods: SPS was detected by repeated performance of light transmission aggregometry and its genetic basis was evaluated by the single nucleotide polymorphism (SNP) analysis of the PEAR1, glycoprotein VI, MRVI1, and GAS6 gene.
Results: Significant relation between SNPs of MRVI1 gene and ischaemic stroke, significantly increased occurence of major haplotype TTAGA in individuals with SPS type I and ischaemic stroke, and increase in the incidence of TTAAG haplotype of glycoprotein VI gene in subjects with SPS type II with venous thromboembolism were detected.
Summary/Conclusion: SNPs of MRVI1, PEAR-1 and glycoprotein VI gene may be associated with the platelet hyperaggregability in patients with SPS.
Key words: sticky platelet syndrome, genetic analysis, single nucleotide polymorphism
Acknowledgement: Authors thank the support of the projects of the Scientific Grant Agency (Vega) 1/0168/16 and Vega 1/0549/19. Informed consent of the patient was obtained.

ECTH 2019
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How could pregnancy in women with sticky platelet syndrome be managed?

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Background: Sticky platelet syndrome (SPS) is the thrombophilic qualitative disorder of platelets defined as *in vitro* increased platelet aggregation following the addition of low concentration of adenosine diphosphate and/or epinephrine and typical for an increased risk of development of thromboembolic events. Its clinical manifestation includes also pregnancy complications (fetal growth retardation, pregnancy loss, pregnancy-related deep venous thrombosis in women), that are diagnosed in 18.7% of all women with this thrombophilia.
Aims: To present the experience of the authors with the thromboprophylaxis in pregnant women with SPS.
Methods: In women with SPS, the most frequent pregnancy complication diagnosed in their past history was the pregnancy loss present in 76.09% of the cases. Further clinical manifestations were deep venous thrombosis, thrombosis and infarction of placental vessels, preeclampsia, intrauterine fetal demise, preterm labour, transient ischaemic attack, migraine attacks with aura and primary infertility.
Results: During the actual pregnancy, the most common acquired thrombophilic state was the increased factor VIII activity and acquired protein S deficiency.
Summary/Conclusion: Thromboprophylaxis in pregnant patients included in this study was effective and clinically without further thrombotic and pregnancy complications. After the detection of the acquired prothrombotic changes in haemostasis, concomitant antithrombotic treatment with antiplatelet agent sused in low doses and low molecular weight heparin was used.
Key words: pregnancy, sticky platelet syndrome, thromboprophylaxis
Acknowledgement: Authors thank the support of the projects of the Scientific Grant Agency (Vega) 1/0168/16 and Vega 1/0549/19. Informed consent of the patient was obtained.

ECTH 2019
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Long lasting prothrombotic state implied by changes of plasma von Willebrand factor parameters (antigen level, collagen binding activity and multimerisation) after radical prostatectomy

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Background: Thromboembolic complications are present in 0.8% - 16.8% of the cases after radical prostatectomy (RP). Association between elevated plasma von Willebrand factor (VWF) levels - as an endothelial activation marker - and increased risk of thrombotic events has been evidenced.
Aims: Upon perioperative plasma VWF levels (VWF:Ag) its collagen binding activity (VWF:CB), multimerisation, and cleaving enzyme (ADAMTS13) of the VWF multimers were quantitated along with FVIII and routine laboratory parameters in this observational pilot study. It aimed to elicit new data on the VWF after RP of prostate cancer patients (CaP) and explore the role of it as a thrombotic risk factor.
Methods: Plasma samples of 24 CaP patients were collected before (-1 day; D-1) and after RP (1 hour, 6 days, 1 month, and 10 months; H1, D6, M1, M10 respectively). The local ethics committee of the University of Debrecen (Hungary) approved the study protocol DE OEC RKEB/IKEB 2884-2008., according to the Declaration of Helsinki, and written informed consent was obtained from each participant before inclusion in the study. VWF:Ag, VWF:CB and ADAMTS13:Ag were measured by ELISA, while the multimer distribution was measured by electrophoresis and quantitative densitometry. FVIII, fibrinogen, D-dimer and other routine laboratory parameters were determined as well. Friedman's and Dunn's post-hoc multiple comparisons tests were applied to analyze the data after D'Agostino and Pearson omnibus normality test. In order to discriminate between clinically normal and clinically abnormal laboratory values receiver-operator characteristic (ROC) curves were constructed and Youden index of the ROC-s were used to select cut off values.
Results: Preoperative values served as baselines, which were compared to controls (24 healthy individuals). VWF:Ag and CB elevated by 122% and 143% respectively at H1 after RP then plateaued at D6 compared to D-1. ADAMTS13/VWF:Ag ratio reduced by 41% at H1, and by 46% at D6, meanwhile the ratio of high molecular weight multimers increased as well. Values returned to baseline at M1 and further reduced to the levels of the controls at M10. When RP samples were compared to controls, the area under the ROCs showed the best separation at H1 and D6 for VWF:Ag, VWF:CB, multimer distribution, FVIII, VWF:Ag/ ADAMTS13, fibrinogen and D-Dimer. At H1 and D6 all of the 24 patients, at M1 14 were in potential prothombotic state according to maximal cut offs of the VWF parameters as indicators.
Summary/Conclusion: Prostate malignancy and then surgical stress, and inflammatory reactions induced release of VWF from the endothelial cells, along with an increasing amount of large multimers and relative reduction of ADAMTS13 level. These changes mark a prothrombotic state even at one month after RP. Evaluation of VWF parameters provides new information about the long term disturbances of primary haemostasis after radical pelvic oncologic surgery like RP. That is why more than one month follow up and prophylactic targeting through the thrombotic and inflammatory activity of the VWF is proposed. To improve the understanding of the differences between physiological and pathological recovery further studies are needed by assessing laboratory parameters.

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PKC-delta-dependent pathways contribute to the exacerbation of the platelet activity in Crohn's disease

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Background: Platelets are widely recognized for their role in the prevention of bleeding and promotion of hemostasis; However, accumulating evidence points to a nonhemostatic role for platelets in chronic inflammatory conditions. This stems from the ability of activated platelets to secrete many immunomodulatory cytokines and chemokines. In addition, it is becoming increasingly clear that an elevated platelet count and reactivity are considered as a marker of inflammatory bowel disease (IBD) activity. Crohn's disease (CD) is one of the most severe gastrointestinal disorders classified as an IBD. PKC-delta (PKCδ) isoform is expressed in platelets and plays distinct roles in regulating platelet function.
Aims: To investigate the functional role of PKCδ isoform and downstream effectors in modulation of molecular inflammatory mechanisms during CD pathogenesis.
Methods: In this study, pharmacological and molecular genetic approaches were used.

Results: In human platelets, pre-treatment with the specific PKCδ inhibitor δ(V1-1)TAT significantly decreased platelet activation in patients with Crohn's disease (CD). Analysis of PKCδ phosphorylation indicates that it is positively regulated by the mitogen-activated protein kinase (MAPK) pathway.
Importantly, PKCδ null mice were refractory to acute dextran sodium sulphate (DSS)-induced colitis, suggesting a target role of the PKCδ isoform during chronic intestinal inflammation pathogenesis.
Summary/Conclusion: These findings highlight a new inhibitory mechanism in the regulation of platelet-induced inflammation that could be the basis for translational research and drug development.

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Filipino cultural beliefs: an input to genetic counseling

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Background: : Genetic Disorder, a genetic problem caused by one or more abnormalities formed in the genome, is greatly being influenced by cultural beliefs, thus it needs to be addressed accordingly. This study explored the Filipino Cultural Beliefs as an input to Genetic Counseling in barangay Siboan-Otong, San Fernando, La Union.
Aims: It aims to provide possible interventions to increase awareness with these Seven Common Filipino Cultural Beliefs – namamana, lihi, sumpa, gaba, pasma, namaligno, and kaloob ng Diyos, to provide culturally appropriate genetic counseling.
Methods: Barangay Health Workers of Siboan-Otong were selected as the participant of the study and reported that majority of the diseases experienced by the community is being influenced by their cultural beliefs causing the patient and its family to become unaware of the proper treatment and scientific explanations behind the genetic disease.
Results: While few of the families are aware of the Genetic Disorders and submitted themselves to a genetic counseling. This revealed that there is a need to conduct Genetic Counseling among the family to completely eradicate such belief.
Summary/Conclusion: So that the community will be more open-minded in dealing with such issues. The researchers recommended to conduct a seminar and an intensive family education program through a house to house campaign in which the researchers will give flyers to each family in Siboan-Otong through the help of the Barangay Health Workers.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Thrombomodulin-bound thrombin induces inflammatory signaling through direct cleavage and activation of protease-activated receptor 2

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Background: Protease-activated receptors (PARs), a small family of four G protein-coupled receptors, evolved to react to extracellular proteolytic activity and therefore, they are crucial in the regulation of chronic as well as acute inflammation. In mammals, PAR1, PAR3, and PAR4 respond to the clotting protease thrombin, whereas PAR2 was assumed to resist activation by physiological thrombin concentrations. The involvement of cell surface thrombin-recruiting co-receptors such as thrombomodulin (TM), which potentially facilitates PAR2 cleavage, has not been addressed so far.
Aims:
In this study, we aimed to examine whether thrombin recruitment to the cell surface by the co-receptor TM results in direct cleavage of PAR2 and whether this cleavage leads to the induction of biological effects such as inflammatory signaling.
Methods: We examined 293T cells overexpressing PAR2 and TM for PAR2 cleavage by TM-recruited thrombin. Cleavage efficiency of overexpressed PAR2 in presence or absence of TM was measured under sustained thrombin stimulation. Thrombin-cleaved overexpressed PAR1 was analyzed as control. To test the required TM–thrombin interactions for PAR2 cleavage and to map cleavage sites on PAR2, mutant constructs of TM's thrombin-binding sites and potential thrombin cleavage sites on PAR2 were engineered and analyzed in the overexpressing system. The biological effects due to thrombin-induced PAR2 activation were investigated by measuring the nuclear factor kappa B (NF-κB) DNA binding activity and the secretion of the pro-inflammatory cytokine interleukine-8 (IL-8) in overexpressing cells and natively expressing PAR2 and TM A549 alveolar epithelial cells.
Results: We showed that, at low to moderate concentrations, thrombin directly cleaved PAR2 at the N-terminal amino acid arginine 36 in a TM-dependent manner. In the presence of TM, thrombin efficiently cleaved both, PAR1 and PAR2, albeit kinetics differed. As expected, the majority of surface expressed PAR1 was rapidly cleaved off, while PAR2 cleavage sustained upon prolonged exposure to thrombin. TM's EGF-like domain 5 was required and TM's proteoglycan sites serine 490 and 492 assisted in PAR2 cleavage. Further, we showed that thrombin-induced activation of NF-κB via PAR2 resulted in release of IL-8 in the overexpression system and A549 cells.
Summary/Conclusion: In this study, we provide a novel concept of how thrombin efficiently cleaves PAR2 in a TM-dependent manner, resulting in induced secretion of the pro-inflammatory mediator IL-8. Our findings of the unexpected role of the co-receptor TM, facilitating direct PAR2 activation by thrombin, lead to a better understanding of the complex mechanisms of protease-PAR interaction, which may result in novel therapeutic options for the treatment of PAR-driven inflammatory and malignant diseases.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
2 - 4 October 2019

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Endothelial-to-mesenchymal transition in venous thrombosis

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Background: Venous thromboembolism (VTE), which encompasses pulmonary embolism and deep vein thrombosis, is a frequent disease and is associated with a significant morbi-mortality. VTE is frequently associated with fibrosis of the venous wall. Endothelial cells and platelets secrete chemokines and cytokines during VTE that may have an important role in vein wall fibrosis such as interleukin (IL)-6 or transforming growth factor (TGF) β. However, the molecular mechanisms of fibrosis development and how they promote recurrent VTE are poorly known. Endothelial-to-mesenchymal transition (EndMT) is characterized by the loss of endothelial markers, the acquisition of mesenchymal markers and the synthesis of extracellular matrix. In adulthood, it has been associated with cardiovascular diseases. Many factors promote EndMT but the most potent inducer of fibrosis is TGFβ. When activated, platelets secrete high amount of TGFβ, which may contribute to fibrosis following VTE. In addition, recent data showed that epigenetic mechanisms, which are associated with gene regulation, modulate EndMT. Our hypothesis is that EndMT is induced during venous thrombosis potentially through TGFβ leading to fibrosis and promoting recurrent thrombosis.
Aims: The aim of this study was to test if the endothelial dysfunction induced by venous thrombosis leads to EndMT.
Methods: Murine endothelial cells and human umbilical vein endothelial cells (HUVECs) were treated with TGFβ, thrombin or both during 72 hours. To study the role of histone deacetylase (HDAC) in EndMT, endothelial cells were also incubate with TGFβ and thrombin in presence of Vorinostat, an HDAC inhibitor. Real-time polymerase chain reaction and western-blot were performed to analyse endothelial (VE-cadherin) and mesenchymal (calponin, alpha smooth muscle actin, collagen) markers expression.
Results: mRNA and protein expression of VE-cadherin was not modify by addition of TGFβ and thrombin. Calponin mRNA expression was increased by TGFβ and thrombin even in the presence of vorinostat. Interestingly, a-smooth muscle actin and collagen expression was increased by thrombin and TGFβ. Addition of vorinostat completely blocked a-smooth muscle actin and collagen mRNA expression.
Summary/Conclusion: In this study, we found that treatment of endothelial cells with TGFβ and thrombin is associated with EndMT. Our preliminary data suggest that HDAC inhibition might prevent fibrosis. This may be due to an increase of antifibrotic factors that counterbalance the pro-fibrotic nature of TGFβ. Current treatments of venous thromboembolism disease are only targeting the coagulation cascade and are associated with a bleeding risk that may be life-threatening. Identification of new biomarkers and new therapeutic targets that are not involved in the coagulation cascade would be instrumental in guiding decisions of treatment for patients with a high risk of recurrent VTE.

ECTH 2019
European Congress on Thrombosis and Haemostasis
Glasgow, United Kingdom
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Interaction of coagulation factor XII with the opportunistic pathogen Candida albicans

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Background: Coagulation pathways are important for early innate immune responses, since they support the inflammatory response and induce entrapment of microbial pathogens in fibrin clots. The plasma contact pathway is comprised of factor XII (FXII) and prekallikrein (PK) with the cofactor high molecular weight kininogen (HK). FXII and PK reciprocally activate when they ''contact'' negatively charged surfaces and function in multiple downstream pathways including coagulation, inflammation, complement and fibrinolysis. The fungus, *Candida albicans* is an opportunistic pathogen that causes a wide variety of infections, including candidiasis, a life-threatening bloodstream infection that kills ~50,000 hospitalized patients a year. On dissemination into the bloodstream, fungal cells rapidly adhere to the endothelial cell layer and form invasive tubular filaments known as hyphae. The hyphal form of *C. albicans* is a key virulence trait, enabling penetration of host tissue, leading to inflammation and sepsis.
Aims: The aim of this study is to investigate potential interactions between FXII and *C. albicans* that might influence the virulence of this pathogen.
Methods: *C. albicans* WT and *mnt*1Δ/*mnt*2Δ hyphal growth was induced using RPMI for 3 h. For confocal microscopy experiments FXII(a) were labelled using DyLight-488 as per manufacturer's instructions. FXIIa activity was detected using S-2302 chromogenic substrate. Human umbilical vein endothelial cells (HUVECs) were seeded at $1x10^5$ and incubated for around 48 h until monolayers formed. HUVECs were incubated with FXII for 1 h before seeding $1x10^6$ *C. albicans* yeast cells. Non-adhered cells were washed off after 20 min, cells were visualised using confocal microscopy and assessed using AxioVision software. *C. albicans* and THP1 cells were seeded at a 2:1 ratio respectively, ±FXII. THP1-*C. albicans* interactions were visualised in real-time using spinning disc microscopy, interactions assessed using Velocity software.
Results: Fluorescently-labelled-FXII binds to *C. albicans* hyphal filaments, but not to the commensal yeast form of the fungus. FXII was unable to bind hyphae of the *mnt*1Δ/*mnt*2Δ mutant, which lacks the ability to *O*-mannosylate secretory proteins destined for the cell surface. This result suggests that *O*-mannan is involved in FXII binding to the hyphal cell wall. Surprisingly, association with hyphae did not elicit FXII auto-activation or downstream coagulation. Although hyphae also bound the activated form of FXII, FXIIa, binding to hyphae inhibited the transition of FXII to FXIIa using activators, such as polyphosphate (polyP) or dextran sulphate. FXII binds to endothelial cells, the most common site of invasion of *C. albicans*. However, the initial attachment of *C. albicans* to HUVECs was independent of FXII. Monocyte recognition of *Candida* is an important step in controlling invasion. Our data show a trend toward increased recognition of *Candida* by THP-1 in the presence of FXII.
Summary/Conclusion: Our data reveals that FXII binds directly to the cell wall of *C. albicans* hyphae, but not to the yeast. Binding was dependent on the presence of *O*-mannan on the hyphal surface. There was no impact of FXII on the interaction of *C. albicans* with endothelial cells, however, there was preliminary evidence to suggest that FXII altered fungal recognition by monocytes. These data suggest that FXII may act as an opsonising agent that increases recognition of *C. albicans* by immune cells, thereby attenuating the pathogenicity of the fungus within the host.

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Investigating the role of neutrophils and NETs in Staphylococcus aureus endocarditis

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Background: In infective endocarditis (IE), the interplay between coagulation and *Staphylococcus aureus* (*S. aureus*) is crucial. IE is characterized by a lesion, or an infected thrombus, containing bacteria, platelets and fibrin. Previously, we have shown that *S. aureus* adheres to cardiac valves via platelets and von Willebrand factor. However, the process of progression from initial bacterial adhesion to a complex vegetation, particularly how bacteria bypass the immune system and thrive in the host environment, remains unclear. Neutrophil extracellular traps (NETs) lie at this interface between host defense and thrombosis.
Aims: We aimed to determine the role of neutrophils and NETs in IE progression using a novel mouse model.
Methods: We intravenously injected mice with *S. aureus* and locally stimulated the endothelium with histamine, resulting in IE lesions that originate on inflamed heart valves. After three days we determined the development of IE on the aortic valves with Gram staining. We investigated the presence of NETs in 14 mice by immunostaining for citrullinated histone H3 (H3Cit), extracellular DNA, and myeloperoxidase. Of these 14 mice, 9 developed endocarditis. In a separate set of experiments, we investigated the role of neutrophils in IE development by injecting a neutrophil-depleting or control antibody 24h before surgery.
Results: Mice with endocarditis had significantly (P=0.005) more detectable H3Cit (9/9) than those without (1/5). More specifically, four mice had H3Cit+ neutrophils within thrombi, indicating early NETosis. Seven mice had an extracellular H3Cit staining pattern within the thrombus. These extracellular H3Cit-positive regions were associated with DNA and myeloperoxidase, indicating the presence of a network of NETs. When we depleted neutrophils, mice developed significantly more endocarditis (7/16 vs. 1/15, P=0.03).
Summary/Conclusion: Endocarditis lesions contained NETs or neutrophils undergoing NETosis, and neutrophil depletion led to increased IE incidence. Further investigating these two players in IE could potentially provide new strategies to combat this deadly disease.

ECTH 2019
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Development of novel imaging tracers of microcalcification for early detection of vulnerable atherosclerotic plaques

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Background: Over 17 million people worldwide die every year as a result of atherosclerosis and associated complications caused by rupture of vulnerable atherosclerotic lesions. A key feature of these plaques is the presence of so called microcalcifications that destabilise the internal structure of the lesion, increasing the likelihood of rupture. To detect these rupture-prone atherosclerotic plaques, development of novel non-invasive imaging tracers is necessary. Gamma-carboxy glutamic acid (Gla)-domains derived from vitamin K-dependent proteins are an interesting source for these novel imaging agents, since they do not only bind to the calcium ions but may also prevent further accumulation of calcium deposits at these sites.
Aims: The main aim of this project is the development of novel imaging agents based on the Gla-domain of existing proteins. As a proof of concept, we will synthesise fluorescent or radio isotope-labeled Gla-domain of protein S for the detection of microcalcifications at sites of atherosclerosis.
Methods: Generation of Gla domain-based imaging agents was performed using chemical protein synthesis. Bioconjugate techniques were used to synthesize dimers and tetramers of the Gla domain constructs which are hypothesized to have higher affinity for microcalcification due to multivalency. Obtained products were characterized using mass spectrometry and purified using high pressure liquid chromatography. The calcification detection ability of our constructs will be assessed in vitro using smooth muscle cell calcification assays.
Results: We have synthesized first the protein S Glu domain, as a control for the Gla domain, wherein all Gla amino acids were replaced by glutamic acids, and have successfully obtained both monomers, dimers and tetramers of this domain. Moreover, we have synthesized the L-Gla amino acid building block necessary for the synthesis of the desired Gla domain, which is currently being performed. In addition, the smooth muscle cell calcification assay as an in vitro assessment method to determine the calcium binding ability and effects on calcification of our constructs has been set up.
Summary/Conclusion: In summary, we have obtained an optimised and standardised production protocol for the generation of our novel Gla domain-based non-invasive imaging tracers and have our in vitro method to assess the diagnostic and therapeutic function of our constructs in place.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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Extracellular vesicles from patients with pulmonary arterial hypertension modulate endothelial function and angiogenesis

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Background: Pulmonary arterial hypertension (PAH) is a severe disease with limited treatment options, characterized by elevated pulmonary vascular resistance and secondary right ventricular failure. Patients with PAH exhibited elevated levels of circulating extracellular vesicles (EVs) whilst their effect and role is poorly understood in relation to diagnosis and therapy of the disease. EVs acts as conveyors of biological information and may modulate functions or fate of target cells. Whether these EVs contribute to the acquisition and/or aggravation of the altered endothelial phenotype is unexplored in PAH patients and draws need for further studies.
Aims: To elucidate whether EVs from patients with PAH differs from healthy individuals and whether these EVs modulate endothelial dysfunction thereby promote angiogenesis *in vitro*.
Methods: 62 patients with PAH and 20 healthy controls were enrolled at the outpatient clinic of Cardiology at Uppsala University hospital (The study was approved by the local ethical committee and was conducted in accordance with the ethical principles of the Declaration of Helsinki). The EVs from plasma of selected PAH patients and controls were purified and characterized by flow cytometry. Purified EVs and primary human pulmonary artery endothelial cells (hPAECs) were used. Effects of EVs on hPAECs were observed using methods such as western blot, qPCR, multiplex electrochemiluminescence assays and angiogenesis assays.
Results: Proangiogenic factors such as PlGF, bFGF and VEGF together with receptor VEGF-R1 were significantly increased in the plasma of PAH patients as compared to healthy controls. Flow cytometric data indicated differences in activated platelet-, endothelial- and erythrocytes derived vesicles in purified EVs among the PAH patients and healthy controls. Human pulmonary artery endothelial cells (hPAECs) incubated with PAH patients plasma derived EVs showed altered endothelial function in terms of increased ICAM-1 and decreased eNOS expression. Moreover, CD41 positive EVs were internalized in hPAECs where PAH derived EVs showed significant extra-lysosomal localization as compared to healthy control derived EVs. Along with endothelial dysfunction, PAH EVs induced significant increased mRNA and protein levels of proangiogenic factors in addition to promote tube like structures by the hPAECs.
Summary/Conclusion: Increased presence of activated platelets-, endothelial- and erythrocyte derived vesicles in EVs purified from PAH plasma indicate avenues to consider further for identification of biomarkers in PAH etiology. Altered congregation of circulating EVs in PAH plasma triggered endothelial dysfunction *in-vitro* and the effect may be partially due to their ability to evade lysosomes, as observed, in target cells. Endothelial dysfunction thus actuated, resulted in elevation of angiogenic markers indicating a potential role of these EVs in the pathogenesis of PAH. Albeit further substantiation warranted, these results shed light on potential involvement of EVs in the diagnosis and pathogenesis of PAH.

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Acute hemorrhagic edema of infancy – is that really a mild, benign disease?

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Background: Acute hemorrhagic edema of infancy (AHEI) is rare leukocytoclastic vasculitis, which presented dramatically as purpuric skin lesions and swelling, in well condition children up to 2 years of age. Approximately 400 cases have been described. The etiology remains unknown. Less than 10% had extra–cutaneous manifestations. With or without treatment, the disease goes to spontaneous recovery within 1–3 weeks, usually without complications. Compartment syndrome because of AHEI has been reported in only one case. We presented unusual case of AHEI with serious complications due to compartment syndrome of the right hand fingers
Aims: A 16–month–old male child, with fever (38.2°C) and sudden appearance and rapidly spread of palpable, painless, non–itching, clearly limited ecchymotic purpura and hematomas, dimension of 0.5–10 cm, on the thigh, cheeks, earlobes, lower legs, forearms, dorsum of hands and feet, with mild edema of these regions, previously treated only with Paracetamol. Psychomotor development, complete systemic examination and vital parameters were normal for age. Regularly vaccinated. Without bleeding disorders in family. He had gingivostomatitis 20 days before disease.
Methods: On admission, laboratory investigations showed mild anemia, with normal number of blood cells, normal blood coagulation screening, inflammation parameters, liver and kidney function tests, electrolytes, serum proteins, albumins, immunoglobulins, complement and urine analysis. Antinuclear, anti–streptolysin O, anti–myeloperoxidase and anti–proteinase 3 antibodies and rheumatoid factor were normal. Abdomen ultrasound excluded visceral involvement. D–dimer was only parameter beyond the reference (7.21 ng/mL). Urine and blood culture and virusology analyses were all negative.
Results: After exclusion of more serious clinical conditions, we ordinated Methylprednisolone. Changes on right forearm and hand expanded on almost entire dorsal side. In next few days, they were spreading on all surface of fingers, with pronounced swelling and bullous lesions. Repeated laboratory tests showed significant anemia, which required transfusion of deplasmated erythrocytes and increased D–dimer. Low–molecular–weight heparin was initiated. Skin biopsy confirmed nonspecific small–vessel vasculitis. Necrotic bullous changes were spreading and cracking. We added Ceftriaxone in therapy.
All that time, child was in good condition, afebrile, with normal laboratory analyses. Other skin changes were in regression. Bullous lesions between fingers macerated and made the compartment syndrome with consequent necrosis of proximal parts of II–IV finger. Dried eschar was formed on dorsal side of the forearm and hand. Debridement of necrotic tissue and amputation of middle and distal phalanges of second and third finger was made and skin from right thigh was transplanted. All wound swabs were sterile. On controls, child has no other skin changes, with adequate healing in month follow–up period.
Summary/Conclusion: Although AHEI is rare entity, early recognition is important to avoid unnecessary investigation and therapy, because of its benign nature. On the other hand, our reported case warns that unexpected complications may occur. Consider treatment with corticosteroids and low–molecular–weight heparin and antibiotics, in case of extensive lesions. Due to extravasation of erythrocytes, regularly follow the hemoglobin values. D–dimer values can be used to monitor disease progression.

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European Congress on Thrombosis and Haemostasis
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The antiangiogenic effects of acetylsalicylic acid in patients with polycythemia vera

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Background: The study of the properties of acetylsalicylic acid (ASA) surprises constantly. This anti-inflammatory and antiplatelet agent has a significantly greater number of effects than is commonly believed.
Aims: To study the effects of ASA in patients with polycythemia vera (PV).
Methods: The study included 54 patients with polycythemia vera who were intaking ASA 75-150 mg/day (group 1; n=33; age 55±9) or not intaking (group 2; n=21; age 59±9). Every patient was examined with parameters for coagulation/anticoagulation, fibrinolysis, endothelial function, platelet aggregation, and other biomarkers including VEGF-A, bFGF, 20-HETE, 15-HETE, tissue's factor, and p-thrombomodulin.
Results: The fact of ASA intaking has correlated positively with the increasing of both VEGF-A (p=0.031) and bFGF (p=0.032), and with the decreasing of 20-HETE (p=0.024), 15-HETE (p=0.041) and p-thrombomodulin (p=0.032).
VWF and ADAMTS-13 have differed significantly between groups but no any associations with ASA were found in the groups.
Summary/Conclusion: We explain obtained results the ASA has effects decreasing angiogenesis in patients with PV. Therefore ASA might to be considered as obligatory part in therapy of such patients.

ECTH 2019
European Congress on Thrombosis and Haemostasis
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2 - 4 October 2019

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Endothelial damage and hypercoagulable state in diabetic patients with gangrene diabeticum

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Background: Diabetes Mellitus (DM) is a chronic metabolic disease characterized by high blood sugar levels and insulin resistance. The diabetic cases is increasing world wide, and Indonesia is ranked 7th in the world. The chronic hyperglycemia will cause damage to endothelial cells, disorders of hemostasis until the occurrence of micro and macrovascular complications. One of the most common complications of DM disease is the occurrence of diabetic foot which can develop into gangrene. and this will increase the morbidty and mortality rate. eNOS, one of the markers of endothelial cells damage is a protein that functions to maintain the stability of endothelial cells under normal conditions. The damage of endothelial cells will activate the coagulation system, hypercoagulable state and resulting in thrombosis.
Aims: This study aims to see the correlation between endothelial cell damage and hemostasis disorders in patients with diabetes with gangrene.
Methods: This is a case control study to study the correlation between endothelial cell damage and hemostasis disorders in patients with diabetes with gangrene. A total of 96 samples were recruited in which 56 were with diabetes and 40 diabetes with gangrene. Study was done after getting informed consent. Blood sugar levels, HbA1C, D-dimer and eNOS were measured. The Statistical Package for Social Sciences (SPSS version 22) was used to perform the statistical analysis.
Results: The HbA1C and D-dimer level were elevated either in the diabetic or diabetic with gangrene. Seventy five percent of the samples were not well controlled (bad), with average of the HbA1C were 9.1 (5.4-14.3), D-dimer cases above normal were 73%, The eNOS level range from 258-4000 (2065 ± 1280), almost the same at both groups. There was statistical significant between HbA1C and d-dimer (p=0.04) but not between HbA1C and eNOS (p=0.85).
Summary/Conclusion: The eNOS levels and D –dimer were elevated in both groups either diabetic or diabetic with gangrene, this showed the endothelial cell damage and hypercoagulable state. HbA1C (glycated hemoglobin) showed statistical significant with d-dimer.