European Congress on Thrombosis and Haemostasis

ABSTRACT BOOK

28 – 30 September 2016

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1 SCIENCE, FAST AND FUROUS

Platelets

ECTH-299

Characterization of two novel RASGRP2 variants leading to defective CalDAG-GEFI- mediated RAP1 activation and platelet dysfunction

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Background: In addition to mutations in *ITG2B* or *ITGB3* genes causing defective aIIb3 expression/function in Glanzmann's thrombasthenia patients, platelet dysfunction can be due to genetic variability in proteins mediating inside-out activation of aIIb3. The *RASGRP2* gene encodes the Ca^{2+} and diacylglycerol-regulated guanine nucleotide exchange factor 1 (CalDAG-GEFI). This protein is strongly expressed in platelets and neutrophils, where it facilitates the activation of the small GTPase Rap1 and subsequent activation of integrins. To date, only three siblings with platelet dysfunction caused by a *RASGRP2* variant have been reported.

Aims: Functional and molecular characterization of three patients from two unrelated families, with bleeding tendency and impaired platelet aggregation, but no mutations in *ITG2B* or *ITGB3*.

Methods: A 9yr-old Chinese child and two Spanish siblings, a 55yr-old woman and 46yr-old man, with suspicion of inherited platelet disorder, were studied. Blood cell counts, platelet function (platelet aggregation and secretion, glycoproteins expression and platelet activation by flow cytometry, clot retraction and platelet spreading), and integrin activation in neutrophils were assessed. DNA from the Chinese boy and the Spanish siblings was analyzed by next generation sequencing (NGS) and whole exome sequencing (WES), respectively. Expression of CalDAG-GEFI protein was quantified by immunoblotting. The GEF activity of purified wild type and mutant CalDAG-GEFI was compared by an in vitro Rap1B activation assay with Bodipy FL GDP.

Results: Patients displayed moderate bleeding tendency, normal platelet count and volume, and mild anemia. Platelet phenotyping showed prolonged PFA-100 closure times and reduced platelet aggregation in response to ADP and low dose collagen. Aggregation with high concentrations of PAR1, collagen, arachidonic acid, or PMA was unaffected. Platelet surface receptor expression was normal. NGS identified a homozygous change c.1142C>T in *RASGRP2,* leading to a p.Ser381Phe substitution in CalDAG-GEFI in the Chinese patient. WES revealed a homozygous c.337C>T (p.Arg113X) mutation in the *RASGRP2* gene of the Spanish siblings. Expression of CalDAG-GEFI, but not Rap1 or the Rap-GAP Rasa3, was markedly reduced in platelets from all patients. Consistent with the aggregation defect, aIIb3 activation was markedly impaired in patient platelets. Only minor defects were observed in granule secretion, clot retraction or spreading on fibrinogen. Patient neutrophils showed normal b2 integrin and reduced b1 integrin expression. b2 integrin activation in patient neutrophils stimulated with fMLP, PMA, or Mn^{2+} was impaired. Neutrophil granule secretion was not affected. Structural modeling suggested that the p.Ser381Phe substitution causes a conformational change affecting both protein stability and nucleotide exchange activity in CalDAG-GEFI. Consistently, purified CalDAG-GEFI p.Ser381Phe protein showed markedly reduced exchange activity towards Rap1B *in vitro*.

Summary/Conclusion: We report three patients with platelet dysfunction resulting from novel mutations in *RASGRP2* that affect the synthesis and/or function of CalDAG-GEFI and cause an impaired platelet aggregation response to select agonists. These findings strengthen the molecular heterogeneity of *RASGRP2* as a cause of inherited platelet disorders.

ISCIII PI14/01956 & CB15/00055; BHF RG/ PG/13/36/30275 & RG/09/007; NIH P01 HL120846 &R01 HL121650.

Platelets

ECTH-471

Light sheet fluorescence microscopy (LSFM) and subsequent quantitative structural analysis of megakaryocytes in intact murine bone

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Background: Megakaryocytes (MKs) are large polyploid cells residing in the bone marrow (BM) which are the progenitors of anucleate blood platelets. According to the current concept, MK development progresses spatially from the endosteal niche in the BM to the vascular niche, where terminal maturation and platelet release into sinusoids occur. However, experimental evidence supporting this concept is limited due to two-dimensionality of avaliable techniques (such as tissue sectioning) as well as unexplored *in silico* investigation of MK behaviour in BM.

Aims: We aimed to study the distribution of MKs in their native environment in 3D by combining light sheet fluorescence microscopy (LSFM) and computer simulations.

Methods: Sterna were antibody-stained for MKs and endothelial cells, paraformaldehyde-fixed, chemically cleared and imaged by light-sheet fluorescence microscopy (LSFM), allowing MK visualization in intact bones. This approach allowed us to quantify MK volume, localization and number in the BM. Based on *ex vivo* data obtained by LSFM, MK distribution in the BM was studied *in silico* using the real vasculature and cell geometries as templates. Further on, two photon microscopy was used to investigate MK motility *in vivo.*

Results: Unexpectedly, in *ex vivo* 3D LSFM we visualized a homogeneous and extremely dense network of blood vessels in the BM, revealing an unexpected spatial limitation for MK migration. LSFM based *in silico* studies on MK localization in BM showed only a minor vessel bias and an essentially random distribution of non-vessel associated cells. Enhanced stimulation of megakaryopoiesis upon induced thrombocytopenia (with the use of anti-GPIb antibodies) had no significant effect on MK number or localization. Further, MK migration was not observed by *in vivo* microscopy, neither before nor after platelet depletion.

Summary/Conclusion: In conclusion, our novel method of imaging intact BM by LSFM complements current *in situ* and *ex vivo* techniques and enables realistic *in silico* studies. The high quality 3D experimental data, combined with *in vivo* and *in silico* studies, challenge the current concept of MK migration and give an in-depth insight into the environmental regulation of MKs and the functional relevance of MK migration for thrombopoiesis.

Clotting

ECTH-370

Risk prediction of recurrent venous thrombosis: validation of three models in a large unselected population

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Background: After a first venous thrombotic event the lifetime risk of recurrence is considerable. Because the bleeding risk associated with anticoagulant treatment is high as well, the decision on its optimal duration is challenging. Accurate assessment of an individual's recurrence risk is therefore essential. Previously, three prediction models for recurrent venous thrombosis have been published, but external validation has hardly been performed, which hampers their practical usefulness.

Aims: To externally validate previously published prediction models for recurrent venous thrombosis (HERDOO2, Vienna, DASH score).

Methods: We used data from the MEGA follow-up study, in which 4731 consecutive patients with a first venous thrombotic event (DVT, PE or both), aged 18-70 years, were followed for recurrence between 1999 and 2010. Until June 2002 blood was sampled approximately three months after discontinuation of anticoagulant treatment (blood available in ~50% of the patients). Missing values on laboratory measurements and other variables were multiply imputed and results were pooled according to Rubin's rules. For the validation analysis we selected patients with an unprovoked first event (i.e. without cancer in the five years before the event, without surgery, trauma, plaster cast, pregnancy or immobilization in the three months before the event, prolonged travel in the two months before the event or hormone use at time of the event). Cox regression analyses were used and the performance of the HERDOO2, Vienna and DASH model in our study was estimated by means of a Harrell's C-statistic. In a sensitivity analysis, C-statistics were estimated for the DASH and Vienna model using the definition of unprovoked thrombosis used in their derivation studies. This was not possible for the HERDOO2 model because of low numbers left after application of its criteria. This study was approved by the Medical Ethics Committee of the Leiden University Medical Center, and all participants gave written informed consent.

Results: Out of 1082 patients with an unprovoked first venous thrombosis 269 developed recurrent venous thrombosis during a mean follow-up of 4.9 years (min 6 days – max 9.1 years), for an incidence rate of 5.2 per 100 persons per year (95%CI, 4.6-5.9). The C-statistic, estimating the discriminative ability of the models, was 0.58 (95%CI, 0.54-0.62) for the DASH-model, 0.54 (95%CI, 0.51-0.58) for the Vienna model and 0.56 (95%CI, 0.49-0.63) for the HERDOO2 model (only women included, n=296). When using the selection criteria for inclusion as reported for the DASH and Vienna model we found C-statistics of 0.65 (95%CI, 0.62-0.68; n=2236) and 0.56 (95%CI, 0.52-0.59; n=1728) for the DASH and Vienna model, respectively.

Summary/Conclusion: The predictive performance of the currently available prediction models for recurrent venous thrombosis seems suboptimal in a large, unselected population.

Platelets

ECTH-459

Thrombotic activity in the acutely ischaemic brain is dramatically reduced in T-cell deficient mice

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Background: Stroke is the second leading cause of death and disability worldwide and mostly caused by thromboembolic occlusion of major brain vessels. The primary therapeutic goal in acute stroke is to achieve recanalization, but, even if successful, only less than one third of patients benefit. The phenomenon of ongoing ischemic lesion development despite recanalization is referred to as *reperfusion injury*. Although numerous contributing factors, like platelet adhesion, degranulation, as well as T-cell responses have been identified, the mechanistic interactions of these factors leading to brain injury remain unknown and their elucidation requires novel *in vivo* imaging technologies.

Aims: We sought to analyze the spatio-temporal characteristics of platelet deposition and thrombus formation in the ischemic brain using light-sheet fluorescence microscopy (LSFM) in wildtype and T-cell deficient mice (*Rag1*$^{-/-}$).

Methods: We subjected mice to transient middle cerebral artery occlusion (tMCAO), a model of ischemic stroke. Brains were antibody-stained for platelets and endothelial cells, paraformaldehyde-fixed and chemically cleared. Entire hemispheres were imaged via LSFM and thrombus formation was mapped.

Results: We established a protocol for antibody penetration, tissue clearing, and triple-color illumination allowing the visualization of platelet deposition in an intact brain hemisphere. This approach allowed us to generate a spatio-temporal map of thrombotic activity in the microcirculation of the ischemic brain, revealing the gradual increase of formed thrombi, while an unexpectedly high number of brain vessels remained perfused. Strikingly, T-cell deficient mice displayed dramatically reduced numbers of thrombi in the ischemic brain, despite normal thrombus formation in models of arterial thrombosis.

Summary/Conclusion: This novel method allows a spatio-temporal mapping of cell-cell interactions during cerebral infarct progression, thereby revealing fundamental pathomechanisms of thrombo-inflammation in the ischemic brain.

Platelets

ECTH-258

Platelet polyphosphate forms solid nanoparticles that are exposed on the cell surface and can trigger contact system activation

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Background: Platelet dense granules contain polyphosphate, which acts on blood coagulation and fibrinolysis. Activated platelets drive coagulation in a factor XII-dependent manner and polyphosphate has been identified as a platelet-derived factor XII activator *in vivo*. Secreted polyphosphate in the supernatant of activated platelets has a chain length of 60-100 residues. However, these short chain molecules only have limited potential for activating factor XII. As such, it has remained unclear how platelet polyphosphate could drive factor XII activation in thrombosis.

Aims: To investigate the nature of platelet polyphosphate. We hypothesized that polyphosphate functions in insoluble form on activated platelet surfaces.

Methods: We studied subcellular localization of polyphosphate in platelets under flow by live-cell imaging, confocal fluorescence microscopy and scanning electron microscopy using differential staining. Biophysical characterization of platelet polyphosphate was performed by ultracentrifugation fractionation, dynamic light scattering, phenol-chloroform extraction, anion-exchange chromatography, DAPI-negative staining and chromogenic substrate assays in plasma.

Results: Live-cell imaging studies revealed that platelets retain polyphosphate on their surface during secretion under flow on immobilized von Willebrand factor. Isolation of polyphosphate showed that platelets harbor both short chain (60-100; secretable) and long chain molecules (>500 units; membrane-associated). Ultracentrifugation fractionation studies pointed out that cell-associated polyphosphate is condensed into stable insoluble spherical nanoparticles. Confocal laser scanning microscopy and scanning electron microscopy confirmed the presence of these nanoparticles on the platelet surface after degranulation. Polyphosphate nanoparticles can be disrupted by chelating agent EDTA, indicating that divalent metal ions are critical for nanoparticle stability. In contrast to short-chain soluble polyphosphate, platelet membrane-associated polyphosphate nanoparticles potently activate factor XII and the contact system in plasma.

Summary/Conclusion: Our findings show that activated platelets secrete soluble short-chain polyphosphate into solution, while they expose insoluble long-chain polyphosphate as nanoparticles on their membranes. Platelet polyphosphate nanoparticles have a strong capacity to factor XII activation, compared to short chain molecules. These findings have potential implications for development of new antithrombotic strategies.

Clotting

ECTH-277

The association of circulating DNA, nucleosomes, and neutrophil extracellular traps with the severity and outcome of venous thromboembolism in patients

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Background: Extracellular DNA and histones promote thrombosis and cytotoxicity in vitro and in vivo. Neutrophils release DNA and histones as extracellular traps (NETs) and aggravate venous thrombosis as well as tissue damage in experimental models. Whether extracellular DNA, histones, and NETs contribute to the severity or outcome of venous thrombosis in patients is poorly understood.

Aims: DNA is elevated in plasma from patients or animals with deep vein thrombosis (DVT). However, the potential of plasma DNA for diagnosis and prognosis in acute thromboembolism (VTE) is unknown. We hypothesized that extracellular DNA, nucleosomes, and NETs in plasma are associated with the severity and mortality of VTE, the combined disease entity of DVT and pulmonary embolism (PE).

Methods: We analyzed plasma from 863 patients of SWITCO65+, a multicenter cohort study that prospectively enrolled consecutive patients aged ≥ 65 years with acute, symptomatic VTE. We detected and quantified extracellular DNA by using specific probes, as well as nucleosomes and NETs by ELISA in citrated plasma samples.

Results: Extracellular DNA and nucleosomes, but not NETs, were associated with the severity of acute VTE as categorized by isolated distal DVT (n = 68), proximal DVT (n = 186), non-massive PE (n = 577), and massive PE (n = 9). Using competing risk and Cox-regression models, adjusting for relevant risk factors, and periods of anticoagulation as a time-varying covariate, we identified extracellular DNA, nucleosomes, and NETs as predictive biomarkers for mortality within 6 months from acute VTE onset.

Summary/Conclusion: Our data on VTE patients corroborates the findings of experimental models that extracellular DNA and histones aggravate venous thrombosis. NETs contribute to circulating DNA in VTE patients and are associated with death. Extracellular DNA may help identifying patients at risk of adverse outcomes within months after VTE diagnosis.

Bleeding

ECTH-481

Characteristics and treatment of vaginal bleeding in women with venous thromboembolism treated with apixaban or enoxaparin followed by warfarin

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Background: Direct oral anticoagulants (DOACs) have been introduced as an alternative for vitamin K antagonists for the treatment of venous thromboembolism, and were found to be at least as effective and safer. Recent studies have shown that abnormal vaginal bleeding is a frequent complication in women receiving DOACs, affecting 20% to 40% of all treated women. Most data have been limited to women using rivaroxaban.

Aims: To investigate and compare the characteristics of clinically relevant vaginal bleeding in patients receiving apixaban and enoxaparin followed by warfarin.

Methods: We performed a post-hoc analysis of the AMPLIFY trial, a randomized controlled double-blind clinical trial, in which the efficacy and safety of apixaban versus enoxaparin followed by warfarin for VTE treatment was evaluated. Of all women with a clinically relevant vaginal bleeding, we collected data on clinical presentation, course, diagnostic procedures, management and outcomes of the bleeds. The investigators who adjudicated and collected the data were blinded to type of treatment.

Results: In the AMPLIFY trial, 1122 women were treated with apixaban and 1106 received enoxaparin followed by warfarin. Major vaginal bleeding occurred in one woman during the use of apixaban (< 0.1%), and in none of those receiving enoxaparin/warfarin. A total of 28 women receiving apixaban (2.5%) experienced a clinically relevant non major (CRNM) vaginal bleeding compared to 24 receiving enoxaparin/warfarin (2.1%). Premenopausal vaginal bleeding events in women on apixaban (79%) were characterized by more prolonged bleeding (i.e. menstrual bleeding for more than 7 days; odds ratio [OR] 2.3; 95% confidence interval [CI] 0.5-11) and anemia (i.e. a hemoglobin value <11.9 g/dL; OR 2.3; 95% CI 0.7-7.8) compared to those using enoxaparin/warfarin (75%). In 64% of the apixaban associated and in 61% of the enoxaparin/warfarin associated premenopausal vaginal bleeds there was an unscheduled contact with a physician. Of both pre- and postmenopausal vaginal bleeds in the apixaban and enoxaparin/warfarin group, 21% and 29% respectively, diagnostic tests were performed (OR 0.7; 95%CI 0.2-2.5). Medical treatment was not deemed necessary in 57% of the apixaban recipients and in 67% of those receiving enoxaparin/warfarin (OR 0.7; 95%CI 0.2-2.1), and in 18% and 19% respectively, a medical intervention was indicated to stop the bleeding in both groups. Anticoagulant treatment was temporarily stopped in 29% and 33%, whereas 7% and 13% stopped anticoagulants permanently in patients receiving apixaban or enoxaparin/warfarin, respectively (temporary or permanent treatment cessation, OR 0.7; 95%CI 0.2-2.0).

Summary/Conclusion: Premenopausal clinically relevant vaginal bleeds occurs at a similar rate in women using apixaban but appear to be more frequently characterized by prolonged menstrual bleeding and subsequent anemia, compared to women receiving enoxaparin/warfarin. Diagnostic procedures and medical treatment or interventions were infrequently applied in both apixaban and enoxaparin/warfarin associated vaginal bleeds, and symptomatic treatment often sufficed. Unscheduled contact with a physician and temporary cessation of treatment were very common in both treatment groups.

Clotting

ECTH-416

Neutrophil extracellular traps in thrombi from patients with acute ischaemic stroke

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Background: Ischemic stroke is caused by blood thrombi that occlude blood vessels in the brain, leading to focal impairment of the downstream blood flow and irreversible damage of the associated brain tissue. For unknown reasons, use of t-PA leads to dissolution of occluding thrombi in some cases, but not in others, Although the thrombus itself is the primary target for acute ischemic stroke intervention, little is known about the exact composition of thrombi that cause ischemic stroke. In particular, neutrophil extracellular traps (NETs) have been reported in various settings of thrombosis but their presence in ischemic stroke thrombi remains unknown.

Aims: To investigate the presence of neutrophil extracellular traps in thrombi from ischemic stroke patients.

Methods: Fifty-three thrombi from acute ischemic stroke patients were collected following thrombectomy. Thrombi were fixed, embedded and sectioned. Besides standard H&E staining, NETs were visualized via immunohistochemistry using antibodies against citrullinated histone H3 (H3Cit) and the granulocyte marker CD66b. Extracellular DNA was visualized via DAPI. Distribution of H3Cit was analyzed and quantified in different sections throughout all thrombi. Per thrombus, the overall mean value of H3Cit quantifications of all analyzed sections was calculated (% NETs).

Results: Positive staining for H3Cit was found in almost all thrombi. H3Cit-positive areas (% NETs) varied from 0.03% to 10.51% in the 53 thrombi. Co-localization of H3Cit with CD66b and extracellular DNA confirmed the presence of citrullinated histones on extracellular DNA released by neutrophils. Strands of extracellular DNA were also observed in neutrophil-rich areas on H&E staining. Different stages of NETosis could be detected, including neutrophils that contain only intracellular decondensated chromatin and neutrophils that have formed extracellular networks of DNA fibers. The majority of collected thrombi were of cardioembolic origin and no significant differences in the amounts of NETs were observed between thrombi with different etiology.

Summary/Conclusion: Stroke thrombi retrieved from patients via thrombectomy provide a unique opportunity to study clot composition. We report the presence of NETs in human ischemic stroke thrombi. Further studies are needed to identify whether NETs could be targeted in order to improve stroke therapy.

2 FOCUS SYMPOSIA ABSTRACTS

Vessel wall
ECTH-201
Silencing of anticoagulant protein C evokes low incident but spontaneous atherothrombosis in Apolipoprotein E deficient mice
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Background: Preclinical studies on atherothrombosis, the major cause of cardiovascular events, are hampered by the lack of proper mouse atherosclerosis models which spontaneously develop atherothrombotic complications. Possible underlying causes are the higher stability of the mouse atherosclerotic plaques and a more efficient plasma anticoagulant activity.
Aims: We hypothesized that (transient) inhibition of anticoagulation may cause murine atherosclerotic plaques to become more prone to (athero)thrombosis.
Methods: Female Apolipoprotein E deficient (*Apoe* -/-) mice were fed a western type diet (WTD) for 9 weeks to provoke development of atherosclerotic lesions. Anticoagulation inhibition was achieved through silencing anticoagulants antithrombin (*Serpinc1*) and/or protein C (*Proc*), using specific siRNAs.
Results: WTD-fed A*poe* -/- mice injected with an siRNA solely targeting *Serpinc1* (si*Serpinc1*) all rapidly developed spontaneous thrombosis, restricted to the venous vasculature. This thrombotic phenotype was similar as described previously for wild type mice injected with siRNAs targeting *Serpinc1* (Safdar et al. *Blood*, 2013). In contrast, mice injected with an siRNA solely targeting *Proc* (si*Proc*) remained fully healthy. Interestingly, although in a first study with small numbers (n=4), microscopic analysis of the aortic root area revealed that one animal displayed a highly unique, organized, and large thrombus superimposed on the atherosclerotic aortic lesion. The thrombus consisted of layers of eosin positive-structures identified as fibrin, and was infiltrated by leukocytes typically at the luminal side. Although at low incidence, similar thrombi at the same location were observed for *Apoe* -/- in a second independent si*Proc* experiment (thrombi for 3 out of 25 si*Proc* treated *Apoe* -/- mice). All thrombi had a composition similar to that observed in the first experiment. si*Proc* treated *Apoe* -/- mice with thrombi superimposed on the atherosclerotic lesions had blood characteristics similar to si*Proc* treated *Apoe* -/- mice without thrombi: Blood platelets numbers (1629 x10^9 platelets/L ± 70 vs. 1832 x10^9 platelets/L ± 58; *P*>0.05, *siProc* treated mice without thrombi vs. *siProc* treated mice with thrombi, resp.), plasma fibrinogen levels (177.8 U/dL ± 8.3 vs. 188.6 U/dL ± 14.3; *P*>0.05), and plasma thrombin-antithrombin complex levels (45.4 ng/L ± 2.9 vs. 43.0 ng/L ± 5.4; *P*>0.05) were comparable to si*Proc* treated *Apoe* -/- without thrombi at the site of the atherosclerotic lesions, and compared to control siNEG injected animals. So far, other organs (liver, lung, kidneys) did not show thrombotic lesions, indicating atherothrombosis was restricted to the heart. Moreover, aortic plaques in si*Proc* mice with thrombi had collagen content (18.61% ± 1.25 vs 15.61% ± 1.92; *P*>0.05), necrotic core size area (20.92% ± 1.05 vs. 23.26% ± 3.89; *P*>0.05) and macrophage content (3.65% ± 0.32 vs. 3.96% ± 0.90; *P*>0.05) comparable to plaques in si*Proc* mice without thrombi.
Summary/Conclusion: Our findings indicate that siRNA-mediated silencing of *Proc* in *Apoe* -/- mice creates a condition that allows the formation of spontaneous atherothrombosis. Although at low incidence, our approach may reveal a condition to study factors modulating atherosclerosis with atherothrombotic complications.

Vessel wall

ECTH-379

Deletion of chromosome 9p21 noncoding cardiovascular risk interval in mice induces a prothrombotic phenotype

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Background: SNPs on chromosome 9p21.3 risk locus have been associated with atherosclerotic coronary heart disease, abdominal aortic aneurysm or myocardial infarction. We have established a direct mechanistic link between 9p21 noncoding risk interval and susceptibility to aneurysm in a mouse model with a targeted deletion of the 9p21 noncoding cardiovascular disease risk interval.

Aims: We determined whether the deficiency of transcripts encoded by this locus predisposes to a pro-thrombotic phenotype and arterial stiffening in this mouse model, describing the function of CDKN2A/B gene products, and in humans with 9p21 DNA variants.

Methods: Carotid blood flow following 7.5% $FeCl_3$ application for 2 minutes was monitored via Doppler profiles (VEVO770, Visualsonics). Platelet aggregation was induced by ADP, collagen or thrombin agonists. Platelet reactivity and glycoprotein expressions at the surface of platelets were quantified by flow-cytometry. Thrombin generation was monitored using calibrated thrombography (CAT) in various conditions (whole blood, platelet-rich and platelet-poor plasmas).

Results: We show that deletion of the orthologous 70-kb noncoding interval on mouse chromosome 4 (chr4$^{\Delta 70kb/\Delta 70kb}$), synthetic to human chromosome 9p21, predisposes to arterial thrombosis. The time to occlusion in a $FeCl_3$-induced carotid thrombosis model was significantly decreased by 30% in the absence of the locus. These results were confirmed by a new model of physiological thrombosis where a higher deposit of fibrin and platelets in chr4$^{\Delta 70kb/\Delta 70kb}$ mice was observed in confocal microscopy. In addition, we observed an effect on the tail bleeding time which is prolonged by 2 fold in chr4$^{\Delta 70kb/\Delta 70kb}$ mice compared to controls. There was no difference between groups in blood pressure, carotid stiffness parameters (diameter and distensibility for a given level of arterial pressure) or in vascular structure. We explored the potential impact of the deletion locus on platelet aggregation and reactivity. Although no difference was observed in platelet aggregation in response to agonists (ADP 10 µM, collagen 2 µg/mL and thrombin 1 U/mL), P-selectin and phosphatidylserine surface expression, and shedding of platelet-derived microparticles were increased as compared to controls. Thrombin generation in whole blood or platelet-rich plasma was significantly increased in chr4$^{\Delta 70kb/\Delta 70kb}$ mice. In 100 apparently healthy postmenopausal women, carriers of the 9p21 risk T allele (sequence variants rs1333040, n= 33 women) display a higher thrombin generation associated with higher procoagulant markers and impaired activated protein C anticoagulant system, and increased aortic arterial stiffness compared with carriers of the C allele.

Summary/Conclusion: These results establish a direct link between variants or deletion in the 9p21 non-coding risk interval and increased platelet reactivity and thrombin generation predisposing to thrombosis in mouse and increased arterial stiffness in aged population.

Vessel wall

ECTH-242

Divergent and convergent PAR1,2,3,4 and histamine-induced signal transduction pathways in endothelial cells uncovered by quantitative phosphoproteomics

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Background: The intimate interplay of the coagulation cascade and the vessel wall is mainly mediated through cleavage of Protease Activated Receptors (PARs) on endothelial cells (ECs) by activated serine proteases, resulting in the activation of downstream signal transduction pathways. We recently dissected thrombin-mediated signaling in ECs (Blood,2014) and showed that this is completely dependent on PAR1 (oral presentation ISTH2015). Other serine proteases of the coagulation cascade, including FVIIa and FXa, target not only PAR1 but also PAR2. The PAR family consists of four G-protein coupled receptors (GPCR). However, it is unknown to what extend all PARs induce similar signaling pathways and how these differ from those induced by other GPCRs, including the histamine receptor family. In addition, PARs can be activated via transactivation by other PARs, However, the molecular details of this transactivation are lacking. Understanding the convergence and divergence of PAR-induced signaling pathways and transactivation mechanisms is particularly relevant for targeting PARs for therapeutic intervention in hemostatic disorders.

Aims: The aim of this study is to map the divergent and convergent signaling pathways of PAR1,2,3 and 4 on endothelial cells using an unbiased phosphoproteomic approach and to compare these to histamine-induced signaling pathways.

Methods: PAR activation peptides were used to selectively activate the different PARs (PAR1: SFLLRN, TFLLR; PAR2: SLIGKV, 2-furoyl-LIGRLO; PAR3: TRFGAP and PAR4: AYPGKF). Endothelial barrier function of Blood outgrowth endothelial cells (BOECs) and platelet aggregation were used as a readout to determine potency and specificity of the PAR activation peptides. Next, BOECs were metabolically labeled using Stable Isotope Labeling with Amino acids in Cell culture and stimulated for 0, 2 or 10 min in a triplicate labeling experiment with each PAR activation peptide (50 µM) or histamine (100 µM). Phosphorylated peptides were enriched using TiO2 precipitation, detected by Orbitrap Fusion Tribrid Mass Spectrometer and analyzed using MaxQuant and Perseus software.

Results: PAR1 and PAR2 activation peptides and histamine all induced endothelial barrier disruption, albeit to a different extend, while PAR3 and PAR4 activation peptides had no effect on ECs. Principal component analysis of quantified phosphosites (>2700) and hierarchical clustering of regulated phosphosites (>600) showed that: (1) PAR1 and PAR2 induced extensive and highly similar phosphoregulation, with limited but significant differences, (2) PAR3 and PAR4 only induced limited phosphoregulation, (3) the initial signaling pathways of PAR1, PAR2 and the histamine receptor are highly similar, whereas after prolonged stimulation their signaling pathways are more diverged, and (4) there is no evidence for transactivation of PAR1 and PAR2 by the PAR3 activation peptide.

Summary/Conclusion: Here, we present for the first time a global overview of the signaling pathways in ECs mediated by the four PARs as well as the histamine receptor family. These data uncover the divergent and convergent signaling pathways of PARs compared to each other and other GPCRs and shed light on the mechanisms of transactivation of PAR activating peptides. In conclusion, our data provide novel insight in the phosphoregulation by PARs and the histamine receptor family in ECs, which may help in a better understanding of PARs as a therapeutic target in the treatment of cardiovascular diseases, hemostatic disorders and cancer.

Clotting

ECTH-154

The role of ADAMTS13 exosites in VWF recognition and proteolysis

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Background: VWF multimeric size is proteolytically regulated in plasma by ADAMTS13, which cleaves the central A2 domain. As ADAMTS13 is secreted as an active protease, it requires a very high degree of specificity to prevent non-specific proteolysis of off-target substrates. ADAMTS13 specificity has been attributed to the multiple exosite interactions between ADAMTS13 spacer, cysteine-rich (Cys), disintegrin-like (Dis) and metalloprotease (MP) domains and complementary binding sites present in the unravelled VWF A2. Although the identity of the interacting exosites is known, the precise contribution of each exosite in the proteolysis of VWF has not been characterised.
Aims: The aim of the project is to characterise how different ADAMTS13 exosite interactions coordinate VWF proteolysis and dictate enzyme specificity and activity.
Methods: VWF96, a 96 amino acid VWF A2 domain fragment (VWF1573-1668) with N-terminal SUMO and C-terminal HSV tags, was expressed and purified. A panel of VWF96 mutants, containing either specific mutations or deletions that ablate each ADAMTS13 exosite interaction individually, were also generated. VWF96, VWF96-MP (L1603N), VWF96-Dis (DIKRD1614/6/7/8/1622AQEET), VWF96-Cys (IWAILI1642/4/7/9/1650/1QYSQQQ), VWF96-Spacer (LVL1664/5/6TNQ), VWF87ΔSpacer (VWF1573-1659) were used as ADAMTS13 substrates in activity assays. A novel ELISA assay was developed to monitor the kinetics of proteolysis. VWF fragments were also used in plate binding assays to measure the binding affinity of ADAMTS13 for each variant.
Results: VWF96 was proteolysed efficiently by ADAMTS13 (k_{cat}/K_m $14x10^5M^{-1}s^{-1}$). C-terminal deletion of the spacer domain exosite (VWF87ΔSpacer) resulted in a 17-fold reduction in proteolysis (k_{cat}/K_m $0.85x10^5M^{-1}s^{-1}$). Interestingly, mutating just 3 hydrophobic residues, LVL1664/5/6TNQ, in the spacer binding region (VWF96-Spacer) resulted in the same (19-fold) reduction in the proteolytic rate (k_{cat}/K_m $0.75x10^5M^{-1}s^{-1}$), suggesting that these 3 residues comprise the spacer domain binding site. Mutation of the Cys domain binding site (VWF96-Cys) caused a ~40-fold reduction in the proteolytic rate (k_{cat}/K_m $0.35x10^5M^{-1}s^{-1}$). Plate binding assays revealed that the reduced rate of proteolysis of VWF96-Cys, VWF96-Spacer and VWF87ΔSpacer was primarily manifest through reduced binding affinity. Proteolysis of VWF96-MP was ~150-fold reduced (k_{cat}/K_m $0.09x10^5M^{-1}s^{-1}$), despite the normal/near normal binding affinity of ADAMTS13 to VWF-MP. However, the greatest reduction (~300-fold, k_{cat}/K_m $0.05x10^5M^{-1}s^{-1}$) in proteolysis was measured when the Dis domain binding site was mutated (VWF96-Dis). Binding of ADAMTS13 to VWF96-Dis was only moderately reduced, and could not account for the 300-fold reduction in proteolysis.
Summary/Conclusion: The kinetic data for the proteolysis of VWF96, VWF96-Cys and VWF87ΔSpacer corroborate previous data. For the first time, we demonstrate that the ADAMTS13 spacer exosite involves interaction with 3 hydrophobic residues (L1664, V1665, L1666) in VWF. Disrupting the spacer and Cys domain interactions resulted in 17- to 40-fold reductions in proteolysis caused by reduced affinity between the two molecules. The ~150-fold effect of mutating the P3 residue in VWF96-MP may be explained by its proximity to the scissile bond. Our results using VWF96-Dis reveal that the ADAMTS13 Dis exosite interaction is the most important in VWF proteolysis, which probably influences the ability of the scissile bond to access the active site, with only moderate influence upon VWF binding.

Bleeding

ECTH-382

High and long-term expression of von Willebrand factor after sleeping-beauty transposon-mediated gene therapy in mice

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Background: Type 3 von Willebrand disease (VWD) is characterized by complete absence of von Willebrand factor (VWF). Current treatment options are limited to VWF/FVIII plasma concentrates, which only provide a short-term solution given the short half-life of VWF (8-12h). Gene therapy for VWD offers the potential for a long-term treatment of VWD.

Aims: To develop an integrative Sleeping Beauty (SB) transposon-mediated VWF gene transfer approach for the correction of VWF deficiency in a mouse model of severe VWD.

Methods: We constructed three different transposon plasmids containing full length murine (m)VWF cDNA. The first two transposon plasmids contained the mVWF cDNA under control of either the ubiquitous CAG or the liver-specific α1-antitrypsin promoter. The third plasmid combined the mVWF cDNA under control of the liver-specific promoter with a sandwich SB transposon design, specifically created to transpose larger transgenes. Each transposon plasmid, together with a plasmid encoding the SB100X transposase, was targeted to the liver of VWF-deficient mice via hydrodynamic gene delivery. Blood samples were taken at several time points after gene delivery to assess plasma levels of VWF. Bleeding diathesis was investigated using a tail-clip bleeding assay and a saphenous vein bleeding model.

Results: Delivery of the SB transposon containing the CAG-promoter resulted in physiological mVWF levels in the first week (135±20%, 3 days after gene transfer, n=24) after which VWF levels stabilized at much lower levels (around 1%) for more than 1 year after gene transfer. Using the more potent liver-specific promoter, VWF-plasma levels stabilized at significantly higher levels (22±4%, 6 months after gene transfer, n=22). Most interestingly, use of the sandwich SB transposon (with the liver-specific promoter) resulted in very high mVWF levels (>2000%) that stabilized around 42 days and remained in the supraphysiological range up to 1.5 year after gene transfer (260±95%; n=9). Transposon-mediated gene integration was confirmed using splinkerette PCR and carbon tetrachloride-induced liver regeneration experiments. Long-term expression of VWF however resulted in a significant reduction of high molecular weight (HMW) multimers (4±1%, 1.5 year after gene transfer, n=5). Most mice had corrected bleeding profiles 1 and 3 days after gene transfer (both $p<0.001$). At later time points, partial correction could still be observed in some treated mice up to 6 months after gene transfer but a larger number of mice were unable to control bleeding. Use of the saphenous vein bleeding model showed similar results. Both tail clip bleeding time and blood loss were inversely correlated with the fraction of HMW multimers (both $p<0.01$).

Summary/Conclusion: The powerful SB transposon system efficiently transposes the large mVWF cDNA into the host genome, resulting in long-term and sustained VWF-expression in a liver-based gene therapy platform. The sandwich SB-transposon resulted in robust and supraphysiological VWF-expression up to 1.5 year after gene transfer. Long-term expression of VWF by hepatocytes however results in reduced amounts of HMW multimers, potentially limiting its long-term hemostatic efficacy.

Bleeding

ECTH-252

Scavenger-receptors LRP1 and SRA display enhanced association to von Willebrand factor clearance mutants p.R1205H (Vicenza) and p.S2179F

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Background: Increased clearance of von Willebrand factor (VWF) has been reported to contribute to low VWF levels in von Willebrand disease (VWD)-types 1 and 2. Recently, we described that increased binding to the clearance-receptor LRP1 explained at least in part the reduced survival of the VWF-type 2B mutants VWF/p.V1316M and VWF/p.R1306Q. However, whether LRP1 or other receptors contribute to the increased clearance of other VWF mutants is unknown.

Aims: To decipher the role of LRP1 and other macrophage-receptors in the increased clearance of VWF mutants.

Methods: Mice deficient for macrophage-LRP1 or Scavenger Receptor-AI (SR-AI) were used to determine VWFpp/VWF:Ag ratios (a measure for VWF clearance) for wild-type (wt)-VWF and mutants VWF/p.R1205H (Vicenza-mutant) and VWF/p.S2169F. Cell- and solid-phase binding studies employing recombinant VWF variants were performed to analyze VWF-receptor interactions.

Results: In sold-phase binding studies, we confirmed that wt-VWF is unable to bind spontaneously to LRP1. However, introduction of the clearance-mutations p.R1205H or p.S2179F was associated with efficient binding to LRP1, suggesting that these mutations decrypt LRP1-interactive sites. This was further examined by expressing wt-VWF and both mutants in control and macLRP1-deficient mice in order to determine VWFpp/VWF:Ag ratios. For wt-VWF, VWFpp/VWF:Ag ratios were 1.3±0.1 and 1.1±0.1 (n=8; p=0.0014) in control and macLRP1-deficient mice, respectively, in agreement with the contribution of LRP1 to VWF clearance. As expected, these ratios were increased for both mutants in control mice (2.9±0.1 and 4.2±0.7 for p.R1205H and p.S2179F, respectively). In macLRP1-deficient mice, the VWFpp/VWF:Ag ratios were significantly reduced to 2.3±0.2 and 2.9±0.4, respectively ($p<0.001$), suggesting that LRP1 contributes to increased clearance of both mutants. However, these VWFpp/VWF:Ag ratios remain significantly higher than for wt-VWF, pointing to other contributing receptors.

In search for an additional scavenger-receptor that potentially could mediate increased VWF clearance we focused on the macrophage-specific receptor SR-AI, given that VWF is able to bind to macrophages also in an LRP1-independent manner. Indeed, binding studies revealed that VWF associates efficiently to the purified SR-AI (half-maximal binding: 6±2 nM) and to SR-AI-transfected HEK293-cells. Binding involves several VWF-domains (D'D3, A1 and D4, but not A2 or A3), is calcium-dependent and is inhibited by 72±4% in the presence of two monoclonal antibodies targeting the A1 and D4 domain. Ristocetin or VWD-type 2B mutations left SR-AI binding unaffected. In vivo, VWFpp/VWF:Ag ratios were decreased to 0.7±0.2 for wt-VWF in SR-AI-deficient mice, indicating a more pronounced role for SR-AI compared to LRP1 in VWF clearance.

As for mutants VWF/p.R1205H and VWF/p.S2179F, we observed enhanced binding of both mutants to purified SR-AI and to SR-AI-expressing cells. In addition, VWFpp/VWF:Ag ratios were significantly reduced in SR-AI-deficient mice (2.1±0.4 and 2.3±0.1 for p.R1205H and p.S2179F, respectively; $p<0.001$ compared to control mice).

Summary/Conclusion: We have identified SR-AI as a novel clearance-receptor for VWF. Both SR-AI and LRP1 display enhanced association to VWF clearance mutants p.R1205H and p.S2179F, which may explain the reduced circulatory survival of these mutants.

Clotting

ECTH-300

Genetic risk factors for venous thrombosis in women using combined oral contraceptives : update of the Pilgrim study

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Background: Venous thrombosis (VT) is a complex disease resulting from the interaction between genetic and environmental risk factors. Combined oral contraceptives (COC) are a widespread environmental risk factor which interaction with thrombophilia is associated with a major increased risk of VT. However identifying women at risk of VT remains extremely difficult and is a major public health issue.

Aims: The aim of the present study was to investigate in women using COC the impact on VT risk of genetic polymorphisms recently found to associate with VT in the general population.

Methods: As part of the PILGRIM (Pill Genetic Risk Monitoring) study, all women included in this work underwent a standardized questionnaire allowing us to collect data on environmental risk factors and family history of VT. A thrombophilia screening and ABO blood group genotyping were systematically performed. Nine polymorphisms, rs710446 (*KNG1*), rs2289252 and rs2036914 (*F11*), rs4524 (*F5*), rs3136516 (*F2*), rs867186 (*PROCR*), rs2066865 (*FGG*), rs78707713 (*TSPAN15*) and rs2288904 (*SLC44A2*) were genotyped. A total of 766 cases (women who experienced an episode of documented VT during COC use) and 464 controls (women with no history of TV under COC at the time of inclusion) were included.

Results: Only F11 rs2289252 was significantly associated with the risk of VT. After adjustment for confounding factors (age, type of COC, COC duration and family history) and four other main risk factors (smoking, obesity, severe thrombophilia, non-O blood group), the F11 rs2289252-A allele was associated with a 1.6 increased risk of VT (95% confidence interval (CI) [1.31-1.91]; p <0.0001). The combination of the risk allele for rs2289252 with non-O blood group was found in 52% of the women population and was associated with an adjusted OR of 4.00 (95% CI [2.49-6.47]; p <0.0001). Around 5% of women harbored the rs2289252-A + non-O blood group + BMI over 30 kg.m-2 (class I obese and above) combination which conferred a 13.1 (95% CI [5.2 – 38.5]; p<0.0001) increased risk of VT compared to non obese women with O blood group and rs2289252-GG genotype.

Summary/Conclusion: The F11 rs2289252 polymorphism was strongly associated with the risk of VT in a specific cohort of women using COC. The consideration of this genetic risk factor for VT, in combination with the previously identified ones, could help to better understand the risk in COC users and so to better guide the prescription.

Clotting

ECTH-125

Mild antithrombin deficiency and risk of recurrent venous thrombosis: results from the MEGA follow-up study

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Background: Mild antithrombin deficiency has been associated with a 2.4-3.5 fold increased risk of recurrent venous thrombosis (VT). This novel finding suggests that duration of antithrombotic therapy should be indefinite in VT patients with mild antithrombin deficiency. However, this result has not been confirmed by other studies.

Aims: Validate whether mild antithrombin deficiency is a risk factor for recurrent VT without increasing the risk of major bleeding by determining this risk in a large group of patients (n=2357) who had a first venous thrombotic event and who were followed for a median of 7.4 years.

Methods: A large population-based, case-control (Multiple Environmental and Genetic Assessment [MEGA] of risk factors for VT) study of 4956 consecutive patients aged 18 to 70 years, with a first deep VT of the leg or pulmonary embolism, between March 1999 and August 2004, at 6 anticoagulation clinics in the Netherlands reported via a questionnaire on risk factors for VT. Three months after discontinuation of the anticoagulant therapy, all patients were interviewed, a blood sample was taken, and DNA was isolated.

Results: Patients (n=2357) with a first VT were stratified according to percentile cut-off antithrombin levels (<5th, 5-10th, $\geq$10th percentile) and functional antithrombin levels (<70%, 70-80%, >80%). During follow-up 361 recurrent events occurred with an incidence rate of 2.5/100 patient-years. We observed an increased risk of recurrent VT in the lowest antithrombin activity category (<5th percentile; < 87%) as compared with antithrombin activity that was $\geq$10th percentile; $\geq$92%), with an adjusted hazard ratio (HR) of 1.5 (95% CI, 1.0-2.3). When we stratified our analyses to the antithrombin cut-off criteria to <70% as compared with patients who had antithrombin activity $\geq$80%, the adjusted HR of venous recurrence was 3.7 (95% CI, 1.4-9.9). Mild antithrombin deficiency was able to predict recurrent VT over at least 8 years of follow-up and the association remained present when the population was stratified to the presence or absence of thrombosis risk factors. The incidence rate of major bleeding was similar in those with mild antithrombin deficiency as in patients who had normal antithrombin levels.

Summary/Conclusion: This study confirms that mild antithrombin deficiency is a risk factor for recurrent venous thrombosis.

Clotting

ECTH-298

Defects of splicing in antithrombin deficiency: much more than expected.

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Background: Mutations affecting splicing are getting more relevance in the development of multiple human genetic diseases. In antithrombin (AT) deficiency only 17 mutations affecting intron splicing signals (IVS) have been described (5.8% of mutations identified in *SERPINC1*), all resulting in type I deficiency without variant AT in plasma.

Aims: To identify and characterize mutations in *SERPINC1* responsible for AT deficiency through a defect of splicing.

Methods: Exons and flanking regions of *SERPINC1* were sequenced in 124 unrelated cases with AT deficiency. Samples with negative findings, MLPA analysis was also done. Plasma AT was studied by functional and western blot assays, purified by FPLC and characterized by proteomic analysis. *In silico* prediction of potential splicing signals was done with the Human Splice Finder software. Recombinant expression of variants created by site-directed mutagenesis was done in HEK-EBNA cells.

Results: We identified 17 patients with potential mutations leading to aberrant splicing. P1-P11 had 9 different IVS point mutations affecting acceptor or donor sequences, 6 not previously described. P12 carried a 31bp deletion affecting the final nucleotide of intron 6 and 30bp of exon 7. These mutations, easily detected using exon flanking primers, caused type I deficiency. P13-P15 carried more distant intronic mutations (IVS2+26 and IVS5-14). *In silico* prediction suggested that IVS5-14G>A, identified in two unrelated cases, created a new acceptor sequence potentially generating a variant with the insertion of four residues. Purification of disulphide linked dimers from plasma of carriers and proteomic analysis confirmed the predicted splicing. P16 had a deletion of exon 4, and P17 carried the first duplication of 193bp comprising exon 6. Interestingly, this duplication was not detected by MLPA and caused moderate AT deficiency (75%) that can be explained by an alternative splicing of the duplicated exon. Finally, i*n silico* prediction of splicing signals potentially affected by all missensense and nonsense *SERPINC1* described in available data bases revealed 9 mutations that might disrupt donor or acceptor splicing signals or create strong cryptic splicing sequences. Two of them: p.Lys254Arg and p.Lys157Arg, the last one identified in a patient from our cohort with type I deficiency, were generated by site-directed mutagenesis in a plasmid containing the cDNA of *SERPINC1* and expressed in HEK-EBNA cells. Both mutants were expressed at similar rates than the wild type and have anticoagulant activity, supporting that the missense mutation does not interfere with the folding, secretion and function of the variant.

Summary/Conclusion: In addition to intronic mutations affecting donor or acceptor sequences of splicing, other genetic defects, including deep intronic mutations, mutations in exons and gross gene defects may cause AT deficiency through an aberrant splicing. Thus, this mechanism may underlie high numbers of cases with AT deficiency (14% of our serie). Moreover, the consequence of an aberrant splicing may be diverse, from type I, type II to even moderate AT deficiency. Finally, our study support that the identification of a mutation affecting a codon does not necessarily imply missense or nonsense consequences. We demonstrate that two theoretically missense mutations cause AT deficiency by an impaired splicing.

Clotting

ECTH-147

Major thromboembolic events and mortality in acquired thrombotic thrombocytopenic purpura: results from the phase 2 study with caplacizumab

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Background: In acquired thrombotic thrombocytopenic purpura (TTP), pathological aggregation of platelets to von Willebrand Factor (vWF) leads to profound thrombocytopenia, hemolytic anemia, and systemic microvascular thrombosis. Despite the current standard of care treatment consisting of therapeutic plasma exchange and immunosuppression, the mortality from an episode of acquired TTP is approximately 20% and patients remain at risk for thrombotic complications until remission is achieved[1]. Caplacizumab is an anti-vWF Nanobody® in development for the treatment of acquired TTP. The efficacy of caplacizumab in conjunction with standard of care was demonstrated in the Phase II TITAN study, in which faster resolution of the acute TTP episode was observed[2].

Aims: A post-hoc analysis was performed to evaluate the impact of treatment with caplacizumab on the incidence of major thromboembolic events during the study drug treatment period and the incidence of TTP-related mortality during this study.

Methods: The Standardized MedDRA Query (SMQ) for 'embolic and thrombotic events' was used to evaluate the occurrence of treatment-emergent major thromboembolic adverse events in the Phase II clinical study database. The total number of events and the proportion of subjects with at least one of these events were summarized and reported per treatment group. This analysis excluded transient episodes, as these were not considered major thromboembolic events. TTP-related mortality during the study was evaluated based on adverse events reporting, with relatedness to TTP as judged by the Investigator.

Results: The safety population consisted of 35 caplacizumab-treated and 37 placebo-treated subjects. Four major thromboembolic events were reported in 4 subjects in the caplacizumab group (1 pulmonary embolism and 3 TTP exacerbations). In the placebo group, 20 major thromboembolic events were reported in 14 subjects (2 acute myocardial infarctions, 1 ischemic and 1 hemorrhagic stroke, 1 pulmonary embolism, 1 deep vein thrombosis, 1 venous thrombosis and 13 TTP exacerbations). Two TTP-related deaths occurred during the study, both in the placebo treatment group (causes of death: refractory TTP and cerebral hemorrhage). In total, 11.4% of caplacizumab-treated subjects versus 43.2% of placebo-treated subjects experienced one or more thromboembolic events or died.

Summary/Conclusion: A lower proportion of subjects treated with caplacizumab had one or more major thromboembolic adverse events or died compared to subjects who received placebo treatment, suggesting that treatment with caplacizumab might reduce the significant morbidity and mortality associated with acquired TTP. A phase III confirmatory study is ongoing in which this clinically meaningful endpoint will be prospectively assessed.

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Platelets

ECTH-326

A novel two-stage FRETS-VWF73 assay reveals a cryptic pool of ADAMTS13 in plasma

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Background: Background: Thrombotic thrombocytopenic purpura (TTP) is a form of thrombotic microangiopathy that is accompanied by low or absent activity of ADAMTS13. This metalloprotease normally degrades von Willebrand Factor (VWF) multimers to control thrombogenicity. To date, it has proven challenging to relate ADAMTS13 activity to TTP disease severity.

The golden standard assay for determination of ADAMTS13 activity makes use of a synthetic peptide substrate (FRETS-VWF73). It contains a single cleavage site for ADAMTS13, flanked by a fluorophore and quencher. Their separation generates a fluorescent signal. The substrate also contains a large binding sequence, which is needed for successful cleavage by ADAMTS13. We previously established that the fibrinolytic enzyme plasmin cleaves VWF, and has protective properties in a mouse model for TTP. The molecular details of this interaction are still unknown.

Aims: We explored the possibility that the FRETS-VWF73 sequence contains a cleavage site for plasmin. We investigated how plasminogen activation in plasma influences the sensitivity of the diagnostic FRETS-VWF73 assay.

Methods: We studied the direct and indirect effects of plasmin activity on the capacity of FRETS-VWF73 substrate to detect ADAMTS13 activity in platelet-poor pooled plasma from healthy individuals (NPP). We either used streptokinase-activated plasminogen (purified from plasma) or alternatively activated plasminogen in plasma by adding streptokinase. In further experiments, we used aprotinin, ε-ACA, or tranexaminic acid to control plasmin activity.

Results: FRETS-VWF73 substrate does not develop fluorescence during direct exposure to plasmin activity. In contrast, plasmin terminates its capacity to detect ADAMTS13 activity in a dose- and time-dependent manner. The deleterious influence of plasmin can be prevented by aprotinin, as well as soluble lysine analogs. These experiments suggested that plasmin cleaves FRETS-VWF73 substrate in its ADAMTS13 binding tail. We subsequently expected that plasmin activity in plasma would disturb diagnostic determination of ADAMTS13 activity by FRETS-VWF73.

Surprisingly, when we triggered plasminogen activation in plasma, we detected a robustly increased ADAMTS13 activity (without plasmin activity: 113.9% +/- 13.9, with plasmin activity: 200.1% +/- 18.3; p=0.0043, Mann Whitney test). This suggests that a pool of ADAMTS13 is unavailable for determination, which can be liberated by plasmin. Based on these findings, we developed a sensitive two-stage protocol for determination of ADAMTS13 activity. In the first stage, plasmin activity is triggered in plasma. In the second stage, plasmin inhibitors and FRETS-VWF73 substrate are added.

Summary/Conclusion: Our experiments suggest the presence of a plasma factor that interferes with the FRETS-VWF73 assay and can be eliminated by plasmin. Interestingly, it was previously reported that ADAMTS13 and VWF form complexes in plasma (ISTH 2011 abstract #O-TU-087). It is to be expected that ADAMTS13 is unable to cleave to FRETS-VWF73 substrate when it is bound to VWF. We, as well as others, identified that plasmin can cleave both VWF and ADAMTS13, although with different kinetics. We hypothesize that controlled induction of plasmin activity in plasma disrupts ADAMTS13/VWF complexes, making ADAMTS13 available for detection by FRETS-VWF73. These findings may provide new insight into the variability in clinical symptoms between patients with low or absent ADAMTS13 activity levels, determined by conventional methods.

Platelets

ECTH-245

Childhood-onset acquired thrombotic thrombocytopenic purpura and long-term outcomes: the French reference centre for thrombotic microangiopathies experience

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Background: Thrombotic thrombocytopenic purpura (TTP) is an uncommon thrombotic microangiopathy (TMA), related to a severe ADAMTS13 deficiency (activity <10%), and characterized with microangiopathic hemolytic anemia, thrombocytopenia and widespread microvascular ischemia. ADAMTS13 deficiency might be either inherited (*ADAMTS13* gene mutations), or acquired (anti-ADAMTS13 autoantibodies, mainly). Patients were diagnosed with acquired TTP when anti-ADAMTS13 antibodies were found, or ADAMTS13 activity recovered during remission. ADAMTS13 phenotypic characterization during remission is crucial to detect a persistent severe and acquired ADAMTS13 deficiency, predictive of TTP relapses. Childhood-onset acquired TTP is rare and initially often misdiagnosed with idiopathic thrombocytopenic purpura, hemolytic uremic syndrome or Evans-syndrome.
Aims: Based on the 16 year-experience of the French TMAs Reference Center, the current study focus on French childhood-onset acquired TTP. Our objectives were: i) to investigate the first TTP bout to provide a demographic, clinical and biological picture of this cohort at presentation; ii) to assess the follow-up of these children and/or young adults, reporting the long-term outcomes (TTP evolution and health-related quality of life).
Methods: A cross-sectional analysis of the French Registry for TMAs was performed from 1999 to 2015 to identify, among childhood-onset TMA patients, those exhibiting acquired TTP. Inclusion criteria were i) a first TMA bout occurring before 18 years old, ii) ADAMTS13 activity <10% at presentation, iii) positive anti-ADAMTS13 autoantibodies at presentation and/or a detectable ADAMTS13 activity in remission. ADAMTS13 activity was measured using FRETS-VWF73 and full-length VWF ELISA; ADAMTS13 autoantibodies were detected with a functional semi-quantitative assay and/or quantified with an ELISA assay (type IgG anti-ADAMTS13) (ADAMTS13-INH ELISA® assay, Technoclone, Austria).
Medical records were extensively analyzed to collect clinical and biological data, during both the acute phase and the follow-up. Informed consent was obtained from each patient and/or his/her parents according to the Declaration of Helsinki.
Results: Forty-five children were enrolled in the current study; 25 patients exhibited an idiopathic presentation while 20 had associated clinical condition at presentation (infections, auto-immune diseases mainly). The median age at diagnosis was 13; sex ratio was 2.5F/1M. At presentation, all patients had a microangiopathic hemolytic anemia (median hemoglobin level 6.7 g/dL) and severe thrombocytopenia (median platelet count 11 G/L); the proportion of anti-ADAMTS13 auto-antibodies was higher in idiopathic TTP (96% *versus* 65% in non-idiopathic TTP). As a curative treatment of the inaugural TTP bout, 39 patients received plasma therapy and 21, curative rituximab. The short outcome showed a mortality rate of 9%. Interestingly, the global relapse rate was of 24%; 16% children benefited from preemptive rituximab. During the follow-up period (from 1 to 14 years, median 4 years), clinical manifestations of systemic lupus erythematosus occurred in two children.

Summary/Conclusion: Acquired childhood-onset TTP is a rare entity within a rare disease (prevalence ~0.3 children /million /year). The global picture of the inaugural childhood-onset TTP bout appears similar to the one of adulthood-onset TTP. Based on our experience, biological, clinical and therapeutic guidelines will be written for childhood-onset acquired TTP management.

3 INTEGRATED SYMPOSIA ABSTRACTS

Direct anticoagulants regulate arterial thrombosis

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The main purpose of anticoagulants is to diminish thrombin activity and fibrin formation, thereby decreasing the risk of venous or arterial thrombosis. Well known targets for effective anti-coagulation treatment are factor Xa (fXa) and thrombin, and direct inhibitors of these proteases have been developed.

However, accumulating evidence suggests that fXa and thrombin are also crucial modulators of cellular mechanisms through the activation of protease-activated receptor (PAR)-mediated signalling. Both fXa and thrombin play significant roles in mediating cellular signalling effects associated with the initial development of atherosclerosis; a chronic inflammatory vascular disease. For instance, thrombin and fXa regulate vascular permeability, migration, and proliferation of smooth muscle cells, recruitment of monocytes into atherosclerotic lesions, inflammation, and apoptosis. As a consequence, changes in levels of circulating thrombin and factor Xa have a direct impact on atherogenesis. This theory is supported by animal models demonstrating that specific and direct inhibition of thrombin or fXa attenuates atherosclerosis. In addition, increased expression and activation of PARs may be associated with atrial fibrillation (AF) and AF-associated thromboembolism. Both, atherosclerosis and AF are associated with hypercoagulability, suggesting that inhibition of thrombin or factor Xa through direct anticoagulants not only attenuates fibrin formation, but may also influence pathophysiological processes like AF and atherosclerosis. Although translation from animal model to clinical patients seems difficult at first sight, effort should be made to fully understand the clinical implications of long-term oral anticoagulant therapy on cardiovascular effects.

GPVI/CLEC-2 function in thrombosis and haemostasis

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The immunoglobulin receptor GPVI and C-type lectin receptor CLEC-2 activate platelets through a shared pathway that is driven by Src, Syk and Tec family kinases. Phosphorylation of a dual or single YxxL motif, known as an ITAM or hemITAM, respectively, is critical for activation by GPVI and CLEC-2, respectively. The major endogenous ligands for GPVI are collagen and fibrin, and for CLEC-2, podoplanin. Patients and mice with a deficiency in GPVI have a mild bleeding diathesis, whereas mice with a deficiency in CLEC-2 have normal haemostasis. In stark contrast, GPVI has been shown to play a critical role in arterial models of thrombosis while CLEC-2 mediates thrombosis at sites of inflammation-driven up-regulation of podoplanin in the venous system. Both receptors represent novel targets for pharmaceutical intervention either by direct receptor blockade or by inhibition of tyrosine kinases (which would target both receptors) with minimal effect on haemostasis.

Targeting GPIb in acute stroke
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Belgium

Stroke is a leading cause of death and long-term disability worldwide. Ischemic stroke is caused by a
blood clot that obstructs cerebral blood flow. Current treatment mainly consists of achieving fast reperfusion, either via pharmacological thrombolysis using tissue plasminogen activator or via endovascular thrombectomy. Unfortunately, reperfusion therapy is only available to a limited group of patients and reperfusion injury can further aggravate brain damage. Hence, there is an urgent need for better understanding of ischemic stroke pathophysiology in order to develop novel therapeutic strategies. In recent years, the pathophysiological importance of von Willebrand factor (VWF) in ischemic stroke has become clear from both clinical and experimental studies. In particular, binding of VWF to platelet glycoprotein Ib (GPIb) has become an interesting target for ischemic stroke therapy. Recent insights show that inhibting the VWF-GPIb interaction could result in a pro-thrombolytic activity improving cerebral reperfusion rates and concurrently reducing cerebral ischemia/reperfusion damage. An overview will be given of recent advances and experimental evidence illustrating the crucial role of the VWF-GPIb axis in ischemic stroke.

The GPVI competitor Revacept: Preclinical and clinical development

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Glycoprotein VI (GPVI) on the platelet surface plays a key role in plaque-induced platelet activation leading to acute coronary syndrome and ischemic stroke. Several pharmacological inhibitory interventions are currently under investigation.
Revacept (GPVI-Fc) is a recombinant, soluble dimeric GPVI and mimicks the binding properties of native GPVI. Collagen-induced static and dynamic platelet aggregation is inhibited by Revacept. Revacept inhibits platelet aggregation induced by human arteriosclerotic plaques (from patients with carotid artery endarterectomy) and plaque-induced thrombus formation at arterial flow conditions. Thrombus formation in vivo after acute vascular injury or in atherosclerotic mice (ApoE -/-) was also grossly inhibited. Revacept improved the outcome in animal models of cerebral ischemic stroke or myocardial ischemia. On the other hand bleeding time was not prolonged in mice with Revacept alone or in combination with a wide range of anti-platelet drugs or anticoagulants.
In a Phase I study in healthy men, Revacept elicited a dose-dependent pharmacokinetic profile and ex vivo efficacy by reversibly inhibiting collagen-mediated platelet aggregation. There were no adverse events - bleeding times in these human volunteers were not prolonged.
In an ongoing randomized double-blind, placebo-controlled Phase II study in patients with symptomatic carotid artery stenosis with recent TIA or stroke, Revacept is currently tested for safety and efficacy. So far, neither spontaneous bleeding complications nor increased bleeding during carotid endarterectomy have occurred. Efficacy data will be available when all data become unblinded and evaluated.
Revacept seems a promising lesion-specific anti-platelet agent to prevent plaque-induced vascular complications without affecting general haemostasis.

Epidemiolomics of complex diseases : Tools and strategies for new genomics risk factors discovery

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Rapid evolution of high-throughput *omics* tools triggered by the ingenious DNA microarray technologies for GWAS investigations ten years ago has led to unprecedented potential for genetics research to impact population health. This technological revolution led to the development of a wide range of tools to better understand the natural history of rare and common diseases, and response to interventions. These technologies allow cost efficient profiling - at an epidemiological (large) scale - of biological processes through whole (meta-)genome, transcriptome, methylome, miRnome, metabolome of various types of biosamples. The availability of several of these *omics*-derived biomarkers within the same individuals part of epidemiological/clinical cohorts offers exceptionally powerful design to address the inter-individual susceptibility to complex diseases : the *epidemiolomics* approach. The key challenge, however, lies in the complexity of these large datasets. To fully capitalize on the potential offered by the *omics* technologies, well-thought-out study design and analytic strategies are needed. These strategies aim to efficiently and simultaneously analyze multi-level *omics* cohort data while integrating information from publicly available resources from both human and experimental models. Data integration in epidemiological studies is key to fulfilling the promise of improved population health through genomics research. In this talk, such strategies will be discussed.

Genetics of coronary artery disease - discovery and translation
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Over the last decade, large-scale GWAS meta-analyses have identified several genetic loci associated with risk of coronary artery disease (CAD). There are at least 58 confirmed loci (reaching genome-wide significance) and a further 202 loci with a high level of evidence. This presentation will describe the current state of the discovery process, discuss what we have learnt and how the findings are being clinically translated.

Risk prediction and missing heritability for multifactorial diseases

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During the last decade, genome wide association studies have been very successful in detecting associations between multifactorial diseases and numerous Single Nucleotide Polymorphisms (SNPs). These associated SNPs are often used for estimating missing heritability and individual risk scores under the assumption of a polygenic additive liability. We will highlight the pitfalls of relying on such a model when there are reasons to suspect etiological heterogeneity and/or departure from the hypotheses on the environmental factor effects.

The main purpose of the genetic study of multifactorial diseases is to understand the etiology of the disease, with the hope that this understanding will contribute to better disease control and prevention. Such an objective is shunted around when assuming that all complex and heterogeneous pathological processes can be summarized by a single simplistic model.

This reductionist view has also led to the widespread belief that any SNP association can be explained by a single nucleotide change in a gene involved in the disease process (“a causal SNP”). The functional variation underneath the observed association at a SNP, however, is likely to be more complex.

We believe that time has come to shift from the polygenic additive model paradigm and to give room to more biologically-driven models of diseases supported by the physiopathology and genetic knowledge specific to diseases.

Gene therapy for Haemophilia: Where does the field stand?

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The curative potential of gene therapy was illustrated by our landmark study in haemophilia B (HB), in which a single administration of a self-complementary serotype 8 pseudotyped adeno-associated viral (AAV) vector resulted in (a) stable therapeutic expression of factor IX (FIX) for >4years enabling discontinuation of FIX prophylaxis; (b) change in bleeding phenotype from severe to mild; (c) improvement in quality of life, and (d) cost saving of >£2M.

Recent data using second generation AAV vectors employing the naturally occurring gain-of-function Padua mutation in the FIX gene, further improves the FIX coagulation activity levels to a remarkable 30% level in severe haemophilia B patients.

Progress has also been made with haemophilia A gene therapy by our group. In a recent BioMarin sponsored study our optimised AAV-FVIII expression cassette mediated Factor VIII at levels ranging between 4-60% in 6 severe haemophilia A patients recruited to the high dose cohort.

Finally, we have commenced building third generation AAV vectors which use synthetic AAV capsids designed to improve efficiency of gene transfer into the human liver. These vectors are likely to enable substantial reduction of vector dose required for therapeutic transgene expression from the current levels of 1013vg/patient, thus enhancing safety, reduce cost, and improve the prospects of more patients benefiting from gene therapy.

Therefore, after decades of failure very strong and rapid progress has been made in haemophilia gene therapy through advances in vector technology. Further developments required to make gene therapy the standard of care for severe haemophilia patients will be discussed.

Effect of emicizumab – a humanized bispecific antibody mimicking FVIII cofactor function – on a variety of assay systems

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Background: The mainstay of hemophilia A treatment is intravenous infusion of FVIII concentrates, which have an average half-life of ~10 hours (up to 19 hours for extended halflife products).[1] Patients who develop FVIII inhibitors are often treated with bypassing agents, which are less effective or predictable than FVIII concentrate use in non-inhibitor patients, and have a half-life of 2-12 hours.[2]

Emicizumab (ACE910), a humanized bispecific antibody that binds simultaneously to FIXa and FX, promotes the formation of FXa in absence of FVIIIa.[3] As its structure is completely distinct from FVIII, it cannot be neutralized by FVIII inhibitors. Emicizumab has a long-half life (4-5weeks) and has been administered weekly subcutaneously in clinical trials.

Aims: Describe the effect of emicizumab on coagulation laboratory tests.

Methods: The impact of emicizumab on activated partial thromboplastin time (aPTT), one-stage FVIII assays, rotational thromboelastometry (ROTEM) analysis, two-stage chromogenic FVIII assays, and thrombin generation testing was evaluated.

Results: In contrast to FVIII bypassing agents, such as rFVIIa and APCC[4], emicizumab has a clear and defined mode of action.[3,5] Emicizumab accelerates the activity of FIXa in the absence of FVIII by 87400 fold[5], which can be detected by intrinsically activated assays, such as aPTT, one-stage FVIII assays and ROTEM analysis. Unlike FVIII, emicizumab does not require an activation step. Therefore, aPTT is normalized at lower emicizumab concentrations, and one-stage FVIII assays when calibrated against FVIII can report very high FVIII concentrations with emicizumab therapy. Emicizumab can be detected using the two-stage chromogenic FVIII methodology using the Hyphen Biophen FVIII:C test. Chromogenic FVIII tests that employ bovine proteins are completely insensitive towards emicizumab. Thrombin generation testing has also shown sensitivity to the effect of emicizumab, mostly using FXIa as the trigger.

Conclusions: Emicizumab has a defined mode of action and a potent effect on FIXa activity, which allows the detection of the effects of emicizumab in a variety of assay systems. Various tests are being evaluated for the diagnostic management of patients receiving emicizumab.

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Potential benefits of Fc fusion beyond half-life extension
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Development of neutralising antibodies (inhibitors) is a serious complication in haemophilia treatment. rFVIIIFc and rFIXFc are human recombinant FVIII and FIX molecules, fused to the Fc domain of human IgG1, produced from a human cell line. Preclinical data from haemophilia A (HA) mice suggest rFVIIIFc to be less immunogenic and to potentially induce tolerance to FVIII[i]. Gene therapy with FVIII immunodominant domains (A2 and C2) on an Ig backbone induced specific tolerance in mice[ii]. Furthermore, maternofoetal transport of Fc fusions of FVIII A2 and C2 domains and rFVIIIFc in HA mice induced FVIII-specific tolerance in the progeny[iii]. No confirmed inhibitor cases were seen in phase 1/2a - 3 studies and to date in the ongoing extension studies in previously treated patients (PTPs)[iv,v]. There have been post marketing reports of inhibitor cases on rFVIIIFc. The safety of rFVIIIFc and rFIXFc in previously untreated patients (PUPs) is currently being studied. Case reports describing experience with ITI and rFVIIIFc in HA patients[vi,vii] have indicated relatively fast reduction of inhibitor titre in some cases[6]. In summary, early preclinical and clinical data suggest that Fc fusion factors may have Fc-mediated immunomodulatory properties allowing tolerance induction in haemophilia patients with inhibitors, but further evidence is needed to support the early findings.

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Use of anticoagulant inhibitors in hemophilia- benefit and risk – how much we know about them?
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Bleeding in haemophila is treated with on-demand factor replacement or prevented by intravenous replacement therapy with FVIII or FIX. However, treatment is suboptimal, particularly in patients with inhibitors, and involves frequent intravenous injections. Amongst emerging experimental therapies targeting natural anticoagulant pathways to reduce activity offers the potential to treat all forms of haemophilia, including rare bleeding disorders, with a single therapeutic agent. This may be particularly attractive in emerging populations who previously did not have access to treatment. Additional advantages are avoidance of development of clinically relevant inhibitors, use in patients with established inhibitors and infrequent subcutaneous administration. Targets under investigation are TFPI, antithrombin and the protein C pathway. However, manipulation of natural anticoagulant pathways may be associated with toxicity profiles not typically expected with conventional procoagulant replacement therapies. Consequently, thrombosis, inflammation and increased susceptibility to sepsis must be de-risked in preclinical development. With new first-in-class biologicals there will also be new challenges with CMC (Chemistry, Manufacturing and Control). Clinical trial design must include monitoring for development of ADAs (anti-drug antibodies), which limit efficacy, as well as detecting major adverse events such as thrombosis, particularly if additive procoagulant factor replacement therapy is required on demand during suppression of natural anticoagulant pathways. The safety profile of an infrequently subcutaneously administered drug that restored the haemostatic balance without the need for replacement of specific procoagulant factors would be paramount but if such a therapeutic could be designed it would be an attractive addition to the expanding number of potential therapeutics for haemophilia.

4 ORAL COMMUNICATIONS

Clotting

ECTH-297

Sex-specific differences in prevalence of pulmonary embolism among patients with venous thromboembolism

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Background: Several sex-specific risk factors of venous thromboembolism (VTE) have been identified. Women are at higher risk during their fertile life years, while men are at highest risk at older age. Furthermore, several risk factors seem to influence the presenting location of VTE. Pregnancy and use of oral contraceptives have been associated with a higher risk of deep venous thrombosis (DVT) than pulmonary embolism (PE). Sex-specific differences in the distributions of DVT, PE and PE with DVT have not been specifically described.

Aims: To explore differences in the distribution of DVT-only, PE-only and DVT with PE between men and women with a first VTE.

Methods: We analysed data of 3 different studies: MEGA-study (case-control study), the HOKUSAI-VTE study (randomized controlled trial), and published data from the RIETE registry. Data of 51701 individuals presenting with a first episode of symptomatic VTE were used (MEGA n=4953; the HOKUSAI-VTE n=6720; RIETE registry n=40028). Differences in distributions of DVT-only, PE-only and DVT with PE among VTE cases between men and women were calculated with their 95% confidence intervals (95%CI). These differences were further explored in different age categories and for unprovoked and provoked events. In the RIETE registry VTE events were only reported as DVT and PE with or without DVT.

Results: In the MEGA study, PE-only was the presenting location in 35.5% of all women and in 29.5% of the men (difference 6.1%, 95%CI 3.5-8.7). In the HOKUSAI-VTE study these proportions were 35.1% for women and 25.2% for men (difference 10.1%, 95%CI 7.8-12.2). In the RIETE registry PE (with or without DVT) was observed more often in women (53.5%) compared to men (47.7%) with a difference in proportion of 5.7% (95%CI 4.7-6.6). The observed higher proportion of PE-only in women was present in all age groups. This sex-specific difference in distributions was most prominent amongst unprovoked VTE events (difference of 15% [95%CI 9.6-20.3] in the MEGA, 10.7% [95%CI 8.1-13.8] in the HOKUSAI-VTE).

Summary/Conclusion: PE-only was more often the presenting location of a VTE event in women than in men. This finding was present for all age groups and most clearly for unprovoked VTE. The underlying mechanism is unknown.

Clotting

ECTH-312

The risk of recurrent venous thrombosis in patients undergoing surgery after a first thrombotic event

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Background: Surgery is a major risk factor for the development of first venous thrombosis (VT). For this reason, routine thromboprophylaxis is strongly recommended around major surgery. Although the risk of a first VT after surgery has been extensively studied, information is lacking about the risk of a recurrent event in patients who need to undergo surgery some time after a first VT.

Aims: To estimate the absolute and relative risk of recurrent VT in patients with a history of VT who underwent various types of surgery.

Methods: A cohort of 4731 patients with a first venous thrombotic event was followed over time for recurrence from 1999-2010 (MEGA follow-up study). Cohort data were linked to the Dutch Hospital Data Register which provided information on all surgical procedures that took place during the study period for all individuals. Follow-up time was estimated from the start of follow-up (stop-date anticoagulant treatment after first VT) until the end of follow-up (death, loss to follow-up, recurrent event, end of study date). The time window of surgery exposure was defined as three months from surgery date onwards. The total follow-up time in which patients were not exposed to surgery was calculated as the total follow-up time minus the exposure time. We calculated incidence rates (IR) by dividing the number of events by total amount of person-time, separately for the exposed and the unexposed time periods. This was done for all surgery, and separately for major and minor procedures. Finally, we calculated an incidence rate ratio (IRR) for recurrent VT comparing exposed with unexposed to surgery.

Results: During a median follow-up of 7.0 years per patient, 601 recurrent events occurred with an IR of 3.4/100 patient-years (95%CI 3.2-3.7/100 ptyrs). In total, 877 patients underwent 1255 procedures during follow-up (672 major, 583 minor). 19 patients developed a recurrent VT within a total of 237 surgery-exposure years (IR 8.0/100 ptyrs [95%CI 4.8-12.5/100 ptyrs]) compared with 582 recurrences in 17215 person-years without surgery (IR 3.4/100 ptyrs [95%CI 3.1-3.1/100 ptyrs]), yielding an IRR of 2.4 (95%CI 1.5-3.7). For major surgery the IRR was 2.9 (95%CI 1.6-5.1) and for minor surgery the IRR was 1.8 (95%CI 0.8-3.7). A limitation of our study is that information on thromboprophylaxis around the procedures was lacking. However, we assume that all patients received thromboprophylaxis according to current guidelines.

Summary/Conclusion: Surgery is a major risk factor for the development of recurrent VT in patients with a history of VT. The high recurrence risk after surgery suggests that anticoagulant treatment strategies for these patients can be further improved.

Clotting

ECTH-216

Association of impaired renal function with venous thrombosis: a genetic risk score approach

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Background: Association between impaired kidney function and venous thrombosis (VT) has been previously reported but supportive data are still sparsed.

Aims: We here wish to strengthen the reported association by investigating whether single nucleotide polymorphisms (SNPs) known to increase the estimated glomerular filtration rate (eGFR), a surrogate marker for renal dysfunction, are associated with a decrease of VT risk.

Methods: Fifty-one SNPs with strong evidence for association with eGFR in the litterature were tested for association with VT in a French case-control collection of 1,953 patients and 2,338 healthy individuals. Stepwise logistic regression analyses were performed to identify SNPs that associated with the risk of disease. Identified SNPs were then entered into a genetic score analysis to assess their cumulative impact on disease. The resulting genetic score was then tested for replication in an independent sample of 1,289 VT patients and 1,049 healthy controls part of the Dutch MEGA study.

Results: A genetic score derived from 12 eGFR-associated SNPs was found significantly ($p = 9.21\ 10^{-4}$) associated with VT risk in the French discovery sample. This genetic score ranged from 1 to 17 according to the number of eGFR-increasing alleles carried by the individuals. One unit increase of the genetic score was associated with decreased VT risk (OR = 0.95 [0.92 - 0.98]) in the discovery sample. In the replication sample, the same genetic score, with similar distribution to that observed in the discovery cohort, was also significantly associated with a decreased risk of disease (OR = 0.88 [0.81 - 0.97], $p = 9.45\ 10^{-3}$). The resulting combined OR was 0.94 [0.91 - 0.97] ($p = 6.67\ 10^{-5}$). We then categorized the genetic score distribution observed in the combined samples into quintiles. Compared to lowest quintile, the protective OR for VT associated with the second, third, fourth and fifth quintiles were 0.87 (p = 0.09), 0.89 (p = 0.15), 0.81 (p = 0.01) and 0.68 ($p = 4.04\ 10^{-6}$), respectively, suggesting a threshold effect.

Summary/Conclusion: In two independent case-control studies, we observed strong statistical association between eGFR associated SNPs and the risk of VT, with an even more pronounced genetic effect in the replication than in the discovery cohorts. Our study that employed a Mendelian-Randomization like strategy then provides new elements supporting the association between impaired renal function and the risk of VT. The present genetic score may help to objectively classify high-risk/ low-risk individuals and thus will allow the best preventive strategies in asymptomatic individuals.

Clotting

ECTH-166

Repeated measures of carotid atherosclerosis and future risk of venous thrombosis

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Background: Previous studies on the relationship between atherosclerosis and venous thromboembolism (VTE) have shown conflicting results. Case-control studies reported a two-fold higher prevalence of atherosclerosis in patients with unprovoked VTE compared to age- and sex-matched controls, whereas prospective studies found no association between atherosclerosis and VTE. Since atherosclerosis is a modifiable risk factor that may develop over time, the lack of association observed in cohorts with one single measurement and long follow-up time could potentially be explained by regression dilution bias. To our knowledge, the association between atherosclerosis and future risk of VTE has not been investigated in a cohort with repeated measurements of intima media thickness (IMT) and total plaque area (TPA).

Aims: To investigate the association between carotid atherosclerosis and VTE using a large prospective cohort with repeated measurements of IMT and TPA in subjects recruited from the general population.

Methods: Study participants were recruited from the fourth (1994-1995), fifth (2001-2002) or sixth (2007-2008) survey of the Tromsø study. Subjects with ultrasound measurements of carotid atherosclerosis and with no previous history of VTE (n=10428) were followed from date of enrollment to the date of an incident VTE, death or migration, or to the end of the study period, 31st of December 2010, whichever came first. In total, 4785 participants attended two or three surveys, and in these, measures of IMT and TPA as well as potential confounders were updated, and could be used as time-varying co-variates. Time-fixed and time-varying Cox-regression models with age as time scale were used to calculate and compare hazard ratios (HR) of VTE across levels of TPA and carotid IMT adjusted for sex and BMI. The study was approved by the Regional Committee of Research Ethics, and all participants provided informed written consent.

Results: There were 309 incident VTE events during a median follow up of 10.3 years. Participants with increasing carotid IMT were on average older and had a less favorable cardiovascular risk profile. TPA increased with increasing IMT (2.1 mm^2 in the lower quartile compared to 24.9 mm^2 in the upper quartile). There was no association between tertiles of increasing TPA and risk of VTE in the time-fixed model, using the group with no plaque as reference (*P* for trend across categories=0.90). Similar results were found in the time-varying analysis (HR upper tertile versus no plaque: 0.97, 95% CI: 0.70-1.35; *P* for trend=0.96). Furthermore, increasing carotid IMT was not associated with increased risk of VTE in the time-fixed (*P* for trend across quartiles=0.9) or time-varying analysis (HR for upper versus lower quartile: 1.23, 95% CI: 0.83-1.82; *P* for trend across quartiles=0.30).

Summary/Conclusion: Increasing carotid TPA and IMT was not associated with increased risk of VTE neither in time-varying nor time-fixed analysis of study participants recruited from the general population. Our findings suggest that atherosclerosis is not a risk factor for VTE.

Clotting

ECTH-441

Protein C activity is associated to early formation of new Q waves and failure to achieve ST segment resolution in patients with acute ST segment elevation myocardial infarction

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Background: The role of protein C system in the development of early necrosis and no-reflow phenomena after successful primary percutaneous coronary intervention (pPCI) in ST segment elevation myocardial infarction (STEMI) patients is potentially important for the function of microcirculation in the condition of severe ischemia.

Aims: To examine association between plasma activity of protein C, antithrombin and coagulation factors II, VII, VIII and fibrinogen to early formation of new Q waves (myocardial necrosis) and ST segment resolution (microcirculatory reperfusion) in patients with STEMI treated with primary percutaneous intervention (pPCI).

Methods: One hundred and seventy one patients with STEMI treated successfully with pPCI were enrolled in the study. Among them 98 patients were represented as an early presenters (the time from the pain onset to reperfusion was $\leq$ 4 hours) and 73 patients were late presenter group. The new Q waves were registered at admission ECG and the significant ST segment resolution ($\geq$50%) were recorded 30 minutes after pPCI. Plasma activity of protein C, antithrombin, coagulation factors II, VII, VIII and fibrinogen was measured from the venous blood samples at the first morning after pPCI.

Results: In the early presenter group protein C activity was significantly lower in 20 (20.4%) patients who had not achieved significant ST segment resolution compared to patients with significant ST segment resolution after pPCI (1.01 vs 1.13 IU/L, p=0.010) and in 37 (37.7%) patients who had Q waves at presentation compared to patients with absent Q waves (1.05 vs 1.14 IU/L, p=0.019). There were no differences between the activities of other haemostasis factors between patients with or without early ST segment resolution and the presence of new Q waves at presentation in both early and late STEMI presenters.

Summary/Conclusion: STEMI patients with early necrosis and no-reflow phenomena have lower activity of plasma protein C levels which may underlie the importance of protein C in the maintenance of microcirculation function.

Clotting

ECTH-323

PDGFB, a new candidate plasma biomarker for venous thromboembolism

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Background: There is a clear clinical need for high specificity biomarkers for predicting risk of venous thromboembolism (VTE), but thus far such markers have remained elusive. Complex interactions between genetic, environmental and acquired risk factors underlie disease development. Recent genetic discoveries as well as established susceptibility genes for VTE have thus far not been translated into a clinically useful setting and the clinical value of such genetic information is debated. We propose that addressing directly the human plasma proteome could be an alternative, and hopefully more efficient, strategy to identify biomarkers that associate with VTE. The development of high throughput technologies has opened up new possibilities for extensive patient plasma protein profiling. As part of the human protein atlas (HPA) project, affinity reagents against a large proportion of the human proteome were developed, which allowed us to extensively analyse plasma samples from large VTE cohorts to identify candidate clinical biomarkers.

Aims: Our aim is to apply an affinity proteomic plasma profiling approach to identify novel plasma biomarkers that may improve current clinical tools for diagnosis and risk prediction in VTE

Methods: We screened plasma samples from 88 VTE cases and 85 controls, collected as part of the Swedish 'Venous Thromboembolism Biomarker Study' (VEBIOS), with multiplexed suspension bead arrays composed of 755 antibodies targeting 408 candidate proteins.

Results: We identified significant associations between plasma levels of HIVEP1, vWF, GPX3, PDGFB and VTE occurrence. For replication we profiled plasma samples of 580 cases and 589 controls from the French FARIVE study. The association of vWF and PDGFB with VTE replicated after correction for multiple testing, whilst only weak trends were observed for HIVEP1 and GPX3. While the plasma levels of vWF and PDGFB were modestly correlated (r ~0.30) with each other, their profiles were independently associated with VTE risk in a joint model in FARIVE (vWF $p < 0.001$; PDGFB $p = 0.002$). PDGFB was verified as the protein target of the HPA antibody by immunocapture mass spectrometry (IC-MS) and sandwich assays.

Summary/Conclusion: In conclusion, we demonstrate that high-throughput affinity plasma proteomic profiling is a valuable research strategy to identify potential candidate biomarkers for thrombosis related disorders.

Moreover, we present the Platelet-derived growth factor (PDGFB), primarily expressed in platelet and endothelial cells, as a novel plasma biomarker for VTE.

Clotting

ECTH-303

Tissue factor expressed by different cell types regulates central nervous system haemostasis and autoimmune inflammation in a mouse model of multiple sclerosis

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Background: Multiple sclerosis (MS) is an autoimmune disease resulting in neuronal demyelination within the central nervous system (CNS). It is associated with a blood-brain barrier damage and activation of coagulation. Increased levels of thrombin generation and extravascular fibrin deposition are seen in the CNS, precede clinical symptoms, and co-localize with areas of demyelination in both patients with MS and mouse model of disease (experimental autoimmune encephalomyelitis – EAE). Thrombin inhibition by hirudin or disruption of interactions between fibrin and microglial cells reduced neuroinflammation and severity of EAE in mice; no bleeding complications have been reported in these studies.
Aims: Tissue factor (TF) is the primary initiator of the extrinsic coagulation cascade. Within the CNS, astrocytes express high levels of TF. Previously, we demonstrated that in mouse models of stroke astrocyte TF is essential for maintaining brain hemostasis but also contributes to microvascular thrombosis and neuroinflammation. The present study investigated the role of and the mechanism by which TF-dependent activation of coagulation contributes to the pathology of MS.
Methods: EAE was induced in 8-10 week old female mice on C57Bl/6J background (n=15-25 per each group) by immunization with myelin oligodendroglial glycoprotein peptide MOG35-55. Clinical score reflecting degree of paralysis was graded on a scale of increasing severity (from 0 to 5) daily for 30 days in a blinded fashion. Thereafter, mice we euthanized and spinal cords were collected to perform histological evaluation of myelin loss, immunohistochemical analysis of inflammatory cells infiltration, and real time PCR analysis of mRNA expression of inflammatory chemokines/cytokines as well as T cell and macrophage markers. The role of the TF-thrombin pathway in EAE was evaluated using genetic and pharmacologic approaches.
Results: EAE mice expressing very low levels of TF (1% of wild type) demonstrated statistically significant reduction of clinical score and neuronal damage which was associated with fewer macrophages and T cells infiltrating into the spinal cord as compared to EAE mice expressing normal levels of TF. Levels of mRNA expression of key inflammatory genes known to contribute to the progression of EAE (CCL2, CCL20, IL-17A, TNFA) were also lower in low TF mice with EAE. A similar beneficial effects were observed in EAE wild type mice treated with the inhibitory anti-TF antibody 1H1 (20mg/kg, ip, every 3 days) or the thrombin inhibitor dabigatran (60 or 120mg/kg, daily, oral gavage) starting on day 10 after induction of EAE. However, low levels of TF expression in all cell types and both anticoagulant treatments also resulted in fatal, bleeding complications in approximately 15-20% of EAE mice. In contrast, astrocyte-specific deletion of TF or total deficiency of PAR-2, dramatically reduced EAE progression and neuroinflammation without any bleeding complications. PAR-1 deficiency had only a modest effect whereas myeloid cell-specific deletion of TF did not affect EAE in mice.
Summary/Conclusion: Total inhibition of TF or thrombin activity significantly reduces neuroinflammation and severity of EAE but also increases the risk of bleeding. Selective deletion of astrocyte TF or PAR-2 deficiency reduces EAE symptoms without impacting CNS hemostasis. We are now investigating if the protective effects of astrocyte TF deficiency are mediated by TF:FVIIa PAR-2 signaling on these cells.

Clotting

ECTH-362

The C-terminus of TFPIα inhibits factor V activation by protecting the Arg1545 cleavage site

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Background: Coagulation factor V (FV) is the inactive precursor of FVa, of which the later acts as an essential cofactor to factor Xa (FXa) in the prothrombinase complex. FV is maintained in the inactive state by the interaction between a basic and acidic region in the B-domain. The C-terminus of tissue factor pathway inhibitor-α (TFPIα) is highly homologous to the FV basic region and also binds to the acidic region of FV. In fact, a large fraction of plasma TFPIα circulates in complex with FV. Thanks to this interaction, FV acts as a cofactor of TFPIα in the inhibition of FXa and TFPIα inhibits prothrombinase complexes containing forms of FVa that retain the acidic region. However, when FV is activated through cleavage at Arg^{709}, Arg^{1018} and finally Arg^{1545} by FXa or thrombin, it loses its anticoagulant properties and becomes a strong procoagulant. Recently, a FV splicing variant (FV-short) that lacks the basic region and binds TFPIα with high affinity has been described. FV-short is present in all individuals and represents ~5% of all plasma FV.
Aims: To gain more insight in the functional implications of the FV-TFPIα interaction.
Methods: We studied the effects of a peptide identical to the TFPIα C-terminus (TFPIα C-term) on thrombin generation in plasma and on FV activation in model systems.
Results: TFPIα C-term prolonged the lag time and decreased the peak height of tissue factor- and FXa-triggered thrombin generation in a dose-dependent manner. These effects were more pronounced at low procoagulant stimuli and in the presence of plasma TFPIα. TFPIα C-term also inhibited thrombin generation in FV-depleted plasma reconstituted with FV, but not in FV-depleted plasma reconstituted with FVa, suggesting an effect on FV activation and/or prothrombinase. In model systems, TFPIα C-term decreased the activation of purified FV by FXa and thrombin in a dose-dependent manner. This could be due to inhibition of FV proteolysis and/or to inhibition of prothrombinase in the assay used to quantify FVa activity. Therefore, FV activation was also followed by SDS-PAGE and Western blotting. This showed that TFPIα C-term interferes with FV activation by both FXa and thrombin, by selectively impairing cleavage of FV at Arg^{1545}, which is located close to the FV acidic region (residues 1493-1537). The effect of TFPIα C-term on FV activation by thrombin was at least 4-fold stronger for FV-short than for full-length FV, in line with their respective affinities for the TFPIα C-terminus.
Summary/Conclusion: In summary, binding of the TFPIα C-terminus to the acidic region of FV inhibits FV activation by FXa or thrombin by blocking access to the Arg^{1545} cleavage site. Since cleavage at this site marks the transition of FV from an anticoagulant to a procoagulant cofactor, this may represent an important new anticoagulant function of TFPIα. The main target of this anticoagulant mechanism is more likely to be FV-short than full-length FV, since FV-short binds TFPIα with high affinity and its plasma concentration (1-2 nM) is closer to the concentration of full-length TFPIα (0.25 nM).

Supported by grant nr. 2014-1 from the Dutch Thrombosis Foundation.

Clotting

ECTH-190

Thrombospondin-1 is elevated in the plasma of patients with antiphospholipid syndrome: implications in the pathogenesis of antiphispholipid syndrome.

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Background: Antiphospholipid syndrome (APS) is an acquired thombophillia characterized by recurrent thromboembolism and
pregnancy morbidity. Thrombospondin (TSP-1) is a matricellular glycoprotein secreted by platelets upon activation with proinflammatory,
antiangiogenic and proapoptotic properties. TSP-1 activates TGF-b_1 and has been shown to be involved in TH-17 response possibly through TGF-b activation.

Aims: We aimed to investigate the role of Thrombospondin-1 in the pathophysiology of antiphospholipid syndrome.

Methods: The study involved 90 patients with APS, 46 healthy controls (HC) and 26 SLE patients. Plasma, serum, and total IgG were isolated from all groups. Monocytes and CD4+ T-cells were isolated from 4 HC.

Human Umbilical Vein Endothelial Cells were isolated from 2 APS patients and 5 HC and cultured with plasma or total IgG from HC or APS patients. Monocytes were stimulated with total IgG and these supernatants were used to stimulate CD4+ T-cells.

Plasma and cell culture supernatant TSP-1, IL-1b, IL-17A and free active TGF-b_1 levels were determined using an ELISA.

Results: APS patients had higher plasma levels of TSP-1 than HCs and SLE patients (APS: mean 390ng/ml vs HC: 144.3 vs SLE: 153.0 $p<0.0001$)

Patient plasma free active TGF-b1 levels were higher and strongly correlated with TSP-1 ($r =0,827$ and $p<0,0001$).

APS HUVECs and HC HUVECs cultured with APS plasma expressed higher levels of TSP-1 than those cultured with HC plasma. (APS=139.4ng/ml vs HC=22.8ng/ml $p=0,0009$). Monocytes stimulated with APS total IgG produced higher levels of IL-1b and TSP-1 compared to the ones stimulated with HC IgG (700pg/ml vs 50pg/ml and 500ng/ml vs 200ng/ml respectively). APS stimulated supernatants induced the expression of IL-17A from T-cells (250pg/ml) whereas the HC had no effect.

Regarding the clinical aspects of APS, there was significant difference between the patients with pregnancy morbidity alone (130.1ng/ml) and those with miscarriages and thrombosis (403.2ng\ml).

Summary/Conclusion: Preliminary results suggest that APS patients have higher TSP-1 plasma levels which correlate with free active TGF-b1. Monocytes and HUVECs treated with APS plasma and APS IgG produce higher levels of TSP-1 and IL-1b and these supernatants induce the expression of IL-17A from naïve T-cells.

All these suggest a possible involvement of TSP-1 in thrombus formation, inflammation and inhibition of angiogenesis that needs further study.

Clotting

ECTH-366

Factor V functions as a synergistic cofactor with protein S in the inhibition of FXa by TFPI

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Background: Tissue factor pathway inhibitor (TFPI) inhibits the initiation of coagulation through direct binding and inhibition of factor Xa (FXa) and the tissue factor/factor VIIa (FVIIa) complex. Protein S (PS) acts as a cofactor for TFPI, enhancing the inhibition of FXa by 4-10 fold. This enhancement is dependent on a direct interaction between the PS SHBG-like domain and TFPI Kunitz domain 3. Through this interaction, protein S lowers the inhibition constant (K_i) for the initial FXa/TFPI binding to below the plasma concentration of TFPI (0.25 nM). In circulation, TFPI also binds factor V (FV) (and also FVa) and is capable of inhibiting prothrombinase through a direct interaction with FXa-activated FV and platelet-derived FV.

Aims: The role of the FV in TFPI anticoagulant action is not fully understood. We therefore aimed to investigate the anticoagulant functions of FV in the TFPI-mediated inhibition of FXa in the presence and absence of PS.

Methods: Recombinant variants of FV, TFPI, PS were expressed and purified. The enhancement of TFPI mediated by protein S and FV was tested in pure component FXa inhibition assays.

Results: While FV alone did not influence TFPI-mediated FXa inhibition, it further enhanced TFPI function in the presence of PS. This was primarily manifest through the enhancement of the formation of the initial FXa/TFPI complex formation. PS (100 nM) reduced the K_i ~4-fold, and FV (30 nM) further enhanced TFPI inhibitory function resulting in an overall ~8-fold reduction in Ki, compared with TFPI alone. Anti-FV and anti-PS antibodies demonstrated that the synergistic effect exerted by FV was specific and highly dependent upon PS. A FV mutant that cannot be cleaved either by thrombin or FXa (R709Q/R1018Q/R1545Q) showed an enhancement of TFPI inhibition in the presence of PS similar to that of wild-type FV. In contrast, thrombin-activated FV (FVa) was not able to enhance TFPI inhibition. A recombinant B-domain deleted FV variant (FVDT, aa 1-810+1492-2196), similar to the previously described FV-short, behaved like FVa and failed to enhance TFPI, both in the presence and absence of PS.

A TFPI variant lacking the basic region of the C-terminal tail (aa 1-249), a region described to interact with the acidic region of the FV B-domain, showed little enhancement by PS alone, but was enhanced by FV in the presence of PS (approximately 3.5-fold compared with TFPI). In contrast, TFPI E226Q, a TFPI variant previously shown to be unable to interact with PS, suggested that the FV-mediated enhancement was entirely dependent on the interaction between PS and the TFPI Kunitz 3 domain. In addition, a PS/growth arrest specific 6 (Gas6) chimera, with the whole sex hormone-binding globulin (SHBG)-like domain (Val243-Ser635) substituted by the corresponding domain in Gas6, was unable to enhance TFPI, alone or in combination with FV, again suggesting that the enhancement of TFPI inhibition shown by FV is dependent on PS, as well as the TFPI/PS interaction.

Summary/Conclusion: Full-length FV (and not FVa) acts together with PS as synergistic cofactor for TFPI in the direct inhibition of FXa.

Clotting

ECTH-264

New modulators of SERPINC1 gene expression. Relevance for antithrombin levels and potential therapeutic usefulness

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Background: Antithrombin (AT) is a crucial anticoagulant serpin whose even moderate deficiency significantly increases the risk of thrombosis. Genetic disorders affecting coding regions of *SERPINC1* causing AT deficiency constitute the strongest thrombophilic defect. However, little is known about regulatory aspects of *SERPINC1*.

Aims: To identify modulators of *SERPINC1* gene expression.

Methods: Patients with unexpected high levels of AT were studied. *SERPINC1* was sequenced. Plasma AT and other hepatic proteins were evaluated and quantified by different methods. The role of multiple factors on the transcriptional control of *SERPINC1* was evaluated in cell (HepG2) and animal models (wild-type and *Serpinc1*KO$^{+/-}$ mice) by qRT-PCR and western blot analysis.

Results: Three patients included in the list of liver transplantation due to hepatic damage caused by biliary atresia presented unexpectedly high levels of AT (anti-FXa: 150-195%; antigen: 110-140%), with parallel reduction of other proteins (prothrombin, PC, PS, FVII, albumin, or α1-antitrypsin). The increased levels of bile salts in these patients together with the transcriptional control that these salts play on multiple genes and the presence in *SERPINC1* of VDRE regulatory elements encouraged to test the effect of different bile salts on*SERPINC1* trascriptional control. Moreover, the role on mRNA and AT levels of other modulators that have been suggested to play a role in *SERPINC1* transcription (vitamin D, dexamethasone, triiodothyronine and GW4064) were also evaluated in HepG2 cell and mice. Different doses and combinations were tested. In human hepatoma cells, we confirmed that dexamethasone (500 nM), GW4064 (1 µM) and triiodothyronine (200 nM) caused a moderate but significant transcriptional activation of *SERPINC1*. Vitamin D (up to 80 ng/mL) significantly increased dose dependently the levels of *SERPINC1* transcripts and AT released to the conditioned medium. From all bile salts evaluated, chenodeoxycholic acid (CDCA), cholic acid (CA), ursodeoxycholic acid (UDCA), lithocolic acid (LCA) and glycochenodeoxycholic acid (GCDCA), only CDCA and UDCA significantly increased the levels of *SERPINC1* transcript. This effect was dose-dependent. Interestingly, the combination ofCDCA, CA and Vitamin D increased up to 3-fold the levels of *SERPINC1* mRNA transcripts.

Treatment of wild type mice with bile salts revealed increased levels of *Serpinc1* transcripts in the liver: 1.5-fold, 2.4-fold and 2.5-fold for CDCA, CA and UDCA respectively. This effect was also observed in *Serpinc1* $^{+/-}$mice, which reached normal levels of *Serpinc1* expression (>100%) when treated with CDCA or UDCA.

Summary/Conclusion: This study confirms the regulatory role of *SERPINC1* transcription of dexamethasone, GW4064, and triiodothyronine, and identifies new modulators of this key anticoagulant: vitamin D and bile salts. This finding explain why accumulation of bile salts due to different disorders may increase the levels of AT in plasma, even though a liver dysfunction. More interestingly, these factors may be explored as new therapeutic agents as an alternative to exogenous AT supplementation to rise endogenous AT levels in cases with congenital or acquired AT deficiency as well as in patients with high risk of thrombosis.

Clotting

ECTH-250

Conformational activation of ADAMTS13: The spacer-CUB/VWF-D4CK domain interactions

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Background: The von Willebrand factor (VWF) cleaving protease, ADAMTS13, circulates in a closed conformation that is maintained by the interaction between its N-terminal spacer domain and its C-terminal domains. This conformation conceals its critical VWF binding exosites in the N-terminal domains. ADAMTS13 must adopt a more open conformation to become fully active. This conformational activation is mediated by the binding of VWF to the ADAMTS13 C-terminal domains, which causes their dissociation from the N-terminal spacer domain.

Aims: We aimed to characterize the interaction of the ADAMTS13 spacer domain and it C-terminal CUB domains to further understand the molecular basis of ADAMTS13 conformational activation.

Methods: Binding between isolated ADAMTS13 CUB domain fragments (CUB1, CUB2 and CUB1-2) and MDTCS (an ADAMTS13 N-terminal fragment lacking its C-terminal tail) was analysed by co-immunoprecipitation and surface plasmon resonance (SPR). The auto-inhibitory potential of the CUB domains was determined by examining their ability to inhibit truncated ADAMTS13 variants (ADAMTS13ΔCUB2, ADAMTS13ΔCUB1-2 and MDTCS), as well as spacer domain variants, in FRETS-VWF73 assays. The binding of ADAMTS13 and its truncated variants (ADAMTS13ΔCUB2 and ADAMTS13ΔCUB1-2) to VWF-D4CK (the binding partner of the C-terminal domains of ADAMTS13) was analysed by SPR to establish which domains in ADAMTS13 bind VWF and in turn facilitate its conformational activation.

Results: Co-immunoprecipitation suggested that both CUB1 and CUB2 domains interact with the spacer domain. SPR analysis confirmed that both CUB1 and CUB2 domains bind to WT-MDTCS and that CUB1-2 exhibited a higher affinity, suggesting that both CUB1 and CUB2 domains mediate an interaction. Binding of both CUB1 and CUB2 is abolished by mutation of the ADAMTS13 spacer domain exosite. The closed, and partially inactive, conformation of ADAMTS13 is retained in the truncated variant ADAMTS13ΔCUB2 but removal of CUB1 in the variant ADAMTS13ΔCUB1-2 leads to an increase in activity similar to that seen in other conformationally active variants. Furthermore, CUB1, but not CUB2, is able to inhibit the conformationally active variant WT MDTCS in FRETS-VWF73 assays.

Finally, we have established that both CUB1 and CUB2 bind to the VWF-D4CK domains. However, the affinity of VWF-D4CK for ADAMTS13ΔCUB2 and ADAMTS13ΔCUB1-2 is similar (K_D of 176.9 ± 31.6 nM and 181.8 ± 25.3 nM, respectively) to its affinity for WT ADAMTS13 (K_D of 148.5 ± 38.1 nM), suggesting that the CUB domains are not alone in binding VWF-D4CK, implicating the other C-terminal domains (TSP2-8) in binding and activation.

Summary/Conclusion: These results suggest that although both CUB1 and CUB2 interact with the spacer domain, only CUB1 is responsible for mediating the partially inactive conformation. Moreover, neither CUB1 nor CUB2 are critical to the interaction between ADAMTS13 and VWF-D4CK and therefore conformational activation of ADAMTS13 by VWF-D4CK is likely to result from its binding to the non-CUB C-terminal domains (i.e within the TSP2-8 domains).

Platelets

ECTH-413

Complete loss of CalDAG-GEFI expression leads to severe bleedings caused by altered platelet function

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Background: Screening for *RASGRP2* mutations in patients suffering severe bleedings with normal platelet count and a putative recessive transmission pattern allowed the identification of three new mutations in two French unrelated families. The first propositus carry a homozygous deletion of two nucleotides (c.199-200delAA) generating an Asparagine to Leucine substitution in position 67 (p.N67Lfs*24) within the Ras-Exchange Motif (REM) and a premature stop codon 25 amino-acids downstream the mutation site. The second patient bears two heterozygous point mutations both affecting the CDC25 domain of the catalytic region of CalDAG-GEFI. One (c.778G>T) generates a stop codon at position 260 (p.E260*) and the other one (c.886T>C) leads to a Cysteine to Arginine transition at position 296 (p.C296R).

Aims: Characterize the platelet function defects in patients carrying these three novel *RASGRP2* mutations.

Methods: Flow cytometry; Platelet aggregation, adhesion under static and flow conditions and spreading; Rap1 GTPase activation assay.

Results: The mutations blunt CalDAG-GEFI protein expression in both transfected GripTite™ 293 MSR cells and platelets isolated from the patients. They strongly dampened the activation of the CalDAG-GEFI prototypical substrate, Rap1, in platelets stimulated with low and intermediate doses of ADP and TRAP-6. PMA leads the same extent of Rap1 activation in patients and controls confirming, the existence of CalDAG-GEFI bypassing pathways dependent on PKC. The mutations affect platelet's ability to perform proper αIIbβ3 integrin inside-out signaling. In homozygous carriers, maximal aggregation in response to all tested doses of ADP is markedly reduced. Among heterozygous patients, ADP-induced aggregation is variably affected. However, in the presence of the P2Y12 blocker, 2MesAMP, platelet aggregation in heterozygous carriers relies more on the ADP-dependent amplification pathway as compared to controls. This suggests that the importance of the ADP-dependent amplification pathway is inversely related to CalDAG-GEFI protein expression level.

Under static and arterial flow conditions, the surface covered by platelets of homozygous patients was strongly reduced and platelet translocation significantly increased. Patients' platelets did not perform proper spreading with normal filipodia formation but reduced lamellipodia elongation. Interestingly, platelets from both homozygous and heterozygous patients were unable to form lamellipodia upon high concentration of TRAP-6 (50µM) despite this dose fully restores platelets aggregation. This suggests that beside its central implication in integrin inside-out integrin signaling, CalDAG-GEFI is also required for outside-in pathways.

Summary/Conclusion: We report here, three new mutations in *RASGRP2* that lead to complete deficiency in CalDAG-GEFI. In addition to our first report, we show that platelet function of heterozygous patients is more dependent on ADP amplification pathway than in controls. Thus, the efficacy of P2Y12 blockers may be impacted by CalDAG-GEFI expression. The demonstration that high doses agonists in heterozygous patients cannot overcome the spreading defect supports the anti-thrombotic effect of partial CalDAG-GEFI inhibition that deserves to be investigated in more depth.

Bleeding

ECTH-169

A fusion protein of interleukin-4 and interleukin-10 protects against blood-induced cartilage damage in vitro and in vivo

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Background: Joint damage upon bleeding causes significant morbidity in patients with hemophilia. Interleukin (IL)-4 and IL-10 have been demonstrated to protect cartilage from blood-induced damage independently. Recently a novel fusion protein of both cytokines, IL4-10 synerkine, has been developed.
Aims: To evaluate whether IL4-10 synerkine protects against blood-induced joint damage similarly as the combination of the individual components, both *in vitro* as well as *in vivo*.
Methods: *In vitro*, human cartilage explants were exposed to 50% v/v whole blood for 4 days to and simultaneously a broad concentration range (0-100ng/mL) of the IL4-10 synerkine. Effects of 10 ng/mL IL4-10 synerkine were compared to the same concentrations of the individual cytokines and the combination. Cartilage matrix turnover was assessed after a recovery period of 12 days. Moreover, effect on IL-1β and IL-6 production was investigated in 4 day whole blood culture.
In hemophilia A mice, a joint bleed was introduced on day 0 and 14 and intra-articular treatment with synerkine (7pmol), IL-4&IL-10 (both 7pmol) or PBS on day 0, 2, 14 and 16. After 5 weeks, joint damage was evaluated by the Valentino score for synovitis and the modified OARSI score for cartilage damage.
Results: *In vitro,* the synerkine prevented blood-induced cartilage damage in a dose-dependent manner up to normalization already at a concentration of 1 ng/mL. At 10ng/mL, the synerkine was equally effective as the combination of the separate cytokines. IL-1β and IL-6 release in whole blood cultures was suppressed. *In vivo*, treatment with the synerkine attenuated cartilage damage upon joint bleeding (difference between experimental and contralateral joint in synerkine group $p=0.201$; IL-4&IL-10 $p=0.008$; PBS $p=0.001$). In all groups, synovial inflammation was statistically significantly increased in the experimental paw, none of the treatments affected this ($p<0.005$ in all groups).
Summary/Conclusion: The IL4-10 synerkine fully prevented blood-induced cartilage damage in a human cartilage tissue *in vitro* model and ameliorated cartilage degeneration upon a repeated joint bleed in hemophilic mice when injected intra-articularly. These data support the need for further investigation of the potency of the IL4-10 synerkine in the treatment of blood-induced arthropathy.

Bleeding

ECTH-248

Matrix metalloproteinase inhibition (CM352) effectively reduces rivaroxaban but not dabigatran associated experimental bleeding

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Background: Direct oral anticoagulants (DOACs) have been used increasingly in clinical settings. Despite their many advantages, bleeding remains a potential complication. Matrix metalloproteinases (MMPs) participate in thrombus dissolution and their inhibition has been assessed for controlling haemorrhage. CM352, a novel antifibrinolytic MMP inhibitor, selected by its MMP10 inhibitory capacity, represents a promising clinical candidate for the acute treatment of bleeding.

Aims: We have studied the effectiveness of the CM352, on bleeding associated with Dabigatran and Rivaroxaban *in vitro* and *in vivo*.

Methods: Functional thromboelastometry assay (ROTEM) was performed with CM352 in the presence of immobilised endothelial cells (EAHy926) to analyse the direct influence of MMP inhibition on the anticoagulant effects of Dabigatran and Rivaroxaban. The resistance of blood clots to fibrinolysis by exogenous tPA was also assessed.

In vivo, fifty mice (n=10/group) received intraperitoneal Dabigatran (4.5 mg/Kg) or intravenous Rivaroxaban (3 mg/Kg) 1h and 1h 30min before removing the tail tip respectively, and bleeding time was measured up to 30 min. CM352 or saline were infused through the femoral catheter five minutes before tail tip excision. Moreover, to illustrate the effect of activated Factor X (FXa) and thrombin on MMPs fibrinolytic activity, fluorogenic and antigenic experiments were performed *in vitro* with recombinant MMP10.

Results: As expected, both anticoagulants significantly prolonged clotting time (CT) in ROTEM analysis as compared to control (1029±28 s Rivaroxaban, 983±23 s Dabigatran and 562±13 s control, both $p<0.01$). However, clot lysis time was significantly reduced by Rivaroxaban (2238±197 s control vs 1469±117 s Rivaroxaban, $p<0.01$) but not by Dabigatran, indicating that Rivaroxaban exerts a profibrinolytic effect. Addition of CM352 did not modify the CT of both DOACs, but delayed lysis time only in the presence of Rivaroxaban (3330±103 s, $p<0.05$). In vivo, administration of CM352 was able to control the bleeding induced by Rivaroxaban but not by Dabigatran (30 min Rivaroxaban vs 18±0.6 min Rivaroxaban+CM352, $p<0.01$ and 29.3±0.5 min Dabigatran vs 29.2±0.8 min Dabigatran+CM352, respectively). In order to unravel the mechanisms involved in the antihemorragic action of CM352 on Rivaroxaban, we analysed the effect of activated Factor X (FXa) and thrombin on MMP10 using purified systems. Western blot analysis and ELISA assays demonstrated that FXa cleaves MMP10 proenzyme (proMMP10) but not its active form (aMMP10), neither it affects aMMP10 activity (fluorimetric assay). These results suggest that inhibition of FXa by Rivaroxaban prevents proMMP10 degradation, favouring its profibrinolytic activity. CM352 would act in this context by inhibiting profibrinolytic MMP10 activity, thus preventing MMP-induced bleeding.

Summary/Conclusion: CM352 could provide an alternative for the management of bleeding events occurring under treatment with FXa inhibitors by inhibiting MMP activity.

Bleeding

ECTH-393

Efficient and allele-specific siRNAs that target von Willebrand factor single nucleotide polymorphisms as a potential personalized therapy for von Willebrand disease

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Background: Treatment of von Willebrand disease (VWD) is based on the increase of plasma von Willebrand factor (VWF) levels by induced secretion of endogenous VWF by DDAVP or by infusion of exogenous VWF concentrates. In both therapeutic approaches, mutant VWF will still be produced which may adversely affect hemostasis. A therapy that targets mutant *VWF* itself may overcome these effects as only normal VWF is expressed. Expression of mutant VWF can be inhibited by small interfering RNAs (siRNAs), but this requires discrimination by the siRNA between mutant and normal *VWF* alleles. The mutant and normal allele can be distinguished by the single nucleotide difference caused by the *VWF* mutation. However, the allele-specific siRNA would then only be applicable for that specific mutation and not for a large group of patients. We therefore suggest to discriminate the mutant and normal allele using heterozygous single nucleotide polymorphism (SNP) alleles linked to the *VWF* mutation. This approach would be applicable for a large group of VWD patients irrespective of the specific mutation.
Aims: We aim to select siRNAs that can distinguish *VWF* alleles by SNP discrimination and efficiently inhibit one allele.
Methods: Three SNPs in *VWF* with a high minor allele frequency in the population have been selected. For each of the two alleles of the three SNPs, two or three siRNAs have been designed. These siRNAs have been tested for their efficiency and allele-specificity in stable HEK293 cell lines expressing either of the SNP variants. The efficiency of the siRNAs was determined by VWF:Ag levels in the medium and lysates. Expression levels were normalized against cells transfected with a scrambled siRNA (siNEG). Allele-specificity was determined by the difference between VWF expression of the targeted SNP-allele compared to the untargeted SNP-allele. The most potent siRNAs have been tested in HEK293 cells co-transfected with the siRNA and the two SNP-alleles. The two alleles were distinguished at the protein level by HA and MYC tags added to the respective VWF constructs, which could be detected by specifically developed VWF-HA and VWF-MYC ELISAs.
Results: Transfections of siRNAs in stable VWF producing HEK293 cells resulted in significant allele-specific inhibition of VWF for at least one siRNA per SNP-allele. The most optimal siRNA showed a knockdown of 77% of the targeted SNP-allele and 34% of the untargeted SNP-allele. Co-transfections of this siRNA with both SNP-alleles showed an even more efficient and more allele-specific knockdown. In co-transfections of siNEG with VWF-MYC and VWF-HA, the ratio between both alleles was approximately 50/50. After co-transfection of these two alleles with the specific siRNA against the SNP-allele conjugated to MYC, this ratio was shifted to 8/92.
Summary/Conclusion: siRNAs designed to target *VWF* SNPs showed efficient knockdown and discrimination of *VWF* alleles transfected in HEK293 cells. A 8/92 ratio of mutant and normal alleles, is very likely to improve VWD phenotypes caused by dominant-negative mutations. The phenotypic effect of this allele-specific knockdown will now be studied on VWF constructs containing dominant-negative mutations, co-transfected with a normal VWF construct and the designed siRNA.

Bleeding

ECTH-261

The correlation between biomarkers of joint cartilage degradation and markers of oxidative stress in patients with severe haemophilia treated by different prophylaxis regimens

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Background: Haemophilic arthropathy is the main cause of morbidity in individuals with severe haemophilia. The precise mechanism which induces haemophilic arthropathy is still unclear but recently studies shown that recurrent bleeding into joints causes degradation of joint cartilage and releasing the biomarkers of joint damage. Moreover, the free iron in joint cartilage causes increased oxidative stress also releasing its markers in physiological fluids

Aims: The aims of this study were to detect correlation between biomarkers of joint cartilage degradation and markers of oxidative stress as well as to estimate the influence of different prophylaxis regimens for severe haemophilia on this process.

Methods: The study included 20 adult patients with severe haemophilia. Written informed consent was obtained from all participiants. Five patients with haemophilia A received prophylaxis with FVIII concentrate in the standard dose of 20 IU/kg three times per week, while another five patients with haemophilia A were given an intermediate dose of FVIII concentrate as prophylaxis, 10-15 IU/kg thrice weekly. Seven patients with haemophilia A and three with haemophilia B, received FVIII/IX concentrate only on-demand. The following biomarkers of joint cartilage degradation were measured: a) serum cartilage oligomeric matrix protein (COMP) and b) urinary C-terminal telopeptide of type II collagen (CTX-II). The estimated oxidative stress markers were following: a) advanced oxidation protein products (AOPP), b) paraoxonase-1 (PON1) and c) sulfhydril groups (SHG). Blood and urine samples were collected initially, before the start of treatment (labelled COMP-1, CTX-II-1, AOPP-1, POM1/1 and SHG-1) and after 3 months follow-up (labelled COMP-2, CTX-II-2, AOPP-2, PNH1-2 and SHG-2).

Results: In the group of patients given standard dose prophylaxis, the mean values of cartilage degradation markers - COMP-2 ($p = 0.043$) and CTX-II-2 ($p = 0.014$) were significantly lower than COMP-1 and CTX-II-2. Also,, the oxidatibe stress markers - AOPP-2 ($p=0.018$), PON1-2 ($p=0.043$) and SHG-2 ($p=0.045$) were significantly lower than those for AOPP-1, PON1-1 and SHG-1. Likewise, the mean values for CTX-II-2 ($p = 0.028$), AOPP-2 ($p = 0.047$) and PON1-2 ($p=0.039$) in the five patients receiving intermediate dose prophylaxis were also decreased when compared to initial values, but COPM and SHG levels were not significantly changed. In patients treated on demand the mean values of all estimated markers were not significantl changed. The results showed the srongest positive correlations between AOPP and both COMP and CTX-II. Namely, lower values of AOPP were significantly associated with decreased levels of both biomarkers of cartilage degradation: COMP ($p = 0.008$) and CTX-II ($p = 0.014$).

Summary/Conclusion: The precise mechanism of joint disease in patients with severe haemophilia remains unknown but probably involves blood-induced increase of oxidative stress, which leads to higher joint cartilage turnover. The most important clinical strategy for management of these patients and prevention of severe arthropathy is treatment by continuous prophylaxis.

Bleeding

ECTH-343

Depletion of prekallikrein improves host defense during Gram-negative pneumonia derived sepsis

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Background: Prekallikrein (PKK) is an essential zymogen component of the contact system. Sepsis is associated with activation of the contact system accompanied with release of bradykinin and initiation of intrinsic coagulation. Knowledge of the role of the contact system in local host defense during bacterial infection and the subsequent development of sepsis is limited.

Aims: To determine the role of PKK in the host response to gram-negative pneumonia derived sepsis.

Methods: We used a common pathogen for pneumosepsis in humans, *Klebsiella pneumoniae*, to infect C57BL/6 mice via the airways (LD100). Mice were pretreated with a PKK antisense oligonucleotide (PKK ASO) to deplete PKK, or a control (Ctrl) ASO. Mice were followed for 10 days after infection, or sacrificed at an early time point (12 h after infection) or during late-stage sepsis (36 h).

Results: PKK ASO pretreatment reduced PKK mRNA levels in the liver and PKK protein levels in plasma by over 85% compared with Ctrl ASO. PKK depleted mice had a strongly prolonged survival compared with mice treated with Ctrl ASO, which was accompanied by significantly lower bacterial loads in the lungs, blood and distant organs . While lung histopathology did not differ between groups, PKK depletion reduced pulmonary levels of proinflammatory cytokines and chemokines in both early and late stage sepsis, and attenuated distal organ damage as reflected by lower plasma levels of transaminases and LDH.

Summary/Conclusion: PKK inhibition improves host defense during *Klebsiella* induced pneumosepsis as indicated by prolonged survival, diminished bacterial growth and dissemination, and attenuated local and systemic inflammation. These results suggest that activation of the contact system is detrimental to the host during pneumonia derived sepsis.

Platelets

ECTH-316

GPV is a central regulator of haemostasis, thrombosis and thrombo-inflammatory brain infarction in mice

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Background: Glycoprotein (GP) V is part of the GPIb-V-IX complex on the platelet surface, which mediates the initial adhesion of platelets to the exposed extracellular matrix upon vessel wall injury via the GPIbα subunit. GPV can be cleaved by thrombin, but – despite the existence of two knockout strains – the function of GPV still remains elusive. The (hem)ITAM receptors GPVI and CLEC2 mediate strong platelet activation. Loss of both receptors results in defective hemostasis and arterial thrombus formation.
Aims: We aimed to elucidate the role of GPV in thrombosis and thrombo-inflammatory processes. Therefore, mice with an inactivating point mutation in the thrombin cleavage site of GPV (*Gp5*$^{kin/kin}$) were generated and analyzed in comparison with *Gp5*$^{-/-}$ mice.
Methods: Platelet function was studied in a broad range of *in vitro* assays and *in vivo* models of hemostasis, arterial thrombus formation and thrombo-inflammatory brain infarction using *Gp5*$^{-/-}$ and *Gp5*$^{kin/kin}$ mice as single mutants or in combination with deficiencies of (hem)ITAM receptors, GPVI and/or CLEC-2 or critical components of their downstream signaling machinery.
Results: In agreement with previous reports, platelets from *Gp5*$^{-/-}$ mice showed a slightly increased activation response to thrombin *in vitro* translating into a very mild acceleration of hemostasis and arterial thrombus formation. Remarkably, however, GPV deficiency completely restored defective hemostasis in mice with deficiencies in (hem)ITAM receptors or defects in their downstream signaling. Moreover, GPV-deficiency fully reverted protection of GPVI-deficient mice from arterial thrombus formation and brain infarction. Platelets from *Gp5*$^{kin/kin}$ mice showed normal activation responses to prominent agonists, including thrombin, *in vitro*, but were completely resistant to GPV cleavage by the latter. Very unexpectedly, these animals reproduced the phenotype of *Gp5*$^{-/-}$ mice in hemostasis and thrombosis in the presence as well as absence of a functional (hem)ITAM receptors.
Summary/Conclusion: These results reveal a central regulatory role of GPV in thrombosis and thrombo-inflammatory processes.

Platelets

ECTH-282

Blocking heteromerization of platelet chemokines CCL5 and CXCL4 reduces inflammation and preserves heart function after myocardial infarction

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Background: Myocardial infarction (MI) is among the most common causes of death in developed countries and its incidence is still increasing. Finding new strategies to prevent and treat this threatening clinical event is thus of high priority. Inhibition of CCL5 was shown to have beneficial effects on the outcome of experimental MI in mice, yet might be accompanied by adverse immunologic side effects. In a previous study, we have demonstrated a pathophysiologic relevance for the heteromer formation of CCL5 and CXCL4 in the progression of atherosclerosis.

Aims: To evaluate a specifically designed compound (MKEY) that blocks the CCL5-CXCL4 interaction in a mouse model of myocardial ischemia/reperfusion (I/R).

Methods: To examine the effect of MKEY in healing following I/R, 8 week-old male mice were intravenously treated with MKEY or scrambled control (sMKEY) from 1 day before, until up to 7 days after I/R. Myocardial function was evaluated using echocardiography and intraventricular pressure measurements and tissue viability, scar formation, leukocyte infiltration and the formation of neutrophil extracellular traps (NETs) was assessed by histology.

Results: MKEY treatment resulted in a significant decrease in infarction size and preserved heart function as compared to sMKEY-treated animals. Moreover, MKEY treatment significantly reduced the inflammatory reaction following I/R, as revealed by specific staining for neutrophils, NETs and monocytes/macrophages.

Summary/Conclusion: Disrupting chemokine heterodimers during myocardial I/R might have clinical benefits, highlighting the therapeutic benefit of blocking the interaction of platelet-derived chemokines, and in addition, reducing the inflammatory side effects while maintaining normal immune defense.

Platelets

ECTH-458

Platelet-derived S1P promotes recovery from anaphylactic shock but is redundant for vascular integrity

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Background: Sphingosine-1-phosphate (S1P) is a signaling lipid with key roles in vascular development and homeostasis. Platelets constitute an abundant source of readily deployable S1P, but the relevance of platelet-derived S1P is unclear as S1P is also continuously provided to plasma by erythrocytes and other cell types. Recent reports have nevertheless suggested essential roles of platelet-derived S1P in the regulation of vascular integrity and thrombosis.

Aims: The aim of this study was to address the impact of platelet S1P deficiency on vascular development and homeostasis.

Methods: We generated mice deficient in platelet S1P by deletion of genes encoding sphingosine kinases (Sphk) 1 and 2 selectively in megakaryocytes. We then addressed the effect of platelet S1P deficiency on platelet function and platelet-endothelial interactions *in vitro*, and compared the effects of platelet S1P deficiency with effects of platelet deficiency or depletion and pan-hematopoietic S1P deficiency on vascular development and vascular integrity in mice.

Results: We observe that combined deficiency of sphingosine kinases 1 and 2 in mouse megakaryocytes nearly eliminates S1P release from platelets, blunts platelet aggregation and spreading and impairs the ability of platelets to modulate endothelial barrier function *in vitro*. Yet it does not sensitize to bleeding, vascular leak or blood-lymph mixing, nor does it protect from arterial thrombosis. Lymph node bleeding in mice with pan-hematopoietic Sphk1 & 2 deficiency, attributed to lack of platelet-derived S1P, is not observed with isolated S1P deficiency in platelets and can be reversed by transfusion of erythrocytes from platelet S1P-deficient mice. Despite its importance in isolated systems, platelet S1P is thus made redundant for protection of vascular integrity by other S1P sources *in vivo*. By contrast, platelet S1P deficiency increases mortality in a model of anaphylactic shock even in the presence of erythrocyte S1P. Aspirin, known to blunt S1P release from platelets, increases mortality in wild-type mice but not in platelet S1P-deficient mice.

Summary/Conclusion: Platelet-derived S1P is dispensable for vascular development and vascular integrity, but plays a non-redundant protective role during anaphylactic shock that is sensitive to aspirin.

Platelets

ECTH-468

Defects in TRPM7 function result in abnormal Ca2+ dependent platelet signaling in mice

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Background: Non-selective cation channel, Transient Receptor Potential Melastatin-like 7 channel (TRPM7) has been implicated to be essential for various cellular processes including development, proliferation, migration, and protein synthesis. Recently we showed that megakaryocyte (MK) and platelet specific deletion of TRPM7 in mice (*Trpm7*$^{fl/fl\text{-}Pf4Cre}$) induces macrothrombocytopenia. Abnormal subcellular localization and degradation of non-muscle myosin IIA heavy chain (NMMIIA) was detected in MKs and platelets. *In vitro* Mg^{2+} supplementation could revert these abnormalities and normalized proplatelet formation. However, the physiological importance of TRPM7 in platelet signaling is still unknown.
Aims: We aimed to understand the platelet signaling function of TRPM7 in normal and pathophysiological conditions.
Methods: To identify the role of the TRPM7 in platelets, *Trpm7*$^{fl/flPf4cre}$ knockout mouse line was generated and analyzed in different *in vitro* and *in vivo* assays of thrombosis and stroke.
Results: In the present study, we observed an increased PLC activity which strongly enhanced Ca^{2+} store release in platelets. Consequently, enhanced Erk-phosphorylation and thromboxane A2 (TxA_2) release to CRP or thrombin stimulation were observed. In line with this, increased aggregation response to thrombin and TxA_2 analogue U46619 was measured. Despite the enhanced Ca^{2+} store release, a defective calcium entry mechanism was detected, which strongly inhibited Ca^{2+} induced phosphatidylserine (PS) exposure and degranulation in *Trpm7*$^{fl/flPf4cre}$ platelets upon activation. However, the observed *in vitro* platelet abnormalities did not translate either into a pro-thrombotic or an anti-thrombotic phenotype in models of arterial thrombosis and stroke.
Summary/Conclusion: Deletion of TRPM7 caused multiple defects in platelet function which did not influence thrombus formation or ischemic brain infraction in mice.

Platelets

ECTH-480

Platelet-thrombi induce an unstable plaque phenotype and arterial stiffening locally at the site of vascular injury

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Background: Blood platelets are increasingly implicated in pathological processes, such as progression of atherosclerosis. Evidence hereof is predominantly based on animal models, in which an overt thrombotic trigger is either lacking or the thrombotic trigger is lethal.

Aims: To characterize the vascular-directed effects of platelets locally at the site of atherothrombosis.

Methods: Plaque rupture was induced by temporary ligation of the carotid artery of *Apoe*$^{-/-}$ mice proximal to the bifurcation. Matrix degrading activity (MMPsense 680) was monitored non-invasively 24 h after ligation using the eXplore Optix imaging system. Carotid extension was measured non-invasively during two weeks via the ultrasound Vevo2100 system. Two weeks after ligation, mice were sacrificed and vascular changes were assessed by immunohistochemistry.

Results: In control animals, ligation led to formation of non-occlusive platelet-rich thrombi that remained present for a prolonged time. Locally at the site of ligation, thrombocytopenic mice displayed **I)** decreased matrix degrading activity, measured after 24 h (-53%, P=0.01), and **II)** decreased arterial stiffening, measured during two weeks (-73% extension, P=0.01). Histological analysis, two weeks after ligation revealed a thicker fibrous cap (+90%, P=0.01), decreased necrosis (-25%, P<0.005), and decreased medial thickening (-90%, P<0.005) with a trend towards higher collagen burden (+89% Sirius Red, P=0.13) in the carotid arteries of thrombocytopenic mice when compared to control mice.

Summary/Conclusion: By applying a mouse model of post-thrombotic vascular remodeling we provide first-time evidence that platelet-thrombi exert MMP-dependent matrix degrading activity in vivo, promote an unstable plaque phenotype and mediate arterial stiffening locally at the site of vascular injury. As in particular an unstable plaque phenotype and vascular stiffening are established risk factors for cardiovascular diseases, our findings have translational importance, for instance for the secondary prevention of arterial thrombosis.

Bleeding

ECTH-493

Platelet function testing in patients with cerebral aneurysms treated with endovascular procedures and dual antiplatelet therapy.

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Background: The main complication after endovascular procedures for cerebral aneurysm treatment is the stent thrombosis and less commonly hemorrhagic complications. Identify and optimal pre-procedure response to antiplatelet therapy is essential to guarantee a success result. Acetylsalicylic acid (ASA), clopidogrel, and the combination of both, have proved very effective; however a high variability was reported in the individual responses to the antiagregant effect, especially with clopidogrel.

Aims: 1) To measure the antiplatelet effect of aspirin and clopidogrel in patients with brain aneurysms who are undergoing endovascular treatment by different laboratory methods (Multiplate assay, Verify now and PFA-100). 2) To compare the results with the different methods.

Methods: 22 patients with cerebral aneurysms, who were to be undergoing endovascular procedure, were included in the study. Subjects had started taking aspirin (100 mg/24 h) and clopidogrel (75 mg/24 h) between 7 and 10 days before performing sensitivity studies to antiplatelet agents.

Samples from each patient were collected previously to procedure and the following functional tests were performed:

· Impedance aggregometry from whole blood (Multiplate): arachidonic acid (AA); ADP and TRAP have been used as agonists. Cut off values for different antiplatelet drugs are not clearly established. We have considered good response to aspirin aggregation an AA <40 U and good response to clopidogrel an aggregation ADP < 47 U.

· VerifyNow: A simplifies platelet aggregation by turbidimetry, used as agonists AA and specific blocking P2Y12. Cut off values are not clearly established. We have considered good response to aspirin < 550 Aspirin Unit Reaction (AUR), and to Clopidogrel < 208 P2Y12 Unit Reactions (PUR).

· PFA-100: for overall assessment of platelet function. Epinephrine-collagen cartridge has been used extensively to identify platelet dysfunction secondary to aspirin.

Results: The most relevant results of platelet function testing of the 22 patients analyzed by three different methods are:

· Verify method showed 4 patients who are resistant to aspirin (3/4 borderline). These results are not consistent with those obtained by PFA-100 and Multiplate assays, which showed a significant aspirin-mediated platelet dysfunction.

· Verify detected 3 patients with low response to clopidogrel consistent with Multiplate results. 9 patients showed excessive response and only 1 of them were reproduced by Multiplate.

· Multiplate detected 13 patients with low response to clopidogrel, these results did not correlate with those obtained by Verify.

Summary/Conclusion: · The effect of aspirin is easily measured by platelet aggregation and/or PFA-100 with col/epi.

· For detecting the clopidogrel-mediated platelet dysfunction by Multiplate with ADP it may be necessary a longer treatment time before the determination (> 10 days) and/or administer a loading dose on the first day of treatment.

· Verify appears to overestimate effect of clopidogrel because hyper-response data are not reproduced by other technique.

· According to the results obtained in this study there is a high interindividual variability in response to clopidogrel.

· Measurement of antiplatelet effect of clopidogrel with a rapid technique remains a challenge.

Vessel wall
ECTH-273
Mechanisms of STXBP5 mediated regulation of Weibel-Palade body release
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Background: Vascular endothelial cells (ECs) contain unique rod-shaped secretory granules, called Weibel-Palade bodies (WPBs). WPBs contain the hemostatic glycoprotein von Willebrand factor (VWF) as their main component, as well as various hemostatic, angiogenic and inflammatory proteins. Exocytosis of WPBs leads to the presentation of their cargo on the endothelial surface or to their release into the vascular lumen. Dysregulation of VWF levels is associated with cardiovascular disease or can result in a bleeding tendency, such as Von Willebrand disease. Thus, tight regulation of WPB release is essential for vascular homeostasis. Several proteins have been identified as mediators of WPB exocytosis, including the small GTPase Rab27A and its effector Slp4-a, but the mechanism remains unclear. Genome wide association studies have related genetic variations in the syntaxin-2 and syntaxin binding protein 5 (STXBP5) genes with differences in VWF plasma levels, suggesting a role for SNARE proteins in regulating VWF release. In agreement with this, we have previously identified the SNARE proteins syntaxin-2 and syntaxin-3 as endogenous Slp4-a binding partners, most likely through the WPB regulator STXBP1.
Aims: In this study we used an iterative interactomic approach to identify new components of the WPB exocytotic machinery and to further elucidate the mechanisms of SNARE-mediated WPB exocytosis.
Methods: Unbiased proteomic interactor screens were performed in primary endothelial cells. Lentivirally expressed mEGFP-tagged fusion proteins were pulled down using GFP-nanobody magnetic beads and specific interactors were identified using mass spectrometry analysis on the Orbitrap Fusion MS. We used siRNA-mediated silencing and lentivirus mediated overexpression of mEGFP-tagged candidates followed by VWF secretion assays to study their role in WPB release.
Results: Our unbiased endothelial interactome analysis of syntaxin-3 identified a number of SNARE proteins, including STXBP2, STXBP5, SNAP23 and NSF. Interactions between syntaxin-3 and STXBP2/5 were confirmed using *in vitro* pull down studies. In accordance with earlier reports, silencing of STXBP5 led to a potentiation of stimulated VWF release. STXBP5 contains a carboxyterminal VAMP-like domain (VLD), which is able to associate with syntaxins. We confirmed that the STXBP5-VLD was able to interact with syntaxin-3. We found that lentiviral overexpression of mEGFP-tagged STXBP5 VLD did not alter the morphology of WPBs or expression of syntaxin-3 but did inhibit stimulated VWF release. Possibly, this inhibitory moiety of STXBP5 inhibits WPB exocytosis by competing with VAMPs and sequestering syntaxins in unproductive SNARE complexes. Subsequent proteomic profiling of the STXBP5 VLD interactome yielded a great number of other SNARE proteins, including several syntaxins, that are currently under investigation.
Summary/Conclusion: Our data provide mechanistic detail on how SNAREs and STXBP5 in particular regulate release of VWF from endothelial cells.

Vessel wall
ECTH-247
Effects of dabigatran-etexilate and warfarin on atherosclerotic plaque vulnerability
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Background: Vitamin K-antagonist (VKA) are the first choice of treatment for patients at risk for arterial or venous thrombosis for over the last 50 years. Nevertheless, VKA have the undesired side effect to accelerate atherosclerotic plaque calcification. The direct oral anticoagulant Dabigatran-etexilate is suitable to replace VKA treatment for antithrombotic therapy, although its effects on plaque calcification remain unknown.
Aims: Therefore, this study *aims* at comparing the effects of Dabigatran-etexilate and warfarin on plaque calcification in an apoE$^{-/-}$ mouse model of atherosclerosis
Methods: Female apoE$^{-/-}$ mice received Western Type Diet (WTD; control) or WTD supplemented with either warfarin (3 mg/g) or Dabigatran-etexilate (7,5 mg/g) for 6 or 20 weeks. An additional group received the first 6 weeks WTD supplemented with warfarin followed by 14 weeks of WTD supplemented with Dabigatran-etexilate. Mice were sacrificed and aortic arches were removed, paraformaldehyde-fixed and analyzed by µCT and immunohistochemistry.
Results: Plaque calcification was significantly increased after 20 weeks of warfarin (61% ± 25) treatment compared to control (33% ± 30) and Dabigatran-etexilate (21% ± 30) treatment. Switching diets from warfarin to Dabigatran-etexilate was unable to prevent increased plaque calcification (48% ± 30) and was significantly increased compared to 20 weeks of Dabigatran-etexilate treatment. Although no calcification was present in any of the groups after 6 weeks of treatment, warfarin treatment significantly increased tissue levels of the nonfunctional calcification inhibitor uncarboxylated MGP in atherosclerotic plaques. Uncarboxylated MGP levels remained increased after 20 weeks of warfarin treatment, suggestive for a pro-calfcification environment. Switching diets from warfarin to Dabigatran-etexilate diminished the increased levels of uncarboxylated MGP, indicative that the initial vitamin K deficiency is restored. Additionally, oxidative stress was significantly increased after 20 weeks of warfarin treatment as compared to all other treatment groups while vascular smooth muscle cells and macrophage content remained similar between groups. The osteochondrogenic marker BMP4 was significantly increased after 20 weeks of warfarin treatment compared control and Dabigatran-etexilate treatment.
Summary/Conclusion: Warfarin treatment aggravates plaque calcification by loss of calcification inhibition, as demonstrated by increased uncarboxylated MGP and increasing osteochondrogenic cell differentiation. Dabigatran-etexilate induced anticoagulation without having undesired vascular side effects. It is tempting to speculate whether co-treatment of Dabigatran-etexilate with vitamin K supplementation might be beneficial by decreasing thrombosis tendency and improving vitamin K status in the vessel wall, thereby inhibiting vascular calcification.

Vessel wall

ECTH-236

Targeting coagulation factor Xa by rivaroxaban reduces the onset and progression of atherosclerosis and enhances plaque stability in ApoE null mice

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Background: Atherosclerosis is a progressive chronic inflammatory vascular disease, complicated by plaque rupture and subsequent thrombus formation. *In vitro* studies indicate that coagulation proteases such as thrombin and factor Xa (FXa) may contribute to pro-atherosclerotic mechanisms mediated through protease-activated receptors (PARs). Whereas experimental thrombin inhibition markedly reduced development and progression of atherosclerosis, the contribution of FXa to atherogenesis has not been fully elucidated.

Aims: To assess the involvement of FXa in atherosclerosis, we investigated the effects of direct FXa inhibition by rivaroxaban on experimental atherosclerosis.

Methods: Female ApoE$^{-/-}$ mice (age 8-9weeks) received high-fat diet or high-fat diet supplemented with rivaroxaban for 8 or 14 weeks (n=10/group). In the second arm, ApoE$^{-/-}$ mice received high-fat diet for 14 weeks, followed by either continuation with high-fat diet or switched to high-fat diet supplemented with rivaroxaban for 6 weeks (n=10/group). Acquired aortic arches and carotid arteries were embedded in paraffin and cut in tissue sections for immunohistochemical staining. Quantitative analysis of atherosclerotic plaque in the aortic arch and carotid artery was performed by use of haematoxylin and eosin staining. Qualitative analysis of plaques was assessed by staining against; macrophages (mac3), collagen (sirius red), necrotic core (toluidine blue), PAR1, PAR2, thrombin, FXa, vascular smooth muscle cells (VSMCs) and matrix metalloproteinases (MMPs).

Results: Direct inhibition of FXa decreased the onset of atherosclerosis in the aortic arch after 8 weeks (-36.92%, $p<0.05$) and 14 weeks (-46.47%, $p<0.05$) as well as in the carotid artery after 8 weeks (-38.14%, $p<0.05$) and 14 weeks (-23.38%, $p<0.05$). Moreover, progression of pre-existing plaque after 14 weeks was reduced in the aortic arch (-20.85%, $p<0.05$) and the carotid artery (-30.26%, $p<0.05$). Immunohistochemical staining also revealed that FXa inhibition reduced plaque vulnerability, as reflected by diminished macrophage infiltration (-38.28%, $p<0.05$), enhanced collagen deposition (+45.30%, $p<0.05$) and a reduced necrotic core (-37.49%, $p<0.05$). These findings were associated with elevated collagen-producing VSMCs and reduced collagen degradation proteins MMP-9 and -13. The qualitative and quantitative improvements were accompanied by reduced expression of PARs and their activators, thrombin and FXa in atherosclerotic plaques.

Summary/Conclusion: Pharmacological inhibition of FXa with rivaroxaban impedes the onset and progression of atherosclerosis and causes increased plaque stability, which is possibly mediated through reduced activation of PARs. These data suggest that direct targeting of FXa may be beneficial in treatment of atherosclerosis; to demonstrate clinical relevance further studies are required.

Clotting

ECTH-239

Venous thromboembolism as a first sign of occult malignancy is associated with an increased risk of recurrent venous thromboembolism

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Background: Venous thromboembolism (VTE) is a chronic disease with a substantial rate of recurrence. Patients with active cancer have a two- to nine-fold higher risk of recurrence when compared to non-cancer patients. Independent predictors of VTE recurrence in cancer patients are uncertain, and previous studies have mainly focused on overt cancer. Accordingly, there is limited knowledge on VTE recurrence in subjects who develop an incident VTE during the occult cancer period.

Aims: We aimed to investigate the risk of VTE recurrence by overt-, occult-, and previous cancer in patients with a first-lifetime VTE derived from a large population-based cohort.

Methods: All incident VTE events (n=773) among participants of the Tromsø 1-6 surveys (source population: n=33 885) were included and followed up from January 1, 1994 until December 31, 2012. Occult cancer was defined as a cancer diagnosed within the first year after a VTE, overt cancer as a cancer diagnosed within two years before the VTE and previous cancer as cancer diagnosed more than two years before the VTE. We calculated hazard ratios (HR) for VTE recurrence across categories of cancer, using VTE patients without cancer as the reference group. The study was approved by the regional committee for research ethics and the patients gave informed, written consent.

Results: Among the 773 patients with incident VTE, 104 had overt cancer, 96 had previous cancer and 42 had occult cancer. There were 125 VTE recurrences during a mean follow-up of 4.2 years. The one-year recurrence risk was higher in subjects with occult cancer (HR 3.86, 95% CI 2.16-6.93) than in subjects with overt cancer (HR 1.63, 95% CI 0.93-2.87) or previous cancer (HR 1.23, 95% CI 0.60-2.54). The risk estimates were only modestly affected by adjustment for patient-related factors such as age, sex, body mass index and common inherited risk factors (F2 [rs1799963], ABO [rs8176719] and F5 [rs6025, rs4524]). In subjects with VTE recurrence during the occult cancer period, the proportion of pro-thrombotic cancers (lung, gastrointestinal and prostate) was high. In 64% of the subjects who developed a first VTE during the occult cancer period, there was some degree of metastasis (regional or distant) at the time of cancer diagnosis. The majority (54%) of the recurrences in the occult cancer group occurred before cancer diagnosis and, therefore, were not treatment-related.

Summary/Conclusion: The risk of VTE recurrence was highest in subjects with occult cancer, and the majority of these recurrences occurred before the cancer was diagnosed. Our findings suggests that the high rate of recurrence in VTE patients with occult cancer is explained by the aggressive nature of the cancer itself rather than other factors.

Clotting

ECTH-430

Thromboembolic events during the treatment of childhood acute lymphoblastic leukaemia

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Background: Thromboembolic events are serious complications of childhood acute lymphoblastic leukemia (ALL) therapy, that may result in significant morbidity and occasionally mortality. Prospective studies including only clinically symptomatic VTE report prevalences between 3% and 14%.
Aims: The mechanism for increased risk of VTE is associated with alterations in the haemostatic system by use of L-asparaginase (ASP) alone or in combination with vincristine or prednisone, presence of central venous lines (CVLs) and/or inherited thrombophilia.The aim of this study is the evaluation of the relationship between thromboembolic events, treatment with steroids and L-asparaginase and the inherited thrombophilia in children with ALL under BFM protocol.
Methods: 429 children with newly diagnosed ALL were recruited in observational cohort study conducted between January 2004 to December 2015. ALL patients were treated with BFM protocol. Due to previous unpleasant experiences central venous catheters were not used. Venous thromboemboli were diagnosed by color doppler sonograpy. Cerebral thromboses were diagnosed using MRI and if possible with MR angiography or venography. Patients with ALL were searched for any accompanying inherited prothrombotic risk factors (protein C, protein S, antitrombin III levels, Factor V Leiden and protrombin 20210 A mutation. All symptomatic thromboembolic events diagnosed were recorded.
Results: Thirteen of 429 patients (3 %) had thrombosis, of them 9 patients (69.3 %) had venous thrombosis, and 4 patients (30.7 %) had arterial thrombosis. Deep venous thrombosis of the lower extremity was present in one case. Four patients had venous thrombosis of upper extremity, only one of them was deep venous thrombosis . Eight patients had cerebral thrombosis (four arterial and four venous). All the cerebral thrombosis had developed at the induction phase of the therapy. Inherited prothrombotic risk factors were detected in 7 of 8 patients (87.5 %) with cerebral thrombosis. One of the patients with arterial cerebral thrombosis had died. This patient had low levels of both protein C and S. One of the patient aged 15 had lower extremity deep venous thrombosis, Inherited prothrombotic risk factors were lacking, similar to the patients with venous trombosis in upper extremity. The rate of death due to thrombosis in children with ALL was found as 0.2 %.
All the cerebral thrombosis had developed at the second half of protocol I, phase I of BFM treatment after 4th dose of asparaginase. Enoxaparin 1mg/kg s.c twice a day was started . After recanalization had occured enoxaparin was decreased to once a day dose and it was stopped during maintanence therapy. None of the patients had complications due to LMWH or recurrences after stopping of LMWH.
Summary/Conclusion: Thrombosis in children with ALL is an important complication with high morbidity and occasionally mortality. As the patients that we follow, when the rate of central venous catheter use has declined, the rate of thrombosis also decrease and then cerebral thromboembolic events become significant relatively. Hereditary prothrombotic risk factors were detected in 7 of 13 patients . Accompanying prothrombotic risk factors to prednisolone and asparaginase therapy may trigger development of thrombosis. So, patients with the inheredited prothrombotic risk factors should be followed- up carefully for the possible emergence of cerebral thrombosis and strategies of antithrombotic prophylaxis should be investigated in this setting.

Clotting

ECTH-139

Risk of venous thrombo-embolism in female malignancies: the Scandinavian Thrombosis and Cancer Cohort

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Background: Cancer is associated with increased risk of venous thromboembolism (VTE) and cancer patients who develop VTE have reduced lifespan and impaired quality of life compared with those who do not develop VTE. Previous studies assessing patient related and cancer specific risk factors for VTE indicated the initial cancer stage to be associated with risk of VTE in overt malignancy.
Aims: To investigate the impact of initial cancer extension on the risk of VTE in female malignancies in a prospective, population based cohort study, the Scandinavian Thrombosis and Cancer (STAC) Cohort.
Methods: The STAC cohort includes data from 144.952 participants included in three large population based cohort studies with enrollment between 1993 and 1997, i.e. the Tromsø Study and HUNT2 study from Norway, and the Danish Diet, Cancer and Health Study. The cohort profile and absolute risks of symptomatic, first time, objectively confirmed VTE events have been published *(Jensvoll, H et al, Clin. Epi. 2015).* Information of cancer diagnosis coded in the ICD-10 system and stage of the disease were obtained by linking to the respective national cancer registries. This study included breast, ovarian, uterine and cervical cancers. Cancer stages coded in the International Federation of Gynecology and Obstetrics (FIGO) staging system and TNM classifications were mapped to summary stages as recommended by the International Cancer Benchmarking Partnership *(Walters, S et al, IJC, 2013).* Relative risks of VTE were assessed by means of Cox regression models. We treated death as competing risk in the analysis.

Results: During follow-up 2.395 women were diagnosed with breast cancer, 130 had cervical cancer, 407 uterine cancer and 311 ovarian cancer. During a median follow-up of 5.1 years, 81 first time VTE events were observed. In breast cancer the incidence of VTE was 3.4 events per 1000 person years (10^{-3} p-y), in cervical cancer the incidence of events was 2.4 * (10^{-3} p-y), in uterine cancer 6.3 * (10^{-3} p-y), and in ovarian cancer 12.5 * (10^{-3} p-y). The age adjusted relative risk (hazard ratio, HR) of VTE in regional spread and distant breast cancer compared with localized disease was 1.3, [95% confidence interval (CI): 0.7-2.6] and 6.3 [2.3-17.4] respectively. Regional spread uterine cancer had a HR of 1.8 [0.5-6.1] and metastatic uterine cancer was associated with a HR of 4.6 [0.5-39.3]. In ovarian cancer a HR of 1.1 [0.1-12-9] was observed in regional spread, while in metastatic disease a HR of 3.5 [0.7-16.4] was observed. In cervical cancer, few events limited estimation of relative risks.
Summary/Conclusion: The risk of VTE was high in ovarian cancer, also high but considerably lower in uterine cancer, and still somewhat lower in breast and cervical cancer. Regional spread of these cancers was associated with no or only a slightly increased risk whereas distant metastasis showed a 3 - 6 times increased risk.

Clotting

ECTH-233

Thrombin contributes to protective immunity in pneumonia derived sepsis via fibrin polymerization and platelet-neutrophil interactions.

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Background: Innate immunity and coagulation are closely linked during sepsis, and their interaction can either enhance host defense or contribute to tissue injury. The specific components of the coagulation system involved herein are beginning to be unraveled, but the role of thrombin has not been fully elucidated.

Aims: We aimed to investigate the contribution of thrombin to the host response during pneumonia derived sepsis.

Methods: Mice treated with the specific thrombin inhibitor Dabigatran or control chow were infected with the common human sepsis pathogen *Klebsiella (K.) pneumoniae* via the airways. In subsequent infection experiments, mice were additionally treated with Ancrod to deplete fibrinogen. *Ex vivo Klebsiella* outgrowth was assessed by incubating human whole blood or specific blood components in various conditions with *Klebsiella*.

Results: Thrombin inhibition by Dabigatran enhanced bacterial outgrowth and spreading and strongly accelerated mortality. Thrombin inhibition did not influence neutrophil recruitment to the lung, neutrophil activation or neutrophil extracellular trap formation. Dabigatran reduced D-dimer formation and fibrin deposition in the lung. Fibrin depletion also enhanced bacterial outgrowth and spreading, and thrombin inhibition had no additional effect herein. Both thrombin and fibrin polymerization inhibited *ex vivo Klebsiella* outgrowth in human whole blood, which was neutrophil dependent and the effect of thrombin required the presence of platelets and platelet protease activated receptor-1. *In vivo* thrombin inhibition reduced platelet-neutrophil complex formation and endothelial cell activation, but did not prevent sepsis-induced thrombocytopenia or organ damage.

Summary/Conclusion: These results suggest that thrombin plays an important role in protective immunity during pneumonia derived sepsis by enhancement of fibrin polymerization and platelet-neutrophil interactions.

Vessel wall
ECTH-426
The role of von Willebrand factor in a malaria-associated lung pathology model
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Background: Malaria is a global health burden, affecting more than 3 billion people, resulting in 200 million clinical cases and around 400,000 deaths each year. The pathophysiological mechanisms of severe malaria are complex and involve factors that remain poorly understood. Recent clinical studies have demonstrated that severe malaria infection is associated with acute endothelial cell activation, accumulation of highly reactive ultra large (UL-) von Willebrand factor (VWF) multimers, and a significant reduction in ADAMTS13 activity. Whether this prothrombotic state constitutes an epiphenomenon, or whether this plays an active role in the pathophysiology of malaria remains to be determined.
Aims: To investigate the role of VWF in malaria using a murine model of malaria-associated lung pathology.
Methods: Wild-type (WT) and VWF knock out (*Vwf*$^{-/-}$) mice on C57BL/6J background were inoculated with 10^4 *Plasmodium berghei* (*P.b.*) NK65-infected erythrocytes via intraperitoneal injection. After infection, blood samples were taken to measure platelet counts and to assess levels and activities of VWF and ADAMTS13. Daily Giemsa-stained blood smears were prepared to measure parasitemia. Lung pathology was assessed by measuring protein levels in broncho-alveolar lavage (BAL) fluid, which is indicative for edema and alveolar leakage.
Results: Plasma VWF levels in infected WT mice significantly increased 3 days after infection (2 fold increase; $p<0.0001$), but normalized afterwards. During the course of infection, VWF multimer patterns remained normal until the end stage (day 8/9) at which a marked decrease in high molecular weight VWF multimers was observed ($p<0.0001$). This was accompanied by a significant reduction of the ADAMTS13 activity/antigen ratio ($p<0.0001$). Interestingly, severe thrombocytopenia was observed in both mouse strains starting from day 5 after infection, indicating a VWF-independent mechanism causing thrombocytopenia following *P.b.* NK65 infection. Overall mouse survival times were slightly but significantly shortened in *Vwf*$^{-/-}$ mice compared to WT mice (9 versus 10 days; $p=0.03$). *Vwf*$^{-/-}$ mice also showed an increased appearance of infected red blood cells, with a marked increase in mature schizont stages starting at day 7 after infection. Alveolar leakage in lungs at day 8 post-infection was significantly lower in *Vwf*$^{-/-}$ mice (*Vwf*$^{-/-}$: 1.8±0.4 mg/mL versus WT: 3.6±0.5 mg/mL; $p=0.02$).
Summary/Conclusion: Our data demonstrate that early *P.b.* NK65-mediated murine malaria infection is associated with elevated levels of plasma VWF, which is indicative for endothelial cell activation and in accordance with human malaria infection. Our findings also show that VWF does not contribute to malaria-associated thrombocytopenia. Furthermore, VWF might influence the development of parasitemia and lung pathology, potentially by interfering with the sequestration of infected red blood cells.

Clotting

ECTH-340

Higher prothrombotic activities in M1 than M2 macrophages in atherosclerotic patients

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Background: The thrombi formation on atherosclerotic plaques plays a key role in acute ischemic cardiovascular events. The thrombotic process is triggered by an atherosclerotic plaque rupture or erosion and the contact of blood with intra-plaque prothrombotic elements, such as tissue factor (TF), the main initiator of thrombin and fibrin generation and PAI-1 (plasminogen-activator inhibitor-1), the main inhibitor of fibrinolysis. These proteins are mainly produced by intra-plaque macrophages. Two subtypes of macrophages have been described in atherosclerotic plaques, the M1 or “classic” macrophages and the M2 or "alternative" macrophages.

Aims: To define the respective roles of these 2 macrophages subtypes on plaque thrombogenicity

Methods: Circulating monocytes-derived macrophages from 36 atherosclerotic patients were cultured in presence of IL-1β (10 ng/ml) or IL-4 (10ng/ml), to induce M1 or M2 macrophages, respectively. The expression of TF, TFPI, the main inhibitor of the tissue factor pathway, and PAI-1 was measured by quantitative RT-PCR, the secretion of TFPI and PAI-1, by ELISA and macrophages overall thrombogenicity, by a thrombin generation test (TGT) using a calibrated automated thrombogram (CAT) system.

Results: We observed that TF expression was higher in M1 macrophages compared to M2 ($p<0,001$) whereas M2 expressed and secreted higher levels of TFPI ($p<0.05$). Finally, the FT/TFPI ratio, representing the haemostatic balance, was higher in M1 vs. M2. Furthermore, M1 macrophages expressed and secreted more PAI-1 compared to M2. Concerning TGT, the profiles shown that the peak of thrombin generated by the M1 occurred earlier than for M2 ($p<0,05$) and the amount of thrombin generated, the peak height and the velocity index were higher for M1 than for M2 ($p<0.05$).

Summary/Conclusion: This study reveals that M1 macrophages display more important prothrombotic properties than M2 macrophages that could explain, in part, the deleterious effects of M1 macrophages in atherosclerotic plaques.

Bleeding

ECTH-268

Predictors of bleeding after low-risk dental procedures in patients on vitamin K antagonists: a cohort study

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Background: Patients on vitamin K antagonists (VKA) often need to undergo dental procedures, such as tooth extractions. Risk factors for bleeding after these procedures and optimal perioperative management are still unclear for patients treated with VKA other than warfarin. Dutch guidelines suggest that VKA can safely be continued in combination with tranexamic acid (TXA) mouthwash in low-risk dental procedures, but lack validation in a real world setting.

Aims: To determine risk factors associated with bleeding after low-risk dental procedures in patients on acenocoumarol or phenprocoumon and to evaluate current perioperative management.

Methods: We evaluated all dental procedures performed in patients of a large Dutch anticoagulation clinic between January 2013 and January 2015. Information regarding perioperative management, procedure, patient characteristics, and outcomes were extracted from patient records. Multivariate backward conditional logistic regression analysis was used to determine risk factors associated with an increased risk of clinically relevant oral mucosa bleeding within 30 days after low-risk dental procedures, based on the Dutch guideline from the Academic Centre for Dentistry Amsterdam (ACTA). Bleedings were clinically relevant if these caused hospitalization or death, required a second intervention or medication, or were reported spontaneously by the patient. Due to the observational study design, a waiver for informed consent was granted by the local ethics committee.

Results: In total**,** 2329 dental procedures in 1845 patients were included for analysis. Of these, 2004 (86.0%) were considered low-risk procedures. Most procedures (n=1431, 71.4%) were extractions of 1-3 elements. Treatment with VKA was continued in 1350/2004 (67.4%) procedures, of which 900 (66.7%) with TXA mouthwash and 450 (33.3%) without. VKA therapy was temporarily interrupted in 654/2004 procedures (32.6%), of which 246 (37.6%) were bridged with low-molecular-weight heparin (LMWH) and 408 without bridging (62.4%). Post-interventional bleeding occurred in 67/2004 (3.3%) low-risk procedures. In these low-risk procedures, continuation of VKA with TXA mouthwash was associated with a lower bleeding risk compared to VKA continuation without TXA mouthwash (OR=0.41, 95% CI 0.23-0.73) or VKA interruption with bridging (OR=0.49, 95% CI 0.24-1.00), and non-inferior compared to VKA interruption without bridging (OR 1.44, 95% CI 0.62-3.64). When VKA therapy was interrupted, bridging was associated with a higher bleeding risk compared to forgoing bridging (OR=2.94, 95% CI 1.14-7.57). Backward conditional modelling revealed the following predictors of an increased risk of bleeding after low-risk dental procedures; perioperative exposure to concomitant platelet aggregation inhibitors [OR 2.40, 95% CI 1.33-4.32], bridging with LMWH [OR 3.19, 95% CI 1.22-8.35], absence of a valid INR result from the anticoagulation clinic within 72 hours before the procedure [OR 1.90, 95% CI 1.10-3.28], last INR result at the anticoagulation clinic before procedure >3.5 [OR 1.75, 95% CI 0.98-3.12] and procedures not reported to the clinic at least 24 hours in advance [OR 2.60, 95% CI 1.52-4.46].

Summary/Conclusion: In a real world setting, most risk factors for bleeding after low-risk dental procedures were specifically related to perioperative management. Standardization of perioperative management and adherence to guidelines may reduce the incidence of bleeding after low-risk dental procedures in patients on VKA.

Bleeding

ECTH-214

Prospective evaluation of bleeding incidence in factor XIII deficiency (Pro-RBD study)

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Background: The PRO-RBDD registry was designed to prospectively collect data on patients with factor (F)XIII deficiency and understand the association of circulating FXIII and clinical severity. Data retrieved at patient enrollment identified a new FXIII activity cut-off of 15% with a very high sensitivity and a promising capability to discriminate patients who may develop a spontaneous major bleeding (*results under submission*).

Aims: The aim of the analysis herein reported was to evaluate the benefits and complication of current treatment regimens.

Methods: Since February 2013, 13 Hemophilia Treatment Centers collected data on 60 FXIII-deficient patients (32 females and 28 males), updating data at least biannually over three years. Bleeding events in patients were analyzed as bleeding incidence. A survival analysis was also used to compare the cumulative incidence of the first bleeding treated with replacement therapy. Analysis was done using R v.3.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results: Patients were followed up for a median of 528 days (IQR: 790-185). Bleeding incidence of 1.19 patient-year^{-1} in on-demand therapy and 0.14 in prophylaxis (9-59U/Kg/month FXIII concentrate) was calculated, with a significant reduction of bleedings in the prophylaxis group (IR=0.12, IC95% 0.09-0.15). A clear decrease of bleeding cumulative incidence using prophylaxis was observed (46%, 95%CI 13-68 on demand *vs* 5%, 95%CI 0-10 on prophylaxis).

Similarly, in patients with FXIII activity <15%, the bleeding incidence was 1.94 patient-year^{-1} in on-demand therapy and 0.15 in prophylaxis with a significant reduction of bleeding episodes in prophylaxis (IR=0.08, IC95% 0.06-0.10). The risk of bleeding requiring replacement therapy is significantly lower in patients on prophylaxis (cumulative incidence: 67%, 95%CI 20-84 on demand vs 5%, 95%CI 0-15 on prophylaxis). No adverse events were observed.

Moreover, 4 deliveries (3 vaginal, 1 cesarean), 14 surgeries (3 major, 7 minor, 4 dental) were performed after prophylactic administration of plasma-derived FXIII concentrate (12-45 U/Kg). Intraoperative bleeding was observed after 1 minor endoscopic surgery and 2 major orthopedic surgeries in only one patient who, on other occasions, underwent the same surgeries with the same prophylaxis without any bleeding complication. Numbers were too small to draw any conclusion.

Summary/Conclusion: The PRO-RBDD is the first post-registration surveillance database in the field of RBDs collecting data on a large group of FXIII deficiency patients observed in a long follow-up period. The beneficial role of FXIII prophylaxis to prevent bleeding episodes is clearly confirmed with no adverse events, making this treatment strongly recommendable, in particular in severe deficiency.

Bleeding

ECTH-309

The necessity of refining the diagnostic evaluation of patients with a bleeding tendency not explained by current routine laboratory testing

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Background: Diagnostic evaluation of patients with a bleeding tendency remains challenging. It is difficult to objectify bleeding tendencies for both bleeding assessment tools and current laboratory techniques have limitations. As a result, in literature 47%>69% of patients with a bleeding tendency do not fulfill diagnostic criteria.

Aims: To assess the diagnostic evaluation of patients with a bleeding tendency.

Methods: Patients referred for either a bleeding tendency or a positive family history between 01-2014 and 12-2015 to the Erasmus University Medical Center/Sophia Children's Hospital were included. Medical history was taken by a (pediatric) hematologist. Bleeding Assessment Tools (BATs) were used to objectify the bleeding tendency. The condensed MCMDM-1 VWD questionnaire was abnormal when ≥4, and the ISTH-BAT when ≥4 for males, ≥6 for females, and ≥3 for children. The diagnosis bleeding disorder was rejected when both medical history/BAT and laboratory tests were considered normal. The diagnosis bleeding disorder was made when fulfilling 1 out of 4 categories: 1)Classic: based on definitions according to national guidelines; 2)Low VWF: von Willebrand factor (VWF) levels between 0.30-0.60 U/ml; 3)Platelet disorder not otherwise specified (NOS): abnormal aggregometry pattern not fitting a known thrombocytopathy; 4) Unexplained bleeding: absence of laboratory abnormalities, but medical history/BAT requires a treatment protocol in case of intervention or bleeding according to the hematologist. A waiver for informed consent was granted by the local ethics committee.

Results: In total 242 patients were eligible, 223 were included. Most patients were female (80%). Median age was 31 years (12-91). A bleeding disorder was excluded in 114 patients (51%), 39 were referred for family screening. In 109 patients (49%) a bleeding disorder was diagnosed by laboratory testing or their bleeding was considered significant but remained unexplained.

Of these 109 patients, 23% had a bleeding disorder based on current guidelines (7 von Willebrand disease, 7 factor deficiencies, 5 known platelet disorders, 6 Hemophilia carriers). In 38% low VWF was found, a platelet disorder NOS in 15% and 25% had an unexplained bleeding tendency.

In 199 patients a bleeding score was obtained, 90% were adults. Bleeding score was normal in 114 patients (57%). When a bleeding disorder was excluded, 75% had a normal bleeding score. Of patients with abnormal laboratory results, 45% had a normal bleeding score ($p<0.001$). More specific, 47% of patients with low VWF levels and 25% of patients with a platelet disorder NOS had a normal bleeding score. In patients with unexplained bleeding the bleeding score was normal in 12%.

Summary/Conclusion: Our study shows that current coagulation tests and definitions confirm a bleeding disorder in 11% (25/223) of patients. Clinical impact of low VWF and abnormal aggregometry pattern is unclear. Furthermore, diagnostic uncertainty remains in patients with platelet disorders NOS and an unexplained bleeding tendency. When no laboratory abnormalities are found, medical history and bleeding score are important tools for physicians as 25% of patients with no bleeding disorder was discharged with an abnormal BAT. Further research is required including investigation of new coagulation tests in areas that are not reflected in current tests and the future role of genetic testing, hereby refining the diagnostic evaluation of patients with a bleeding tendency.

Platelets

ECTH-417

Stefin A, a novel megakaryocyte and platelet protein regulated by the metabolic status

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Background: Platelets play a role in atherogenesis and its thrombotic complications. Increased platelet activity occurs during type 2 diabetes mellitus (T2DM). Even though antiplatelet therapies are useful strategies to improve cardiovascular outcomes, T2DM patients remain at higher risk of adverse cardiovascular events as compared to non-diabetic ones.

Aims: Our goal was to identify and characterized new dysregulated pathways in platelets in the setting of T2DM. We also aim to characterize their regulation *in vivo* and *ex vivo* in rats and humans.

Methods: We compared gene expression profile in bone marrow-isolated MKs from obese and diabetic (*db/db*) and wild-type (Wt) mice using the Agilent platform array with the whole mouse genome chip (8 x 60k) and subsequent qPCR. We analyzed protein expression in serum and/or platelet lysates obtained from obese/diabetic patients and from rats fed a high-sucrose diet by ELISA and Western-blot.

Results: Whole genome analysis revealed an increased expression of *Stefin A* in *db/db* MKs as compared to Wt mice (fold-change [FC]: 9.7, $p < 0.001$). This up-regulation was further confirmed by qPCR (FC = 13, $p = 0.0047$). *Stefin A*, the mouse homolog for the human *Cystatin A (CSTA)*, encodes a cysteine proteinase inhibitor, implicated in matrix remodelling, apoptosis and cancer.

In humans, CSTA mRNA and protein expression increase during differentiation of $CD34^+$-derived MKs. Transmission electron microscopy on human platelets, shows that CSTA is localized within the membrane of α-granules. Furthermore, CSTA is released from human platelets upon activation with a maximum at 60 minutes (control: 0.73 ± 0.06; Trap-6: 1.64 ± 0.16; PMA: 1.73 ± 0.15; $p = 0.02$; ng/ml, mean ± SE). GM6001 markedly inhibited released of CSTA. In order to explore the role of the metabolic status on CSTA regulation, we measured serum CSTA levels in diabetic/obese patients. CSTA concentrations were significantly ($p < 0.0001$) increased in obese patients (1.11 ± 0.09 ng/ml) as compared to control lean individuals (0.64 ± 0.07 ng/ml). A significant decrease in serum CSTA levels, which correlates with glucose indexes, was also observed twelve months after bariatric surgery (0.83 ± 0.12 *vs.*1.83 ± 0.22 ng/ml at baseline, $p < 0.0001$).

We further looked at a possible stefin A regulation in an environmental model of metabolic syndrome in rats. Stefin A levels is significantly increased in platelets from high-sucrose fed rats as compared to controls (4.9 ± 0.4 *vs.* 6.3 ± 0.4 A.U., $p = 0.004$). These levels correlate positively with body weight, glucose intolerance and fat mass and negatively with lean mass.

Summary/Conclusion: We identified Stefin A/CSTA as a new obesity-regulated protein in platelets and MKs. Indeed, we found elevated levels of Stefin A in MKs from obese mice and in platelets isolated from rats that correlate with metabolic syndrome indexes. Serum CSTA levels are increased in obese/diabetic patients and reduced after bariatric surgery. We suggested that circulating CSTA arises, at least in part, from activated platelets. We are currently investigating the role of CSTA in atherothrombotic disease.

Bleeding

ECTH-348

ADAMTS13 containing the naturally occurring mutation p.arg1177gln adopts an open conformation

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Background: Thrombotic thrombocytopenic purpura (TTP) is caused by a deficiency in the metalloprotease ADAMTS13, due to mutations in the *ADAMTS13* gene (congenital TTP) or presence of anti-ADAMTS13 autoantibodies (acquired TTP). Mutations found in the *ADAMTS13* gene of TTP patients are valuable to gain more insight into the working mechanism of the enzyme. Recently, we and others demonstrated that ADAMTS13 adopts a closed conformation through interaction between its distal and proximal domains. Cryptic epitopes in the proximal ADAMTS13 domains become exposed upon addition of activating anti-distal domain antibodies or upon binding of its substrate von Willebrand factor. The presence of three linker regions in the distal domains of ADAMTS13 seem to account for the flexibility and conformational changes of the enzyme. Our in house developed monoclonal antibody 6A6, which recognizes a cryptic epitope in the proximal metalloprotease (M) domain of ADAMTS13, is a unique tool to distinguish between the open and closed ADAMTS13 conformation. Whether conformational activation of ADAMTS13 induces proteolysis by other coagulation proteases remains to be investigated.

Aims: In this study we aimed at fully characterizing ADAMTS13 in a TTP patient. In addition, we investigated whether the identified novel p.Arg1177Gln mutation induces a conformational change in the enzyme.

Methods: Patient plasma ADAMTS13 antigen levels and the presence of anti-ADAMTS13 antibodies were determined. In parallel, the exons of the *ADAMTS13* gene were sequenced and site-directed mutagenesis was used to introduce substitutions in the ADAMTS13-pcDNA6.1 expression vector. Wild type (WT) and mutant proteins were produced through stable HEK293 cell lines. ADAMTS13 activity and conformation were analyzed using the FRETS VWF-73 assay and 6A6-ELISA.

Results: We performed a detailed laboratory analysis of a patient clinically diagnosed with TTP. Undetectable ADAMTS13 activity, decreased ADAMTS13 antigen level (0.27 ± 0.06 µg/ml, n = 3) and the presence of inhibitory anti-ADAMTS13 antibodies led to the diagnosis of acquired TTP. In addition, we identified the p.Ala900Val polymorphism and a novel p.Arg1177Gln mutation in the patients' *ADAMTS13* gene. Since both substitutions are heterozygous, their effect on TTP pathophysiology was expected to be minimal. Interestingly however, the novel p.Arg1177Gln mutation is situated in the third linker region of ADAMTS13 between its distal T8 and CUB1 domains. As we recently demonstrated that removal of this linker region resulted in a more active, open ADAMTS13, we next investigated the effect of this naturally occurring mutation on the activity and conformation of ADAMTS13. Using the FRETS VWF-73 assay we demonstrated that the p.Arg1177Gln mutant was 2.15 times more active than WT ADAMTS13. In line with this, the p.Arg1177Gln mutant had a more open conformation as 6A6 did recognize the p.Arg1177Gln mutant in contrast to WT ADAMTS13.

Summary/Conclusion: We here show for the first time that a naturally occurring mutation in the third linker region of ADAMTS13 induces a more open conformation and hence increases its proteolytic activity. Whether this open conformation makes ADAMTS13 more susceptible to proteolysis, thereby contributing to the TTP phenotype remains to be determined.

Platelets

ECTH-270

Age-related increase of thromboxane B2 and risk of cardiovascular disease in atrial fibrillation

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Background: Aging is strictly associated with an increased incidence of cardiovascular events (CVEs) in the general population. Mechanisms underlying the risk of CVEs are still unclear. Platelet activation contributes to the onset of cardiovascular complications. The incidence of atrial fibrillation (AF) increases with age, and the natural history of AF is often complicated by CVEs.

Aims: Aim of the study was to prospectively investigate the relationship between age, urinary thromboxane (Tx) B_2, which reflects platelet activation, and CVEs in a cohort of AF patients

Methods: We included 833 non-valvular AF patients treated with oral anticoagulants. Baseline urinary TxB_2 was measured in all patients. The occurence of CVEs at follow-up was registered. Only the first CVE was used for the analysis.

Results: Mean age of patients was 72.8±8.4 years, and 45.6% were women; 86.7% had hypertension, 19.2% diabetes, and 15.5% heart failure (HF). Moreover, 15.2% had a previous stroke/ transient ischemic attack (TIA), 19.3% a previous myocardial infarction (MI)/coronary heart disease (CHD), and 8.9% smoked.

Median TxB_2 level was 120 [66-200] ng/mg of urinary creatinine. At multivariable linear regression analysis, age (B: 0.097, p=0.005) and previous MI/CHD (B: 0.069, p=0.047) were associated with log-TxB_2 levels. When we divided our population into age classes (i.e. <60, 60-69, 70-79, ≥80 years), we found a significant difference in TxB_2 levels across classes (p=0.005), with a significant elevation at 74.6 years. During a mean follow-up of 40.9 months, 128 CVEs occurred; the rate of CVEs significantly increased with age classes (Log-rank test, p<0.001). TxB_2 levels were higher in patients with compared to those without CVEs in patients aged 70-79 (p<0.001) and ≥80 (p=0.020) years.

Summary/Conclusion: In conclusion, TxB_2 levels enhance by increasing age, suggesting that platelet activation contributes to CVEs in elderly patients with AF.

Platelets

ECTH-387

Haemostatic abnormalities in patients with Ehlers-Danlos syndrome

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Background: Any type of Ehlers-Danlos syndrome (EDS) is associated with an increased bleeding risk, but a comprehensive study of hemostasis in patients with EDS is lacking.

Aims: To study primary and secondary hemostasis in a large cohort of patients with EDS and to correlate the results of such tests with a validated method to assess bleeding tendency (International Society of Thrombosis and Hemostasis Bleeding Assessment Tool [ISTH-BAT])

Methods: 100 patients (M/F 22/77 median age 34) were included in the study after signing the informed consent. ISTH-BAT was administered by a trained physician. A careful pharmacological history was recorded and patients who took drugs known to interfere with hemostasis (antiplatelets and anticoagulant drugs)were excluded. The following tests were performed: complete blood count, prothrombin time (PT ratio), activated partial thromboplastin time (aPTT ratio), fibrinogen, von Willebrand factor ristocetin co-factor (RICOF), endogenous thrombin potential (ETP), platelet aggregation and platelet secretion studied by lumi-aggregometry. If PT or aPTT were prolonged, coagulation factors were tested.

Results: ISTH-BAT was above 5 (significative for an hemostatic problem) in 48 patients (48%). Platelet count was in the normal range in all patients. PT and/or aPTT were slightly prolonged in 10 patients, all due to mild coagulation factors deficiencies. RICOF was above 45% in all patients and did not correlate with ISTH-BAT. ETP was normal in all patients. In 60% of patients a reduction of platelet aggregation with at least a single aggregating agent (mostly ADP) was found. Similarly, 40% of patients had a secretion defect to at least one aggregating agent. The impairment of platelet aggregation correlated with ISTH-BAT: the odd ratio to have an abnormal response to ADP in patients with ISTH-BAT>5 compared with patients with normal ISTH-BAT was 2.2 (95% c.i. 1.01-5.54).

Summary/Conclusion: In the majority of EDS patients with an ISTH_BAT >5, an abnormal platelet aggregation was found. Coagulation tests were normal or non-significantly altered.

Clotting

ECTH-346

Inhibition of TAFI and PAI-1 reduces ischaemic stroke brain damage

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Background: Despite recent advances in stroke prevention and therapy, ischemic stroke is still one of the leading causes of death and disability worldwide. Progressive stroke is often observed in patients despite successful rapid revascularization of the occluded blood vessel. Cerebral ischemia and subsequent reperfusion is associated with activation of the coagulation cascade and fibrin(ogen) deposition in cerebral microvessels contributing to the no-reflow phenomenon. Both thrombin activatable fibrinolysis inhibitor (TAFI) and plasminogen activator inhibitor 1 (PAI-1) attenuate fibrinolysis and are therefore attractive targets for the treatment of cerebral ischemia/reperfusion injury.

Aims: To dissect the roles of TAFI and PAI-1 in the setting of cerebral ischemia/reperfusion injury and to evaluate their potential as therapeutic targets.

Methods: Mice were subjected to 1h of cerebral ischemia followed by 23h of reperfusion after which neurological and motor outcome, brain infarct size and cerebral fibrin(ogen) deposition were assessed. Treatment was given five minutes after the start of reperfusion and consisted of anti-TAFI MA-TCK26D6 (25mg/kg; 6mg/kg or 1mg/kg), anti-PAI-1 MA-33H1F7 (6mg/kg or 1mg/kg) or a combination of both (1mg/kg).

Results: Both anti-TAFI (25 mg/kg) or anti-PAI-1 (6 mg/kg) MAs significantly decreased cerebral fibrin(ogen) deposition 24 h after stroke compared with an IgG control MA ($p < 0.01$). Decreased fibrinogen deposition was accompanied by a two-fold reduction in cerebral infarct size (37.0 ± 5.6 mm^3 and 36.0 ± 6.0 mm^3, respectively vs. 80.6 ± 11.1 mm^3; $p < 0.01$ and $p < 0.05$, respectively). Concurrently, both animal neurological and motor performance was improved after blocking PAI-1 or TAFI. Interestingly, combined targeting of TAFI and PAI-1 using low, and by themselves ineffective, doses of antibodies also reduced cerebral fibrin(ogen) deposition and infarct sizes by 50% (42.5 ± 8.0 mm^3, $p < 0.05$). Importantly, no cerebral bleeding was observed in any of the mice treated.

Summary/Conclusion: Inhibition of TAFI or PAI-1 is protective in a mouse model of cerebral ischemia/reperfusion injury by attenuating fibrin(ogen) deposition. Combined inhibition has a cooperative effect that could become useful in ischemic stroke therapy.

Clotting

ECTH-385

Low haematocrit thrombi form more rapidly and are more resistance to fibrinolytic degradation as a result of increased cross-linking by factor XIIIa

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Background: The participation of red blood cells (RBC) in coagulation and thrombus formation has been considered insignificant, yet their sheer abundance in blood means that they are a dominant element in the resulting clot. Patients with anaemia are at increased risk of thrombosis, however the mechanisms by which RBCs influence this outcome remain unclear.
Aims: To examine the influence of haematocrit (HCT) on coagulation parameters, clot firmness and resistance of thrombi to fibrinolysis.
Methods: Whole blood drawn from healthy volunteers was separated into plasma and RBC constituents by centrifugation. Samples were reconstituted with 35% platelet rich plasma and RBCs to produce 20%, 40% and 60% final HCT. Coagulation parameters were assessed using thromboelastometry (ROTEM) and thrombus lysis using the Chandler loop method. Thrombi were formed under continuous flow for 90 min from reconstituted blood in the presence of FITC-labelled fibrinogen. Lysis was induced by bathing thrombi in 1 µg/ml tPA and samples taken at 30 min intervals. Venous blood from anaemic and non-anaemic myeloproliferative patient volunteers was also processed using the Chandler loop method.
Results: Thromboelastometry revealed shorter clot time, clot formation time and increase in the α-angle at 20% HCT vs 60% HCT; indicative of faster clot formation at lower HCT. An increase in maximum clot firmness was detected at 20% HCT. Chandler model thrombi formed at 20% HCT were longer than those formed at 60% HCT ($p<0.05$) and demonstrated increased resistance to lysis with tPA ($p<0.005$). Inclusion of a transglutaminase (TG) inhibitor, to inhibit factor XIIIa (FXIIIa), significantly augmented lysis of 20% HCT thrombi ($p<0.001$) and to a lesser degree 40% HCT thrombi ($p<0.01$). In contrast, inclusion of the TG inhibitor had no impact on lysis of 60% HCT thrombi ($p=0.113$). Neutralization of FXIIIa resulted in equivalent lysis rates at all HCT analysed. Western blots demonstrate an increase in cross-linked fibrin and the inhibitor $a_2$antiplasmin (a_2AP) at low HCT. An ELISA for a_2AP confirmed higher concentrations of the inhibitor in thrombi formed at 20% HCT vs 60% HCT ($p<0.0001$) and less FXIII was detected in the serum of thrombi formed at 20% HCT vs 60% HCT ($p<0.01$). Preliminary results from anaemic myeloproliferative patients show that thrombi lyse slower and are longer in length than non-anaemic volunteers ($p<0.01$); thereby supporting the observations in manipulated HCT thrombi.
Summary/Conclusion: HCT has a dramatic impact on thrombus formation and stability, with low HCT enhancing clot formation, resulting in thrombi with increased firmness and resistance to fibrinolysis. The antifibrinolytic impact of FXIIIa on thrombi is dependent on RBC content with increased cross-linked fibrin and $a_2$antiplasmin at low HCT. Consistent with this thrombi formed from anaemic myeloproliferative patients are longer and more resistant to fibrinolytic degradation. These differences in fibrinolytic resistance of thrombi formed at low HCT may contribute to the increased risk of thrombosis in patients with anaemia.

Clotting

ECTH-479

Development of a method for in vivo detection of active thrombi in mice

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Background: Every year about 40.000 people in The Netherlands develop an ischemic stroke. During the first 4.5 hours of thrombus formation, an ischemic stroke is best treated by thrombolysis using tPA, whilst response to this treatment is inversely correlated with age of the thrombus. In active thrombi, activated factor XIII (FXIIIa) cross-links fibrin chains and cross-links α_2-antiplasmin to fibrin. This is a transglutamination process, in which α_2-antiplasmin and FXIIIa form an intermediate thioester complex, to eventually couple α_2-antiplasmin to fibrin. Using an α_2-antiplasmin based contrast agent (A14) that can be covalently linked to fibrin by FXIIIa, the activity of fibrin cross linking can be visualized and potentially used to discriminate active thrombi from old thrombi. In this study we will validate the application of ^{111}In-DTPA–A14 as SPECT/CT probe in two murine models of thrombosis.

Aims: Development of a molecular imaging agent for *in vivo* detection of active thrombi by SPECT/CT.

Methods: Thrombus formation was induced in male C57Bl6 mice >12 weeks of age, by endothelial damage or disruption of blood flow. In the FeCl3 model, the inferior vena cava (IVC) was exposed by midline incision and subjected to 10% $FeCl_3$ for 3 minutes. Two, six or 24 hours post-surgery (hps), mice were injected with 100 µL/25 gr ExiTron™ nano 12000 (Viscover/Miltenyi Biotech). In the stasis model, the IVC was exposed by midline incision, and a partial ligation was placed on the infrarenal IVC to create ≈90% stenosis. Six, 24 or 48 hps mice were injected with 100 µL/25 gr ExiTron™ nano 12000.
Mice were scanned using a CT to visualize thrombus formation and to obtain anatomical information at the time points above. After scanning, the IVCs were excised and embedded in paraffin for histochemical analysis. Carstairs-, DAPI- and H&E-staining was used to visualize platelets, leukocytes and fibrin in the different models. Mice models were validated to assess binding and visualization of the newly synthesized ^{111}In-DTPA–A14 probe in SPECT/CT.

Results: Stable thrombi using FeCl3 after two hours and in the stenosis model occlusive thrombi are formed after six hours. Clot formation in the vena cava is visible by negative CT contrast. Histochemical analysis shows that in the ferric chloride model, the thrombus is mainly composed of platelets, while thrombi that occur after stenosis mainly consist of fibrin and red blood cells. In the stenosis model, clot formation in the vena cava corresponded with a SPECT hotspot using ^{111}In-DTPA–A14 molecular imaging agent.

Summary/Conclusion: The fibrin-targeted A14 peptide was shown to specifically bind murine thrombi in a model of venous thrombosis. Thus, the use of specific and covalently binding fibrin probes might enable clinical non-invasive imaging of thrombosis.

Clotting

ECTH-255

Granulocyte clone size and eculizumab impact clot structure in patients with paroxysmal nocturnal haemoglobinuria

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Background: Paroxysmal nocturnal haemoglobinuria (PNH) is a haematopoietic stem cell disorder in which a somatic mutation of the *PIGA* gene results in an increased susceptibility of red cells to complement-mediated lysis. Patients suffer from extensive intravascular haemolysis leading to a variety of pathologies, the most serious of which is thrombosis, which remains the leading cause of death. Diagnosis is made by flow cytometry and the disease is defined by the size of an individual's mutated PNH granulocyte clone. Previous studies demonstrated patients with thrombosis form denser, thinner-fibred fibrin clots that incur greater resistance to fibrinolysis and we aimed to study whether similar changes to clot architecture may contribute to thrombosis in PNH. Furthermore, treatment with eculizumab, which inhibits complement factor C5, reduces thromboembolic events in PNH, indicating an important role of complement activation in the pathophysiology of thrombosis in PNH.

Aims: To characterise the structure of the fibrin clot in patients with varying PNH clone sizes and determine the effect of eculizumab on clot structure.

Methods: Informed consent was obtained from all participants. Thirty-five patients from the PNH National Service in Leeds were recruited into the study and grouped according to PNH granulocyte clone size; small (less than 50%, n=15), and large (greater than 50%, n=20). Plasma samples were obtained and *ex vivo* fibrin clot structure and fibrinolysis rates were analysed by permeation, turbidimetry and confocal microscopy. P values of <0.05 were taken to indicate significance.

Results: Turbidimetric assays demonstrated a significantly lower maximum absorbency in patients with large clones (0.32 0.09) than small clones (0.39 0.10), *P=0.039.* Comparison of participant clinical characteristics showed 12 of the 14 patients receiving eculizumab had large clones, which led to analysis of results based upon treatment; those receiving no treatment (n=15), eculizumab (n=14), or other anticoagulation (n=6). Patients on eculizumab had a significantly lower maximum absorbency and lower fibre count/100µm than patients receiving no treatment; (0.30 0.09 versus 0.40 0.09, *P=0.0286*) and (18.47 4.42 versus 23.9 4.21, *P=0.006*) respectively. There was a trend towards higher Ks values in patients on eculizumab compared to other treatment groups, however this did not reach significance, P=0.286. There were no differences in lysis times.

Summary/Conclusion: Patients with large PNH clone sizes form clots composed of thinner fibrin fibres than those with small clones. Patients treated with eculizumab also showed a lower average fibre thickness than those receiving no treatment or other anticoagulation, as well as a less dense clot structure. Due to the overlap of patients with large clones who were also receiving eculizumab, it was not possible to determine the exact effects of clone size on clot structure independent of treatment. However, our results indicate that a large clone size is associated with thinner fibrin fibres, while complement inhibition with eculizumab alters clot architecture, accounting for a less dense clot structure. These effects of eculizumab on clot structure may contribute to the antithrombotic effects of this drug in patients with PNH.

Bleeding

ECTH-220

Efficacy and safety of recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in previously treated adult and adolescent patients with haemophilia B

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Background: IDELVION®, a recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP), has recently been approved in the US and Canada for prophylaxis and on-demand treatment of children and adults with hemophilia B. IDELVION® has an improved pharmacokinetic profile, which allows dosing every 7–14 days.

Aims: A global study evaluated the safety and efficacy of rIX-FP for prophylaxis treatment every 7, 10- and 14-days and on-demand treatment of bleeding episodes in previously treated male patients (12–61 years old) with severe hemophilia B (FIX activity ≤2%).

Methods: Previously treated male hemophilia B patients in the on-demand arm received on-demand treatment for 6 months and then switched to 7-day prophylaxis treatment. Subjects in the prophylaxis arm received 7-day prophylaxis treatment for 6 months, and eligible subjects switched to a 10- or 14-day prophylaxis treatment interval. Annualized spontaneous bleeding rates (AsBR) were compared between on-demand treatment and prophylaxis treatment periods (on-demand arm), and between 7-day prophylaxis treatment and 10- or 14-day prophylaxis treatment (prophylaxis arm). Treatment efficacy was evaluated by the number of injections to achieve hemostasis. Safety was evaluated by the number of inhibitors to FIX, and the development of antibodies to rIX-FP. The treatment period was 12 to 18 months. Written informed consent was obtained from all subjects or their legal guardians.

Results: A total of 63 subjects were enrolled from Europe, Japan, Israel and US. The PK profile of rIX-FP was improved in comparison with standard FIX products, with a half-life of 102 hours. In the on-demand arm, 19/23 subjects switched to 7-day prophylaxis treatment after completing 6 months on-demand treatment. The median (Q1, Q3) AsBR during on-demand treatment and prophylaxis treatment was 15.43 (7.98, 17.96) and 0.00 (0.00, 0.96), respectively, a reduction of 100% ($p<0.0001$). Twenty-one subjects extended their treatment interval to 14-day prophylaxis interval. All prophylaxis subjects (n=40) on 7-, 10- and 14-day prophylaxis treatment had a median AsBR of 0.00. Subjects on 14-day prophylaxis treatment (50-75 IU/kg) reduced consumption by 50% over their prior FIX product. A total of 98.6% of bleeding episodes were successfully treated with ≤2 injections of rIX-FP, 93.6% with 1 injection. Compliance with the longer treatment intervals was excellent, with 94.7%, 90.7% and 97.2% compliance for 7-, 10- and 14-day prophylaxis regimens, respectively.

No subjects developed inhibitors to FIX or antibodies to rIX-FP and there were no related serious adverse events during the study.

Fifty-two subjects continued their prophylaxis regimen in the ongoing extension study of the PROLONG-9FP program. Currently, 89% of subjects have switched to a prophylaxis treatment interval longer than 7 days.

Summary/Conclusion: This phase 3 study demonstrated the clinical efficacy of rIX-FP for routine prophylaxis every 7-, 10- and 14-days and on-demand treatment of bleeding episodes. Furthermore, rIX-FP demonstrated favorable long-term safety and tolerability, and longer prophylaxis treatment intervals

had high compliance among patients. Longer treatment intervals are being evaluated in the ongoing extension study.

Bleeding

ECTH-353

Variation in baseline factor VIII concentration in mild and moderate hemophilia A patients carrying the same F8 mutation

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Background: In patients with mild and moderate hemophilia A (MHA) the bleeding phenotype is inversely associated with the residual plasma concentration of factor VIII (FVIII). Within a group of patients with the same *F8* missense mutation the FVIII baseline plasma concentration (FVIII:C) may still vary, as other determinants also influence baseline FVIII:C. In healthy individuals von Willebrand factor (vWF) levels, ABO blood group and age are known to influence baseline FVIII:C. In patients with MHA, our pathophysiological understanding on how the causative genetic event leads to reduced baseline FVIII:C is still limited.

Aims: This study aimed to estimate the variation and determinants of baseline FVIII:C among MHA patients with the same *F8* missense mutation.

Methods: 346 patients carrying mutations that were present in at least 10 patients were selected from the INSIGHT and the RISE study, cohort studies together including data of 3534 MHA patients from Europe, Canada and Australia. The baseline FVIII:C used for this analysis was measured by one-stage clotting assay. Levene's test, univariate and multivariate linear regression analysis were used to analyse the variance in the lowest baseline FVIII:C of all patients. A mixed model was constructed to address the determinants of inter- and intra-individual variation of all baseline FVIII:C measurements that were available.

Results: In this retrospective cohort of 346 patients with 13 different *F8* missense mutations we found that the observed variation in lowest baseline FVIII:C was explained for 59% by age and genotype. Nine *F8* mutations significantly associated with lowest baseline FVIII:C, but the other four mutations did not significantly associate with lowest baseline FVIII:C. Intra-individual variation explained 45% of the observed variance in baseline FVIII:C among patients with the same *F8* missense mutation. Subsequently, the linear mixed model for each separate mutation group showed differences in the effect of age, that was strongest in patients with the Arg612Cys mutation compared to patients with the Arg2169His or Asn637Ser mutation . Age at time of baseline FVIII:C measurement explained 0.5%, 5% and 13% of the observed variation in Asn637Ser, Arg2169His and Arg612Cys respectively.

Summary/Conclusion: Although in this cohort age and genotype explain 59% of the observed lowest baseline FVIII:C variance in patients with MHA, for four out of 13 *F8* missense mutations other factors were stronger determinants of baseline FVIII:C. For example, intra-individual explained almost half of the observed variation in our largest three mutation groups. Age exerted a variable effect for each of the largest mutation group. Our results indicate that baseline FVIII:C levels are not exclusively determined by *F8* genotype in MHA. These findings emphasize the need for individual patient-tailored treatment in mild and moderate hemophilia A.

Bleeding

ECTH-465

Non-neutralizing antibodies against factor VIII and the risk of inhibitor development in patients with severe haemophilia A

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Background: The development of anti-Factor (F) VIII neutralizing antibodies (inhibitors) is a major complication in hemophilia A. Non-neutralizing antibodies (NNA) have been detected in hemophilia patients and unaffected individuals

Aims: The aim of this study is to assess the prevalence of NNA in previously untreated or minimally treated patients affected with hemophilia A and to evaluate whether or not their presence is associated with the development of inhibitors.

Methods: In the SIPPET trial 251 patients with severe hemophilia A who were previously untreated or minimally exposed to blood components patients were randomized to receive plasma-derived or recombinant FVIII concentrate. Plasma samples collected before any exposure to such concentrate were available for 237 patients and were analyzed for the presence of anti-FVIII NNAs

Results: NNAs were found in 18/237 (7.6%) of patients, with a clear age-gradient. Of those with NNA, seven subsequently developed an inhibitor for a cumulative incidence of 45.4% (95% confidence interval (CI95) 19.5-71.3%), whereas among those without NNA, 64/219 developed an inhibitor (cumulative incidence 34.0%, CI95 27.1-40.9%). In Cox regression patients with NNA at screening had a 83% higher incidence of inhibitor development than patients without NNA (hazard ratio (HR) 1.83, CI95 0.84-3.99). For high-titer inhibitors the rate was almost 3-fold increased (HR 2.74, CI95 1.23-6.12). The associations did not materially change after adjustment

Summary/Conclusion: The presence of anti-FVIII NNAs in patients with hemophilia A not previously exposed to any FVIII concentrates is associated with an increased incidence of high-titer inhibitors.

Bleeding

ECTH-386

Next-generation sequencing for haemophilia A and B genotyping in Lille university hospital

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Background: The identification of molecular defects in haemophilia is essential for the optimization of patient treatment and the formal characterization of female carriers. The Sanger method is the gold standard for sequencing *F8* and *F9* genes but is time-consuming and expensive.
Aims: We aimed to develop a high-throughput method to genotype haemophilia A (HA) and B (HB) patients using the Next-Generation Sequencing (NGS) technology for an exhaustive and less expensive analysis of *F8* and *F9* genes.
Methods: We developed a small panel containing *F8* and *F9* genes. We used two different methods for library preparation (AmpliSeq™, Life Technologies™ and HaloPlex™, Agilent™), performed in the same PCR emulsion system (Ion One Touch 2™, Life Technologies™) and sequenced with a Ion 316™ chip in a PGM™ Ion Torrent sequencer, or a Ion PI™ chip in a Proton™ sequencer (Life Technologies™) respectively. NGS analysis was first performed in 62 samples previously characterized for *F8* or *F9* mutations by Sanger method or Multiplex Ligation-Probe Amplification (MLPA). All types of mutations were studied and were distributed in all exons of *F8* and *F9*. NGS analysis was further performed in 42 haemophilia patients with unknown mutation status. All patients were included by the local Comprehensive Care Haemophilia Center of Lille University Hospital after written informed consent. Data were analyzed with SeqNext™ software (JCI Medical System™). A Normalized Reads Depth (NRD) ratio was used to detect exons deletion/duplication.
Results: In previously-genotyped patients, 92% (57/62) of *F8* and *F9* mutations were detected by AmpliSeq™ and 85% (53/62) by HaloPlex™. The detection rate of small insertion/deletion in homopolymers of exon 14 in *F8* was only 20% (1/5) with both methods and 71% (5/7) in other exons of *F8.* Four deletions and one duplication of exons accounting for a severe haemophilia phenotype were identified. In 5 uncharacterized patients by Sanger method, neither AmpliSeq™ nor HaloPlex™ were able to find a mutation. In never-genotyped patients, a mutation was detected in 90% (38/42) of cases. A duplication of exons 10 to 14 was also detected in a severe HA patient and was confirmed by MLPA. In NGS negative patients, no mutation was found in promoter or 3' regions. Of the ten candidate mutations identified in our cohort, seven were predicted to be deleterious. No mutation was found in 10% (4/42) of never-genotyped patients with mild haemophilia A, in consistence with the available data for the mild phenotype. The technical development and laboratory protocol was easier and less expensive ($530 *vs* $602 including reagents and technical/medical staff) with AmpliSeq™ than HaloPlex™.
Summary/Conclusion: NGS is able to detect the main types of mutations in *F8* and *F9* genes, albeit with a lower mutation detection rate with HaloPlex™ compared to AmpliSeq™. AmpliSeq™ seems also an interesting screening method for the detection of exons deletion/duplication using the NRD ratio. However, both strategies fail to detect small insertion/deletion located in homopolymers of exon 14 in *F8*, whom identification will still rely on Sanger sequencing. AmpliSeq™ protocol performed in the PGM™ sequencer appears as a new interesting tool in genotyping of HA and HB patients of the Lille University Hospital.

5 POSTER SESSIONS ABSTRACTS (incl. board No)

Vessel wall

ECTH-249

Board No. 1: Selective increase of endothelial and cardiomyocyte extracellular vesicles after experimental myocardial infarction

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Background: Extracellular vesicles (EVs) are small vesicles that can be potentially released from all cell types and are capable of transferring biological information to target cells. Moreover, they express markers that indicate changes in apoptosis or activation state of their cell or organ of origin. Therefore, they should be considered as intercellular messengers, as well as biomarkers of cell injury or activation. Interest in EVs has rapidly risen in the cardiovascular field, as they have been shown to predict the future risk of cardiovascular events in some patients. However, the dynamics of their release and clearance is still unclear, as many studies only measure them at a single time point.
Aims: 1) to determine changes in EV numbers and cellular origin at different time points after myocardial infarction in WT mice, including those of cardiac origin, and 2) to assess in vitro whether cardiomyocyte derived EVs modify endothelial function.
Methods: WT mice were subjected to myocardial infarction by the ligation of left-descending coronary artery. Plasma obtained at baseline and 3, 15 and 30 days post-ischemia, was separated from whole blood by double centrifugation 20 minutes at 1560×g to remove residual cells. EV numbers and cell origin were measured in PPP by flow cytometry (FACSCantoII) using antibodies against CD41 (platelets), CD31 (endothelial cells), CD45 (leukocytes), TER119 (erythrocytes) and Connexin-43 (cardiomyocytes). To assess in vitro whether cardiomyocyte derived EVs influence endothelial function, EVs were purified from murine cardiomyocytes (HL-1 line) and added to endothelial cultures (MS1 cell line) to study their internalization by confocal microscopy.
Results: EV numbers were similar at baseline (806±12 EVs/mL) and after ischemia (EVs/mL: 741±108 Day 3, 670±84 Day 15, 704±84 Day 30). No changes were observed in leukocyte, platelets or erythrocyte derived EVs among different time points post-myocardial infarction. Endothelial derived EVs increased by 50 % 3 days post ischemia compared to baseline, and gradually returned to control levels, while cardiomyocyte derived EVs increased by 35 % early after infarction, remain high 15 days post-ischemia (216±16 EVs/mL, p=0.037 compared to baseline) and start to decrease at day 30 (200±13 EVs/mL). In vitro, endothelial monolayers were able to internalize EVs purified from HL-1 cell line.
Summary/Conclusion: EVs of cardiac and endothelial origin, present in blood under normal conditions, increased early after myocardial ischemia and gradually returned to baseline, suggesting a dynamic release of EVs to the circulation according to organ damage. In vitro, internalization of cardiomyocyte derived EVs by endothelial monolayers, suggests that EVs may have pathophysiological implications.

Vessel wall
ECTH-265
Board No. 2: Endogenous plasmin activity in acute thrombotic thrombocytopenic purpura
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Background: A severe functional deficiency of ADAMTS13 is related to the life-threatening disorder thrombotic thrombocytopenic purpura (TTP), where patients suffer from episodes of thrombotic microangiopathy associated with widespread microvascular ischemia. We previously demonstrated that plasmin can cleave von Willebrand factor (VWF) multimers in the absence of ADAMTS13, and that exogenous activation of plasmin serves as a potential new treatment strategy for TTP. Interestingly, we also showed that endogenous plasmin is generated in patients during acute TTP episodes, but it is currently not known if and how plasmin-mediated digestion of VWF can influence the outcome of a TTP episode.
Aims: Elucidate the role of endogenous plasmin activity in acute TTP.
Methods: ADAMTS13 and plasminogen activator inhibitor (PAI-1) activity was blocked using monoclonal antibodies in either *α2-antiplasmin*$^{-/-}$ or wild type mice. TTP was triggered using 2000 U/kg recombinant (r)VWF. *In vivo* cleavage of platelet decorated VWF strings was visualized in $FeCl_3$ injured mesenteric venules, after labeling platelets with Rhodamine 6G.
Results: First, we determined whether excess amounts of endogenous plasmin are able to regulate acute TTP symptoms. Therefore, we used mice deficient in α2-antiplasmin with an acquired deficiency in PAI-1 and ADAMTS13. These mice thus lack the main plasmin (activation) inhibitors and therefore have unrestrained plasmin activity. Upon injection of rVWF, the mice suffered temporarily from TTP symptoms such as thrombocytopenia (146 ± 36 x10^3 platelets/μL) and recovered after 24 hours (681 ± 232 x10^3 platelets/μL; n=10). Littermate controls with normal α2-antiplasmin and PAI-1 levels, but with depleted ADAMTS13, were thrombocytopenic during the complete time period (153 ± 16 x10^3 platelets/μL after 24 hours; n=10). Additionally, mice with unrestrained plasmin activity revealed an accelerated VWF string cleavage after $FeCl_3$ injury of the mesenteric venules (4.3 ± 0.4 sec compared to 8.9 ± 0.9 sec in controls, n=7). This demonstrates that when plasmin activity is unrestrained, a faster recovery from acute TTP symptoms is observed.
We next investigated if plasmin is also generated during normal acute TTP episodes in mice. When mice with inhibited ADAMTS13 were triggered with rVWF, plasmin levels, measured by PAP (plasmin-α2-antiplasmin)-complexes, were indeed 5.9 fold elevated (n=10). However, as these mice did develop TTP, it is clear that increased endogenous plasmin levels are not enough to attenuate acute TTP episodes. These data are in agreement with our patient data where we also measured increased PAP-complexes (9.1 fold elevated compared to remission; n=27), which did not prevent the TTP episode. Interestingly, we also found increased PAI-1 levels in both mice (19 fold elevated; n=10) and TTP patients (6.7 fold elevated, n=27) during the acute phase.
Summary/Conclusion: We here showed that unrestrained plasmin activity can attenuate TTP symptoms and induce a rapid recovery in ADAMTS13 deficient mice by cleaving VWF. However, in conditions where plasmin generation is restricted, the increased endogenous plasmin levels are not high enough to attenuate TTP in both mice and TTP patients. Further investigation is required to determine to what extent increased PAI-1 levels, as observed during acute TTP in both mice and humans, are limiting endogenous plasmin activity during acute TTP.

Vessel wall

ECTH-342

Board No. 3: Identification of the core human endothelial transcriptome using a systems approach

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Background: Endothelial cells (EC) line blood vessels and regulate haemostasis, inflammation and blood pressure. Proteins critical for endothelial function tend to be enriched or specifically expressed in EC across vascular beds. Currently, there is no definitive description of the human pan EC enriched transcriptome.

Aims: To use a systems approach to identify a comprehensive panel of human endothelial-enriched genes using global, body-wide transcriptomics.

Methods: We performed RNA-seq tissue transcript profiling of 124 samples collected from 32 human organs as part of the Human Protein Atlas Project (www.proteinatlas.org). We selected three transcripts that encode for proteins that are known to be EC enriched across different vascular beds; c-type lectin domain family 14, member A (*CLEC14A*), von Willebrand factor (*vWF*) and CD34 (*CD34*) and calculated correlation coefficient values between the FKPM values of these transcripts and those of the other >20,000 mapped protein-coding genes. A high correlation value with all 3 EC reference genes indicated tissue wide EC-enriched expression of the gene(s) in question. We used antibody-based profiling to confirm protein expression of identified transcripts across vascular beds and measured expression in cultured EC *in vitro*.

Results: We identified a panel of 232 human pan EC-enriched transcripts, which contained both well-described EC transcripts (e.g. *CDH5*, *ESAM*, *KDR*, *FLT1*) and a number that encode for novel or uncharacterised EC proteins (e.g. *CXorf36*, *FAM110D*). The majority of identified transcripts could be detected in cultured EC from various vascular beds, and we observed maintenance of relative expression in early passage cells.

Summary/Conclusion: We describe a widely applicable method to determine cell type specific transcriptome profiles in a whole organism context, based on differential abundance across tissues. We identify potential vascular drug targets or endothelial biomarkers, and highlight candidates for functional studies to increase understanding of the endothelium in health and disease.

Vessel wall
ECTH-226
Board No. 4: A novel cell-surface proteomics approach identifies IL-1ß and TNFα-induced changes on the endothelial surface and reveals a conformational change in ITGA5
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Background: Endothelial dysfunction is associated with a variety of vascular diseases, including hypertension, hypercholesterolemia, diabetes and atherosclerosis. The molecular mechanisms that underlie endothelial dysfunction are poorly understood, but are often associated with endothelial activation by pro-inflammatory cytokines. Although transcriptional profiling has revealed cytokine-induced regulation of mRNA levels of various proteins, including cell surface molecules Vascular Cell Adhesion Molecule 1 (VCAM1) and Intercellular Adhesion Molecule 1 (ICAM1), cell surface alterations at the protein level have remained largely unexplored. Unraveling these is key to identify novel inflammation-markers and putative therapeutic targets to treat endothelial dysfunction.
Aims: Therefore, the aim of this study is to probe cytokine-induced changes in endothelial cells, with a focus on cell surface alterations.
Methods: To assess cytokine-induced cell surface changes, we have developed a novel quantitative cell surface proteomics-based method by combining metabolic labeling and cell surface footprinting. Briefly, Blood Outgrowth Endothelial Cells (BOECs) were metabolically labeled using Stable Isotope Labeling with Amino acids in Cell culture (SILAC) and Interleukin 1β (IL-1ß), Tumor Necrosis Factor α (TNFα) or mock-treated for 24 hours. Available extracellular lysine residues were labeled using a non-membrane permeable *N*-hydroxysuccinimido-biotin label. Pooled cell lysates were processed into peptides and biotinylated peptides were enriched and subjected to high resolution LC-MS/MS.
Results: Using this approach, 1080 biotinylated peptides were quantified, of which the majority showed an unaltered cell surface presence. However, for 129 peptides the SILAC ratio was more than twofold altered upon cytokine stimulation. Gene Ontology (GO)-term analysis of the corresponding proteins revealed an enrichment of the GO terms associated to the plasma membrane and extracellular region, supporting the applied approach. Most proteins affected in one condition are also altered in the other condition, indicating that the effect of both stimulants is very similar.
These data were combined with the cellular proteome in order to obtain a more broad picture. In the proteome 59 proteins were regulated, whereas this was true for 53 proteins in the cell surface proteome. Only 14 proteins were significant in both, demonstrating the added value of these methods. Analysis of all regulated proteins reveals enrichment for various processes, including angiogenesis, coagulation, cell adhesion, lymphocyte-mediated immunity and antigen presentation.
As expected, the proteins most prominently altered were ICAM1 and VCAM1. However, levels of many other proteins were altered, including adhesion molecules (NRCAM, BCAM and ITGA6), proteins involved in antigen presentation (HLA-A, -B and –C and B2M), enzymes (LIPG, PLAU) and receptors (ANTXR2 and PROCR). Most remarkably, differential labeling within integrin α5 was detected, indicating a conformational change in this protein.
Summary/Conclusion: In conclusion, we have developed a novel mass spectrometry-based method to quantify cytokine-induced cell surface alterations. Combined with conventional proteomics, this allows for detailed quantification both at the cell surface and within the cell. We have identified various novel targets for intervention of endothelial activation and dysfunction and our method allows for detecting stimulus-induced conformational changes.

Vessel wall
ECTH-140
Board No. 5: Differential effects of vitamin K antagonists vs direct oral anticoagulants: consequences to the genesis, progression and vulnerability of atherosclerotic plaque
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Background: Vitamin K-antagonists (VKA) are the most widely used anticoagulant drugs to treat patients at risk of arterial and venous thrombosis. Although being on the market for more than 50 years, unfavorable pharmacokinetics of VKA stimulated the development of alternative anticoagulant drugs, resulting in direct oral anticoagulant (DOAC) drugs, which specifically target coagulation factor Xa and thrombin. The anticoagulant effect of VKA is by limiting the carboxylation of vitamin K-dependent coagulation factors. The inhibition of carboxylation by VKA is not limited to coagulation, but affects all vitamin K-dependent proteins. As a consequence, VKA have detrimental side effects by enhancing vascular and cardiac valve calcification. This effect is most likely via vascular smooth muscle cell synthesized matrix Gla-protein, one of the strongest inhibitors of vascular calcification. Vascular calcification is an important independent risk factor for the development of myocardial infarction, stroke, and renal disease. If symptomatic cardiovascular disease is already apparent, the extent of calcification is a potent indicator of unfavorable outcome.

Aims: Previously we analysed effects of warfarin and dabigatran etexilate on calcification in vivo. Warfarin significantly increased calcification of atherosclerotic plaques as compared to Dabigatran treated animals. It became clear that not only the amount and density of calcification is a predictor of outcome, but that active micro-calcification in the atherosclerotic plaques renders plaque vulnerability. Detecting micro-calcification using CT is a challenge since the detection limit is 250mm. Recently, $Na^{18}F$ was shown to visualize the vulnerable plaque on the basis of microcalcifications in patients (Joshi et al. Lancet 2014). We hypothesized that apoE-/- animals on warfarin diet have increased $Na^{18}F$ uptake as compared to animals on dabigatran etexilate diet, indicating that warfarin induces a vulnerable plaque phenotype and that dabigatran etexilate favors a stable atherosclerotic plaque phenotype.
Methods: ApoE-/- mice (n=6 / group) were fed Western type diet (WTD) food supplemented with either dabigatran etexilate or warfarin in order to induce atherosclerosis. $Na^{18}F$ and micro-CT imaging was performed in vivo and aortas are excised and analysed ex vivo using histochemistry. Thirty minutes before the PET-scan measurement, 15MBq of $Na^{18}F$ was injected intra-venous. The PET-scan was performed under anesthesia using 2% isoflurane.

Results: Using the PET probe $Na^{18}F$ we show that active mineralization correlates with plaque vulnerability. In line, using histochemistry we demonstrated that ApoE mice treated with VKA had significantly more vulnerable atherosclerotic plaques as compared to dabigatran etexilate.

Summary/Conclusion: VKA induce a vascular vitamin K deficiency resulting in atherosclerotic plaque vulnerability by inducing vascular calcfication. Accumulating evidence demonstrates vascular benefit from high vitamin K intake. Several clinical preclinical and clinical trials are initiated to verify the beneficial effect of vitamin K on the arterial vessel wall. The discovery that vitamin K-dependent processes are involved in the inhibition of vascular calcification has boosted our mechanistic understanding of this process and has opened up novel avenues such as combining DOACs and vitamin K for both benefiting coagulation and calcification.

Vessel wall
ECTH-160
Board No. 6: Nestin is a novel endothelial specific protein in the nonproliferative adult human vasculature, and has potential links to cardiovascular disease
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Background: The intermediate filament protein nestin is widely expressed during development, but adult expression was thought to be mostly limited to stem cells or those undergoing active proliferation. Recently however, we have identified nestin as endothelial cell (EC) specific across multiple tissue beds in the human adult.
Aims: To determine whether nestin expression in the human adult vasculature is restricted only to angiogenic vessels, and to investigate its function and possible role in cardiovascular disease (CVD).
Methods: We used RNA-seq tissue transcript profiling of 124 samples collected from 32 human organs to perform correlation analysis between nestin (*NES*) transcript expression and those for the proliferation markers *PCNA*, *Ki-67* and *CDK2*. Tissue sections from multiple organs were stained for these proteins by IHC, and primary EC were cultured *in vitro* to examine nestin expression under proliferative and non-proliferative conditions, static culture or exposure to shear stress. To establish whether EC nestin could be linked with CVD we searched the literature for genetic associations and used expression quantitative trait loci (eQTL) analysis in EC for SNPs of interest.
Results: There was no positive correlation observed between *NES* and *PCNA*, *Ki-67* or *CDK2*, and nestin protein was detected in the vasculature of multiple human tissues by IHC in the absence of the proliferation markers. Nestin mRNA and protein was detected in cultured primary EC , and expression was not altered when low serum conditions were used to inhibit proliferation. When cultured under flow conditions EC nestin was reorganised from a juxtanuclear subcellular location into a cell-spanning filamentous network. Published work has previously reported SNPs in the nestin locus were associated with coronary heart disease, we found, using eQTL analysis, that these SNPs were also associated with nestin expression levels in EC.
Summary/Conclusion: Contrary to current opinion, nestin is widely expressed in non-proliferating adult EC. There is evidence to suggest nestin could be important in CVD, and our data indicates that this may be linked to its expression in the vasculature.

Platelets

ECTH-467

Board No. 7: Defective magnesium transport enhances receptor-operated calcium entry in platelets thereby accelerating thrombosis and stroke

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Background: Magnesium (Mg^{2+}) is *the second* most abundant cation in platelets and proposed as a natural “platelet antagonist” since elevated cytosolic Mg^{2+} concentration inhibit platelet aggregation. Mg^{2+} influx has been described in megakaryocytes and platelets but the responsible transporter or channel proteins and their signaling functions are unknown. Altered Mg^{2+} homeostasis has been described in patients suffering from various heart diseases and ischemic brain infarction. Mg^{2+} supplementation is frequently used as a treatment in these pathophysiological conditions. The current understanding is that supplemented Mg^{2+} counter-regulates Ca^{2+} overload in different cell types of the hematopoietic system and also in brain and heart tissues. In agreement with this, Mg^{2+} supplementation in ischemic brain infarction improves brain function recovery, pointing to a therapeutic benefit of Mg^{2+} uptake in ischemic neurons.

Aims: We aimed to understand the role of Magnesium transporter 1 (MagT1) in platelet Mg^{2+} homeostasis and in thrombo-inflammatory diseases.

Methods: To elucidate the role of Mg^{2+} transport in platelets, we analyzed mice lacking MagT1 (*Magt1*$^{-/-}$) using different *in vitro* and *in vivo* assays of thrombosis and stroke.

Results: Our report gives the first *in vivo* evidence, that interference to Mg^{2+} transport activity in mice by genetic ablation of *Magt1* results in an aberrant Mg^{2+} efflux in activated platelets. Imbalanced Mg^{2+} homeostasis accounts for increased Ca^{2+} influx through receptor-operated Ca^{2+} entry mechanism, which strongly modulates platelet pro-coagulant activity and aggregation responses under flow. Consequently, *Magt1*$^{y/-}$ mice displayed accelerated occlusive arterial thrombus formation *in vivo*, shorter bleeding time, and a dramatically worsened brain damage after focal cerebral ischemia. Mg^{2+} supplementation normalized all the observed deleterious outcomes *in vitro* and *ex vivo* conditions.

Summary/Conclusion: Our results reveal that decreased intracellular Mg^{2+} concentration in platelets, due to the defective MagT1 function, can be a risk factor for the development of arterial thrombosis and stroke.

Platelets

ECTH-407

Board No. 8: Deletion of junctional adhesion molecule A from platelets increases early stage neointima formation after wire injury in hyperlipidaemic mice

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Background: Platelets play an important role in the pathogenesis of vascular remodeling after injury. Junctional Adhesion Molecule A (JAM-A) was recently described to regulate platelet activation. Specific deletion of JAM-A from platelets resulted in increased reactivity and in accelerated progression of atherosclerosis.

Aims: The aim of this study was to investigate the specific contribution of platelet-derived JAM-A to neointima formation after vascular injury.

Methods: Mice with or without platelet-specific (tr)JAM-A-deficiency in an apolipoprotein e (apoe–/–) background underwent wire-induced injury of the common carotid artery.

Results: Ex vivo imaging by 2-photon microscopy revealed increased platelet coverage at the site of injury in trJAM-A–deficient mice, 1 hour after wire injury. Cell recruitment assays showed increased adhesion of monocytic cells to activated JAM-A–deficient platelets than to control platelets. Up to 4 weeks after wire-injury, intimal neoplasia and neointimal cellular content were analyzed. Neointimal lesion area was increased in trJAM-A–/– apoe–/– mice and the lesions showed an increased macrophage accumulation and proliferating smooth muscle cells compared with trJAM-A+/+ apoe–/– littermates 2 weeks ($3.01 \pm 1.1 * 10^4$ vs. $1.96 \pm 0.49 * 10^4$, n=9, $P<0.05$), but not 4 weeks after injury ($6.89 \pm 1.9 * 10^4$ vs. $7.53 \pm 1.8 * 10^4$, n=11). Re-endothelialization was decreased in trJAM-A–/– apoe–/– mice compared with controls 2 weeks after injury, yet it was complete in both groups after 4 weeks.

Summary/Conclusion: A platelet gain-of-function by deletion of JAM-A accelerates neointima formation only during earlier phases after vascular injury, through an increased recruitment of mononuclear cells. Thus, the contribution of platelets might become less important when neointima formation progresses to later stages.

Platelets

ECTH-185

Board No. 9: Full activation of mouse platelets requires an ADP secretion pathway regulated by SERCA3 ATPase-dependent calcium stores

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Background: Cytosolic calcium (Ca^{2+}) is central to platelet activation and signaling. Upon agonist platelet stimulation, Ca^{2+} rises in the cytosol due to either mobilization from internal stores or to entry from the extracellular medium (Ca^{2+} influx). The sarco-endoplasmic reticulum calcium (Ca^{2+})ATPases (SERCA)2b and SERCA3 down-regulate Ca^{2+} mobilization by pumping cytosolic Ca^{2+} back into internal stores, but their specific role remains poorly explored.

Aims: To better define the role of SERCA3 in platelet physiology by the study of a $SERCA3^{-/-}$ mouse model.

Methods: We have analyzed hemostasis and thrombosis *in vivo* (tail clip assay, ferric chloride-induced thrombosis of mesenteric vessels) and *in vitro* (thrombus formation under flow, aggregation, secretion, $\alpha_{IIb}\beta_3$ activation, Ca^{2+} mobilization) in a $SERCA3^{-/-}$ mouse model.

Results: First, we show that $SERCA3^{-/-}$ mice exhibit prolonged tail bleeding time and re-bleeding. Thrombus formation was delayed both in arteries and venules in an *in vivo* ferric chloride-induced thrombosis model. Defective platelet adhesion and thrombus growth over a collagen matrix was confirmed *in vitro*. ADP removal by apyrase diminished adhesion and thrombus growth of control platelets to the level of $SERCA3^{-/-}$ platelets (unaffected by apyrase). Aggregation, dense granule secretion and Ca^{2+} mobilization of $SERCA3^{-/-}$ platelets induced by low collagen or low thrombin concentration were weaker than controls. Accordingly, $SERCA3^{-/-}$ platelets exhibited a partial defect in total stored Ca^{2+}, and in Ca^{2+} store re-uptake following thrombin stimulation. Ca^{2+} influx was slightly increased, consistent with the known role of SERCA3 in restoring internal Ca^{2+} stores from store-operated Ca^{2+} entry (SOCE). Importantly ADP but not serotonin, another weak agonist released from dense granules, rescued aggregation, secretion and Ca^{2+} mobilization in $SERCA3^{-/-}$ platelets, strongly suggesting the effect of ADP was specific. Dense granules appeared normal upon electron microscopy, mepacrine staining, and total serotonin content, ruling out a dense granule defect. ADP induced normal platelet aggregation, excluding a defect in ADP activation pathways. Importantly, the SERCA3-specific inhibitor tBHQ diminished both Ca^{2+} mobilization and secretion of control platelets to the level of $SERCA3^{-/-}$ platelets. In contrast the SERCA2b inhibitor thapsigargin did not inhibit ADP secretion. These results confirmed the specific role of catalytically active SERCA3 in ADP secretion. Accordingly, SERCA3-dependent Ca^{2+} stores appeared depleted in $SERCA3^{-/-}$ platelets. Finally $\alpha_{IIb}\beta_3$ integrin blockade did not affect SERCA3-dependent secretion, therefore proving it is not dependent on $\alpha_{IIb}\beta_3$ engagement.

Summary/Conclusion: Altogether these results show that SERCA3-dependent Ca^{2+} stores control a specific ADP secretion pathway required for full platelet secretion induced by agonists at low concentration, and independent of $\alpha_{IIb}\beta_3$.

Platelets

ECTH-355

Board No. 10: Mice lacking GARP on platelets display unaltered thrombus formation

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Background: Glycoprotein-A Repetitions Predominant protein (GARP, or LRRC32) is a receptor for latent TGF-β1 and is present on among others platelets and endothelial cells. Evidence for its positive involvement in thrombus formation was suggested in GARP knockdown zebrafish.
Aims: To evaluate the role of GARP in murine platelet physiology and in thrombus formation using platelet specific GARP knock out mice (GARP cKO).
Methods: Cre expression driven by the PF4 promoter was used to generate transgenic mice specifically lacking GARP expression on megakaryocytes and platelets. In PF4-GARP cKO and littermate controls, hematological parameters (platelets, mean platelet volume, white and red blood cells, hemoglobin and hematocrit) and platelet surface glycoprotein expression (GPIb, GPVI and integrin αIIb) were determined. Platelet function was measured ex vivo by flow cytometry (P-selectin expression, integrin αIIbβ3 activation and fibrinogen binding), spreading analysis and aggregometry using PAR4-activating peptide and collagen related peptide as agonists. Additionally, collagen-induced adhesion and aggregation under flow and clot retraction were analysed. *In vivo* tail bleeding time and occlusion time of the carotid- and mesenteric artery after $FeCl_3$-induced thrombosis were determined in PF4-GARP cKO and control mice.
Results: GARP cKO mice had normal hematological parameters and their platelets had normal surface GPIb, GPVI and integrin αIIb glycoprotein expression. GARP cKO platelets furthermore displayed normal agonist induced activation, spreading on fibrinogen and aggregation responses. Absence of GARP did not influence clot retraction and had no impact on thrombus formation on collagen-coated surfaces under flow. In line with this, platelet GARP deficiency did neither affect the tail bleeding time nor change the occlusion time in the carotid- and mesenteric artery after $FeCl_3$-induced thrombosis.
Summary/Conclusion: Although previous zebrafish studies have indicated an important role for GARP in thrombus formation, the present results provide evidence that platelet GARP is not important in hemostasis and thrombosis in mice. Whether GARP on endothelial cells might influence thrombus formation is currently under investigation.

Platelets

ECTH-448

Board No. 11: TMEM16F-mediated platelet pro-coagulant activity is critical for haemostasis and thrombosis but not for infarct progression after ischaemic stroke

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Background: Activated platelets support coagulation by providing a procoagulant surface rich in negatively charged aminophospholipids to support the production of thrombin. The transmembrane protein TMEM16F, mutated in patients with the bleeding disorder Scott syndrome, is important for the scrambling of phosphatidylserine (PS) to the platelet surface upon platelet activation. Thus, it plays an essential role in the platelet pro-coagulant response. However, the contribution of TMEM16F and the pro-coagulant activity of platelets to thrombo-inflammation after ischemic stroke is unknown.

Aims: To investigate the pathophysiological role of TMEM16F-mediated platelet pro-coagulant activity in the setting of ischemic stroke

Methods: A platelet and megakaryocyte specific TMEM16F knockout (KO) mouse was generated by targeted deletion of exon 3 in the *Anoctamin6* gene. The mice were assessed in the transient middle cerebral artery occlusion (tMCAO) model of ischemic stroke in addition to models of thrombosis and hemostasis, as well as in vitro platelet analyses.

Results: TMEM16F KO platelets exposed significantly less PS as compared to wildtype (WT) platelets and also failed to acquire a ballooned morphology after stimulation with ionomycin, indicating a reduced pro-coagulant potential. Likewise, thrombinoscope measurements to assess thrombin production over time showed that both, the time to initiation of thrombin generation (lagtime) as well as time taken to reach peak thrombin concentration, were significantly delayed in KO platelet rich plasma (PRP), as compared to WT PRP. KO mice displayed significantly prolonged tail bleeding times, and were protected in a model of ferric chloride induced thrombosis in the carotid artery, confirming previous reports. These results highlighted the TMEM16F KO as a suitable model with which to investigate the pathophysiological significance of pro-coagulant platelets in cerebral infarct progression. In the tMCAO model of ischemic stroke, KO mice had infarct volumes similar to WT mice and also comparable neurological outcomes as well as motor function and coordination outcomes.

Summary/Conclusion: These results show that TMEM16F mediated procoagulant activity of platelets is not important for the thrombo-inflammatory role of platelets in ischemic stroke. This adds to the current understanding of the role of platelets in cerebral thrombo-inflammation.

Platelets

ECTH-197

Board No. 12: Evaluation of isoflavonoids antiplatelet potential in relation to their structural modifications

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Background: Isoflavonoids were suggested to have positive effects on human health in particular on cardiovascular diseases. These data are also supported by lower incidence of cardiovascular diseases in Asian countries with high consumption of soya products.

Aims: The aim of this study was to test antiplatelet activities of 18 isoflavonoids, define structure-activity relationship and identify the mechanism of their action.

Methods: Screening of the antiplatelet activity was investigated by impedance aggregometry. Inhibition of cyclooxygenase-1 and thromboxane synthase was analysed by measurement of products formation (prostaglandin H_2 and thromboxane A_2, respectively) by use of ELISA method. Antagonism at the thromboxane A_2 receptors was measured by turbidimetry.

Results: The screening in the whole human blood has shown that 12 isoflavonoids blocked platelet aggregation induced by arachidonic acid. The structure-activity relationship revealed that the 7,4′-dihydroxyl group represented the most efficient functional core of the isoflavonoids. Substitution of hydroxyl group(s) at those positions by (a) methoxyl group(s) led to a reduction of the effect while glucose in position C-7 was associated with almost complete loss of the activity. Presence of a 5-hydroxyl group seemed to be beneficial, as well as, the co-presence of a 6-methoxy group which markedly enhances the effect.

Summary/Conclusion: Two of the tested compound acted as inhibitors of cyclooxygenase-1 while the effect of other active isoflavonoids appeared to be based mainly on the antagonism at thromboxane receptors. Isoflavonoids could possess even higher antiplatelet activity than acetylsalicylic acid, the standard antiplatelet drug.

Acknowledgement: The study was supported by Charles University in Prague (SVV 260 293) and grant project GAUK (170/50/55003).

Platelets

ECTH-352

Board No. 13: Elevated levels of neutrophil extracellular traps markers are associated with severity of stroke

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Background: Neutrophil extracellular traps (NETs) are formed by DNA, histones and proteolytic enzymes, and are produced by activated neutrophils through different mechanisms. NETs can activate platelets favoring thrombotic processes and have been detected in venous and arterial thrombosis, but not in stroke.
Aims: To study the presence of NETs in patients with stroke, and their correlation with markers of disease and progression.
Methods: 243 patients with stroke were included in the study. Clinical and demographic data were registered, including scores of neurological damage at the onset of event and at discharge. As markers of NETs cell free DNA (cfDNA), nucleosomes and citrullinated histone 3 were determined in plasma samples.
Results: NETs were present in plasma of patients with stroke and elevated as compared with healthy subjects. Values were specially elevated in >80 years and in those patients with history of atrial fibrillation, inflammation (elevated neutrophil to lymphocyte ratio) or high glucose. NIHSS score>12 at onset positively correlated with elevated concentrations of NETs, which also predicted a longer stage and a worse neurological index at discharge.
Summary/Conclusion: NETs formation participates in the pathopysiology of stroke, and is associated with the gravity of the stroke. These results open new avenues of research in the diagnosis and treatment of stroke.
IIS Carlos III. Fondos FEDER PI13/00016; Red Cardiovascular [RD12/0042/0003]; Beca de la FETH.

Platelets

ECTH-401

Board No. 14: Storage pool disease with mild thrombocytopenia associated with two novel FLI1 mutations

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Background: Non syndromic storage pool diseases (SPD) represent a heterogeneous group of inherited platelet disorders for which genetic diagnosis needs to be improved.
Aims: Our goal was to determine the genetic etiology of autosomic dominant SPD in two families explored at the bleeding and thrombosis exploration center (CHU Timone, Marseille). The first propositus suffers excessive bleeding (score: 16), prolonged bleeding time (> 30 min) and shows a subnormal platelet count (130 to 154.10^9/L) whereas the second one did not experience bleeding but presents moderate thrombocytopenia (100 to 130.10^9/L). In these families, coagulation, fibrinolysis and von Willebrand factor levels were normal.
Methods: Aggregation and flow cytometry were used to assess platelet function. Electron microscopy and determination of serotonin and PAI-1 levels has allowed granule evaluation. Protein expression levels were investigated by western-blot. Mutations were screened using high-throughput sequencing technologies (308-gene panel or whole exome sequencing). The effects of the identified mutations on subcellular localization (epifluorescence microscopy) and on transcriptional regulatory properties of the FLI1 protein (luciferase reporter system assay) were investigated in transfected GripTite™ 293 MSR cells. Peripheral $CD34^+$-derived proplatelets were quantified after 12 days of culture in serum-free medium supplemented with thrombopoietin and stem cell factor.
Results: The affected members (n = 3) of both families exhibited similar platelet phenotype: reduced ADP-induced aggregation (maximum intensity, mean ± SD: 44 ± 19; normal value range: 75-95), low platelet serotonin level (mean ± SD: 0.24 ± 0.02 ng/mL; normal range: 0.30 - 1.20 ng/mL) but normal serum PAI-1 antigen levels. Platelet receptor levels (GPIIbIIIa, GPIba, and CD62P) were within the normal range except CD63 levels upon thrombin stimulation (50µM) (MFI, mean ± SD: 0.74 ± 0.3; normal range: 1.30 - 4.23). Electron microscopy revealed a 80% reduction in dense granule number (whole mount) and large fused alpha granules (12 - 14% platelets with granule diameter > 400 nm). In addition, non-muscle myosin heavy chain IIB (MYH10) was detected in the platelets of all affected members by western-blot. Our gene screening strategy revealed two heterozygous mutations in the *FLI1* gene, unidentified so far: c.1010G>A and c.1033A>G, predicting p.R337Q and p.K345E substitutions respectively. Both mutations affect the highly conserved ETS DNA-binding domain without altering FLI1 protein expression in either the patients' platelets or transfected cells overexpressing *FLI1* mutants. The two FLI1 protein variants exhibited a reduced nuclear accumulation as compared to the native form (-80% and -50%). Accordingly, these mutants exhibited significantly reduced transcriptional activity (-61% and -73%). Both *FLI1* mutations are associated with a dramatic defect of *in vitro* proplatelet formation.
Summary/Conclusion: These results identified and characterized two novel germline mutations in *FLI1* gene as causes for SPD with mild thrombocytopenia. Of note, these *FLI1*-associated bleeding disorders strongly differ between these two families indicating variable effects of *FLI1* mutations which require further explorations.

Platelets

ECTH-263

Board No. 15: ADAMTS13 reactive CD4+ T-cells in autoimmune thrombotic thrombocytopenic purpura

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is a severe and life threatening disorder characterized by the formation of auto-antibodies against ADAMTS13. Several groups have reported that HLA-DRB1*11 provides a risk factor for development of acquired TTP. We have previously shown that CUB-2 domain derived peptides with core-sequence FINVAPHARIA and ASYILIRD are presented by antigen presenting cells expressing HLA-DRB1*11 or HLA-DRB1*03. CD4+ T cells recognizing these CUB-2 derived peptides were detected in patients with acquired TTP (Verbij et al., Blood, 2016). These results suggests that these two peptides contribute to the onset of acquired TTP.

Aims: Here, we explored whether ADAMTS13 specific CD4+ T cells derived from patients with acquired TTP also recognizes peptides located outside the CUB-2 domain.

Methods: Splenocytes of a patient with acquired TTP were seeded in serum-free AIM-V medium (Life Technologies, Carlsbad, CA) in a 96-well plate at 2×10^6 cells per well. Cells were allowed to rest for 24 hours and were then stimulated with pools of overlapping peptides. The previously identified FINVAPHAR peptide and full-length ADAMTS13 (FL-ADAMTS13) were also included in this analysis. *Staphylococcus aureus* enterotoxin B (SEB) (1 µg/mL; Sigma-Aldrich, St Louis, MO) was used as a positive control. Samples were stimulated for 24 hours and cultures were supplemented with 1 µg/mL blocking αCD40 antibody to prevent down-regulation of CD40L. Overlapping peptides composed of 15 amino acids were obtained from JPT Peptide Technologies. ADAMTS13 was expressed in HEK293 cells and purified as described previously.

Results: Splenocytes of a patient with acquired TTP were stimulated for 24 hours and activation of T cells was monitored employing the activation marker CD40 ligand (CD40L). Unbiased screening of peptide reactivity of CD4+ T cells was performed using 4 pools composed of in total 354 overlapping peptides covering the entire coding sequence of ADAMTS13. Pool 1 contained peptides derived from Met1 (signal peptide) to Pro395 (TSP-1), pool 2 Gly385 (TSP-1) to Tyr779 (TSP-3), pool 3 Cys769 (TSP-3) to Gln1163 (CUB-1) and pool 4 Tyr1153 (CUB-1) to Tyr1427 (CUB-2). CD4+ T cells responding to peptides present in pool 2 (0.062%), 3 (0.054%) and 4 (0.043%) were detected. Also, CD4+ T cells responding to the previously described FINVAPHAR peptide were observed (0.048%). Upon stimulation with recombinant full length ADAMTS13 a small population of CD4+ t cells upregulated CD40L expression (0.051%). No significant numbers of reactive T cells were observed upon stimulation with pool 1 (0.028%) or the medium control (0.019%). CD40L expressing CD4+ T cells were single cell sorted; 150 clones were obtained which will be screened for their reactivity with individual peptides.

Summary/Conclusion: We present a novel platform for the unbiased analysis of CD4+ T cells in patients with acquired TTP. Our initial data indicate that CD4+ reactive T cells recognizing non-CUB2 domain peptides are also present in patients with acquired TTP.

Platelets

ECTH-163

Board No. 16: The role of the endothelium and platelets in the development of neonatal sepsis

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Background: In the western world, *Streptococcus agalactiae*, commonly known as Group B Streptococcus (GBS), is a major causative organism of sepsis in neonates. GBS, a normal commensal of the gastrointestinal and vaginal tract can be transmitted from mother to baby during delivery which can lead to sepsis. Neonatal sepsis carries a high mortality rate as the underdeveloped immune system of the child cannot cope with the high level of infection. A common feature of patients with sepsis is the formation of micro-thrombi and endothelial dysfunction. Currently, there is paucity of data on the role of platelets in the pathophysiology of GBS-induced sepsis. The molecular interactions by which GBS induces endothelial cell dysregulation and platelet activation are not completely understood.
Aims: The aim of this study was to investigate the molecular interaction between human vascular endothelial cells, platelets and GBS.
Methods: Human aortic endothelial cells (HAoECs) sheared at 10dynes/cm^2 were incubated ± 10ng/ml TNF-α with GBS in the presence of human plasma, fibrinogen or Immunoglobulin G (hIgG). Adherence of GBS to HAoECs was measured by fluorimetry. Assessment of the ability of 20 GBS clinical isolates to induce platelet aggregation, with or without inhibitors, was carried out using light transmission aggregometry. Whole blood was perfused over monolayers of GBS under low shear (200s^{-1}) or high shear (800s^{-1}) conditions.
Results: Using a clinically relevant model of infection, GBS adhered to sheared HAoECs. Adhesion to sheared HAoECs was not dependent on plasma proteins as removal of plasma from the model failed to affect binding (P=NS). Of the 7 GBS serotype strains tested, strains representing Ia, Ib, II, III, IV and V induced platelet aggregation in a donor-dependent manner but all the strains representing serotype VI failed to induce aggregation. Platelet aggregation was all or nothing with a mean of 60.3±17.4% (P<0.001) for aggregating strains. Aggregation was dependent on the thromboxane pathway as blocking cyclooxygenase inhibited the aggregation (P<0.001). Inhibition of FcRgIIa but not TLR2 inhibited platelet aggregation. Consistent with this observation, addition of IgG was critical for aggregation using washed platelets (P<0.001). Micro aggregates formed on monolayers of GBS under low shear conditions, however no interaction was observed under high shear conditions.
Summary/Conclusion: Here, we show that GBS has the ability to interact directly with the human endothelial cells. Attachment of GBS to the endothelial cells presents the bacteria to patrolling platelets. Platelet aggregation induced by GBS is host-dependent and critically non-serotype dependent. We also show that platelet FcRgIIa/ IgG may contribute to GBS-induced aggregation. A better understanding of the interactions between GBS, endothelial cells and platelets could lead to the development of novel therapies to treat neonatal sepsis.

Platelets

ECTH-304

Board No. 17: Clinical outcomes of argatroban versus fondaparinux in heparin-induced thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is a serious immune-mediated adverse drug event that occurs following unfractionated heparin (UFH) or low-molecular-weight-heparin (LMWH) exposure. The treatment of HIT requires both the immediate discontinuation of all heparins and administration of nonheparin anticoagulant such as argatroban (direct thrombin inhibitor) and fondaparinux (synthetic indirect factor Xa inhibitor).
Aims: The current study compares the safety and efficacy of intravenous argatroban with subcutaneous fondaparinux in HIT patients
Methods: A retrospective analysis conducted in King Abdulaziz Medical City, large tertiary care academic medical centre, Riyadh, Saudi Arabia. Clinical (pre-test probability 4Ts scoring system) and laboratory (enzyme-linked immunosorbent assay, ELISA) findings between June 1st, 2013 and December 31st, 2014 were used to confirm the positive HIT cases. We used Chi-square tests to compare categorical variables, Student's t-tests to compare differences in means and Mann Whitney U tests to compare differences in medians. All p-values were two-tailed and statistically significant at an alpha of <0.05.
Results: A total of 33 patients were identified as a HIT positive and received nonheparin anticoagulants, either argatroban or fondaparinux. Argatroban was given to 21 patients where as fondaparinux was administered in 12 subjects. The main demographic characteristics of the argatroban and fondaparinux groups were comparable and no statistical differences found. The majority of patients received UFH in both groups. The admitting services and pre-test probability 4Ts scoring system were similar for both groups. The average length of hospital stay of patients treated with the argatroban was longer than the fondaparinux group but not statistically significant. The mean time (in days) for the resolution of thrombocytopenia was 3.5 (±1.8) for patients who received argatroban compared with 3.7 (±1.7) patients received fondaparinux (P=0.843). Ultimately, HIT complications were found similar in both groups.
Summary/Conclusion: Heparin-induced thrombocytopenia with or without thrombosis is a potentially catastrophic complication of heparins therapy. Argatroban and fondaparinux were similar in achieving and maintaining therapeutic anticoagulation goals, clinical outcomes, and safety in the management of patients with HIT. Prospective controlled trials need to be conducted to determine the optimal strategy to treat HIT.

Platelets

ECTH-447

Board No. 18: Major cardiovascular events prediction after primary percutaneous coronary intervention: do fibrinogen and von Willebrand factor add value to number of stents and poor anti-platelet therapy response?

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Background: Prediction of major cardiovascular events (MACE) significantly improves outcomes after primary percutaneous coronary intervention (PPCI). Number of stents and poor anti-platelet agents response are associated with high risk for stent thrombosis. Hyperfibrinogenemia and high von Willebrand factor (vWf) activity generally predispose to arterial thrombosis.

Aims: Study investigated whether fibrinogen and vWf levels measured on hospital admission are associated with post-PPCI MACE risk as well as whether their combination with number of stents and poor dual anti-platelet therapy response (acetyl salicylic acid and clopidogrel) potentiates the above-mentioned risk.

Methods: During 11 months 126 patients (84 men and 42 women, median age (min-max) 58 (23-85) years) were included. Fibrinogen and vWf were measured on hospital admission, using standard kits (Siemens Healthcare, reference range 2-4 g/L for fibrinogen and 70-150 % of normal for vWf). Platelet function was assessed after 6-48 h after therapy initiation by impedance aggregometry (Multiplate®, Roche Diagnostics). Poor anti-platelet therapy response was defined by the following AUC results: ASPI>600 or ASPI/TRAP>0.5 for acetyl salicylic acid and ADP HS>500 or ADP HS/TRAP>0.5 for clopidogrel. MACE occurrence was monitored during one-month follow-up. Uni- and multivariate logistic regression analysis was used for statistic evaluation.

Results: MACE frequency was 15.9%. Non-fatal re-infarction as the most common form (11.9% patients) and lethal outcome was recorded in 1.6% of participants. Majority of patients (69.8%) had one stent, while maximum number of stents was five (2.4%). Resistance frequency was 14.3% for acetyl salicylic acid and 47.6% for clopidogrel in patients. Mean fibrinogen concentration (min-max range) was 5.1(2.4) g/L, while for vWf value was 150(16.5) %. Following variables were identified as significant MACE predictors (relative risk; 95% confidence interval): number of stents (6. 8; P<0.001), ASPI>600 (4.6; P=0.007), ASPI/TRAP>0.5 (3.5; P=0.014), ADP HS>500 (5.6; P=0.004) ADP HS/TRAP>0.5 (6.8; P=0.003) and fibrinogen level (1.3; P=0.010). After multivariate logistic regression relative risk remained significant only for number of stents (7.7; P<0.001) and ADP HS>500 (6.7; P=0.006).

Summary/Conclusion: In investigated group of patients fibrinogen concnetration was associated post-PPCI MACE risk, while the same was not observed for vWf activity. Nevertheless, their levels did not increase the risk conferred by the number of stents and poor response to anti-platelet therapy.

Bleeding
ECTH-347
Board No. 20: N-acetylcysteine in preclinical animal models for thrombotic thrombocytopenic purpura
Claudia Tersteeg[*1], Walter J Van Rensburg[2], Charlotte Dekimpe[1], Nele Vandeputte[1], Inge Pareyn[1], Aline Vandenbulcke[1], Seb Lamprecht[2], Hans Deckmyn[1], Simon F De Meyer[1], Jan Roodt[2], Karen Vanhoorelbeke[1]
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Background: Thrombotic thrombocytopenic purpura (TTP) is a microangiopathic disorder diagnosed by thrombocytopenia and hemolytic anemia, and associated with a deficiency in von Willebrand factor (VWF) cleaving protease ADAMTS13. Current treatment is based on fresh frozen plasma infusion for congenital TTP, or plasma exchange, often in combination with immunosuppressive agents, for acquired TTP. However, new treatment methods are highly warranted. N-acetylcysteine (NAC), an FDA-approved anti-mucolytic agent, could be a possible new treatment strategy for acute TTP patients as it was demonstrated to reduce disulfide bonds in VWF, thereby decreasing VWF multimer size and hence its prothrombotic potential.
Aims: In the current study, we investigated whether NAC, without concurrent plasma exchange and immunosuppressive therapy, is effective in treating TTP symptoms in well-established murine and baboon models for TTP.
Methods: *Adamts13*$^{-/-}$ mice were triggered with 2000 U/kg rVWF and received 800 mg/kg NAC 15 minutes before (prophylactic) or one and 12 hours after (treatment) injection of rVWF. Baboons (*Papio ursinus)* were injected intravenously every 48 hours with 600 µg/kg inhibitory anti-ADAMTS13 antibody 3H9. TTP symptoms developed within 72 hours, at which time treatment was initiated by intravenous injections of 400 mg/kg NAC every 12 hours for five days.
Results: In mice, prophylactic administration of NAC was effective in preventing the onset of TTP. After 24 hours, 492 ± 181 x10^3 platelets/µL were measured compared to 226 ± 113 x10^3 platelets/µL in the control group (both n=12). This was supported by in vitro data, demonstrating the VWF-reducing properties of NAC in solution. Nonetheless, in both mice and baboons, treatment with NAC was not effective in curing TTP symptoms; thrombocytopenia, hemolytic anemia and organ damage could not be restored when the animals suffered from a TTP bout. In mice receiving NAC, severe thrombocytopenia persisted as the platelet count after 24 hours was 267 ± 123 x10^3 platelets/µL compared to 245 ± 149 x10^3 platelets/µL in non-treated mice (both n=10). Also baboons did not recover from TTP after receiving NAC for 5 days as their platelet count remained low (38 ± 11 x10^3 platelets/µL (n=4), compared to 39 ± 16 x10^3 platelets/µL in control baboons (n=4)). In mice and baboons treated with NAC, a reduction in VWF multimers was observed, demonstrating that NAC was, as expected, efficient in reducing disulfide bonds in circulating VWF multimers. Nevertheless, NAC was not able to break down existing VWF/platelet agglutinates in vitro, indicating that NAC is not able to induce thrombus resolution.
Summary/Conclusion: In conclusion, NAC is effective in preventing TTP in mice but not effective in resolving acute TTP symptoms in neither mice nor baboons.

Bleeding

ECTH-240

Board No. 21: Bleeding tendency measured by thrombin generation in patients with severe vitamin K antagonist overdose

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Background: Vitamin K antagonists (VKAs) are the most widely used oral anticoagulants. They have an individual dosage and a narrow therapeutic index. Patients spend more than one third of their time outside the therapeutic range. VKA treatment is monitored using the international normalized ratio (INR) calculated from the prothrombin time (PT). Bleeding is one of the most important complications of VKA treatent. Although the risk of bleeding increases sharply when the INR exceeds 5, in extremely over-anticoagulated patients, when the INR cannot be determined (INR>10), the exact bleeding risk in the individual patient is difficult to define. Thrombin generation (TG) is a global haemostasis assay that measures the overall capacity of the plasma to form thrombin after initiating coagulation with tissue factor. It is decreased in patients on VKA treatment and shows an inverse, but not linear correlation with INR.
Aims: The aim of this study was to measure TG in extremely over-anticoagulated patients and correlate it with their bleeding symptoms.
Methods: Twenty patients receiving vitamin K antagonist treatment with INR>10 were included. Based on the clinical evaluation of the bleeding symptoms, bleeding severity was classified as life threatening, major, minor, and non-relevant. Blood was collected on the day of overdose and one day after. TG was measured by the Calibrated Automated Thrombogram method. In order to achieve thrombin formation in the highly anticoagulated plasma, a modified test using high concentration tissue factor was applied.
Results: Three of our patients had major and three had minor bleeding symptoms. The rest of the patients did not have any relevant bleeding. In the three patients with major bleeding symptoms, TG was considerably lower than in the other patients. We found no difference in TG parameters between the minor and non-relevant bleeding groups.
Summary/Conclusion: In patients with severe vitamin K antagonist overdose, the actual risk of bleeding is unknown. Thrombin generation can be measured in the highly anticoagulated plasma (INR>10) using a modified protocol. This way, it may provide an estimation of the individual bleeding tendency.

Bleeding

ECTH-135

Board No. 22: Identification of a novel mutation in intron 7 of the F8 gene

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Background: Over the past decades, severe hemophilia has changed from a debilitating disease to a condition with a good quality of life. Along with the progress in diagnosis and treatment, genetic studies in hemophilia have played a major role to the better understanding of its biology, the detection of carriers, and prenatal diagnosis, and even prediction of inhibitor development.
Aims: Here, we present a case of a 16 months boy affected by a severe hemophilia A (FVIII C: <0.01 IU/mL). He was diagnosed at the age of 5 months due to bruises. He is on secondary prophylaxis since his mouth bleed. There was still no evidence of inhibitors to *F8* protein.
In order to establish the carrier status of the mother and planning for her next conception, the mutation studies was carried out in our laboratory.
Methods: F8 gene rearrangements of intron 22, type 1, type 2 and intron 1 inversions were carried out by using inverse shifting PCR. Screening of all 26 exons and the flanking intronic regions were analysed by Sanger sequencing.
Results: No inversion in introns 1 and 22 was observed. Therefore, whole *F8* gene sequencing was carried out. An acceptor splice site mutation IVS7 1010-2 Ahttp://www.factorviii-db.org and http://www.hgmd.cf.ac.uk. The same mutation was observed in heterozygous state in the mother. Furthermore, we detected the same mutation in the grandmother and the grand-grandmother of this individual in heterozygous state. This proves that most probably this mutation segregates within the maternal pedigree of this family.
Summary/Conclusion: The putative role of this point mutation needs to be further investigated in the mRNA of the *F8* gene. However, it is important to identify the underlying mutations in order to better understand the pathogenesis and develop appropriate treatment strategies to effectively treat the disease.

Clotting

ECTH-290

Board No. 23: The need for vitamin K antagonists in patients with haemophilia A: nature does not protect all

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Background: It is unknown whether patients with haemophilia A (PWH) require treatment with vitamin K antagonists (VKA) to the same extent as the normal population. This is an emerging issue in PWH with atrial fibrillation.

Aims: to compare the haemostatic potential in PWH and patients on VKA using thrombin generation (TG).

Methods: 133 patients with severe (FVIII <1%, n=15) and non-severe (FVIII 1-50%, n=118) haemophilia A, 97 patients on VKA with an international normalized ratio (INR) ≥1.5 and a plasma pool of healthy controls were compared. All subjects were male adults. TG, initiated with 1pM tissue factor, was measured with a Calibrated Automated Thrombogram. Endogenous Thrombin Potential (ETP in nM*min) was compared according to FVIII level (<1%, 1-14% and 15-50%) to healthy controls, patients with subtherapeutic INR (1.5-1.9) and therapeutic INR (≥2.0). Medians and interquartile ranges (IQR) were calculated.

Results: Compared to healthy controls, both PWH and patients on VKA had a lower median ETP (898 versus 304 and 176 respectively) ($p<0.001$). ETP was quite similar in severe PWH (185 (116-307)) and patients with therapeutic INR (156 (90-225); $p=0.08$). Compared to patients with therapeutic INR, ETP in patients with FVIII 1-14% and patients with FVIII 15-50% was significantly higher at 290 (199-413) and 397 (235-615) respectively ($p<0.001$). All patients with therapeutic INR had an ETP <400. Considering this threshold, 93% of severe PWH, but only 73% of patients with FVIII 1-14% and 53% of patients with FVIII 15-50% had an ETP <400.

Summary/Conclusion: In severe PWH, haemostatic potential was comparable to that on a therapeutic INR, but in approximately one third of non-severe PWH haemostatic potential was significantly higher. These results suggest that anticoagulation therapy should be considered in a substantial proportion of patients with non-severe haemophilia and atrial fibrillation.

Bleeding
ECTH-244
Board No. 24: Time-dependent biodistribution of rIX-FP following intravenous application to rats and hemophilia B mice
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Background: IDELVION®, a recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP), has recently been approved in the US and Canada for prophylaxis and on-demand treatment of children and adults with hemophilia B.
Aims: Aim of our first study was to explore the general tissue distribution of rIX-FP in rats using a whole body autoradiography (QWBA) method. Thereafter, a follow up study was initiated, evaluating the time-dependent biodistribution of rIX-FP on protein and activity levels in selected tissues of rats and FIX-deficient mice (hemophilia B mice).
Methods: In the first study, rats were intravenously (i.v.) administered with [^{3}H]-rIX-FP, [^{3}H]-rFIX or [^{3}H]-albumin at a dose of approx. 400 µCi/kg followed by QWBA imaging up to 240 hours (h). In the subsequent study, rIX-FP was administered to rats and hemophilia B mice at an i.v. dose of 2000 IU/kg. Thereafter, plasma and selected tissues (based on QWBA data) were harvested at time points up to 72 h post dosing (p.d.), processed to tissue homogenates and quantitatively analyzed for FIX antigen (rats and mice) and activity levels (mice only) using an ELISA- or chromogen-based method, respectively.
Results: The tissue distribution of [^{3}H]-rIX-FP and [^{3}H]-rFIX (but not of [^{3}H]-albumin) was comparable in rats, both penetrating predominantly into bone and well-perfused tissues. Detailed knee-joint analyses indicated rapid presence of [^{3}H]-rIX-FP within the bone marrow and synovial or mineralized regions mostly localized to the zone of calcified cartilage within the growth plate regions of long bones. Intriguingly, [^{3}H]-rIX-FP-derived radioactive signals were detectable up to 240 h p.d., while [^{3}H]-rFIX-derived signals declined already after 1 h p.d.. Data of the follow up study in rats demonstrated that rIX-FP distributed into all tissues analyzed with peak antigen levels between 1-7 h p.d., thereby matching the QWBA data. In hemophilia B mice, rIX-FP tissue distribution was comparable to rats. Furthermore, FIX antigen levels correlated with FIX activity readouts.
Summary/Conclusion: Our data show prolonged retention of rIX-FP within relevant target tissues following i.v. administration compared to rFIX. Importantly, it was demonstrated that rIX-FP available in tissues retains its functional activity and can thus facilitate its therapeutic activity at the site of potential injury.

Bleeding

ECTH-143

Board No. 25: Guardian™5: a multi-centre non-interventional study of safety and efficacy of turoctocog alfa during long-term treatment of severe and moderately severe haemophilia A (FVIII ≤2%)

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Background: Turoctocog alfa, a B-domain truncated recombinant coagulation factor VIII (rFVIII) product developed by Novo Nordisk for the prevention and treatment of bleeding in people with haemophilia A, is being assessed in an ongoing multinational clinical trial programme, guardian™. This trial programme has been designed to investigate the pharmacokinetics, safety and effectiveness of turoctocog alfa. guardian™5 (NCT02035384) is a non-interventional, prospective, post-authorisation safety study (PASS), designed in accordance with the European Medicines Agency guidelines to ensure consistency between outcomes from pre-authorisation clinical trials and routine clinical use.
Aims: This study was designed to provide real-world evidence of the safety and effectiveness of turoctocog alfa in the treatment of patients with congenital severe to moderately severe haemophilia A (FVIII ≤2%).
Methods: guardian™5 is recruiting approximately 80 patients for baseline screening from countries within Europe and North America (approximately 70 patients will be included in the study). Currently 13 countries are contributing to the study: Austria, Belgium, France, Germany, Greece, Hungary, Italy, the Netherlands, Slovenia, Spain, Sweden, Switzerland and USA. Male previously treated patients (>150 exposure days [ED]) with congenital severe or moderately severe haemophilia A (FVIII ≤2%) are being enrolled. The age distribution of the male patients was well balanced and they were eligible for inclusion if they had a negative FVIII inhibitor test not more than four weeks prior to first dosing with turoctocog alfa. Patients with a history of inhibitors may participate if they have been successfully immune-tolerised. Patients receive turoctocog alfa on-demand or as prophylaxis and are evaluated until ≥50 patients have reached ≥100 ED, which is expected to take approximately 4 years. The primary outcome measure is the incidence rate of FVIII inhibitors (≥0.6 Bethesda Units/ml). Secondary outcome measures include adverse reactions, haemostatic effect during treatment of bleeds and during surgery. The annualised bleeding rate is also evaluated for both preventative and on-demand treatment. Patient recruitment for this study began in June 2014 and is ongoing.
Results: Ongoing.
Summary/Conclusion: guardian™5 will provide information on real-world, long-term safety and effectiveness of turoctocog alfa when used to treat patients with congenital severe and moderately severe haemophilia A in routine clinical practice.

Bleeding

ECTH-217

Board No. 26: The efficacy and safety of recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in previously treated children with hemophilia B: results of a Phase 3 pivotal clinical trial

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Background: IDELVION®, a recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP), has recently been approved in the US and Canada for prophylaxis and on-demand treatment of children and adults with hemophilia B. IDELVION® has an improved pharmacokinetic profile, which allows dosing every 7–14 days.

Aims: A global study evaluated the long-term safety and efficacy of rIX-FP for both prophylaxis and on-demand treatment of bleeding episodes in children with moderate or severe hemophilia B (FIX activity ≤2%).

Methods: Previously treated male hemophilia B patients (<12 years) received weekly prophylaxis with 35–50 IU/kg rIX-FP; dose could be adjusted as required while maintaining weekly prophylaxis. Bleeding events were treated on demand with rIX-FP. The PK of both rIX-FP and previous FIX product were assessed at the beginning of the study. Primary endpoints of the study were PK of rIX-FP and the number of subjects developing inhibitors against FIX. Annualized spontaneous bleeding rates (AsBR) were calculated. Treatment efficacy was evaluated by number of injections to achieve hemostasis and investigator assessment. Written informed consent was obtained from the subject's legal guardian; subjects gave informed assent.

Results: A total of 27 subjects enrolled and received prophylactic treatment with rIX-FP for a mean of 62 weeks. The PK profile of rIX-FP was improved in comparison with standard FIX products, with a >5-fold longer half-life (91.4 vs 18.6 hours); half-life of rIX-FP was similar between younger (<6 years [n=12]) and older (6–11 years [n=15]) age groups. Clearance was 1.11 ml/hr/kg, 6.4-fold slower than previous FIX treatment. Following a single intravenous dose of 50 IU/kg rIX-FP mean FIX activity levels in all patients remained above 5 IU/dl through day 10 and above 2 IU/dl through day 14, supporting a prophylaxis treatment interval of 7 to 14 days. While on weekly prophylaxis patients maintained a median trough FIX activity level of 13.4 IU/dl.

Median (Q1, Q3) AsBR was 0.00 (0.00, 0.91), and was similar between younger and older age groups. Mean weekly consumption of rIX-FP was more than 50% lower than with prior FIX treatment (47 vs 107 IU/kg). Of 106 bleeds treated during the study, 97% were treated with 1 or 2 injections of rIX-FP (95% CI: 92% to 99%), and 96% of treatments were rated effective (excellent or good) by the investigator. All 27 subjects (100%) were compliant with their weekly prophylaxis treatment schedule.

No subjects developed inhibitors to FIX or antibodies to rIX-FP or CHO proteins. There were no related adverse events.

Twenty-four subjects continued their prophylaxis regimen in the ongoing extension study. Of these, 63% have switched to a prophylaxis treatment interval of 10 or 14 days.

Summary/Conclusion: This phase 3 study demonstrated the favorable PK and safety profile of rIX-FP in children. rIX-FP was also efficacious both as weekly prophylaxis and for treatment of bleeding episodes. This trial also demonstrated high compliance with weekly prophylaxis. PK results support a prophylaxis interval of 14 days; this is being investigated further in the extension study.

Bleeding

ECTH-213

Board No. 27: Results of a Phase III pharmacokinetics, efficacy and safety study in children less than 12 years of age with severe haemophilia A treated with rVIII-singlechain

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Background: rVIII-SingleChain, a novel B-domain truncated recombinant Factor VIII (rFVIII) comprised of covalently bonded FVIII heavy and light chains, was designed to have a higher binding affinity to von Willebrand Factor (VWF).

Aims: This Phase III study investigated the safety, efficacy and pharmacokinetics (PK) of rVIII-SingleChain in previously treated pediatric patients <12 years of age with severe hemophilia A.

Methods: Participants were <12 years of age, with severe hemophilia A (endogenous FVIII <1%) and more than 50 previous exposure days (EDs) to FVIII products who received either on-demand or prophylactic infusions of rVIII-SingleChain.

Results: Of the 88 patients screened, 84 met study eligibility criteria (0 to <6 years, N=35; ≥6 to <12 years, N=49). PK was evaluated in 39 patients (0 to <6 years, n=20; 6 to <12 years, n=19); FVIII activity profiles and PK parameters were similar between age groups. Mean PK parameters for the younger and older groups, respectively, were AUC_{inf}: 1080 and 1170 IU*h/dL, clearance: 5.07 and 4.63 mL/h/kg, and half-life: 10.4 and 10.2 h. 81 patients were assigned to prophylaxis and 3 to an on-demand regimen. The predominant prophylaxis regimens were twice weekly (n=43 [53%], median starting dose 35 IU/mL) and three times weekly (n=24 [30%], median starting dose 32 IU/mL). Hemostatic efficacy was rated excellent or good in 96.3% of the 347 treated bleeds evaluated by the investigator. The median annualized spontaneous bleeding rate (AsBR) was 0.00 (Q1, Q3: 0.00, 2.20), and the median annualized bleeding rate (ABR) was 3.69 (Q1, Q3: 0.00, 7.20) across all prophylaxis regimens. The total cumulative exposure during the study was 5239 ED, with 65 participants reaching >50 ED (0 to <6 years, N=27; ≥6 to <12 years, N=38). No participant developed an inhibitor in this study.

Summary/Conclusion: rVIII-SingleChain is a novel rFVIII molecule, showing excellent hemostatic efficacy and a positive safety profile in a clinical study in children <12 years of age with severe hemophilia A.

Bleeding

ECTH-177

Board No. 28: Pharmacokinetic-guided daily dosing: a significant reduction in weekly clotting factor VIII consumption

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Background: As early as 1997, Carlsson et al. showed that pharmacokinetic (PK)-guided dosing of factor VIII (FVIII) is a promising strategy to individualize prophylactic dosing and to concomitantly reduce FVIII concentrate consumption without an increase in bleeding events. As factor concentrate constitutes more than 90% of costs of care in hemophilia, PK-guided dosing may be an important intervention to implement in selected patients.

Aims: Our objective was to simulate potential effects of PK-guided dosing on FVIII consumption in different prophylactic regimens.

Methods: Twenty-one (moderate) severe hemophilia A patients on prophylactic treatment were included (baseline FVIII:C: twenty patients < 0.01 IU mL^{-1} and one 0.03 IU mL^{-1}). All patients underwent individual PK profiling with a FVIII bolus infusion of 50 IU kg^{-1} followed by three FVIII:C measurements at 4, 24 and 48 hours (Bjorkman et al., 2010). Subsequently, a prophylactic FVIII dosing regimen was calculated with 24, 48 and 72 hours infusion intervals, aiming for FVIII:C trough levels >0.01 IU mL^{-1} without taking vial size into account. Analyses were performed by Bayesian analysis using NONMEM® software and based on the FVIII (Advate®) population PK model (Bjorkman et al. 2012). The study was approved by the Medical Ethics Committee and informed consent was obtained according to the Declaration of Helsinki.

Results: Patients had a median age of 37.0 years, [IQR 42.5 years] and median body weight of 74.0 kg, [IQR 31.8 kg]. Calculated median prophylactic FVIII dosages were hypothetically: 834 IU/week [IQR 673 IU] (in case of dosing every 24 hours), 2851 IU/week [IQR 2646 IU] (every 48 hours) and 8989 IU/week [IQR 13356 IU] (every 72 hours). Total weekly dosages varied significantly between dosing intervals ($H(2) = 33.69$, $p < 0.001$). In addition, the median weekly dosages increased with longer dosing intervals ($J = 1156$, $z = 6.24$, $r = 0.79$). Based on these results, prophylactic dosing every 48 hours could hypothetically save 68% of weekly factor concentrate costs when compared to dosing every 72 hours. Moreover, costs of factor concentrate could hypothetically be reduced with 91% with dosing every 24 hours.

Summary/Conclusion: Simulations show that more frequent prophylactic dosing may strongly reduce FVIII concentrate consumption and therefore costs of treatment. However, when considering more frequent dosing, evaluation of bleeding events and quality of life measures under novel dosing regimens is essential. Therefore, prospective studies in a large group of patients are needed to prove its feasibility.

Bleeding

ECTH-384

Board No. 29: Long-term safety and efficacy of recombinant factor VIII Fc (rFVIIIFc) for the treatment of severe haemophilia A: European subgroup interim analysis of the Aspire study

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Background: The ongoing rFVIIIFc extension study, ASPIRE (#NCT01454739), evaluates the long-term safety and efficacy of rFVIIIFc in adults, adolescents, and children with severe haemophilia A.

Aims: To report interim outcomes for European (EU; n=67) subjects in ASPIRE (n=214).

Methods: Upon completing A-LONG or Kids A-LONG, eligible subjects could enrol in one of 4 treatment groups in ASPIRE: individualized prophylaxis (IP)(25-65 IU/kg every 3-5 days, or 20-65 IU/kg on D1 and 40-65 IU/kg on D4 if twice weekly); weekly prophylaxis (WP)(65 IU/kg every 7 days); modified prophylaxis (MP)(to further personalize and optimize treatment when needed); or episodic treatment (ET). Subjects could change treatment groups at any time. Subjects <12 yrs participated only in individualized and modified prophylaxis groups. Primary endpoint: development of inhibitors. Secondary outcomes included annualized bleeding rate (ABR) and rFVIIIFc exposure days (EDs).

Results: Sixty-seven subjects (36 from A-LONG; 31 from Kids A-LONG) enrolled from UK, Ireland, Austria, Italy, Germany, Poland, France, Spain, Belgium, Sweden, Switzerland and The Netherlands. As of the interim data cut (8 Dec 2014), the median time on ASPIRE was 129 (A-LONG) and 70 (Kids A-LONG) wks; 97.2% (A-LONG) and 96.8% (Kids A-LONG) of subjects had ≥100 cumulative rFVIIIFc EDs. Majority of A-LONG subjects didn't change treatment groups upon enrolment into or during ASPIRE; all Kids A-LONG subjects stayed on individualized prophylaxis in ASPIRE. No inhibitors were observed; adverse events were typical of the general adult and paediatric haemophilia A populations. The majority of patients from A-LONG (n=29) were in IP and had a low median ABR (IQR) of 0.81 (0.00, 2.48). ABRs (IQR) in remaining subjects were WP 2.49 (0.67, 3.50) [n=8]; MP 3.30 (0.00, 6.59) [n=2]; ET 21.41 (12.37, 30.46) [n=2]; Kids A-LONG; IP 1.95 (0.00, 3.57) [n=31]. Overall, most subjects treated prophylactically during the parent study did not experience changes to their total weekly prophylactic dose or dosing interval during ASPIRE. For subjects who enrolled from A-LONG and Kids A-LONG, 80.3% and 73.3% of all bleeding episodes during ASPIRE, respectively, were controlled with one injection.

Summary/Conclusion: Interim data from EU subjects in ASPIRE are consistent with those of the phase 3 parent studies and the overall ASPIRE interim analysis. The individualized prophylaxis arm included the majority of EU subjects (80% of adults and 100% of kids). Results from ASPIRE confirm the long-term safety of rFVIIIFc and the maintenance of a low ABR with extended interval prophylactic dosing in individuals with severe haemophilia A.

Bleeding

ECTH-307

Board No. 30: A multicentre international study on perioperative treatment with replacement therapy in hemophilia B: should we B more precise? (Opti-clot studies)

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Background: Perioperative management with clotting factor IX (FIX) concentrate in hemophilia B is challenging. Both underdosing and overdosing, should be avoided as they are associated with a risk of bleeding and unnecessary costs, respectively.

Aims: To identify the extent and possible predictors of underdosing and overdosing of perioperative replacement therapy in moderate/severe hemophilia B patients.

Methods: Hemophilia B patients (FIX <0.05 $IUml^{-1}$) undergoing elective, minor or major surgical procedures between 2000-2015 in ten Academic Hemophilia Treatment Centers in the Netherlands and United Kingdom were included. Perioperative infusion of FIX concentrate and achieved (steady state and trough) FIX plasma concentrations were collected and compared to target factor levels according to National guidelines. Predictors of underdosing <24 hours after surgery and (excessive) overdosing were analyzed by logistic regression analysis. Excessive overdosing was defined as FIX target level plus median deviation of >0.20 $IUml^{-1}$. Bleeding complications were defined according to International Society of Thrombosis and Hemostasis guidelines. Bleeding complications requiring re-operation or red blood cell transfusion were defined as clinically relevant. The study was approved by local Medical Ethics Committees; in the United Kingdom, an opt-out informed consent procedure was used; in the Netherlands, the study was not subject to the Medical Research Involving Human Subjects Act, as patient data were analysed anonymously; one center requiring prior patient informed consent.

Results: Two hundred fifty nine surgical procedures in 117 patients were included (median age 41 years, median body weight 79 kg). Surgical procedures were mainly orthopedic and (re)placement of central intravenous catheters. Depending on postoperative day, 9- 60% of achieved FIX plasma concentrations were under and 18-59% were above predefined FIX target ranges. In addition, 60% of FIX plasma concentrations <24 hours after surgery were under target level with a median deviation of 0.22 $IUml^{-1}$ [IQR 0.12-0.36]; while 59% of FIX plasma concentrations were above target >six days after surgery with a median deviation of 0.19$IUml^{-1}$ [IQR 0.10-0.39]. Fifty bleeding complications were observed (19%); however only seven were clinically relevant (2.7%). Bleeding complications were not associated with lower FIX plasma concentrations. Replacement therapy by bolus as opposed to continuous infusion was predictive of underdosing <24 hours after surgery (OR=6.1 95%CI 2.8-13.4). In the total perioperative period, both treatment by bolus infusion and a minor surgical procedure were predictive of underdosing (respectively OR=5.4 95%CI 3.5-8.3, OR=2.0 95%CI 1.2-3.2). Patients treated by bolus infusion were at lesser risk of overdosing (OR=0.3 95%CI 0.2-0.5). Children received higher amounts of FIX concentrate in comparison to adults when corrected for body weight and duration of hospitalization (p<0.001). Mode of infusion, type of product (plasma derived or recombinant) and severity of surgical procedure did not lead to differences in FIX concentrate consumption.

Summary/Conclusion: Despite well-defined dosing guidelines, targeting of FIX plasma concentrations in the perioperative setting is clearly complex and not optimal. Quality of care and potentially cost-

effectiveness of treatment can be significantly improved by alternative dosing strategies based on a wide variety of individual factors such as patient-, surgical- and treatment characteristics.

Bleeding

ECTH-187

Board No. 31: Arthropathy in patients with moderate and severe von Willebrand disease

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Background: The 'Willebrand in the Netherlands' study group has previously published that almost a quarter of patients with moderate and severe Von Willebrand disease (VWD) reported joint bleeds. Those bleeds had a negative impact on health related quality of life and joint integrity, according to patient-reported and retrospective medical file data. However, besides that, little information is available for VWD patients on the prevalence and severity of arthropathy and its influence on joint function and daily life activities.

Aims: To assess the prevalence and severity of arthropathy and its impact on joint function and daily life in moderate and severe VWD patients (trial number NTR 4548).

Methods: Dutch patients with moderate and severe VWD (VWF activity<30IU/dLand <10IU/dL, respectively) and documented treatment for at least one joint bleed were invited to participate. The same number of controls (moderate and severe VWD patients without joint bleed treatment) were selected and matched for age (±2 years), gender and FVIII level (±10%). A single experienced physiotherapist conducted the Haemophilia Joint Health Score (HJHS, 0-124). X-rays were made from all joints with prior bleeds, contralateral joints and one control joint. One radiologist scored the X-rays according to Pettersson (PS, 0-13 per joint). Arthropathy was defined as a clinical HJHS score >3 or PS>0.
All participants completed the Haemophilia Activity List (HAL, 0-100) questionnaire. The Visual Analogue Score (VAS, 0-10cm) was used to assess joint pain.

Results: 48 patients and 48 controls were included, 60% males, mean age 46 years (range 18-80). Mean FVIII levels were 26 IU/dLin the patients and 31IU/dL in the controls(p=0.19). More patients had type 3 VWD (19/48 vs. 3/48 controls). In the control group of patients without documentation on joint bleed treatment, 14/48 patients did report one or more joint bleeds but none of them more than five. In contrast, 56% of the 48 patients had more than five joint bleeds. Arthropathy occurred in 37/48 (77%) patients and 35/48 (73%) controls (p=0.51). Overall, arthropathy occurred in both severe (47/64) and moderate VWD (25/32) and in all three VWD types (22/28 type 1; 30/46 type 2; 20/22 type 3). The median HJHS was significantly higher in the patients compared to the controls (5 vs. 1.5, p<0.01, maximum score 47 vs. 29). PS>3 occurred in 2 controls compared to 12 patients (p<0.01) and overall most in type 3 VWD patients (9/22 type 3 vs. 3/46 type 2 vs. 2/28 type 1). The total HAL score as well as the scores on the three separate HAL components were significantly lower for the 48 patients compared to the controls (median scores HAL Sum: 88 vs. 100, p<0.01; Upper Extremity Activities: 93 vs. 100, p=0.01, Basic Lower Extremity Activities: 87 vs. 100, p<0.01; Complex Lower Extremity Activities: 80 vs. 100, p<0.01). Clinically relevant joint pain (mean VAS score >3) was reported by 17 patients and 9 controls (p=0.07).

Summary/Conclusion: Arthropathy, according to our stringent definition, was seen in 77% of patients with moderate and severe VWD treated for joint bleeds. Notably, arthropathy was also found in 73% of matched control VWD patients without joint bleed treatment. Joint function and –integrity in the VWD patients treated for joint bleeds was affected, consistent with higher HJHS and joint X-rays scores. These patients experienced a significant impact on daily life activities (HAL, both upper and lower extremities) and 35% also reported clinically relevant joint pain (VAS).

Bleeding

ECTH-319

Board No. 32: Endothelial injury markers are associated with an increased risk of major bleeding in patients treated with vitamin K antagonists: a case-cohort study

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Background: Major bleeding is a common and severe side effect of anticoagulant treatment that occurs in 1 to 3 per 100 patients on anticoagulant treatment per year. Existing prediction scores for major bleeds in anticoagulated patients perform moderately, necessitating the need for better predictors for major bleeding. Here we delineated the role of the endothelium on the risk of major bleeding in patients using vitamin K antagonists (VKAs).

Aims: To assess whether soluble thrombomodulin (sTM) and von Willebrand propeptide (VWFpp), both markers of endothelial damage, are associated with an increased risk for major bleeding in VKA patients.

Methods: A cohort study of 16706 eligible (and 16570 enrolled) patients starting VKA treatment between January 2012 and December 2014 was formed, and plasma (leftovers following INR analyses) was collected three weeks after the initiation of VKA therapy. Patients were followed until a major bleed, the end of VKA treatment, death, or December 31st 2014, whichever came first. From the cohort, we assembled a case-cohort study that included all 326 cases with a major bleeding and a random sample of 652 patients at baseline (subcohort). Plasma sTM and VWFpp levels were measured by ELISA and stratified by the 25th, 50th, 75th and 90th percentiles. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by means of weighted Cox regression and adjusted for age, sex, diabetes mellitus and hypertension.

Results: Plasma was available from 263 cases and 578 controls. Cases were on average 76 years old (standard deviation 11 years), 56% was male, and the indication atrial fibrillation occurred most frequently (200 patients, 76%). The average age of the subcohort was 70 years (standard deviation 13 years), 55% was male, and the indication atrial fibrillation was most frequent (392 patients, 68%). Adjusted HRs increased dose dependently with increasing sTM levels, from 1.28 (95%CI 0.72-1.74) in the 25th to 50th percentile to 2.07 (95%CI 1.19-3.59) above the 90th percentile as compared with under the 25th percentile. The hazard ratios of VWFpp levels to major bleeding risk were 1.07 (95%CI 0.69-1.72) in the 25th to 50th percentile and 1.24 (95%CI 0.69-2.25) above the 90th percentile as compared with under the 25th percentile. Patients with sTM and vWFpp levels above the 50th percentile were at increased risk for major bleeding as compared with patients with both measures below the 25th percentile (HR 1.33 to 3.50).

Summary/Conclusion: Increased sTM and VWFpp levels are associated with major bleeding during VKA treatment, suggesting a role for endothelial injury markers as predictors of major bleeding.

Bleeding

ECTH-405

Board No. 33: Oral bleeding symptoms and outcome of prophylactic treatment in children with moderate and severe von Willebrand disease – from the WIN study

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Background: Oral cavity bleeding and bleeding after dental extraction are common in children with von Willebrand disease (VWD). However, limited information is available on the burden of oral cavity bleeding in daily life and the use and efficacy of prophylactic treatment.

Aims: To assess the burden and treatment of oral cavity bleeding and the outcome of prophylaxis given for dental extractions in a paediatric cohort of patients with moderate or severe VWD.

Methods: We analysed 113 children aged 0-16 at the start of the "Willebrand in the Netherlands"(WiN) study with von Willebrand factor (VWF) antigen (VWF:Ag)and/or activity (VWF:Act) ≤30 IU/dL (type 1 VWD n=60; type 2 VWD n=44; type 3 VWD n=9). The ISTH Bleeding Assessment Tool (ISTH-BAT) was administered to assess oral cavity bleeding and dental extractions, including outcome of prophylactic treatment. Analyses of association were performed with Spearman's rho or logistic regression analysis. Medical Ethics Committee approval and informed consent were obtained.

Results: Sixty-four percent (72/113) of patients reported previous oral cavity bleeding, of which lip, cheek or tongue bites (72%), teeth eruption or exfoliation (50%) and teeth brushing (22%) were the most common causes. Oral bleeding occurred less than once per year in 68% of type 1 patients, but 11% of type 2 and 22% of type 3 patients reported at least weekly oral bleeding. Frequency of oral bleeding correlated with VWF:Ag, VWF:Act, and coagulant factor VIII activity (FVIII:C), and most strongly with collagen binding (VWF:CB; Spearman's rho -0.493, $p<0.001$). In 46% of patients oral cavity bleeding usually stopped spontaneously. However, 27% of patients usually required treatment with tranexamic acid and 9% usually needed desmopressin or clotting factor concentrate (CFC).

Of 113 patients, 109 were old enough to have had primary teeth exfoliation. Although bleeding after exfoliation was reported by 53% of type 1, 69% of type 2, and 78% of type 3 VWD patients (p=NS), prophylaxis (usually tranexamic acid) was administered in only 12% of patients.

Forty children had a total of 65 dental extractions. Bleeding was reported after 24 (37%) extractions with a similar bleeding prevalence in all VWD types. Prophylaxis was administered in 79% of extractions and usually consisted of tranexamic acid (59%) or desmopressin/CFC (41%). Overall, prophylactic treatment was associated with a lower bleeding risk: odds ratio (OR) 0.23, 95% confidence interval (CI) 0.07-0.81. Considering only the first dental extractions, prophylaxis was also associated with decreased bleeding risk (OR: 0.18, 95% CI: 0.04-0.88). Tranexamic acid (with or without additional prophylaxis) was associated with a decreased bleeding risk (OR: 0.32, 95% CI: 0.11-0.94). Prophylaxis with desmopressin or CFC was associated with a non-significant lower bleeding risk (OR: 0.58, 95% CI: 0.19-1.78).

Summary/Conclusion: Oral cavity bleeding is common in children with VWD but is usually self-limiting. Although bleeding after dental exfoliation is very common in children with VWD, prophylaxis is rarely given. Prophylaxis is administered in the majority of dental extractions and is associated with a lower bleeding risk. Tranexamic acid is still underused, and future studies are needed to optimise the use of desmopressin and CFC.

Bleeding

ECTH-462

Board No. 34: Intracranial bleeding as an initial manifestation of haemophilia : long term follow up of inhibitor development

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Background: Bleeding in the central nervous system(CNS) causes significant morbidity and mortality in patients with hemophilia. Moreover other life threatening bleeding events (bleeding within internal organs such as liver, spleen, renal, retroperitoneal, bleeding in a large muscle group such as iliopsoas and bleeding within vital sutructures such as head , neck or intrathoracic region and gastrointestinal bleeding) can ocur as an initial manifestation of hemophilia which later contribute a high risk of inhibitor development.
Aims: Our aim was to retrospectively evaluate hemophiliac patients in respect to initial manifetation and inhibitor development.
Methods: Our database revealed 80 hemophilia A(43 severe hemophilia A, 21 moderate and 16 mild hemophilia A and 15 hemophilia B (6 severe, 9 mild& moderate) patients diagnosed at Hacettepe University Pediatric Hematology Department since 2003.
Results: Life treatening bleeding was occur in 5 / 43(11%)of hemophilia A patients and in 4/6 of hemophilia B patients. Cumulative insidans of inhibitor development in patients with severe hemophilia A was found to be 10/43(23%) and 2 out of 6 patients with hemophilia B. Furthermore 4 out of 5 patients with an initial manifestation of intracranial bleeding were all developed inhibitor antibodies within a median follow up time,1 died because of CNS hemorrage. Due to small number of hemophilia B patients it seems that CNS hemorrage was more common 4/6 in this group however interestingly none of the 4 alive patients developed inhibitor.
Summary/Conclusion: It was previosly shown that high dose intensive factor VIII treatment increases the inhibitor development in patients with severe Hemophilia A on which our data support this finding.

Bleeding

ECTH-433

Board No. 35: Bleeding symptoms and patterns in recessively inherited coagulation disorders patients versus haemophiliacs

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Background: Hemophilia A and B are the most frequent inherited bleeding disorders. Together with von Willebrand disease, a defect of primary hemostasis associated with a secondary defect in coagulation factor VIII (FVIII), these X-linked disorders include 95% to 97% of all the inherited deficiencies of coagulation factors Deficiencies of coagulation factors other than factor VIII and factor IX that cause bleeding disorders are inherited as autosomal recessive traits and are rare, with prevalences in the general population varying between 1 in 500 000 and 1 in 2 million for the homozygous forms. Bleeding pattern may considerably vary between affected individuals of rare bleeding disorder s i.e. from deficiency to deficiency and from patient to patient.As a consequence of the rarity of these deficiencies, the type and severity of bleeding symptoms, the underlying molecular defects, and the actual management of bleeding episodes are not as well established as for hemophilia A and B.

Aims: To compare the bleeding symptoms in patients with recessively inherited coagulation disorders (RICDs) and haemophiliacs

Methods: A cross sectional study was conducted in National Institute of Blood Disease (NIBD) and data from 49 patients with bleeding disorders (Factor I, V, VII, VIII, IX, XIII, vitamin K deficiency and von Willebrand disease) were retrospectively included. Information regarding bleeding symptoms like epistaxis, cord bleeding, hemarthrosis, hematuria, oral cavity bleed, IC bleed and GI bleed were collected. Data was entered and analyzed in SPSS version 21. Chi-square test was applied to compare the bleeding symptoms in RICDs and hemophiliacs

Results: Out of 49 patients, 34/49 (69.4%) were haemophiliacs while 15/49 (30.6%) were RICDs. Mean number of visits in RICDs patients were 5.38 ±6.23 while mean number of visits in haemophiliacs were 14.81 ±18.49 (p-value 0.081). Cord bleeding was found significantly higher 2/15 (13%) in RICDs (all factor XIII) as compared to haemophiliacs 0/34 (0%) (p-value 0.030). Epistaxis was found significantly higher 9/15 (60%) in RICDs as compared to haemophiliacs 7/34 (21%) (p-value 0.007). Hemarthrosis was found significantly higher 12/34 (35%) in haemophiliacs as compared to RICDs 1/15 (7%) (p-value 0.036) whereas insignificant difference was observed for hematuria (p-value 0.162), oral cavity bleed (p-value 0.479), IC bleed (p-value 0.544) and GI bleed (p-value 0.094). No symptoms were higher (29.4%) in haemophiliacs as compared to RICDs (13.3%) (p-value 0.228).

Summary/Conclusion: Bleeding symptoms that endanger life or cause long-term handicaps appear to be less frequent in patients with RICDs as a whole than in haemophiliacs

Bleeding

ECTH-145

Board No. 36: The effect of tranexamic acid on blood loss and maternal outcome in the treatment of persistent postpartum haemorrhage: a nationwide cohort study

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Background: Although evidence for improved maternal outcome is limited, tranexamic acid (TXA), an antifibrinolytic agent, is often used in the treatment of major obstetric haemorrhage.

Aims: The aim of this study was to quantify, among women suffering from persistent severe postpartum haemorrhage, the association between early administration of TXA compared to late or no administration of TXA, and the incidence of severe acute maternal morbidity and mortality in these groups.

Methods: We performed a cohort study among consecutive women with persistent obstetric haemorrhage who had received at least four units of donor red blood cells or fresh frozen plasma and/or platelets in addition to red blood cells between 1 January 2011 and 1 January 2013 in 65 hospitals in the Netherlands. The primary outcome was a combined endpoint of maternal mortality and severe acute maternal morbidity (arterial embolization, admission into an intensive care unit and emergency peripartum hysterectomy).

Results: The early TXA group contained 251 women, versus 1009 women in the no/late TXA group**.** After adjustment for confounding the odds ratio for reaching the composite endpoint of morbidity and mortality for early TXA versus no- or late TXA was 0,92 (95%CI 0.66 to 1.27). Despite the fact that women in the early TXA group had bled more severely at baseline (1300mL vs 800mL), their additional blood loss after administration of first line therapy was smaller: adjusted difference minus 177 mL (95%CI -509.4 to +155.0). In both groups, the total number of units of blood products transfused was 6, adjusted difference -0.6 (95%CI -1.7 to +0.5).

Summary/Conclusion: Our results show a statistically non- significant reduction of additional blood loss of 177 mL after initial therapy in women with persistent obstetric haemorrhage who were treated with early TXA. Early treatment with TXA during postpartum haemorrhage did not demonstrate a significant favourable effect on the combined outcome of severe acute maternal morbidity and mortality.

Clotting
ECTH-266
Board No. 37: Synergistic enhancement of APC association to phospholipids by protein S and activated factor V
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Background: Activated factor V (FVa), a cofactor inthe prothrombinase complex,can beproteolytically cleaved/inactivated by activated protein C (APC).The inactivation of FVa depends upon APC binding to phospholipid surfaces and is greatly enhanced by its cofactor,protein S.Residues Gla36 and Asp95 within the protein S Gla and EGF1 domains, respectively, have previously been shown to be essential for the enhancement of APC. The molecular mechanism by which protein S enhances APC-mediated cleavage of FVa hasnot yet been fully established, butit has been hypothesised that protein S enhances the binding of APC to negatively charged phospholipids.Results from functional assays have suggested thatFVa,together with APC and protein S, form tri-molecular complex on the phospholipid surface. However, the actual formation of this complex, and the relative roles of FVa and protein S in complex assembly with APC havenot yet been investigated.
Aims: To investigatethe mechanism underlying APC-mediated FVa inactivation by studyingthe assembly of the tri-molecular complex formed between APC, protein S and FVaon phospholipid surfaces. Furthermore,to evaluate the role of protein S residuesessential for APC anticoagulant function in the formation of the complex.
Methods: Association ofactive-site-labelled (FITC)APC to phospholipid coatedbeads in the presence and absence of protein S and/or FVawas evaluated using flow cytometry.The APC cofactor function by protein S was evaluated using plasma based thrombin generation assays and pure component FVa inactivation assays.
Results: The association of 50nM APC to phospholipids by itself was modest, consistent with its low affinity for these surfaces. Protein S (100nM) enhanced the binding of APC to phospholipids by~2-fold, suggesting that protein S does not appreciably augment APC binding to these surfaces by itself.WhileFVa(25nM) alone did not increase associationofAPC to phospholipids,in combinationwith protein S,it enhanced APC-phospholipid binding by ~6-fold.The synergistic enhancement by FVa and protein S on APC binding to the phospholipid membrane confirms the formation of a tri-molecular APC/protein S/FVa complex. Anti-protein S and anti-FV antibodies specifically inhibited the enhancementof APC binding induced by protein S and FVa. As previously described, protein S E36A and D95A showed little enhancement of APC in functionalthrombin generation and FVa inactivation assays. Interestingly, the enhancement of APC association to phospholipids by the variants was severely reduced both in the presence and absence of FVacompared to wild-type protein S. This suggests that protein S Gla36 and Asp95 are essential for formation of the APC/protein S/FVa tri-molecular complex on phospholipid membranes. Analysis of a direct role of these residues in the complex formation is currently underway.
Summary/Conclusion: FVa, together with protein S, synergistically enhance the association of APC to phospholipid membranes, confirming theformation of an APC/protein S/FVatri-molecular complex.This complex only appears to form efficiently when enzyme, cofactor and substrate are present. Protein S residues Gla36 and Asp95 are essential for the formation of this complex, explaining their importance for the APC cofactor function in functional assays.

Clotting

ECTH-314

Board No. 38: Hepatic tissue factor-mediated intrahepatic fibrin deposition drives liver regeneration after partial hepatectomy in mice

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Background: The potential of the liver to regenerate is unique and requires a complex interplay of multiple mediators and pathways. Experimental and clinical evidence suggests a central role for platelets and platelet-derived factors in liver regeneration. Platelets accumulate in the liver remnant following partial liver resection in mice, and liver regeneration is significantly delayed when platelets are depleted or functionally impaired. In previous studies we noted that rapid intrahepatic fibrin(ogen) deposition accompanies platelet accumulation in the liver remnant after partial hepatectomy (PHx), suggesting activation of the coagulation cascade is paramount to platelet accumulation and function after PHx. We hypothesized that fibrin deposition was initiated by hepatic tissue factor, which has been previously shown in models of chronic and acute liver injury.

Aims: To assess the role of fibrin(ogen) deposition and platelet-coagulation cross-talk in liver regeneration.

Methods: Liver regeneration was induced by performing a 2/3 PHx in wild-type (WT) mice, liver tissue factor deficient (liver $TF^{-/-}$) mice, $TF^{flox/flox}$ mice (used as control mice for liver $TF^{-/-}$ mice), protease activated receptor-4 deficient ($PAR\text{-}4^{-/-}$) mice and fibrinogen-depleted mice. The sham surgery involved the gentle manipulation of the liver lobes without the removal of liver tissue. To deplete fibrinogen, WT mice received a subcutaneous injection of 1.5U of ancrod two hours prior to resection. Mice were subsequently treated with 1.5U of ancrod twice daily to deplete newly synthesized fibrinogen. Mice were sacrificed on day 3 after PHx and livers were collected. Liver sections were stained for Ki-67 to assess hepatocyte proliferation, and stained for platelets and fibrinogen to assess platelet and fibrin deposition.

Results: Liver $TF^{-/-}$ mice had impaired hepatocyte proliferation compared to $TF^{flox/flox}$ mice as the percentage of Ki-67 positive nuclei was significantly reduced (28.4%±7.0% versus 40.1%±11.7% p=0.03). Hepatocyte proliferation was comparable between WT mice and $PAR\text{-}4^{-/-}$ mice (39.8%±8.5% versus 47.1%±11.9%, p=0.21). Hepatocyte proliferation was also impaired in fibrinogen-depleted mice compared to control mice (30.0%±11.0% versus 60.1%±1.1%, p=0.0003). There were no differences in proliferation between sham operated animals. There was a reduction in hepatic fibrin deposition in fibrinogen-depleted mice and liver TF-deficient mice compared to the control groups, but no difference was found between controls and $PAR\text{-}4^{-/-}$ mice. Platelet deposition was similar between the groups.

Summary/Conclusion: The results suggest that activation of liver TF results in intrahepatic activation of coagulation and intrahepatic deposition of fibrin(ogen). Generation of fibrin rather than PAR-4-mediated platelet activation by thrombin drives coagulation-mediated stimulation of liver regeneration. Platelet and coagulation-mediated stimulation of liver regeneration appear to be separate processes.

Clotting
ECTH-360
Board No. 39: Inhibition of coagulation factor Xa attenuates myocardial ischaemia reperfusion injury in mice
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Background: Myocardial infarction accounts for over 10% of all mortalities and is thereby one of the leading causes of death worldwide. Ischemic/reperfusion (I/R) injury is known to have a substantial effect on the outcome of a myocardial infarct. Several processes are involved in I/R injury including the complex interaction between coagulation and pathways of inflammation and apoptosis. Although there is great progression in reperfusion treatment, long term morbidity in patients with coronary syndrome is still substantial. A limitation of the current treatment options for myocardial infarction is the lack of prevention regarding I/R events. Coagulation proteases such as thrombin or factor Xa (FXa) play a central role in the crosstalk between the coagulation and inflammatory pathway. *In vivo* studies demonstrate that inhibition of coagulation proteases can attenuate myocardial ischemic/reperfusion (I/R) injury, mediated by protease activated receptors (PARs). Although *in vivo* (indirect) thrombin inhibition decreased I/R injury, FXa's involvement in I/R injury is less evident.
Aims: To elucidate FXa's role in I/R injury after a myocardial infarction, we studied the effect of FXa inhibition by rivaroxaban on myocardial I/R injury in an I/R mouse model.
Methods: In an experimental model, male WT c57BL/6 mice (age 8-9 weeks) underwent an intervention in which a ligature was placed around the left anterior descending coronary artery. After seven days of post-surgical recovery, myocardial ischemia was induced by tightening the ligature for one hour followed by loosening the ligature for four (n=16) or twenty-four (n=16) hours to induce reperfusion. Mice in both groups (four and twenty-four hours of reperfusion) where split in two and received an intervention: a control group versus a rivaroxaban treated group. The intervention consisted of one injection of 100 µl rivaroxaban (400 ng/100ml) or placebo (0.9%NaCl) in the jugular vein after fifteen minutes of ischemia and after five minutes of reperfusion. After reperfusion, mice were injected with Evans blue to differentiate between the area at risk (AAR) and the area that was not at risk. AAR is the area exposed to ischemia. Heart tissue was collected and stained with triphenyl tetrazolium chloride to identify viable tissue and differentiate between the AAR and area of infarction (AOI). The area of infarction is the area with irreversible damage.
Results: Rivaroxaban treated mice showed significant differences between AOI/AAR when compared to controls after 4h and 24h of reperfusion. A reduction in AOI/AAR of 19% (95% confidence interval 7.5-31, p=0.0007) was observed after 4h of reperfusion and a decrease of 17% (95% confidence interval 5.7-28, p=0.0055) after 24h of reperfusion.
Summary/Conclusion: Direct inhibition of FXa by Rivaroxaban significantly reduces myocardial I/R-injury after one hour of ischemia followed by either four or twenty-four hours of reperfusion in mice. These observations suggest that it might be beneficial to prevent I/R-injury after a myocardial infarction. Although it has great clinical potential, future research is needed to elucidate the mechanisms of action.

Clotting

ECTH-181

Board No. 40: Anti-beta2-glycoprotein I antibodies cause lupus anticoagulant and activated protein C resistance through an interaction with coagulation factor V

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Background: Lupus anticoagulant (LA) is an acquired risk factor for thrombosis and is characterized by a prolongation of coagulation time caused by autoantibodies against the plasma protein beta2-glycoprotein I. The mechanism behind this paradoxical association between thrombosis and a prolonged coagulation time is not understood. It is assumed that anti-beta2-glycoprotein I antibodies prolong coagulation time by competing with coagulation factors for anionic phospholipids, but this cannot explain the prothrombotic phenotype in patients with LA.

Aims: We investigated how anti-beta2-glycoprotein I antibodies prolong coagulation *in vitro* and how this relates to a prothrombotic state.

Methods: We analyzed the effects of monoclonal anti-beta2-glycoprotein I antibody 3B7 on coagulation with purified coagulation factors in normal and coagulation factor V depleted plasma. Effects of 3B7 on the prothrombinase complex were determined with purified coagulation factors and binding studies were performed with ELISA.

Results: Addition of 3B7 to plasma caused a dose-dependent prolongation of the coagulation time when coagulation was initiated with the factor X activator from Russell's Viper Venom, or with factor Xa itself. The effects of 3B7 were attenuated when an excess of phospholipid was used, confirming LA activity of the antibody. In contrast, 3B7 did not influence coagulation initiated with the combination of factor Xa and factor Va, or with Taipan Snake Venom, which contains a prothrombinase-like enzymatic complex. This suggests that 3B7 interferes with the assembly of the prothrombinase complex, not with prothrombin cleavage. Addition of 3B7 to factor V-depleted plasma supplemented with factor V resulted in a 1.6-fold increased coagulation time. Experiments with patient-derived human monoclonal anti-beta2-glycoprotein I antibodies BK117 and EM6 showed similar results. We further explored the effects of anti-beta2-glycoprotein I antibodies on the prothrombinase complex and found that complexes of 3B7 and beta2-glycoprotein I inhibited the activation of factor V by factor Xa by 50% when the phospholipid concentration was limiting, but not in the presence of excess phospholipids. Moreover, analysis of prothrombinase reaction rates showed that 3B7 lowered the Vmax independent of the phospholipid concentration. Binding studies showed that beta2-glycoprotein I binds to both factor V and factor Va. Next, we investigated whether anti-beta2-glycoprotein I antibodies interfere with the inactivation of factor Va by activated protein C. Antibody-beta2-glycoprotein I complexes attenuated the inactivation of factor Va by activated protein C and caused a 2-fold increase in activated protein C sensitivity ratio.

Summary/Conclusion: Anti-beta2-glycoprotein I antibodies cause LA by interference with the assembly of the prothrombinase complex. This interference is mediated via a direct interaction of beta2-glycoprotein I and factor V(a). This effect is associated with activated protein C resistance, a well-known risk factor for thrombosis.

Clotting

ECTH-293

Board No. 41: Evaluation of global coagulation balance among PMM2-congenital disorder of glycosylation patients, using thrombin generation assay

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Background: Congenital disorders of glycosylation (CDG) are rare metabolic diseases characterized by an enzymatic deficiency in the glycosylation process. Phosphomannomutase 2 deficiency (PMM2-CDG) is the most frequent CDG. PMM2-CDG patients present neurological involvement, which may be associated with multivisceral failure. In childhood, acute vascular events can occur such as stroke-like (transient cerebral vascular accident), bleeding or thrombosis. Stroke-like is the most frequent acute event. These events occur in patients younger than 15 years, especially during fever. The biological findings include different coagulation defects such as clotting factors FIX and FXI, and coagulation inhibitors, in particular antithrombin and protein C. Coexistence of anticoagulant and procoagulant factor deficiencies creates an imbalance between hemorrhagic and thrombotic risk. So far, no correlation was reported between coagulation abnormalities and vascular events.

Aims: To evaluate the coagulation balance in PMM2-CDG patients presenting combined coagulation factor impairments, we used a global test, the thrombin generation assay. This coagulation test, when performed in the presence of soluble thrombomodulin (sTM), takes into account both procoagulant factors and coagulation inhibitors.

Methods: 10 patients with PMM2-CDG (7 girls and 3 boys) with a median age of 11 years (range, 3-16 years) were enrolled in the study. In this cohort 4 CDG patients (3 girls, 1 boy) have already presented single or recurrent vascular events, as stroke-like. They were compared to 10 controls matched in age and sex. Thrombin generation was assessed using the calibrated automated thrombogram method, with PPP reagent (tissue factor 5 pM and phospholipids 4 µM). Experiments were conducted with and without sTM (7.5 nM).

Results: Thrombin generation, measured without sTM, was increased in PMM2-CDG patients compared to controls. Indeed, both endogenous thrombin potential (ETP) and Peak were increased (1322 [1092-2251] vs. 970 [915-1108] nM.min; p=0.003 and 270 [221-288] vs. 210 [167-244] nM; p=0.0433 respectively). However, no significant difference in thrombinogram parameters (ETP and Peak) was observed between PMM2-CDG patients who suffered from stroke-like episodes and PMM2-CDG patients free of stroke-like, even though a tendency for a more procoagulant state was observed (2069 [1476-2390] vs. 1153 [1077-1756] nM.min, p=0.1143; and 283 [280-355] vs. 235 [197-271] nM; p=0.0667 respectively). When the test was performed in the presence of sTM, ETP and Peak were not significantly different between patients and controls (942 [319-1270] vs. 339 [248-589] nM.min; p=0.123 and 168 [87-251] vs. 99 [68-155] nM; p=0.166 respectively). Interestingly, ETP was significantly higher in patients that experienced stroke-like events than the other patients (1270 [1098-1771] vs. 329 [258-916] nM.min; p=0.0381). Altogether, our data suggests that the global coagulation balance in PMM2-CDG patients is more hypercoagulable particularly in those who had suffered from stroke-like events.

Summary/Conclusion: Despite decrease in both procoagulant and anticoagulant factors, our results show a global hypercoagulant profile in CDG patients using a thrombin generation assay. This procoagulant balance is even more pronounced in patients that experienced stroke-like events because of an altered sensitivity to the protein C system. These preliminary results should be confirmed in a larger study, even though PMM2-CDG is a rare disease.

Clotting

ECTH-317

Board No. 42: Genetic determinants of thrombin generation and clot lysis time: results of a targeted exome sequencing study

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Background: The thrombin generation (TG) potential and clot lysis time (CLT), which represent the ability to form and dissolve a clot, respectively, are well-established risk factors for venous thrombosis. Despite the heritability of these traits, few genetic determinants have been identified. Exploring the role of protein coding variants could provide insight into the genetic architecture and biology underlying these traits.

Aims: We aimed to identify novel genetic determinants of TG and CLT using targeted exome sequencing.

Methods: We included 296 controls (without venous thrombosis) from two population-based, case-control studies on venous thrombosis (MEGA and THE-VTE). Individuals were selected using the following criteria: European ancestry, no surgery or malignancy, no natural anticoagulant deficiency, and no factor V Leiden or prothrombin 20210A carriership. Targeted sequencing was performed of the coding regions and boundaries of 740 genes involved in thrombosis and hemostasis or closely related pathways. We investigated the association of 3,002 low-frequency, common (minor allele frequency (MAF) ≥1%) variants with CLT and four parameters of TG: endogenous thrombin potential, peak height, time-to-peak, and lag time. Linear regression models were adjusted for age, sex, country of the study, and additionally for oral contraceptive use in the TG analyses. To account for multiple testing, we calculated false discovery rates (FDR).

Results: CLT and three of the TG parameters showed no association with any of the genetic variants observed, with the lowest FDR (22%) observed for an association between CLT and a missense variant in *TLR5*. We identified a common synonymous variant in *COL4A1* which was associated with time-to-peak (c.1815T>C, MAF=6.7%, β=0.65 per z-score increase, $P=1.92*10^{-5}$, FDR=6.2%). *COL4A1* encodes a major component of the type IV collagen of the basement membrane, and it is a highly replicated locus for cerebro- and cardiovascular disease risk.

Summary/Conclusion: We did not identify any variants associated with either CLT or three of the four TG parameters, which is likely attributable to our small sample size. However, we identified a variant in *COL4A1* as a novel determinant of time to thrombin peak. Replication and functional studies are warranted to further unravel the implication of *COL4A1* in TG and, potentially, venous thrombosis risk.

Clotting

ECTH-175

Board No. 43: Myocardial infarction and future risk of cancer in the general population – the Tromsø study

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Background: Myocardial infarction (MI) and cancer are major causes of morbidity and mortality worldwide, but the association between MI and future risk of incident cancer is scarcely investigated.
Aims: We aimed to study the risk of cancer after a first-time MI in a large cohort recruited from a general population with validated information about MI, cancer and potential confounders.
Methods: Participants in the Tromsø study (surveys 4-6) without a previous history of MI or cancer (n=28 763) were included and followed from baseline to date of cancer, death, migration or study end. Baseline information was collected by physical examination, blood samples and self-administrated questionnaires. Cox regression models with age as time scale and MI as a time-varying co-variate were used to calculate hazard ratios (HR) for cancer after MI adjusted for sex, body mass index (BMI), diabetes, smoking, blood pressure, HDL-cholesterol, physical activity and education. The regional ethical committee approved the study and all participants gave their informed written consent.
Results: In total, 1 747 (6.1 %) subjects experienced a first-time MI, and of these 146 (8.4 %) developed subsequent cancer during a median follow up of 15.7 years. The most frequent types of cancers were colorectal cancer (22 %), prostate cancer (22 %) and lung cancer (16 %). In the multivariable-adjusted model, MI patients had a 46 % (HR 1.46; 95 % CI: 1.21-1.77) higher risk of cancer compared to those without MI. The risk estimates for tobacco-associated and not-tobacco-associated cancers were similar for both groups. The incidence of cancer in subjects with MI changed over time after the MI event. The highest incidence of cancer was found during the first 6 months after the MI diagnosis (IR 29.0 per 1 000 person-years). The relative risk of cancer was 2.2-fold higher the first six months after MI (HR 2.15; 95 % CI: 1.29-3.58) compared to subjects without MI, followed by a time period from 6 months to 3 years after MI without any increased cancer risk. In the period more than 3 years after MI, the cancer risk was 60 % (HR 1.60; 95 % CI: 1.27-2.03) higher than in subjects without MI. The short- and long-term risk estimates for cancer by MI were higher in women than in men.
Summary/Conclusion: Patients with MI had a short- and long-term increased risk of cancer compared to subjects without MI. The short-term risk may be explained by occult cancer or surveillance/detection bias, while the long-term risk of cancer after MI may be explained by shared genetic or environmental risk factors other than smoking, obesity, and physical inactivity.

Clotting

ECTH-203

Board No. 44: Reduced ADAMTS13 levels in patients with acute and chronic cerebrovascular disease

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Background: Von Willebrand Factor (VWF) plays a major role in thrombosis and hemostasis. Its activity and thus thrombogenicity is controlled by the metalloprotease ADAMTS13. From animal models it has become clear that absence of VWF is protective in ischemic stroke whereas absence of ADAMTS13 worsens disease outcome. Increasing evidence shows a clear association between VWF levels and acute ischemic stroke in patients but this association is less clear for ADAMTS13.

Aims: To compare plasma levels of ADAMTS13 between acute ischemic stroke patients, patients with a chronic cerebrovascular disorder and healthy controls.

Methods: In total 115 patients with an acute ischemic stoke (AIS) or transitory ischemic attack (TIA), 133 patients with a chronic cerebrovascular disease (CCD) and 97 healthy volunteers (HV) were included. Blood was drawn within 24h of symptom onset in patients with AIS or TIA and once in HV and CCD patients. ADAMTS13 levels were measured by ELISA and correlated with demographic and clinical parameters by multivariate linear regression and Kruskal-Wallis analysis. VWF:ADAMTS13 ratio's were determined using VWF levels that were measured in a previous study.

Results: Acute stroke patients had significantly reduced ADAMTS13 levels (82.6% ± 21.0%) compared with HV (110.6% ± 26.9%; $p < 0.0001$). Interestingly, also CCD patients had significantly lower ADAMTS13 levels compared with HV (99.6% ± 24.5%; $p < 0.03$), however this was still higher compared with the acute stroke patients ($p < 0.0001$). After adjustment for age and sex, these results remained highly significant. No association was found for ADAMTS13 levels with age ($p = 0.101$), disease modality (TIA vs. AIS; $p = 0.780$), stroke etiology (TOAST criteria; $p = 0.668$), anti-platelet therapy ($p = 0.198$) or thrombolysis ($p = 0.255$). ADAMTS13 levels were associated with sex ($p = 0.018$). Lower ADAMTS13 levels were found in the patients with the most severe stroke as assessed with the National Institutes of Health Stroke Scale (NIHSS), the modified Rankin Scale (mRS) and Barthel Index (BI) although this was found to be not significant.

In particular, the combination of high amounts of VWF with low levels of ADAMTS13 could be harmful. We therefore also assessed the VWF:ADAMTS13 ratio in our study cohorts. A significantly higher VWF:ADAMTS13 ratio was found in stroke patients (2.7 ± 1.9) compared with HV (1.1 ± 0.5; $p < 0.0001$) and patients with chronic cerebrovascular disease (1.7 ± 0.7; $p < 0.0001$). Interestingly, the VWF:ADAMTS13 ratio was significantly associated with stroke severity (NIHSS: $p = 0.048$; mRS: $p = 0.015$ and BI: $p = 0.004$) and stroke modality (AIS or TIA; $p = 0.023$).

Summary/Conclusion: Both in acute stroke and chronic cerebrovascular disease patients ADAMTS13 levels were significantly decreased, with the lowest ADAMTS13 levels found in the acute stroke patients. This difference was even clearer when the ratio of VWF:ADAMTS13 was considered. These results confirm the importance of the VWF/ADAMTS13 axis in acute ischemic stroke and put forward the ratio VWF:ADAMTS13 as a potential biomarker for stroke risk and severity.

Clotting

ECTH-337

Board No. 45: Procoagulant state in young females with severe early-onset obesity

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Background: Obesity has been associated with disturbances in inflammatory and coagulation system, and is linked to increased thrombotic risk.
Aims: This study aimed to explore the relationship between early-onset obesity and markers of inflammation and a prothrombotic state at young adulthood.
Methods: In this case-control study, young adults with severe early-onset obesity (weight-for-height >60% before age 7 yrs; n= 54, median age 18 yrs; median body mass index, BMI, 41 kg/m^2) were compared with controls (n=63, median age 19 yrs; median BMI 22 kg/m^2). We analysed anthropometric parameters (BMI and waist circumference), and laboratory markers of coagulation, inflammation and metabolism (prothrombin fragment F1+2, fibrinogen, factor VIII, fibrin degradation products, high sensitive-CRP, leptin and insulin). Gender had a significant effect on the analyses and thus males and females were analysed separately.
Results: In obese females, F1+2 was higher compared with controls, medians being 171 pmol/L (range 113-486 pmol/L) vs. 150 pmol/L (70-639 pmol/L) (p=0.042). F1+2 exceeded the reference range (229 pmol/L) in 30% of obese females and in 15% of female controls. In females, F1+2 correlated with BMI (r=0.29, p=0.02), waist circumference (r=0.29, p=0.03), high sensitive (hs)-CRP (r=0.44, p<0.001), leptin (r=0.29, p=0.03), and insulin (r=0.31, p=0.02). Use of oral contraceptives did not affect the results. In males, F1+2 did not differ between the obese and controls and no correlation was found between F1+2 and markers of obesity and inflammation.
Fibrinogen was significantly higher in obese females compared with control females, with medians of 4.5 g/L (2.7-6.5 g/L) vs. 2.9 g/L (2.3-4.9 g/L) (p<0.001). Fibrinogen exceeded the reference range (4.0 g/L) in 72% of obese females and in 8.3% of control females. In females, fibrinogen correlated with BMI (r=0.66), waist circumference (r=0.65), hs-CRP (r=0.85), leptin (r=0.73) and insulin (r=0.62), (p<0.001). Fibrinogen was also increased in obese males compared with control males, medians being 3.5 g/L (2.4-5.5 g/L) vs. 2.5 g/L (2.2-5.9 g/L) (p=0.002).

In obese females, factor VIII was higher when compared with control females (median 147% (98-205%) vs.119 (74-240%), p=0.01) but did not differ between obese and control males.

Fibrinogen degradation products were generally low in all groups and no correlation was found between fibrin degradation products and markers of obesity and inflammation.
Summary/Conclusion: Young obese females presented with elevated F1+2, a marker of thrombin generation and coagulation system activation. Additionally, fibrinogen and factor VIII were high in obese females. In females, F1+2 and fibrinogen associated with hs-CRP, leptin, insulin and anthropometric measurements. Thus, the prothrombotic and proinflammatory state is already present at young age especially in females and may increase susceptibility to a more adult-like coagulation profile and the risk of early development of cardiovascular disease.

Clotting

ECTH-368

Board No. 46: Coagulation factor VIII, white matter lesions, and cognition in the Cardiovascular Health Study

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Background: Cognitive decline is related to several cardiovascular risk factors, as well as the presence of white matter lesions (WML) on MRI. WML have been linked to small vessel disease caused by vascular brain injury, which can either be overt, when the patient presents with clinically defined stroke, or covert. Coagulation factor VIII (FVIII) is thought to affect the risk of overt clinical stroke, but whether it is also related to covert WML and cognitive decline is unknown.

Aims: To investigate the relationship between high levels of FVIII and (1) the presence and worsening of WML and (2) cognitive test scores and the rate of cognitive decline.

Methods: Data come from the population-based, longitudinal Cardiovascular Health Study (CHS) with 5,888 participants aged ≥65 years. Two cranial MRI scans taken roughly 5 years apart were used to assess WML, graded on a 0 (least) to 9 (worst) scale. Cognitive function and its decline were assessed using annual scores on the Modified Mini-Mental State Exam (3MSE) and the Digit Symbol Substitution Test (DSST). The effect of FVIII was evaluated per increase in standard deviation (SD) and using 3 categories, with the 25th and 75th percentiles as cutoffs and the middle category as the reference. Regression techniques including logistic, ordinal, and linear mixed models were used to determine the cross-sectional and longitudinal relationship between FVIII and both WML and cognition. We adjusted for demographic and traditional cardiovascular (CV) risk factors to address confounding.

Results: High levels of FVIII were associated with the burden of WML (grades 2-9) on the first MRI scan [crude OR_{p75}=1.36, 95%CI 1.12-1.65]. Adjustment for demographic factors followed by additional adjustment for CV risk factors weakened this association [OR_{p75}= 1.28, 95%CI 1.04-1.56; OR_{p75}= 1.24, 95%CI 1.00-1.53]. Per SD increase in FVIII, WML likelihood increased 1.18-fold [95%CI 1.08-1.28]. Ordinal analyses revealed no association between FVIII and WML worsening over time.

Linear regression revealed an association between high levels of FVIII and lower baseline cognitive test scores [$3MSE_{p75}$ = -0.66, 95%CI -1.05 to -0.27; $DSST_{p75}$ = -2.81, 95%CI -3.68 to -1.94]. Per SD of FVIII, lower 3MSE and DSST scores were also observed [3MSE = -0.49, 95%CI-0.66 to -0.33; DSST = -1.84, 95%CI -2.20 to -1.48]. Adjustment for confounding attenuated these associations.

Linear mixed models analyses showed an association between high FVIII levels and lower cognitive test scores during follow-up. [$\Delta 3MSE_{p75}$ = -1.17, 95%CI -1.73 to -0.61; $\Delta DSST_{p75}$ = -2.46, 95%CI -3.32 to -1.61]. A similar pattern was revealed when per SD increase in FVIII levels were analyzed [$\Delta 3MSE_{SD}$ = -0.64, 95%CI -0.87 to -0.40; $\Delta DSST_{SD}$ = -1.59, 95%CI -1.94 to -1.23]. After adjustment for demographic risk factors, these associations remained more pronounced for DSST [$\Delta DSST_{SD}$= -0.35, 95%CI -0.66 to -0.05] than for 3MSE [$\Delta 3MSE_{SD}$= -0.04, 95%CI -0.25 to 0.17].

Summary/Conclusion: In this study, we did not observe a strong association between FVIII and the presence or worsening of WML over time. If anything, higher FVIII is mildly associated with lower cognitive test scores at baseline and over time, especially as assessed by the DSST.

Clotting

ECTH-377

Board No. 47: Assessment of platelet function by thromboelastography-based platelet mapping in cardiac patients on aspirin and clopidogrel

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Background: Standard antiplatelet therapy with aspirin and clopidogrel is the mainstay of treatment in patients with coronary artery disease to reduce the risk of thrombotic events. However, despite apparently adequate antiplatelet treatment some patients experience ischemic events. There is no routine monitoring of platelet inhibition in these patients coupled with the fact that there is no ideal testing method for assessing the antiplatelet effects of these drugs and to predict clinical outcome in such patients.
Aims: This study was planned to assess the platelet inhibition in response to dual antiplatelet drugs and to know the incidence of ischemic events using Thromboelastography Platelet Mapping (TEG-PM) in patients undergoing non emergent percutaneous coronary intervention (PCI).
Methods: A total of 100 patients were included in the study after taking consent and institute ethics approval. All patients were aged >18 years, had been on dual antiplatelet therapy for more than 10 days. A 600 mg clopidogrel loading dose was administered at least 12 h before the procedure. The patients were maintained on aspirin 150 mg/day and clopidogrel 75 mg twice daily after the procedure. Before the PCI, patient's sample was run on hemostasis analyzer (TEG-PM) using platelet mapping kit and platelet inhibition % for aspirin (MA_{AA}) and clopidogrel (MA_{ADP}) was ascertained. The TEG-PM assays measure the reduction in platelet function due to antiplatelet drugs. Specifically, MA_{ADP} measures the inhibition by clopidogrel of the ADP receptor and MA_{AA} measures the inhibition by aspirin of TXA2 receptor. In TEG-PM the maximum platelet function ($MA_{thrombin}$) is a reference point against which percentage of inhibition is compared. Aspirin hyporesponsiveness was defined as > 50% platelet aggregation after stimulation by 1-mmol/l AA as measured by TEG. Clopidogrel Hyporesponsiveness was defined as ≥70% ADP induced aggregation with 2-µmol ADP as measured by TEG . Laboratory testings were performed in department of Transfusion Medicine while coronary interventions were done in Cardiology.
Results: Mean (±2SD) values of 100 patients for TEG-MA, TEG-R, TEG-K, TEG-angle, were 67.3 ± 6.6 mm, 5.4 ± 2.6 min, 1.7 ± 1.0 min, 66.8 ± 10 degree respectively. In our study for aspirin mediated platelet inhibition, 11 out of 100 patients (11%) showed hyporesponsiveness (≤ 50% platelet inhibition). Similarly 10 out of 100 patients (10%) showed hyporesponsiveness for clopidogrel (≤ 70% platelet inhibition). Also three patients showed hyporesponsiveness for both clopidogrel as well as aspirin therapy. During follow up 11 patients were rehospitalized for various ischemic events. Stent thrombosis was seen in a patient who was non responder to Clopidogrel.
Summary/Conclusion: Results of our preliminary study are based on the use of single TEG-PM test done before PCI however the presence of high platelet reactivity (less platelet inhibition) in a subset of patients which was associated with ischemic events after PCI supports the need for early testing of platelet function ideally before the procedure to intensify antiplatelet treatment if needed. We conclude that TEG-PM is likely to be helpful for diagnosing non responders to aspirin and clopidogrel and tailoring antiplatelet treatment in individual patients undergoing PCI.

Clotting

ECTH-406

Board No. 48: Intrinsic coagulation antigen and activation levels, headache and the risk of young ischaemic stroke

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Background: Migraine with aura is a risk factor for ischemic stroke. Hypercoagulable states in migraine patients may play a role in the pathophysiological mechanisms of this association.
Aims: This study aims to provide more insight into this possible connection of migraine, ischemic stroke and the intrinsic coagulation pathway.
Methods: We included patients from the RATIO study, a Dutch population-based case-control study including young women (age 18 to 50 years) with ischemic stroke and healthy controls. We constructed a migraine proxy variable based on several questions on headache history and treatment. Intrinsic coagulation proteins were measured through both antigen levels (FXII, FXI, prekallikrein, HMWK) and protein activation, determined by measuring activated protein complex with C1esterase inhibitor (FXIIa-C1-INH, FXIa-C1-INH, Kallikrein-C1-INH) or antitrypsin inhibitor (FXIa-AT-INH). Odds ratios and corresponding confidence intervals were calculated using logistic regression and adjusted for potential confounders. We performed an interaction analysis which assessed the increase in stroke risk associated with high levels of intrinsic coagulation (>75th percentile), the presence of headache, as well as the combination of both factors.
Results: Data were available for 114 ischemic stroke cases and 612 healthy controls. In total, 194 (18%) patients had a history of headache. We found that the combination of headache and elevated intrinsic antigen and activation (all but FXII antigen level and FXIa-AT-INH) lead to a greater stroke risk than could be expected based on their two individual risks. The most pronounced effects were observed for kallikrein. For example, higher levels of KAL-C1-INH only increased the risk twofold, headache increased the risk 2.5-fold, whereas the combination increased the risk 8.5-fold.
Summary/Conclusion: Our data show that headache and elevated intrinsic coagulation parameters may biologically interact increasing risk for ischemic stroke. Within the headache group, migraine may be mainly responsible for this relation, though further research on this topic is needed.

Clotting

ECTH-454

Board No. 49: Annexin M2 haplotype and adverse pregnancy outcomes - a perfect match?

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Background: Hereditary thrombophilias are well-known to impair placental function and cause adverse pregnancy outcomes (APO) such as recurrent pregnancy loss, pre-term labour, intrauterine growth restriction, small for gestacional age newborns or hypertensive disorders of pregnancy. Mutations in coagulation factors II and V, alterations in protein C and its co-factor protein S are commonly accepted as hereditary thrombophilias. Furthermore alterations resulting in reduced fibrinolysis have also been associated with APO. More recently conflicting data on the possible involvement of M2 Annexin A5 (ANXA5) haplotype have been published, with evidence that supports its involvement in embryonic-induced anticoagulation.

Aims: Assess the presence of M2 ANXA5 haplotype in a population of women with a previous history of APO.

Methods: Retrospective case-control study including women referred to the Pregnancy and Thrombophilia Consultation in our Center, between January 2012 and December 2015, with a previous history of APO. Detailed previous medical history was reviewed. Patients with other hereditary thrombophilias, anti-phospholipid syndrome, auto-immune diseases, polycystic ovary syndrome or uterine anomalies were excluded. The presence or absence of the M2 ANXA5 promoter haplotype was assessed. The possible relationship between M2 and APO was evaluated by comparing our case group with our Center's control group and with two independent control groups. Odds ratio (OR) with 95% confidence intervals (CI) and chi-squared tests were calculated. Statistical significance was defined as $p<0.05$.

Results: We included 110 women with a previous history of unexplained APO, with a mean age of 33.5 years (17-41), median gravidity of 2 (0–7) and median parity of 1 (0–2). Forty-one women had experienced a total of 109 pregnancy losses (median 3; range 1–5), 89.9% of which in the first trimester. Fifty patients had hypertensive disorders of pregnancy (HELLP syndrome, pre-eclampsia and eclampsia). There were 13 cases of intrauterine growth restriction and 10 of fetal deaths. The remainder had other APO and some women had more than one different type of APO. Cardiovascular (CV) risk factors were distributed as follows: smoking habit 13.6% (n=15), dyslipidemia 10.9% (n=12), hypertension 10.9% (n=12) and 1 had diabetes. Mean body mass index (BMI) was $25.21 \pm 4.00 kg/m^2$. All but one were Caucasians. Seven patients had history of thrombotic events. Presence of M2 ANXA5 promoter haplotype was observed in 31% (n=34) of our cases. When comparing the study group with our Center's controls we found a statistically significant association between the presence of M2 haplotype and the occurence of unexplained APO (OR 2.13, 95%CI 1.01–4.46; p=0.0466). When comparing with a general control population and a group of fertile female controls we also found significant association: OR 2.4, 95%CI 1.47–3.77, $p<0.001$ and OR 4.80, 95%CI 2.86-8.05, $p<0.0001$, respectively. No differences in age, BMI, CV risk factors were identified when comparing carriers of M2 *versus* non-carriers. No association between carriage status for M2 haplotype and subtype of APO was identified.

Summary/Conclusion: A higher frequency of the M2 ANXA5 haplotype was found in our case group, when compared with our controls and the 2 independent control populations, suggesting a possible relationship between this specific genotype and unexplained APO. Genotyping ANXA5 M2 status seems to be a well oriented approach in the diagnostic of women with unexplained APO.

Clotting

ECTH-336

Board No. 50: Venous thromboembolism predictive capability of genetic risk scores in subjects with afamily history of venous thromboembolism

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Background: Positive family history of venous thromboembolism (FH) is a strong risk factor for a first idiopathic venous thromboembolism (VTE) and it is suggested to be a valid means to assess the risk of VTE. The clinic-genetic risk score Thrombo inCode (TiC) has shown to have a better predictive capability of VTE than known genetic risk factors like Factor V Leiden mutation and the prothrombin G20210A mutation (FVL+PT).

Aims: To investigate whether TiC provides a better prediction of VTE than the FVL-PT score in individuals with FH.

Methods: This is an approved by the institutional Ethic Committee prospective case-control study including 179 subjects with FH referred to the Coagulation laboratory at Karolinska University Hospital and who provided informed consent. Two VTE risk scores were used, TiC (included FVL, PT, rs118203906 and rs118203905, rs1801020, rs5985, rs121909548, rs2232698, rs8176719, rs7853989, rs8176743, rs8176750, FH, age, diabetes smoking and BMI) and FVL-PT. Clinical utility was assessed in terms of discrimination (AUC), sensitivity, specificity, positive likehood ratio (+LHR), negative likehood ratio (-LHR) and odd ratio (OR).

Results: Of 179 patients, 79 had developed VTE**.** FVL and PT were identified in 29.11% and 5%, respectively of subjects with VTE and 27% and 6.33%, respectively of the subjects without VTE. The presence of the allele AB0 A1 was identified in 54.4% and 42% of subjects with and without VTE, respectively. FVL-PT showed a non-significant AUC 0.513, p=0.712. TiC showed a significant AUC 0.613, p=0.009 which is significantly higher than for FV-PT (p=0.005). Investigation of FVL-PT only resulted in a sensitivity of 34.2%, specificity 68.0%, +LHR 1.07 and –LHR 0.97, OR 1.1. TiC showed a higher sensitivity 86.1% (p<0.001), but a lower specificity 32%, (p<0.001) than FVL-PT, and better + and –LHR 1.26 and 0.43, respectively, OR was 2.91.

Summary/Conclusion: The presence of FVL in subjects with FH and VTE is similar to the previously published results. However, in our study in subjects with FH but without VTE, FVL is more common than previously reported suggesting that FVL is highly associated with FH in this population and additional risk factors are required to develop VTE. According to our results, in populations with a high prevalence of FVL, this genetic variant together with PT may not to be useful in the discrimination of patients at risk of developing VTE (the AUC is not significantly different to 0.5). By the contrary, TiC with more thrombophilic relevant variants and clinical data showed a higher VTE predictive capability than FVL-PT alone in patients with FH. The AUC was significantly different to 0.5 and higher than that for FVL-PT, clinical sensitivity of TiC was significantly higher than that showed by FVL-PT what confers TiC with a high clinical utility Therefore, in patients with FH the use of TiC may provide clinically useful predictive information. Remains to be elucidate whether the inclusion of more clinical data could improve the performance of TiC .

Ferrer inCode has provided financial support. Gendiag.exe has performed genotyping and contributed preparing the abstract. Abstract final version was a decision of the non-company authors.

Clotting

ECTH-219

Board No. 51: Association of disease prevalence and failure rate in diagnostic management studies in suspected pulmonary embolism: towards a new tailored standard for future studies

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Background: Traditionally, the accepted failure rate of pulmonary embolism (PE) diagnostic studies is ≤2.7%, based on a meta-analysis of pulmonary angiography studies performed in the 1990's. However, the disease prevalence in PE studies has decreased considerably over the past decade and differs between geographical regions. Considering Bayes' theorem, this should imply that the failure rate margin of contemporary diagnostic tests or algorithms should be lowered as well.

Aims: We set out to evaluate the association of PE prevalence and diagnostic failure rate in prior studies in order to set a new standard for future studies.

Methods: We selected all high-quality diagnostic studies in acute PE from 1990 on, that included consecutive patients who were followed for at least three months and were subjected to an appropriate diagnostic standard, i.e. (an algorithm consisting of) a validated clinical decision rule combined with a highly sensitive D-dimer test, multi-slice CT pulmonary-angiography, ventilation/perfusion scintigraphy in accordance to the PIOPED criteria, and/or conventional pulmonary angiography.

Results: Fifty studies including 28,848 patients were included, with a mean baseline PE prevalence of 23.3% (95%CI 20.5-26.1, range 0.28-44.5%). The reference line calculated by linear regression analysis adjusted for study sample size of the graph plotting failure rate against PE prevalence corresponded to a mean sensitivity of 99.6% across the studies. The background 3-month incidence of PE (virtual population with 0% PE at baseline, cross point of the y-axis) was 0.69%. The formula of the upper 95% confidence interval of the reference line is '1.*85+ 0.0041*prevalence*'.

Summary/Conclusion: We propose that future PE diagnostic studies should incorporate this formula as reference standard for the minimum diagnostic accuracy of new diagnostic tests or algorithms and thus customize their power analysis to the expected PE prevalence in the studied cohort.

Clotting

ECTH-183

Board No. 52: Homocysteine levels and risk of first venous thrombosis: the influence of (unmeasured) confounding factors

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Background: Whether there is an association between hyperhomocysteinemia and venous thrombosis (VT) is controversial. Meta-analyses have reported increased risks in individuals with hyperhomocysteinemia but could not fully take confounding into account or exclude publication bias, whereas randomized trials of homocysteine levels were negative.

Aims: The aim of this study was to investigate the effect of homocysteine levels on the risk of VT, overall and stratified by sex and subgroups of type of events.

Methods: Fasting homocysteine levels were measured in 1689 patients with first VT, 787 partner controls, and 939 random digit-dialing (RDD) population controls in a single large case-control study (the MEGA study). Patients were compared with population controls to estimate odds ratios (ORs) by unconditional logistic regression. Results were adjusted for age, sex, BMI, smoking, statin use, history of arterial disease, and regular sports activities. Patients were matched to their partners to additionally adjust for unmeasured confounding factors using conditional logistic regression.

Results: After adjustment for putative confounders, elevated homocysteine levels were not associated with an increased risk for VT when comparing patients to RDD controls, neither as a continuous variable (OR:1.00, 95%CI:0.99,1.01), or in terms of 5 µmol/L increase (OR:0.99, 95%CI: 0.93,1.05), or when levels were >18 µmol/L vs <12 µmol/L (OR:1.02, 95%CI: 0.77,1.34). Similar results were obtained when patients were compared with their partners. Stratification by sex, deep vein thrombosis, pulmonary embolism, provoked and unprovoked VT also provided no evidence of an association.

Summary/Conclusion: In this population-based study, homocysteine levels were not associated with increased risk of venous thrombosis.

Clotting

ECTH-434

Board No. 53: Homozygous prothrombin 20210A mutation among children with deep venous thrombosis

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Background: The G20210A variant in the prothrombin gene (FII G20201A) is associated with a risk of venous thromboembolism. Heterozygotes for the variant have an odds ratio for VTE ranging from 2 to 4. Some conflicting results have been reported for the risk of arterial thrombotic events in G20210A variants as well. Homozygote prothrombin mutation is very rare and clinical data on homozygous 20210A patients with regard to the severity of their thrombotic episode are lacking.

Aims: Our aim is to evaluate homozygous FII G20201A incidence and clinical features among children with thrombosis in our center.

Methods: We retrospectively evaluate our database in children with trombosis at Hacettepe University Pediatric Hematology Department since 2004 and find a total number of 662 children with thrombosis. From those patients 412 had a complete clinical data and 348 out of 412 had an information about FII G20201A mutation. Only 4/348 (1.1%) was found to be homozygote FII G20201A mutation. We also evaluate other prothrombotic risk factors, treatment and prognosis in this group.

Results: Case 1 is 13 years old-girl with DVT and postphlebitic syndrome without other known prothrombotic risk factors. Case 2 is a 15 years-old boy with a left cardiac thrombosis and pulmonary thromboembolism who received tPA and complete resolution occured after 6 months of treatment. Case 3 is a 2 months old boy with an underlying disorder(gastroschisis) and he had had other protrombotic risk factors who experienced catheter related DVT. Case 4 is an 13 years old boy having diagnosed of a CNS tumor and DVT, he had a pulmonary thrombosis as well with other prothrombotic risk factors.

Summary/Conclusion: Four patients having homozygous mutation was found in this study ;two of our homozygous patient having no other underlying prothrombotic risk factors however two have.And these patients were closely follow up for the risk of recurrens and they all received long term anticoagulation.

Clotting
ECTH-126
Board No. 54: Long-term clinical implications of residual pulmonary vascular obstruction after pulmonary embolism: a ventilation-perfusion lung scan follow-up study at two timepoints
Romain Chopard*, Bruno Genet[1], Fiona Ecarnot[1], Marion Chatot[1], Gabriel Napporn[2], Agnès Hyvert[3], Katy Didier-Petit[4], Francois Schiele[1], Nicolas Meneveau[1]
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Background: Background: The long-term impact of persistent pulmonary vascular obstruction after pulmonary embolism (PE) remains unknown.

Aims: Objectives: Based on ventilation-perfusion (V-Q) lung scan performed at discharge and 3 months after a first pulmonary embolism (PE), we aimed to investigate the prognostic value on 5-year adverse events of (1) residual pulmonary vascular obstruction (RPVO) at discharge (DIS-RPVO), (2) RPVO at 3 months (3M-RPVO), and (3) relative change in RPVO between the two scans (RC-RPVO).
Methods: Prospective, multicenter cohort study from 01/2007 to 12/2009 including patients who survived at least 3 months after a PE. RC-RPVO was defined as (DIS-RPVO – 3M-RPVO)/DIS-RPVO. The primary endpoint was a combined endpoint at 5 years, composed of all-cause death, recurrent venous thromboembolism, chronic thromboembolic pulmonary hypertension, heart failure (HF) and rehospitalization for cardiac causes. Receiver-operating characteristic curves were computed to define thresholds of DIS-RPVO, 3M-RPVO and RC-RPVO predictive of the primary combined end-point at 5 years.
Results: Overall, 241 patients were included (high-risk PE:11.2%, intermediate-risk PE:51.8%, low-risk PE:37%). Mean DIS-RPVO was 27.9±15.1%, mean 3M-RPVO was 10.3±10.8% and mean RC-RPVO was 61.7±33.4%. At 5 years, 112 patients (46.5%) experienced the combined end-point. Both 3M-RPVO³15% and RC-RPVO≤37.5% were independently related to the occurrence of the combined end-point at 5 years (p=0.01 and, p=0.02, respectively). DIS-RPVO did not predict long-term adverse events.
Summary/Conclusion: RC-RPVO£37.5% and 3M-RPVO³15% were independently related to the occurrence of adverse events 5 years after a first PE. Special attention should be paid to follow-up planning and care in these patients at risk of long-term adverse events.

Clotting

ECTH-383

Board No. 55: Preeclampsia and risk of a first and recurrent venous thromboembolism

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Background: Preeclampsia occurs in 2-8% of all pregnancies and has been associated with a thrombogenic state and an increased risk of cardiovascular disease. Population-based studies have estimated an increased short term risk of venous thromboembolism (VTE) after preeclampsia.
Aims: To assess the risk of first and recurrent VTE after preeclampsia.
Methods: From a large population based case-control study (MEGA study) women who were pregnant in the year before the index date (time of VTE for cases and time of enrollment for controls) were selected. Detailed information on hospital admission dates of preeclampsia which are encoded according to International Classification of Diseases-9 codes was obtained by linkage to the data of Statistics Netherlands. In the MEGA follow-up study, VTE cases from the MEGA study were followed for recurrence.
First, in a case-control analysis, the relative risk of a first VTE related to a pregnancy that was complicated by preeclampsia was explored, compared to the risk of pregnancy without preeclampsia. Conditional logistic regression models were used to estimate the odds ratio (OR) with 95% confidence intervals (CIs).
Secondly, in a follow-up analysis, the relative risk of a recurrent VTE after a VTE related to a pregnancy that was complicated by preeclampsia was estimated compared to the recurrence risk after a VTE related to a pregnancy without preeclampsia. Observation time in person-years (PY) from the first VTE until recurrence, death or end of study (when vital status was retrieved) was determined. Incidence rates for recurrent VTE were calculated. Cox regression models were used to estimate hazard ratios (HR) with 95%CIs.
Results: A total of 191 VTE cases and 141 control subjects had been pregnant in the year before VTE or index date. Of these, 10 cases and 3 controls were admitted for preeclampsia before the VTE (or index date). The corresponding OR (adjusted for age) was 2.5 (95%CI 0.7-9.2). Of the 191 pregnant VTE cases 183 (>95%), 9 with preeclampsia and 174 without preeclampsia, participated in the MEGA-follow-up study. After a follow-up of 53 PY in the exposed group (preeclampsia-related VTE) and 989 PY in the unexposed group (pregnancy-related VTE), there were 1 and 14 recurrences, respectively. Incidence rates were 19.2/1000 PY in the preeclampsia-related VTE group and 14.2/1000 PY in the pregnancy-related VTE group. The HR (adjusted for age) for a VTE recurrence after an initial VTE in a pregnancy complicated by preeclampsia was 1.3 (95%CI 0.2-13.2) compared with women who had a pregnancy-related VTE without preeclampsia.
Summary/Conclusion: Preeclampsia appears to increase the risk of a first VTE on the short term. Larger studies are necessary to estimate the risk of VTE recurrence after a first VTE related to pregnancy complicated by preeclampsia.

Clotting

ECTH-376

Board No. 56: Performance of the Khorana score in patients with pancreatic cancer

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Background:
Patients with pancreatic cancer have a 10% annual risk of developing venous thromboembolism (VTE), but routine thromboprophylaxis in these patients is not recommended. The Khorana score is a clinical prediction score to identify ambulatory cancer patients at high risk of VTE who may be eligible for thromboprophylaxis. This score has been validated in various populations with cancer, but its performance in patients with pancreatic cancer is less clear.
Aims: To evaluate the performance of the Khorana score in patients with pancreatic cancer receiving chemotherapy.
Methods: This is a single center, retrospective cohort study in which consecutive, ambulatory patients with pancreatic adenocarcinoma who started neoadjuvant or palliative chemotherapy at our center between 2003 and 2014 were included. Patients were assigned points according to the Khorana score based on tumor site (pancreatic cancer; +2 points), pre-chemotherapy hemoglobin below 6.25 mmol/L or use of erythropoietin stimulating agents (+1 point), pre-chemotherapy white blood cell count over 11 x 10^9/L (+1 point), pre-chemotherapy platelet count of 350 x 10^9/L or more (+1 point), and body mass index 35 kg/m^2 or more (+1 point). Subsequently, patients were classified as ‘intermediate risk’ (2 points) or ‘high risk’ (3 points or more). The primary outcome was the composite of objectively confirmed symptomatic or incidental lower extremity deep vein thrombosis (DVT) or pulmonary embolism (PE) during 2-year follow-up. The cumulative incidence was estimated using competing risk analysis in which death from any cause was treated as a competing risk for the primary outcome. Competing risk regression analysis was used to evaluate the difference between ‘intermediate risk’ and ‘high risk’ patients. Multiple imputation was used to avoid the bias associated with missing data.
Results: The study group comprised 178 ambulatory patients with pancreatic cancer who started neoadjuvant (7%) or palliative chemotherapy (93%). The mean age was 62 years and 51% was female. At presentation, 7% of patients had a resectable or borderline resectable tumor, 31% had an irresectable tumor, and 62% had distant metastasis. The Khorana score classified 57% of patients as ‘intermediate risk’ and 27% as ‘high risk’; the score could not be calculated in 17% due to missing data. During a median follow-up duration of 234 days, 22 of 178 patients (12.4%) developed lower extremity DVT or PE. The cumulative incidence at 6 months was 8.2% in ‘intermediate risk’ patients and 9.5% in ‘high risk’ patients (subhazard ratio 1.23; 95%CI 0.41-3.65). At 2 years, the cumulative incidence was higher in ‘intermediate risk’ patients (15.3%) than in ‘high risk’ patients (10.1%).
Summary/Conclusion: In our study, the Khorana score was not able to discriminate between patients with pancreatic cancer at intermediate risk and high risk of VTE, and its use can therefore not be recommended in this population. This study confirms the high risk of VTE in patients with pancreatic cancer. Physicians should have a low threshold of starting thromboprophylaxis in patients with pancreatic cancer, regardless of the Khorana score.

Clotting

ECTH-243

Board No. 57: The effect of GPVI rs1613662 and platelet count on cancer-related venous thrombo-embolism

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Background: Cancer is a risk factor for venous thromboembolism (VTE). The GP6 gene encodes a platelet membrane glycoprotein that acts as a receptor for collagen and plays a vital role in platelet adhesion and activation, processes that are essential for thrombus formation. Pre-cancer platelet count has previously been identified as a risk factor for VTE in patients with cancer but not in those without cancer.

Aims: We aimed to investigate (i) the effect of the GP6 rs1613662 single nucleotide polymorphism, and (ii) the combined effect of GP6 rs1613662 and pre-cancer platelet count, on cancer-related VTE in a case-cohort study with subjects recruited from the general population.

Methods: Cases with a first VTE (n=604) and an age-weighted sub-cohort (n=1793) sampled from the Tromsø 4 (1994-95), 5 (2001-02) and 6 (2007-08) surveys were included and followed up until the end of 2012. Active cancer was defined as the period from six months before a cancer diagnosis until 18 months after. Cox regression was used to estimate hazard ratios (HR) of VTE with 95% confidence intervals (CI) using non-cancer subjects with no risk alleles as the reference group. Platelet count was dichotomized by the using the median value in the cohort (246 x 10^9/L) as the cut-off. The study was approved by the Regional Committee of Research Ethics, and all participants provided informed written consent.

Results: Of the 2397 subjects, 586 were heterozygous and 57 were homozygous for GP6 rs1613662 (g allele). During follow-up, 463 subjects were diagnosed with cancer and, among these 114 had active cancer-related VTEs. Cancer-free subjects with 1 or 2 risk alleles at rs1613662 had a reduced risk of VTE (HR 0.76, 95% CI 0.60-0.95 and HR 0.53, 95% CI 0.23-1.20, respectively). The risk of an active cancer-related VTE was 8-fold higher (HR 8.29; 95% CI 6.49-10.59) in subjects with no risk alleles compared to those without cancer. Among subjects with active cancer, those with 1 risk allele had a 10-fold (HR 10.36; 95% CI 7.13-15.07) higher risk of VTE, and those with 2 risk alleles a 17-fold (HR 17.03; 95% CI 7.03-41.24) higher risk of VTE, compared with those with no risk alleles. In subjects with active cancer and ≥1 risk alleles, those with a baseline platelet count <246 x 10^9/L had a HR of 7.79 (95% CI 4.43-13.70) and ≥246 x 10^9/L a HR of 10.46 (6.70-16.31) compared to non-cancer subjects without risk alleles and lower platelet count. This effect, however, was not seen in subjects without cancer and risk alleles at the site: HR 0.68 (0.49-0.95) for platelet count <246 x 10^9 and HR 0.57 (0.42-0.78) for ≥246 x 10^9.

Summary/Conclusion: Our findings demonstrate that there is an additive effect of risk alleles at GP6 rs1613662 and active cancer on the risk of VTE. Higher platelet counts further increased the risk of a cancer-related VTE in subjects with risk alleles at GP6. This suggests that both platelet count and platelet activity play a key role in the pathogenesis of VTE in cancer patients, but not in cancer-free subjects.

Clotting

ECTH-174

Board No. 58: Hospital-related first venous thrombosis and risk of recurrence

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Background: Venous thromboembolism (VTE) is particularly common among hospitalized patients, with incidence rates exceeding 100-times greater than in community residents. Hospitalization in itself is an interim exposure, and may therefore be considered as a transient risk factor assumed to yield a low risk of recurrence. However, the risk of recurrence after a first hospital-related VTE, particularly hospitalization for conditions other than cancer or surgery, has not been extensively studied. Furthermore, as comorbidities and hospital admissions are associated with higher mortality rates, the cumulative incidence of recurrence may be influenced by the competing risk of death.
Aims: To investigate the risk of recurrence and mortality among patients with a first hospital-related VTE, and to compare the impact of transient and persistent hospital-related factors such as surgery, cancer or other medical conditions on the risk of recurrence in models with and without death as a competing event.
Methods: The study included 822 patients with objectively confirmed first-lifetime VTE among participants in the Tromsø study (source population: n=33885), Norway, in the period 1994-2012. Information on hospitalization, co-morbidities and clinical characteristics was collected by thorough review of medical records. Recurrent VTEs and deaths were recorded through December 31, 2012. Hospital-related VTE was defined as active cancer, surgery, or hospitalization for a medical condition within 8 weeks preceding the VTE.
Results: In total, 132 experienced a recurrent event during a median of 2.79 Years of follow-up. A hospital-related VTE per se was not associated with increased risk of recurrent VTE (HR: 0.99, 95% CI: 0.69-1.41). However, stratification on hospital-related factors revealed considerable differences. After 5 years, the cumulative incidence of recurrence was 27.4%, 11.0% and 20.0% in patients with a first VTE event associated with cancer, surgery or other medical illness, respectively, whereas patients with a non-hospital related first VTE had an 18.4% risk of a recurrence. High mortality rates and risk of death was found for all subgroups of hospital-related VTE, except for surgery related events. Consequently, the cumulative incidence of recurrence dropped in the presence of competing risk by death, and patients with a first VTE related to hospitalization for other medical illness, cancer or surgery had a cumulative incidence of recurrence of 15.0%, 10.9% and 10.4% after 5-years, respectively.
Summary/Conclusion: The risk of recurrence after a hospital-related first VTE was highly dependent on the reason for hospitalization. Patients suffering from incident VTEs related to hospitalization for medical illness other than cancer or surgery had a high risk of recurrence, even after competing risk by death was taken into account.

Clotting
ECTH-257
Board No. 59: Effect of tumoral lysis on the haemostatic system: parallel and massive increase of circulating DNA and D-dimer without significant changes in anticoagulants: relationship to the risk of thrombosis
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Background: The risk of venous thrombosis significantly increases with chemotherapy treatments. However, the mechanisms and the effect that these treatments might have on the haemostatic system are not well known.
Aims: To study the haemostatic system of patients with chemotherapy treatments and the relationship to the risk of thrombosis.
Methods: Prospectively, we studied 13 patients with acute leukemia (5 lymphoblastic and 8 myeloblastic). Serial samples (basal, before initiation of chemotherapy until day +10) were collected. Circulating DNA levels were quantified by a fluorogenic method. D-dimer levels by a turbidimetric method. Fibrinogen, antithrombin, Protein C and S, FII, FV, FVII, FIX, FX, FXI, PAI-1 and TFPI were evaluated by functional or immunological assays and/or by western blot. The *in vitro* formation and degradation of the clot induced with thromboplastin and recombinant tissue plasminogen activator (rt-PA) from plasma samples of patients and controls was also evaluated. In this *in vitro*model, the effect of supplementation with purified DNA was also studied.
Results: At early moments of the induction treatment, D-dimer, circulating DNA and LDH reached very high levels: 4238 ng/mL [1671-30105]; 0.25 ng/µL [0.07-0.60] and 507 U/L [156-7176], respectively. Values of these parameters were down during evolution to reach those of healthy subjects. There was a significant and positive correlation of these three parameters ($p<0.001$). However, no one, particularly D-dimer associated with thrombosis. Actually, the patient with the highest D-dimer (30105 ng/mL) did not developed thrombosis, and the 2 patients who developed thrombosis, both associated with catheter, reached relatively moderate D-dimer levels (3918 ng/mL and 1764 ng/mL) and thrombosis did not occurred when D-dimer levels were high. Despite very high D-dimer values are suggestive of increased thrombin generation, levels and/or electrophoretic features of anticoagulants were nearly normal. Actually, no significant changes of all hemostatic factors evaluated in the study were detected, except for a moderate reduction of FXII and fibrinogen levels. Activation of FXII was also assayed due to the suggested effect of nucleic acids on the contact pathway. However, no FXIIa was detected, even when plasma contained high circulating DNA levels. The possible interference of nucleic acids in the D-dimer test was discarded with treatment of plasma with DNase and RNase. Purified DNA did not interfere in the formation or degradation of the fibrin clot *in vitro*, but free DNA and FXII levels were reduced after thrombus formation.
Summary/Conclusion: The tumor lysis induced by chemotherapy in patients with acute leukemia causes a release of nucleic acids and intracellular content that parallels with one of the higher elevation of D-dimer described (up to 100 times higher than that of patients with venous thrombosis). However, this response is not associated with significant changes in anticoagulants or procoagulant elements, but with mild decrease of FXII and fibrinogen levels. Moreover, none of these parameters associated with the development of thrombosis, suggesting that the very high levels of D-dimer may be a response of the fibrinolytic system. These patients can provide an excellent model for identifying new regulatory mechanisms of the haemostatic system.

6 POSTER VIEW & DISCUSSION ABSTRACTS (incl. Board No)

Bleeding
ECTH-338
Board No. 61: High incidence of postpartum haemorrhage in carriers of haemophilia in a single-centre cohort
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Background: Carriers of haemophilia are at increased risk for bleeding tendency such as postpartum haemorrhage (PPH). Knowledge on the physician-reported incidence of PPH in carriers of haemophilia and the influence of risk factors may provide clues to optimize obstetric management in these women.
Aims: The aim of this study was to assess the incidence and risk factors of primary PPH in an unselected single-center cohort of carriers of haemophilia.
Methods: Data on deliveries between 2002 and 2014 of haemophilia A (HA) and B (HB) carriers were collected from patient charts and delivery reports. Both carriers aware and unaware of their carrier status were included. Primary PPH was defined as blood loss of ≥500 mL and severe primary PPH as blood loss of ≥1000 mL, both within 24 hours after labour.
Results: In total, data of 64 carriers (54 HA, 10 HB) who had 87 deliveries were included. The incidence of primary PPH was 28% and of severe primary PPH 10%. All deliveries in aware carriers (n=75) were managed according to guidelines, stating that carriers with a factor VIII/IX level of <50 IU/dL in the third trimester require prophylactic treatment to normalize their clotting factor to >100 IU/dL just before delivery. No differences could be found in the incidence of PPH between carriers aware of their carrier status during pregnancy (27%) and carriers unaware of their carrier status (33%, p=0.73). The incidence of PPH in carriers with a baseline FVIII/IX level <100 IU/dL (n=73) seemed higher (32%) compared to the PPH incidence in carriers with a baseline value >100 IU/dL (n=12, 8%), although not statistically significant (p=0.17) due to the low number of women. In the third trimester, 5 carriers still had FVIII/IX levels <50 IU/dL and were treated with clotting factor concentrate, of which 2 carriers developed PPH. Following the guidelines, known carriers with a FVIII/IX level >50 IU/dL in the first trimester were not retested in the third trimester. In this group (n=31) the incidence of PPH was 39%.
Summary/Conclusion: Despite haemostatic treatment according to guidelines, carriers of haemophilia are at increased risk for primary PPH compared to the general population. In order to get more information about haemostasis during and post-delivery and thus ensure optimal obstetric management, factor VIII/IX levels should be checked in the third trimester. Further research is needed to determine whether higher factor activity levels are needed for the haemostatic challenge of delivery in carriers of haemophilia.

Bleeding

ECTH-130

Board No. 62: Overall haemostatic potential and thrombin generation in haemophilia A compared with haemophilia B

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Background: Hemophilia A and hemophilia B are inherited bleeding disorders characterized by impaired thrombin generation, disturbed clot formation and stability, as well as by increased fibrinolysis. Despite the disorders having been traditionally regarded as clinically indistinguishable, recent studies have shown that hemophilia A actually may have a worse clinical phenotype than hemophilia B. These studies have primarily been focused on clinical endpoints and there is a lack of laboratory studies comparing the disorders.

Aims: To compare platelet-poor plasma from patients with hemophilia A and B with the global hemostatic methods overall hemostatic potential and thrombin generation.

Methods: 36 patients, 18 with hemophilia A and 18 with hemophilia B were recruited after informed consent at the tertiary hemophilia centers in Stockholm, Sweden and Belgrade, Serbia. The patients with each diagnosis were matched according to factor concentration levels and the samples were compared with the in house method overall hemostatic potential (OHP) and the thrombin generation test endogenous thrombin potential (ETP). The study was approved by the Regional Ethics Committee in Stockholm. Financial support was given by the Swedish Society for Medical Research, the Swedish Doctors Society, the Karolinska Institute Research Foundation, the Tore Nilson Foundation, the Johan & Jakob Soderberg foundation and an unrestricted grant from Baxter Healthcare Corporation.

Results: There was no significant difference between the groups for either of the methods ($p>0.05$). Overall hemostatic potential in the patients with hemophilia A was 6.23 Abs-sum (range 0.0 – 15.80 Abs-sum) and in the hemophilia B patients 5.91 Abs-sum (range 0.0 – 26.90 Abs-sum). Thrombin generation, measured with the endogenous thrombin potential method, was 279.30 mA (range 24.00 – 433.80 mA) in the hemophilia A group and 171.30 mA (range 34.19 – 472.90 mA) in the hemophilia B group. The median level of FVIII was 0.081 kIE/L (range <0.01 – 0.34) for the patients with hemophilia A and FIX was 0.085 kIE/L (range <0.01 – 0.36) for the patients with hemophilia B.

Summary/Conclusion: No difference could be found between the hemophilia A and B groups. More and larger studies are needed to improve the understanding of this field.

Bleeding
ECTH-204
Board No. 63: FVIII plasma activity of rVIII-singlechain can be measured in both the one-stage and chromogenic substrate assays: results of an international field study
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Background: rVIII-SingleChain is a novel B-domain truncated recombinant factor VIII molecule. Activity measurements exhibit a discrepancy between the chromogenic substrate (CS) and one-stage (OS) assay formats. Pharmacokinetic evaluation of rVIII-SingleChain in 27 male subjects revealed a strong linear relationship between the CS and OS assay results, allowing for reliable interpretation of either method.
Aims: An international, multicenter, randomized and blinded field study, designed to determine the intra-laboratory and inter-laboratory variability of measurements of both rVIII-SingleChain and octocog alfa across assay methods was initiated.
Methods: Plasma samples were spiked at 0.04, 0.3, 0.6 and 1.0 IU/mL for rVIII-SingleChain and octocog alfa, blinded, and distributed to local laboratories to be assayed by the OS and CS assays. In this study, laboratories followed their routine laboratory practices and used their own routine in-house standards and reagents, including the routine in-house FVIII standard, FVIII-deficient plasma and assay reagents. In order to allow for recalculation of results against a common standard, as well as a product specific standard, the sample kit was supplemented with a Standard Human Plasma standard.
Results: Data from 23 laboratories were included in this study. These comprised 13 from the United States, 8 from the EU, 1 from Canada and 1 from South Africa. Results demonstrate that intra-laboratory and inter-laboratory variability in OS assays were similar for both products. When comparing within the OS assay format across different aPTT reagents, there was a similar and reagent-correlated variability in response to different activators for both rVIII-SingleChain and octocog alfa. Results were near the target value at all spiked levels when using the CS assay. The OS assay values underestimate rVIII-SingleChain by approximately 45%; however, this underestimation is highly predictable and consistent across the complete range of investigated FVIII plasma concentrations.
Summary/Conclusion: This data shows a consistent underestimation of rVIII-SingleChain plasma activity when using the OS assay. Due to the consistency and reproducibility, this underestimation is predictable, suggesting that the activity of rVIII-SingleChain from patient samples can be reliably measured with both the CS and OS assay methods. These results support an approach to provide clinical guidance on interpretation of rVIII-SingleChain plasma activity measurements when the OS assay is used for monitoring.

Bleeding

ECTH-207

Board No. 64: Efficacy and safety of rVIII-singlechain in 21 major surgeries

Claudia Djambas Khayat[1], Johnny Mahlangu[2], Ross Baker[3], Cindy Leissinger[4], Elena Santagostino[5], Maria Fernanda Lopez Fernandez[6], Faraizah Abdul Karim[7], Donna Boruchov[8], Johannes Oldenburg[9], Stephanie P'ng[10], Alex Veldman*, Katie St. Ledger[11], Ingrid Pabinger[12]

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Background: rVIII-SingleChain, a novel recombinant Factor VIII, has been designed as a B-domain truncated construct with a covalent bond between the heavy and light chain, resulting in a higher binding affinity to von Willebrand Factor.

Aims: The surgical sub-studies of the Affinity program investigate the safety and efficacy of rVIII-SingleChain to control hemostasis in pediatric, adolescent and adult patients with severe hemophilia A undergoing surgery.

Methods: The studies were approved by the relevant Ethics committee and national authorities. They were conducted according to GCP and the Declaration of Helsinki. 18 patients underwent a total of 21 major surgical procedures (a surgical procedure that required general, spinal or regional anesthesia). Dosing was guided by the WFH recommendations. rVIII-SingleChain was administered either as continuous or as a bolus infusion.

Results: A range of different surgical procedures were performed, including orthopedic surgeries and a variety of other procedures: knee replacement (5), knee arthroscopy, elbow replacement, ankle arthroplasty, lengthening of the achilles tendon combined with straightening of the toes, extraction of multiple teeth (2), abdominal hernia repair, cholecystectomy, circumcision (5), excision, curettage and bone grafting of radius/ulnar pseudo tumor, open reduction internal fixation right ankle fracture and hardware removal right ankle. Investigators rated the efficacy of rVIII-SingleChain to provide hemostasis during surgery as excellent (defined as hemostasis not clinically significantly different from normal) in all cases but two in which they were rated as good (defined as hemostasis normal or mildly abnormal in terms of quantity and/or quality e.g., slight oozing). No related adverse events or serious adverse events were observed during the surgery period.

Summary/Conclusion: rVIII-SingleChain is able to provide very effective and safe control of hemostasis during a wide range of surgical procedures when dosed either by continuous infusion or by bolus infusion.

Bleeding
ECTH-193
Board No. 65: Biochemical profile of pooled, prion cleared and solvent-detergent treated plasma after thawing with different thawing devices
Andrea Heger*, Patrik Wenz[1], Manfred Karlovits[1], Katharina Pock[1], Jürgen Römisch[1]
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Background: The most common way for the thawing of plasma, such as the pooled, prion cleared & solvent/detergent treated plasma (SDP) is a water bath with good circulation at 37°C. Alternative systems for the thawing of plasma include dry ovens or microwave ovens.
Aims: The aim of this study was to investigate the quality of SDP after thawing using different thawing devices available on the market.
Methods: SDP bags manufactured by Octapharma AB (Stockhom, Sweden) were thawed using the following thawing devices: water bath circulator (MB-13A, Julabo GmbH), Quick Thaw water bath (DH4, Helmer), Plasmatherm dry tempering system (Barkey), SAHARA-III dry tempering system (Transmed Medizintechnik GmbH) and Transfusio-therm 2000 microwave oven (EIC Umwelt- und Medizintechnik Ltd). In the first part of the studies, optimized thawing conditions were defined. In the second part of the studies, using the different thawing devices and selected thawing conditions, 7 batches of SDP of different blood groups were thawed and tested on product release parameters.
Results: All SDP bags thawed by different devices and optimized thawing conditions were clear and free of solid and gelatinous particles. There were no significant differences observed in the global coagulation parameters, as well as important coagulation factors and protease inhibitors during thawing by different devices. In addition, there was no coagulation activation observed in any of the plasma bags thawed. All parameters were after thawing within the product release specification levels.
Summary/Conclusion: Thawing devices tested enable standardized thawing and warming of plasma bags. Our study demonstrated that SDP can be thawed using all above listed devices without any negative influence on the plasma quality, presupposed that optimized settings defined for this plasma product are used.

Bleeding

ECTH-196

Board No. 66: A prospective diagnostic accuracy study evaluating rotational thromboelastometry and thromboelastography in 100 patients with von Willebrand disease

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Background: Rotational thromboelastometry (ROTEM) and thromboelastography (TEG) are increasingly used in the perioperative and emergency assessment of bleeding tendencies. The diagnostic value of ROTEM and TEG in Von Willebrand disease (VWD) remains to be established.

Aims: To investigate whether ROTEM and TEG can discriminate patients with VWD and healthy volunteers.

Methods: ROTEM and TEG whole blood coagulation profiles were compared between VWD patients (n=100) and healthy controls (n=89). Measures of diagnostic accuracy were calculated, including sensitivity, specificity, and receiver operating characteristic (ROC). All participants gave written informed consent

Results: TEG R-time had a positive and negative predictive value (PPV, NPV) of 0.84 and 0.68, respectively. TEG clotting index (CI) had a PPV of 1.00 and a NPV of 0.60. Both TEG R and CI had a high specificity and accurately discriminated VWD patients from healthy controls, with an ROC area under the curve of 0.85 and 0.99, respectively. The ROTEM coagulation profiles of VWD patients did not differ from healthy controls.

Summary/Conclusion: TEG accurately discriminated VWD patients and healthy controls. The test's performance may be improved by implementing local test threshold adjusted to a reference population. ROTEM was of limited diagnostic value in VWD.

Acknowledgements

D.E.S. was supported by a scholarship from the German National Academic Foundation (Studienstiftung des Deutschen Volkes). A.Å. received an unrestricted grant from CSL Behring for the present study.

Bleeding

ECTH-516

Board No. 67: Anti-xa activity after a reduced therapeutic dose of nadroparin in renally impaired patients using a dosage guideline of the dutch federation of nephrology

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Background: Low molecular weight heparins (LMWHs) are mainly excreted by the kidneys and may accumulate in patients with renal impairment, leading to an increased anti-Xa activity (aXa) associated with a 2-3 fold increased risk of bleeding complications. Therefore, in renally impaired patients the Dutch federation of Nephrology (NfN) recommend a first standard dose and a 25% dosage reduction in patients with an estimated glomerular filtration rate (eGFR) 30-60 ml/min and 50% dosage reduction in patients with an eGFR < 30 ml/min, followed by monitoring of the aXa in patients treated more than three days. The evidence supporting this recommendation is sparse and only based on studies with enoxaparin, dalteparin and tinzaparin. Furthermore, because the pharmacokinetic response to renal impairment may differ among the various LMWHs due to differences in the mean molecular weight, the findings of these studies cannot be directly extrapolated to other LMWHs.

Aims: In this study we determine the equivalence of the mean aXa in patients with an eGFR < 60 ml/min treated with a reduced therapeutic dose of nadroparin using the dosage guideline of the NfN and patients with an eGFR > 60 ml/min receiving a standard therapeutic dose of nadroparin.

Methods: This prospective, observational, multicentre, cohort study was conducted in Medical Centre Leeuwarden, Isala Zwolle and Jeroen Bosch Hospital and Bernhoven. Patients were included between July 2014 and April 2016 if they met de following inclusion criteria: age > 18 years, a therapeutic dose of nadroparin, subcutaneous administration for at least three days and written informed consent. Exclusion criteria were: receiving dialysis, participation in another study and use of anti-Xa inhibitors other than nadroparin or four-factor prothrombin complex concentrate within seven days before the start of the study or during the study. After at least three adjusted doses on the third day of therapy a blood sample was drawn four hours after the administration of nadroparin (therapeutic range: 0.6-1.0 IU/ml).

Results: In total 97 respectively 100 patients with an eGFR < 60 ml/min and > 60 ml/min were included. The mean aXa in the renally impaired group respectively normal renal function group was 0.63 IU/ml and 0.62 IU/ml. The 90% confidence interval of the exact difference of 0.0047 IU/ml lies entirely within the a priori defined equivalence margin of -0.090 IU/ml to 0.090 IU/ml ($p = 0.015$) and therefore equivalence can be assumed. In the renally impaired group 51.6%, 11.6% respectively 36.8% of the patients achieved a sub-, supra- and therapeutic aXa compared with 46.5%, 7.1% respectively 46.5% in the normal renal function group ($p = 0.30$).

Summary/Conclusion: The results in this study show that in the daily clinical practice both patient groups achieve an equivalent mean aXa low within the therapeutic range. Furthermore, both groups show a similar distribution of sub-, supra- and therapeutic aXa. Based on these results we conclude that in renally impaired patients a dosage reduction of therapeutic nadroparin using the dosage guideline of the NfN results in equivalent treatment compared to patients with normal renal function treated with a standard dose.

Bleeding
ECTH-363
Board No. 68: The p.arg498cys mutation in the cysteine rich domain of ADAMTS13 results in a secretion deficiency
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Background: Congenital thrombotic thrombocytopenic purpura (TTP) is characterized by mutations in the *ADAMTS13* gene causing defects in secretion, substrate binding and/or enzyme activity. To date, over 140 mutations have been identified but the contribution of each mutation to the TTP phenotype is not always known.
Aims: We aimed at determining ADAMTS13 antigen (Ag) and activity in a congenital TTP patient, at identifying the mutation(s) in his *ADAMTS13* gene and at studying the effect of the mutation(s) on ADAMTS13 Ag and activity *in vitro*.
Methods: ADAMTS13 Ag and activity, the presence of anti-ADAMTS13 autoantibodies, von Willebrand factor (VWF) Ag and VWF multimer distribution were determined on patient's plasma samples from an acute TTP episode and a remission phase. ADAMTS13 Ag and activity was also determined in the plasma of the patients' parents. Genomic DNA was isolated from the propositus and his parents and all 29 *ADAMTS13* exons and intron-exon boundaries were sequenced. Patient mutations were introduced in the wild type (WT) ADAMTS13 pcDNA6.1 expression vector via site-directed mutagenesis. CHO cells were transfected with WT and mutant *ADAMTS13* expression plasmids and ADAMTS13 Ag present in the expression medium was determined. Cotransfections were performed with a GFP expressing plasmid to determine transfection efficiency.
Results: The patient had no detectable ADAMTS13 Ag during the acute phase and very low Ag during the remission phase (0.04±0.039 µg/mL). ADAMTS13 activity was always below the detection limit. His parents both had ADAMTS13 Ag of 50% and the activity was 71% and 64% for his mother and father respectively. Total VWF Ag was increased in the propositus (193.5±22.4%, $p<0.05$) in the acute TTP episode but not in remission (101.5±14.9%, ns). As expected an increase in high molecular weight VWF multimers compared with normal human plasma (NHP) was observed during remission (43.78±3.46% vs. NHP 34.48±3.74%, $p<0.05$) but not in the acute phase (27.96±2.98%, ns). No (inhibitory) anti-ADAMTS13 autoantibodies were detected. We identified 1 novel heterozygous insertion c.775_776insCGGCGCC in the metalloprotease domain (p.G259PfsX391) resulting in a premature stop codon in the thrombospondin type 1 repeat and detected the previously defined c.1492C>T mutation (p.Arg498Cys) localized in the cysteine-rich domain of the *ADAMTS13* gene. The parents were each carrier of one of the mutations. Whereas construction of the c.775_776insCGGCGCC mutant is currently ongoing, the c.1492C>T mutation was successfully introduced in the WT ADAMTS13 pcDNA6.1 expression vector via site-directed mutagenesis. Transient transfection of WT and p.Arg498Cys expression plasmids revealed that the p.Arg498Cys mutation resulted in a secretion defect as no mutant ADAMTS13 protein was detected in the expression medium. Transfection efficiency was similar for both WT and mutant expression plasmids (32.43±9.44% and 33.42±8.82% respectively) and resulted in 0.04 µg/mL expression of WT ADAMTS13.
Summary/Conclusion: The patient is compound heterozygous for the c.1492C>T (p.Arg498Cys) and the c.775_776insCGGCGCC insertion (p.G259PfsX391). *In vitro* expression of mutant p.Arg498Cys ADAMTS13 revealed a severe secretion deficiency. ADAMTS13 Ag is absent in patient's plasma, it is likely that the c.775_776insCGGCGCC insertion which results in a premature stop codon in the *ADAMTS13* gene will also lead to a secretion deficiency. In conclusion, both mutations explain the absence of ADAMTS13 Ag in the patient's plasma.

Bleeding

ECTH-141

Board No. 69: The use of turoctocog alfa for the prevention and treatment of bleeds in patients with haemophilia A: efficacy data from European countries included in the Guardian™2 clinical trial

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Background: Turoctocog alfa is a B-domain truncated recombinant factor VIII product developed for prophylaxis and treatment of bleeds in haemophilia A. Turoctocog alfa's long-term safety and efficacy are being investigated in the guardian™2 clinical trial. Variability in annualised bleeding rate (ABR) between countries has been previously noted in this trial; however, an earlier analysis to explore whether prior treatment may underpin this finding was inconclusive.
Aims: Here we have investigated whether ABR variation in European countries may be related to differences in patient age between countries.
Methods: Data were analysed from previously treated patients without inhibitors (<0.6 Bethesda Units) participating in guardian™2 as of the interim cut-off (31 December 2013). Patients received turoctocog alfa for prophylaxis (adults/adolescents: 20–40 IU/kg every second day or 20–50 IU/kg 3x/week; children: 25–50 IU/kg every second day or 25–60 IU/kg 3x/week) and treatment of bleeds. ABRs were determined per European country and age.
Results: Of 200 patients, 98 were from Europe (Croatia, Germany, Italy, Latvia, Lithuania, Macedonia, Poland, Russia, Serbia, Spain, Switzerland and UK). The majority of patients were adults (n=60); six countries included paediatric patients (n=29), while four had adolescent patients (n=9). ABR following prophylaxis with turoctocog alfa ranged from 0.09 (Poland) to 5.40 (Macedonia). For adult patients only, ABR ranged from 1.09 (Croatia) to 7.17 (Russia). While data from Poland (lowest ABR) were from paediatric patients only (n=5), data were from adult patients only in Croatia (second lowest ABR). No significant association between ABR and patient age was observed. No patients developed FVIII inhibitors.
Summary/Conclusion: While ABRs were low, variation was observed across European countries. As this variability cannot be explained by differences between countries in patient age, further analyses on an individual level may be needed.

Bleeding

ECTH-388

Board No. 70: Recombinant von Willebrand factor-based enzyme-linked immunosorbent assay and antibodies detection in 31 patients with acquired von Willebrand syndrome

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Background: Acquired von Willebrand Syndrome (AVWS) is an uncommon bleeding disorder defined by an acquired qualitative or quantitative von Willebrand Factor (VWF) deficiency. Diagnosis is difficult but essential since severity, treatment strategies and their efficacy differ, depending on VWF deficiency mechanism. Anti-VWF antibodies should be investigated as their patho-physiological role is yet unclear, and they can be associated with several underlying disorders. However, there is no gold standard for auto-antibodies research, as neutralizing mixing studies show poor sensitivity and their interpretation can prove to be difficult. Furthermore, Enzyme-linked immunosorbent assay (ELISA) studies using plasma derived-VWF (pVWF) is poorly standardized and causes false positive results in blood group O patients.
Aims: Our aims were to develop an ELISA test using recombinant VWF (rVWF) identifying anti-VWF antibodies free of blood group interference, and to apply it to patients with AVWS.
Methods: Thirty-one consecutive patients from 16 French hospitals were included, for whom an anti-VWF antibody research was prescribed because of AVWS suspicion. Clinical and biological data were recorded. After analytical conditions determination of our rVWF direct coating-based ELISA, we calculated a fixed Optical Density (OD) cut-off (mean+1.645DS), in order to classify samples either as reactive or negative, and a fixed Optical Density Difference (ODD) cut-off, used to confirm signal specificity, thereby determining positive samples among "reactive samples". Cut-off value was determined using 41 healthy volunteers' plasma samples, among whom 20 women and 21 men, 21 of O blood group and 20 of non O blood group (A, B or AB). Quantitative variables were compared, using a Mann-Whitney test. Qualitative variables were compared using Fisher's exact test.
Results: Clinical features were comparable to the ISTH registry, published in 2000. The most frequent underlying disorder is MGUS, followed by Waldenstrom's macroglobulinemia. No IVIg failure was reported and VWF concentrates were unsuccessful when administered, which was in favour of an accelerated clearance of VWF through auto-antibodies in these patients. Blood group was O in 15 patients and non O in 15 patients. There was no statistical difference between cut-off values among O blood group and non-O blood group healthy subjects. Proportions of positive samples were comparable between O and non-O blood group patient' samples. Fifteen plasma samples were considered "reactive". Among them 9 were positive (i.e. 28% of cases). Positive anti-VWF antibody patients had significantly higher OD, lower FVIII:C and VWF:Ag, and higher VWF:pp/VWF:AgF ratio. Interestingly, previous pVWF ELISA testing showed both false positive and false negative results when compared to rVWF. The low frequency of anti-VWF antibodies might be explained by the presence of plasmatic anti-VWF/VWF immune-complexes, their clearance being too rapid to find free antibodies in plasma samples.
Summary/Conclusion: In conclusion, we developed an ELISA test for anti-VWF antibody research in AVWS using rVWF, independent of blood group epitope interference, contrary to the previous pVWF test, and appearing to be more sensitive than pVWF ELISA.

Bleeding
ECTH-390
Board No. 71: Effects of fibrinogen and platelet transfusion on clot formation and platelet aggregation in cardiac surgery patients with ongoing bleeding
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Background: Excessive bleeding occurs in approximately 10-15% of cardiac surgery patients. The bleeding may be caused by surgical factors and/or an impaired haemostasis. Transfusion of fibrinogen and platelet concentrate is used clinically to improve haemostasis and reduce bleeding. However, a systematic investigation of the effects of fibrinogen and platelet transfusions on clot formation and platelet function in cardiac patients with ongoing bleeding has previously not been performed.
Aims: The aim of this study was to examine the effects of fibrinogen and platelet transfusion on clot formation and platelet aggregation in cardiac surgery patients with ongoing bleeding.
Methods: In a prospective observational study, 51 patients with ongoing significant bleeding after open heart surgery were included. Patients received either fibrinogen concentrate (median dose 2(range 1-3) g) (n=16), platelet concentrate (median dose 2(1-3) units) (n=15), or both fibrinogen and platelets (median doses 2(1-4) g and 2(1-3) units, respectively) (n=20). Blood samples were collected immediately before and 15-30 minutes after the transfusion was completed. Clot formation was analysed with modified rotational thromboelastometry using extem and fibtem tests with tissue factor as initiator. Platelet aggregation was assessed with impedance aggregometry induced with ADP, arachidonic acid or thrombin receptor activating peptide-6 (TRAP). In addition, fibrinogen concentration, platelet count and bleeding volumes were measured before and after the transfusion. The study was approved by the regional Research Ethics Committee, who waived individual patient consent, and was performed according to the Declaration of Helsinki.
Results: Transfusion of fibrinogen concentrate increased fibtem maximum clot firmness with 2.8±0.8 mm (mean ± standard error of the mean, p=0.008) and increased the plasma concentration of fibrinogen with 0.5±0.1 g/L (p=0.001), but had no effect on platelet aggregation. Platelet transfusion shortened extem clotting time by 4.1±1.7 seconds (p=0.019), improved platelet aggregation induced by arachidonic acid by 25±8.2 U (p=0.011) and increased the platelet count with $43±9.9×10^9$/L (p=0.001). Transfusion of both fibrinogen and platelet concentrate shortened extem clotting time by 16±3.4 seconds (p<0.001), increased fibtem maximum clot firmness by 3.9±0.6 mm (p<0.001), increased fibrinogen concentration with 0.5±0.1 g/L (p<0.001) and increased the platelet count with $53±10×10^9$/L (p<0.001). In addition, platelet aggregation was improved by transfusion with fibrinogen and platelets. The increase was 7.4±2.5 U (p=0.012), 39±9.0 U (p=0.001) and 15±3.8 U (p=0.003) for ADP-, arachidonic acid- and TRAP-induced aggregation, respectively.
One third of the 51 patients (n=17) proceeded to re-exploration because of sustained excessive bleeding, and surgical cause of bleeding was found at re-exploration in 11/17 patients. Transfusion of fibrinogen, platelets, or both, decreased bleeding from 172±24 mL/h during the last two hours before transfusion vs 109±20 mL/h during the first two hours after transfusion (p<0.001).
Summary/Conclusion: The results suggest that transfusion of fibrinogen and/or platelet concentrate to bleeding patients after open heart surgery improves haemostasis significantly and reduces bleeding.

Bleeding

ECTH-221

Board No. 72: Efficacy and safety of long-acting recombinant fusion protein linking factor IX with albumin (rIX-FP) in haemophilia B patients undergoing surgery

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Background: IDELVION®, a recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP), has recently been approved in the US and Canada for prophylaxis and on-demand treatment of children and adults with hemophilia B. IDELVION® has an improved pharmacokinetic profile, which allows dosing every 7–14 days.

Aims: The PROLONG-9FP clinical trial program assessed the pharmacokinetics, safety and efficacy of rIX-FP across five clinical trials; a sub-study was included in three of these trials to assess the hemostatic efficacy of rIX-FP during surgical procedures.

Methods: Patients with moderately severe to severe hemophilia B (FIX activity ≤2%), participating in three Phase III clinical trials, one in adult and adolescents (NCT02053792), one in pediatrics (NCT01662631) and an extension study (NCT02053792), and undergoing a minor or major surgical procedure were included. For major surgeries, a single bolus dose of rIX-FP was given preoperatively to a target activity of 80–100 IU/dL; subsequent intraoperative dosing of rIX-FP was guided by FIX activity and surgery type. From wound closure to 14 days, rIX-FP was dosed in order to maintain FIX activity in line with WFH postoperative guidelines. Hemostatic efficacy was assessed by the investigator for up to 72 hours after surgery; additional efficacy assessments included re-bleeding events within 72 hours and rIX-FP consumption during the 14-day postoperative period. Safety measurements included adverse events (AEs), inhibitors to FIX and antibodies against rIX-FP. Written informed consent was obtained from all patients or their legal guardians.

Results: Twenty-one surgeries were performed in 19 patients, including 9 major orthopedic surgeries in 8 subjects. Hemostatic efficacy was rated as excellent (n=17) or good (n=4) in all surgeries; in the 9 orthopedic surgeries, hemostatic efficacy was rated as excellent in 7 and good in 2. Intraoperative hemostasis was maintained by a single preoperative dose in 20 of 21 surgeries, and in 8 of the 9 orthopedic surgeries. In 7 of the 9 surgeries, first postoperative dose was more than 24 hours after surgery. Overall, rIX-FP consumption during the 14-day postoperative period was low; median total consumption (including prophylaxis doses) was 375.3 IU/kg. For orthopedic surgeries, median rIX-FP consumption was 87 IU/kg preoperatively and 375 IU/kg overall. All AEs were mild or moderate, and were not considered to be related to rIX-FP administration. No subject developed inhibitors to FIX or antibodies to rIX-FP.

Summary/Conclusion: For the majority of surgeries, a single preoperative dose of rIX-FP maintained FIX activity levels throughout the surgical procedure without the need for additional intra- or postoperative dosing on the day of surgery. Total consumption of rIX-FP was low and less than that reported with conventional FIX replacement therapies.

Bleeding

ECTH-408

Board No. 73: Acquired von Willebrand disease associated with mantle cell lymphoma: a case report

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Background: Acquired von Willebrand disease (aVWD) is a rare bleeding disorder, associated with a variety of underlying disorders. Due to similar clinical and laboratory manifestations, differentiation of congenital and acquired VWD can be challenging. A negative family history and onset of bleeding symptoms later in life make an aVWD more likely. When the suspicion of aVWD is high, a search for the underlying cause is frequently initiated.

Aims: To demonstrate a rare case of aVWD associated with a mantle cell lymphoma and to make clinicians aware that the absence of inhibitors in mixing studies does not reject the diagnosis of aVWD.

Methods: We conducted a thorough analysis of the patient file and carried out an extensive literature search in the Pubmed database.

Results: A 61-year-old male suffered from recurrent epistaxis, hematuria, spontaneous hematomas and rectal bleeding since a few months. Besides bleeding after a transurethral resection of his prostate in 2003, no bleeding complications occurred during and after multiple surgical procedures. Family history was negative for bleeding disorders, although his father died suddenly from an intestinal bleeding. Initially, physical examination was normal. Laboratory studies revealed a low factor VIII-activity (0.43 IU/ml) and VWF antigen level (0.31 IU/ml). Ristocetin cofactor activity and collagen binding activity were markedly reduced (0.09 IU/ml and 0.10 IU/ml respectively). The highest molecular weight VWF multimers were absent. A diagnosis of VWD type 2A was made, but no genetic mutation was found. Because of a high suspicion of aVWD, mixing tests were done. No inhibitory antibodies directed against VWF or factor VIII were detected. A few months later, physical examination showed slightly enlarged cervical, axillary and inguinal lymph nodes. Factor VIII-activity was 0.07 IU/ml and VWF antigen 0.22 IU/ml. Again, no auto-antibodies were found. A CT-scan confirmed multiple enlarged lymph nodes. A clonal B-cell population matching a mantle cell lymphoma was detected in the bone marrow. Initiation of treatment with prednisone and R-CHOP resulted in a rapid decrease of bleeding problems and complete normalization of factor VIII activity and VWF antigen.

Pathogenic mechanisms of aVWD are highly heterogeneous. In patients with lymphoproliferative disorders, aVWD can be caused by the development of auto-antibodies. However, the detection rate of inhibitors is low due to the unavailability of standardized assays, saturation of antibodies in immune complexes and the presence of non-neutralizing auto-antibodies that increase VWF clearance. Other mechanisms in lymphoproliferative diseases include tumor-cell expression of the platelet VWF receptor (GPIb) and selective binding of VWF onto these malignant cells. Increased proteolysis of VWF and, rarely, reduced synthesis of VWF have been reported in association with a variety of other underlying disorders.

Summary/Conclusion: We describe a rare case of a patient with aVWD caused by a mantle cell lymphoma. Initiation of the appropriate treatment with immune-chemotherapy completely corrected the acquired coagulopathy. Because of difficulties in the detection of auto-antibodies and the fact that different mechanisms contribute to the pathogenesis of aVWD, negative mixing studies do not reject the diagnosis of aVWD.

Bleeding

ECTH-271

Board No. 74: Validation of a tissue plasminogen activator induced thromboelastometry (tPA-rotem) in chemotherapy induced thrombocytopenic patients

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Background: Prophylactic platelet transfusions are often given at a total platelet count of $<10x10^9$/L in haemato-oncological patients with chemotherapy induced thrombocytopenia (CIT). However, platelet count alone is a poor predictor of bleeding. In search for other predictors of bleeding, we try to determine variables for clot stability and bleeding in these patients. In this observational study, clot stability and resistance to fibrinolysis in patients with CIT is tested using a tissue plasminogen activator induced thromboelastometry (tPA-ROTEM) (Kuiper et al. Thrombosis Journal. 2016; 14:1).
Aims: The aim of this observational study is to validate the tPA-ROTEM in chemotherapy induced thrombocytopenic patients.
Methods: Between October 2015 and April 2016, citrated blood samples were taken from chemotherapy induced thrombocytopenic patients on the haematology ward of the Maastricht University Medical Centre+ (MUMC+). Patients were excluded if they had an active bleeding, splenomegaly or used anticoagulation medication. Results were compared to data from 40 healthy volunteers (Kuiper et al. Thrombosis Journal. 2016; 14:1). The maximum clot firmness (MCF), the lysis onset time (LOT), defined as time to initiation of clot lysis (85% of MCF) and the lysis time (LT), defined as completion of lysis (10% of MCF), were measured. Furthermore, 100-10% lysis speed (MCF-LT), 100-85% lysis speed (MCF to LOT) and 85- 10% lysis speed (LOT to LT) were calculated in mm/min and %(of MCF)/min.
Results: tPA-ROTEM results were obtained from 79 measurements in 54 patients with CIT. We compared the results of these patients to the results of the tPA-ROTEM of 40 healthy individuals. MCF is significantly lower in thrombocytopenic patients compared to healthy individuals (median MCF 26mm vs. 60mm, $p < 0.05$, 95%CI 31-37). MCF in thrombocytopenic patients highly correlates with platelet count ($r^2=0.75$), not with fibrinogen levels ($r^2=0.04$). LOT and LT are significantly shorter in thrombocytopenic patients compared to healthy individuals (median LOT 1479s vs. 2148s, $p < 0.05$ 95%CI 429-780; median LT 2404s vs. 3060s, $p < 0.05$ 95%CI 444-994). Lysis speed 100-85% was significantly faster in patients with CIT compared to healthy individuals when measured in mm/min and %/min (median LS100-85% 0.92mm/min vs. 0.51 mm/min, $p < 0.05$ 95%CI 0.15-0.25; median LS100-85% 1.27%/min vs. 0.88 %/min, $p < 0.05$ (0.38-0.74). Lysis speed 100-10% and 85-10% calculated in mm/min was significantly slower in patients with CIT compared to healthy individuals (median LS100-10% 0.80mm/min vs. 1.61mm/min, $p < 0.05$ 95%CI 0.47-0.89; median LS85-10% 1.15mm/min vs. 2.75mm/min, $p < 0.05$ 95%CI 1.27-2.06). However, when calculated in %/min there was no significant difference. Within the group of patients with CIT high variability is seen in tPA-ROTEM results.
Summary/Conclusion: Patients with chemotherapy induced thrombocytopenia have weaker clots (lower MCF) probably due to low thrombocyte count. Their clots are also more susceptible to fibrinolysis, showing in a higher lysis speed during the start of fibrinolysis (100%>85% of MCF). We also see high variability within the group of patients with CIT. Further investigation is needed to find out why these differences occur and if these difference might explain why some patients with CIT bleed and some do not bleed.

Bleeding

ECTH-503

Board No. 75: Changes in blood coagulation and in blood rheology and in properties of fibrin clot during the first month of treatment with ibrutinib

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Background: It is known that ibrutinib treatment leads to hemorrhagic complications in 1-5% of cases due to ibrutinib impacts negatively to platelets functions. The nature of this influence is not clearly revealed.

Aims: The aim was to study changes in activity of blood clotting, in blood rheological properties and in properties of fibrin clot during the first month of treatment with ibrutinib.

Methods: We examined 14 patients with chronic myeloid leukemia (CML) treated at Moscow City's Hematological Centre of City's Clinical Hospital n.a. S.P.Botkin. Two patients (14,3%) developed petechiae during followup. All patients were study by hematological parameters, hemocoagulation screening, thromboelastography in three types, platelet aggregation and hemorheologic assays before treatment and within intervals 1-2 and 3-4 weeks of treatment.

Results: Under treatment all patients showed nonsignificant raise in leukocytes, monocytes, lymphocytes and significant increase of neutrophils. No significant differences were for platelet counts between the stages of follow-up. However patients with petechiae had somewhat low platelet count in comparison to other patients.

ADP-induced platelet aggregation increased as after the start of treatment as well as during treatment. The same trends were revealed for ristomycin-induced platelet aggregation.

No significant changes were for relative blood viscosity values and for activity of erythrocyte aggregation/disaggregation and for erythrocyte deformability.

Under ibrutinib APTT was prolonged, another screening hemocoagulation parameters including fibrinogen and D-dimer did not change significantly. It was found the improvement of fibrin clot properties (density, elasticity and firmness) mainly due to platelet activation. Data analysis revealed interrelation between clot properties and collagen-induced platelet response.

Summary/Conclusion: Thus we have performed the first such study in Russia. It can be assumed that the cause of the petechiae developed in 2 patients was individually low platelet count which had not enough compensated from other reserves of the blood coagulation. The increases of ADP- and ristomycin-induced platelet aggregation, apparently, can be considered like an inflammatory response. The correlation between clot parameters and collagen-induced platelet aggregation suggests that ibrutinib affect platelet adhesion. In any case unclear nature of ibrutinib effects to hemocoagulation have requires to continue the study.

Bleeding
ECTH-344
Board No. 76: The decrease in fibrinogen during cardiopulmonary bypass consistently and independently predicts the risk of red blood cell transfusion in cardiac surgery patients
Felipe Guerrero[*], François Labaste[1], Hélène Charbonneau[1], Sophie Leclerc[1], Amel Daboussi[1], Violaine Calmels[2], Vincent Minville[1], Nicolas Savy[3]
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Background: Many cardiac surgery patients bleed in excess with an increased risk of morbidity and the need for blood transfusions. Several prediction scores have been developed over more than a decade in order to identify high risk patients. Despite these efforts transfusion remains frequent. Fibrinogen supplementation has become a first line treatment to control acquired bleeding. However fibrinogen is not considered as a prognostic risk factor in the main prediction scores developed in this setting.
Aims: Evaluate the role of the patient's fibrinogen concentration in the risk stratification of blood transfusion.
Methods: We performed a prospective observational study in three cohorts of consecutive cardiac surgery patients operated in Rangueil Hospital in Toulouse France: 79 patients in 2013 (derivation cohort), 171 in 2014 (validation cohort) and 206 in 2015 after an important change in the bleeding management that was adopted by all anesthesiologists and surgeons (confirmation cohort). The patient was considered transfused if he received at least one blood cell pack during his hospital stay. Risk factors where identified with multivariate logistic regression, prognostic information was estimated with ROC curves, AUC-ROCs were compared by DeLong's test and estimates were validated by bootstrapping. The study was approved by the local ethics committee.
Results: The risk of transfusion can be predicted (multivariate analysis) with two variables: first, the difference between fibrinogen measured before surgery and at the end of cardiopulmonary bypass (OR=3.9 for a 1g/L difference, p=0.03); second, a preoperative hemoglobin concentration below 12g/dl (OR=11, p<0,001). The AUC-ROC for transfusion using these two variables is 0.81 (IC95=0.71-0.90). These results were confirmed in the validation cohort (OR=4.1 for a 1g/L difference in fibrinogen, p=0.002; OR=4.4 if hemoglobin <12g/l, p<0.001; AUC-ROC=0.75, IC95=0.68-0.83; p=0.34 for AUC-ROC comparison between the 2 cohorts). The decrease in fibrinogen is directly correlated to the preoperative fibrinogen concentration and to the volume (ml) of perfusions administered before the end of cardiopulmonary bypass adjusted to the patient's body surface (m²) (p<0,001 for both variables, Pearson's coefficient = 0.54 for the linear model). In the confirmation cohort, a preoperative fibrinogen concentration above 4 g/L increases the risk of transfusion (OR=2.8, p=0.02, IC95=1.2-6.3) independently of preoperative hemoglobin concentration.

Summary/Conclusion: The decrease in fibrinogen concentration during cardiopulmonary bypass independently predicts the risk of red blood cell transfusion in most cardiac surgery patients. The preoperative fibrinogen concentration predicts the decrease in fibrinogen. The relation between fibrinogen and transfusion is independent of the bleeding management and of hemoglobin concentration. This surprising strong correlation between the patient's preoperative fibrinogen, fibrinogen consumption and the risk of bleeding warrants further study. These correlations need to be confirmed by multicenter studies.

Bleeding

ECTH-251

Board No. 77: D-dimer measured at diagnosis is associated with risk of major bleeding events during the 1-year after incident venous thrombosis

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Background: Accurate prediction of bleeding-risk in patients with acute venous thromboembolism (VTE) is pivotal to guide appropriate decisions on length of anticoagulant treatment. Discovery of novel risk factors for bleeding is warranted as currently available tools, mainly based on clinical parameters, do not provide accurate predictions of bleeding risk in these patients. D-dimer is measured in patients referred to hospitals under the suspicion of VTE to exclude the diagnosis. However, elevated d-dimer has also been reported in conditions associated with bleeding, such as acute aortic dissection and disseminated intravascular coagulopathy.

Aims: We aimed to investigate whether d-dimer measured at VTE-diagnosis was associated with major bleeding events during the first year after an incident VTE.

Methods: There was 498 out-of-hospital patients that developed an objectively confirmed first-lifetime VTE and had d-dimer measured at time of diagnosis among participants in the Tromsø study (1994/95-2007/08, n=33885) during 1994-2012. Information on hospitalization, co-morbidities and clinical characteristics was obtained from medical records. Major bleeding was defined according to the ISTH recommendation and included fatal and/or symptomatic bleeding in critical area or organ, and/or bleeding causing two units of blood transfusion, and/or a fall in hemoglobin concentration >20.0 g/L. Major bleeding events, death, and migration were registered during the following one year after the incident VTE event. The study was approved by the Regional Committee of Research Ethics, and all participants provided informed written consent.

Results: Out of the 498 patients with incident VTE, 44 developed a major bleeding event during the first year. The one-year incidence rate of major bleeding was 10.7 per 100 person-years (95 % CI: 7.98-14.4). The hazard ratio (HR) of major bleeding per one standard deviation (6.0 mg/L) increase in d-dimer was 1.57 (95% CI: 1.21-2.03) in the multivariable model adjusted for age, sex, body mass index, treatment duration (3, 6 or >6 months), and hypertension. In category-based analyses, VTE patients with d-dimer in the upper 20th percentile (≥9.9 mg/L) at diagnosis had a 3.1-fold higher risk (HR 3.05; 95% CI 1.42-6.56) of bleeding compared with those in the lower 40th percentile (d-dimer ≤2.7 mg/L). The risk estimates remained essentially similar in analyses taking competing risk by death into account, but was somewhat attenuated when patients with overt cancer was excluded from the analyses.

Summary/Conclusion: Our findings suggest that high plasma d-dimer concentration measured at first-time VTE diagnosis is associated with major bleeding events during the following year, and should be considered when treatment duration for first VTE is decided.

Bleeding
ECTH-345
Board No. 78: Are factor IX inhibitors in haemophilia B neglected compared with factor VIII inhibitors in haemophilia A? A developing country experience
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Background: Development of inhibitors in hemophilia A and B is a major concern in hematology field. Inhibitors can bind to coagulation factor VIII and X in the blood and neutralize them. Hence, in patients with hemophilia and inhibitor, no suitable outcome is seen after infusion of coagulation factor VIII and IX concentrates. The existence of inhibitors do not increase the frequency of bleeding episodes, but the management of hemorrhages in patients with inhibitors is more complicated. Owing to more coagulation factor concentrates are used during treatment of bleeding in the existence of inhibitors, these therapeutic regimens are usually more expensive and impose financial load to health provider system. It is recommended by world federation of hemophilia (WFH) to screen patients with hemophilia every 3 months. It seems that in developing countries, there are more reports about inhibitors in hemophilia A and less attention has paid to hemophilia B. To examine current hypothesis, a comparison was done on reported frequency in Iran, a developing country, with more than 8000 registered cases with bleeding disorders.
Aims: The aim was to test whether detection and frequency of inhibitors in hemophilia B is as equals as the frequency of inhibitors in hemophilia A?
Methods: A literature review was done in medical search engines of PubMed, Scopus, Google Scholar, ScienceDirect and domestic medical search engine Iranmedex and sid.ir. The keywords included:" hemophilia A + inhibitor + Iran " and " hemophilia B + inhibitor + Iran". There were no time and language limitations.
Results: After doing a literature review, 13 relevant papers that had paid to inhibitors in hemophilia A and in Iran were found and studied. The relevant full texts were retrieved. Among them, nine papers were selected. Six full papers had paid to inhibitors in hemophilia A and 3 papers to inhibitor B. Overall, the status of inhibitor against factor VIII and IX has been reported in 1717 patients with hemophilia A and 149 patients with hemophilia B. The minimum, maximum and mean of reported percentages of factor VIII inhibitors were 4%, 19.6%, and 14.8% respectively. The minimum, maximum and mean of reported percentages of factor IX inhibitors were 0%, 11.8%, and 6% respectively.
Summary/Conclusion: Given that the frequency of hemophilia B is estimated to be 1/3 of hemophilia A frequency and on the other hand, the frequency of factor IX inhibitors is lower in comparison with frequency of factor VIII inhibitors in hemophilia A, it seems inhibitors in hemophilia B is not neglected point and has paid to it as parallel to hemophilia A in Iran.

Bleeding
ECTH-421
Board No. 79: Binding of von Willebrand factor to membrane microdomains on dendritic cells
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Background: Hemophilia A is an X-linked bleeding disorder caused by reduced levels of blood coagulation factor VIII (FVIII). Approximately 25% of the severe hemophilia A patients develop inhibitory antibodies following treatment with FVIII. The initial step in FVIII inhibitor formation is the endocytosis of FVIII by professional antigen presenting cells such as dendritic cells (DCs). Von Willebrand factor (VWF) may affect FVIII immunogenicity by modulating its uptake by antigen presenting cells. We have recently demonstrated that VWF binds to the surface of dendritic cells. Unexpectedly, VWF was not internalized by dendritic cells but remained bound to the surface of immature DCs. We also showed that in contrast to VWF, FVIII-derived peptides were presented on MHC class II. Therefore, surface bound VWF not only reduces the uptake of FVIII but also modulates the repertoire of FVIII-derived peptides on MHC class II. Deciphering how VWF interacts with DCs will give new insight on the pathogenesis of the anti-FVIII immune response.
Aims: To identify membrane components contributing to the surface binding of VWF to dendritic cells.
Methods: Immature DCs (iDCs) derived from monocytes of healthy donors were used for all experiments. Lipid rafts were enriched from iDCs using an OptiPrep density gradient and ultracentrifugation method. Enrichment of lipid rafts was validated by Western blot analysis. The enriched micro-domains were separated by SDS-PAGE and subsequently analyzed by mass-spectrometry. To identify the binding partners of VWF on dendritic cell membranes, recombinant VWF was pre-incubated with iDCs. Confocal microscopy was employed to determine whether VWF colocalized with membrane constituents expressed on iDCs.
Results: Confocal imaging revealed that VWF binds to the surface of iDCs but was poorly endocytosed. Interestingly, the punctual staining pattern observed suggested that VWF is concentrated in lipid rafts. To address this issue we performed co-stainings with flotilin-1, an established marker of lipid rafts. Both VWF and flotillin-1 were found to be organized in microdomains but staining of VWF did not overlap with flotillin-1 staining. These findings show that VWF is not bound to lipid microdomains that are enriched for flotillin. Then we isolated the lipid microdomains employing density gradient fractionation. Membrane fractions were subsequently analyzed by mass spectrometry. Using this approach, we identified several potential binding partners for VWF, including galectin-1 and -3. Morphological analysis revealed that galectin-1 and -3 are expressed in membrane microdomains in iDCs. Co-staining with flotillin-1 revealed that the membrane microdomains containing galectin-1 or 3 did not overlap with flotillin-1 containing lipid rafts. Surprisingly, VWF did not co-localize with either galectin-1 or 3 on iDCs suggesting that galectin-1 or 3 are not a major binding partner for VWF on iDCs.
Summary/Conclusion: VWF associates with membrane microdomains on iDCs that are distinct from flotillin-1 containing lipid rafts. Also membrane microdomains consisting of galectin-1 and 3 do not contribute to the binding of VWF on the surface of iDCs.

Bleeding

ECTH-267

Board No. 80: A patient with no detectable plasma factor V, two mutations identified in the Factor 5 gene and no bleeding history

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Background: A woman of Italian origin was investigated for a possible bleeding disorder following a heavy postpartum haemorrhage. She was shown to have total factor V deficiency. Previously she had undergone dental extraction and adenoidectomy without excessive blood loss and had no history of abnormal bruising or bleeding.
Aims: To characterise the bleeding phenotype and determine the molecular basis of the factor V deficiency.
Methods: Prothrombin time, activated partial thromboplastin time (APTT), one stage coagulation factor assays, calibrated automated thrombography (CAT) and tissue factor activated rotational thromboelastometry testing were performed. Free tissue factor pathway inhibitor (TFPI) levels were quantified by ELISA. Factor 5 gene (F5) mutation analysis was performed by fluorescent sequence analysis of exons 12 and 16 of the F5 gene.
Results: The prothrombin time was 34 seconds (normal range 9.8-12.6 seconds) and the APTT was 81 seconds (normal range 25.8-35.0 seconds). Factor V activity was < 0.01IU/mL (normal range 0.67-1.28IU/mL). Rotational thromboelastometry data showed a slightly increased Clot Time of 686 seconds (normal range 262-502 seconds) with other parameters being within normal reference limits. Similarly CAT showed prolonged Lag Times for platelet poor plasma (PPP) of 11.6 minutes (normal range 1.8-3.2 minutes) and 16.3 minutes (normal range 2.9-8.4 minutes) for platelet rich plasma (PRP). The Endogenous Thrombin Potential was minimally reduced for PPP 936nmol/L.min (normal range 1121-1816nmol/L.min) although normal for PRP 1415nmol/L.min (normal range 1263-1841nmol/L.min). Free TFPI levels were slightly reduced at 7.6ng/mL (normal range 7.7-26.5ng/mL). Factor 5 gene sequencing showed that she was a compound heterozygote for two pathogenic variants: a c.5408A>G, p.His1803Arg mutation, and a novel duplication c.1830_1831dupGC, p.His611ArgfsTer13.
Summary/Conclusion: A novel two nucleotide duplication was identified within the F5 gene sequence that causes a frame shift and a premature stop codon. This factor V null allele was co-inherited with a mutation previously described and associated with factor V deficiency. Given the low factor V level we anticipate that the p.His1803Arg mutation is a folding defect causing a lack of factor V secretion. However given the CAT results we anticipate that any secreted factor V protein is functional. Further investigations are currently being performed.

Bleeding
ECTH-162
Board No. 81: Next-generation sequencing of the whole F8, F9 and VWF genes allows establishing a molecular diagnosis in haemophilia A and B
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Background: Current diagnosis in hemophilia requires molecular diagnosis in order to: distinguish von Willebrand disease (VWD); highlight the excess of risk inhibitor development associated with specific mutations; and, enable carrier testing for female relatives and prenatal or pre-implantation genetic diagnosis. Currently, Next-Generation Sequencing (NGS) allows simultaneous investigation of several entire genes despite they could span very extensive regions.
Aims: The goal of this study was to evaluate the usefulness of a molecular algorithm incorporating a NGS approach to sequence the whole *F8*, *F9* and *VWF* genes.
Methods: The proposed algorithm includes the detection of inversion of introns 1 and 22, NGS custom panel (*F8*, *F9* and *VWF* whole genes), and Multiplex Ligation-dependent Probe Amplification (MLPA) analysis.
Results: A total 102 samples, ninety-seven HA and HB patients, and five female carriers, were included in the study. IVS-22 screening found eleven out of twenty severe HA patients and one female carrier. IVS-1 analysis did not find any alteration. NGS approach provided a positive result in 89 cases, allowing a differential diagnosis between mild/moderate hemophilia A and VWD in eight cases. In addition, 2 genetic variants were found in deep intronic regions, in 4 mild hemophilia A patients. MLPA confirmed large exons deletions. Only one case did not show pathogenic mutations. The overall success rate of the proposed algorithm was 99%.
Summary/Conclusion: This evaluation demonstrates that this algorithm can reliably identify pathogenic variants and accurate diagnosis of patients with HA, HB or VWD.

Bleeding

ECTH-504

Board No. 82: Assessment of new definition of tarjet joint proposed by international society on thrombosis and haemostasis (isth) in patients with hemophilia.

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Background: In the last decades several definitions for a target joint (TJ) have been proposed, all of them define a TJ as one in which at least three or four bleeds have occurred within a 3–6-month period. The definition proposed by the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) Group is as follows: three or more spontaneous bleeds into a single joint within a consecutive 6-month period.

Aims: This observational study was designed to identify the TJ, according to the new definition proposed by ISTH Group, in patients with hemophilia attending at Hemophilia Unit during the last 2 years.

Methods: We analyzed 29 patients diagnosed of severe hemophilia attending at our Hemophilia Center. We collected data on demographic and clinical characteristics of patients, type and severity of hemophilia, factor replacement products, type of joint affected by bleeding and radiologic findings in TJ.

Results: Median age was 21 years (range: 1-50 years). Out of total 29 cases 25 were diagnosed as hemophilia A and 4 cases of hemophilia B. According to their factor level all cases had severe disease. 28 patients with severe hemophilia were primarily treated with different schemes of prophylaxis (8 patients received factor clotting every 48 hours, 10 patients 3 times per week, 9 patients 2 times per week and 1 every 2 weeks) and one patient was primarily treated on demand. Median number of joint bleeds was 1 (range: 0-6).

Ankle joint was the predominant joint affected in 11 cases followed by knee joint in 10 cases and elbow joint in 7 cases. Out of total 3 patients of severe hemophilia had developed target joint according to the new definition of TJ by ISTH Group. Ankle joint was the predominant TJ in 2 cases and knee joint in 1 case. All these patients had adequate adherence to prophylaxis program at time of the events. Radiological findings of hemophilic arthropathy were detected in 7 patients by Magnetic Resonance: 3 cases (27.3%) correspond to TJ according to ISTH and 4 cases (36.3%) without clinical findings of TJ.

Summary/Conclusion: TJ definitions based solely on clinical criteria underestimate the true impact of TJ in patients with severe hemophilia. It would be advisable to incorporate radiological parameters together with clinical criteria to evaluate TJ without clinical joint manifestations in hemophilic patients.

Bleeding
ECTH-507
Board No. 83: Comorbidities in older patients with hemophilia. A retrospective study
Gonzalo Ferrer Garrido[1], Natalia Espinosa Lara[1], Laura Lacalle Aicua[1], Adrian Segura Díaz[1], Elivira Salvador Rupérez[1], Marta Villalba Montaner[1], Nuria Fernández Mosteirin[1], José Manuel Calvo Villas*[1], Pilar Delgado Beltrán[1]
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Background: Life expectancy for persons with hemophilia has increased significantly during the last decades resulting in a potentially normal life span. As a consequence, a growing number of hemophilic patients develop age-related comorbidities, such as cardiovascular disease, cancer and chronic diseases.
Aims: To identify and estimate the prevalence of age-related comorbidity associated with middle-aged and elderly hemophilia patients attending at Hemophilia treatment center.
Methods: In this retrospective study, we analyzed 24 hemophilia A or B (HA/HB) patients older than 40 years who were attended at least once during the last 2 years. We collected data on demographic and clinical characteristics of patients, type and severity of hemophilia, factor replacement products, survival and non-hemophilic-related morbidity. We defined comorbidity as the presence of one or more diseases/conditions, other than the patient's hemophilia disease. The presence of cardiovascular disease, infections and liver disease, malignancies, renal disease and chronic renal failure (CRF), joint disease and general comorbidity was reviewed. Data were obtained from the patient's medical charts.
Results: Of the 24 patients who met the inclusion criteria, 19 patients (71%) had HA. Median age in HA patients was 55 years (40-77) and 54 years (47-58) in HB patients. Besides, these patients were treated on demand and 19 had mild hemophilia (16 HA and 3 HB). Age distribution: 40-55 years: 12 cases, 56-70 years: 9 patients, >70 years: 3 patients. The main comorbidities were 2 cardiovascular diseases, 13 hemophiliacs (54%) had HCV infection, 3 of them developed liver cirrhosis, 5 malignancies, 6 renal diseases (5 CRF) and 16 joint diseases. The most common conditions identified were 6 arterial hypertension, 8 obesity / dyslipidemia, 2 chronic obstruction pulmonary disease, 3 diabetes and 5 cases of smoking and 2 patients chronic inflammatory bowel disease. Factor concentrates were administered prior to surgery / invasive procedures in 5 cases: 2 mild HA prior to surgery of basal cell carcinoma, 1 mild HA prior to coronary angiography (currently treated with aspirin), 1 mild HA with gonarthrosis prior to implantation of a prosthetic knee, 1 moderate HB patient with myelodysplastic syndrome prior bone marrow aspiration. One patient died during the study period due to HCV cirrhosis.
Summary/Conclusion: The prevalence of comorbidities in patients with hemophilia older than 40 years are similar to those reported in the literature, although we observed a lower prevalence of liver cirrhosis compared to other studies. Despite of the prevalence of cardiovascular risk factors (hyperlipidemia or hypertension) is high (20-30%), the low prevalence (<10%) of cardiovascular disease in our series suggests a protective effect of hemophilia. The increase of the life expectancy among patients with hemophilia determines the onset of age-related comorbidity and require greater care in managing their health needs and caring.

Bleeding
ECTH-409
Board No. 84: Von Willebrand disease: analysis of von Willebrand factor multimers
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Background: Von Willebrand disease (vWD) is the most common inherited bleeding disorder caused by quantitative or qualitative abnormalities of von Willebrand factor (vWF). vWF is a glycoprotein synthesized in megakaryocytes and endothelial cells. vWF is a mediator of platelet adhesion to subendothelium, the FVIII carrier, which protects it from proteolysis. The analysis of vWF multimers is of fundamental importance in diagnostics and treatment of vWD. HMW (high molecular weight multimers are necessary for the effective function of the vWF multimers. Willfact is usually used for the prevention and treatment of surgical or other bleeding in patients with vWD when treatment with desmopressin (DDAVP) is ineffective or contraindicated.
Aims: To present our results related to the research and analysis of vWF multimers.
Methods: The principle of the multimer analysis is the electrophoretic separation of vWF multimers on SDS agarose gels based on their molecular weight, followed by non-radioactive visualization using an alkaline phosphatase conjugated antibody system.
Results: We compared the vWF mutimer analysis of the sample in patient with vWD before and after treatment with concentrate of Willfact. In the sample of patient treated with Willfact the vWF multimer analysis discovered the forms of multimers (including HMW) visualized each as clearly separated strips confirming efficacy of the substitution therapy.
Summary/Conclusion: vWF multimers analysis is a method for analysing the concentration and distribution of vWF multimers present in plasma. Depending on the methodology used the sample can be visualized with 10 – 20 multimers. The structure of vWF multimers in plasma vWF complex results of metabolism and its individual processes – synthesis, post-translational modifications, secretion, proteolysis, and clearance. The change of one or more processes leads to the characteristic changes in vWF multimers. Even though the vWF multimer analysis is time-consuming and costly, it is important in the diagnosis of vWD and monitoring of treatment response.
Acknowledgement: This work was supported by projects APVV 0222-11, Virtual and simulation education (IMTS 26110230071) and BioMed Martin (ITMS 26220220187), which are co-financed from EU sources.

Bleeding

ECTH-412

Board No. 85: Resolution of acquired haemophilia A in a patient with HCV/HIV coinfection after hepatitis C treatment by sofosbuvir and daclatasvir

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Background: Acquired haemophilia A can be idiopathic or associated with modification of immune system in conditions like post-partum, lymphoproliferative disorders, long term immunosuppressive treatments, HIV/HCV infections etc. HCV chronic infection has been associated with immunological disorders and could be involved in anti-FVIII auto-antibodies production, independently of interferon alpha treatment.

Aims: We report here the case of an HIV/HCV co-infected patient who developped acquired haemophilia A.

Methods: Longitudinal clinical and biological follow-up of the patient.

Results: Acquired haemophilia A was diagnosed fortuitously on coagulation testing, with FVIII at 2% and auto-antibody titer of 5 Bethesda units. At this time, HIV was controlled by a highly active antiretroviral therapy associating abacavir, lamivudine and efavirenz. A previous treatment by interferon alpha and ribavirin for HCV had been arrested for five years, because of haematological toxicity. Considering the infectious risk in this context of HIV/HCV coinfection, a unique corticosteroid therapy was introduced to treat acquired haemophilia A, but was not followed by the patient. After a first event of post-traumatic deep hematoma several months later, for which activated prothrombinic complex concentrates were administrated, a corticosteroid therapy was reintroduced. Despite a slight increase of FVIII level to 36% after five months of treatment, a relapse was observed (FVIII at 6% one month later). A new event of hematoma associated with severe deglobulisation occurred, leading to a few day-use of another by-passing agent, recombinant activated factor VII. A treatment associating corticoid and cyclophosphamid for at least 3 months was introduced. Indeed, in this context of acquired haemophilia A therapeutic failure and genotype 1a HCV induced cirrhosis, the patient benefited from the new C hepatitis treatment by an association of direct antiviral agents: sofosbuvir and daclatasvir for 3 months. After only one week of treatment, HCV RNA decreased from 543 645 to 983 UI/mL. Concomitantly, FVIII began to increase, from 5 to 9%, and reached 66% four months later. One month after the end of the anti-HCV treatment, a relapse was observed (HCV RNA at 958 115 UI/mL). A sofosbuvir/daclatasvir therapy was reintroduced for 6 months inducing a control of HCV replication. Finally, HCV RNA was still undetectable 10 months after the end of the anti-HCV therapy, FVIII level had normalized and anti-FVIII auto-antibodies were no longer detectable.

Summary/Conclusion: In the case reported here, the control of HCV was strongly associated with acquired haemophilia resolution. This observation highlights the involvement of HCV by itself in this coagulopathy, although the role of cyclophosphamid in the resolution of this case of acquired haemophilia can not be excluded.

Bleeding

ECTH-211

Board No. 86: Feasibility of desmopressin and pharmacokinetic-guided FVIII concentrate combination treatment in mild haemophilia A: a proof of concept (David study)

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Background: In non-severe hemophilia A (HA) patients (FVIII ≥0.01 IU/mL), surgery is the main reason for treatment. Treatment consists of either desmopressin (DDAVP) or FVIII concentrate. Treatment with FVIII concentrate is effective, but expensive. Moreover, FVIII concentrate treatment dosing often leads to FVIII levels above target levels when based on an in vivo recovery of 0.02 IU/mL per IU/kg. In addition, desmopressin is cheap and widely available. Desmopressin however does not produce a sufficient increase in FVIII levels in most non-severe HA patients to undergo surgery.

Therefore, we hypothesized that combination treatment of desmopressin and FVIII concentrate may be just as efficacious and less expensive than FVIII concentrate alone. Endogenous FVIII plasma levels, temporarily increased by desmopressin, can theoretically be supplemented with FVIII concentrate to reach perioperative FVIII target levels. Pharmacokinetic (PK) modeling, previously proven effective in the prophylactic treatment of (moderate) severe HA patients, can be applied to accurately dose combination treatment. We have developed two population PK models for non-severe HA patients: one for desmopressin and one for perioperative treatment with FVIII concentrate.

Aims: To show the feasibility of desmopressin and FVIII concentrate combination treatment in non-severe HA patients using PK-guided dosing of FVIII concentrate in the perioperative setting.

Methods: To proof the concept of combination treatment, two non-severe HA patients were treated with a combination of desmopressin and FVIII concentrate in the perioperative setting. Dosing of FVIII concentrate was determined by using two population models describing the PK of FVIII in non-severe HA patients after desmopressin and after perioperative administration of exogenous FVIII. The individualized dose was based on patient characteristics and the FVIII increase after a previous administration of desmopressin or FVIII concentrate. To guarantee safety and validate the predictions, FVIII levels after administration of desmopressin and FVIII concentrate were monitored.

Results: Two non-severe HA patients undergoing dental surgery were included. Patient 1, a 22 year old male (FVIIII 0.08 IU/mL), received preoperative administrations of 0.3 µg/kg desmopressin and 28 IU/kg FVIII concentrate. FVIII levels increased to 0.78 IU/mL after desmopressin and 1.35 IU/mL after FVIII concentrate. Predicted FVIII levels were 0.57 IU/mL after desmopressin and 1.20 IU/mL after FVIII concentrate, differing 37% and 13% respectively. The FVIII trough level was 0.39 IU/mL, 35% lower than the predicted 0.60 IU/mL.

To increase the precision of the predicted FVIII levels, previous increase of FVIII after administration of FVIII concentrate was used in patient 2. This patient, a 53 year old male (FVIII 0.04 IU/mL), received pre-operative administrations of 0.3 µg/kg desmopressin and 25 IU/kg FVIII. This resulted in FVIII levels of 0.33 IU/mL after desmopressin (predicted 0.29 IU/mL), 1.21 IU/mL after FVIII concentrate (predicted 1.10 IU/mL) and a trough level of 0.53 IU/mL (predicted 0.57 IU/mL). Perioperative FVIII levels differed 7-14 % from the predicted FVIII levels.

Summary/Conclusion: Perioperative combination treatment of non-severe HA patients with desmopressin and PK-guided dosing of FVIII concentrate is feasible. A multicenter prospective trial has been initiated to validate this concept: DDAVP treatment combined with clotting FVIII concentrate in mild hemophilia A patients (DAVID-study).

Bleeding

ECTH-170

Board No. 87: First prospective results of joint distraction in severe haemophilic ankle arthropathy

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Background: In hemophilia nowadays most intra-articular bleedings occur in the ankle, resulting in an indication for joint fusion at a young age. Joint distraction, an effective treatment in ankle osteoarthritis, has the advantage of preservation of the original joint without compromising subsequent conservative surgeries like arthrodesis, if still needed. In three cases evaluated in retrospect, good clinical and structural efficacy in hemophilic ankle arthropathy was demonstrated.

Aims: To gather prospective data on clinical effectiveness and tissue structure changes in ankle joint distraction in hemophilia.

Methods: Hemophilia patients (n=10; ≥18 and <55 years) were eligible in case of severe complaints of arthropathy in the tibiotalar joint causing functional limitations, despite analgesics and conservative treatment. Ankle joint distraction using an Ilizarov external fixator was performed during 10 weeks. Clinical effectiveness was evaluated using standard questionnaires and physical examination. Functional tests, X-ray and MRI examination were performed at baseline and 1-year follow-up.

Results: At the moment, a 12 months follow-up is available in 4 patients, three with severe hemophilia A and one with severe hemophilia B, age 21 to 33 years. During distraction, none of the patients experienced bleeding. Pin tract infection, commonly seen with external frame use, occurred in 3 patients, and was treated effectively with oral antibiotics.

Pain (visual analogue scale) decreased from 67 (47-79)mm at inclusion to 27 (7-84)mm at 6 months and 15 (1-43)mm at 12 months follow-up. Functional limitations, measured by the Haemophilia Activities List and the Ankle Osteoarthritis Scale, improved in three patients at 6 months, and in all four patients at 12 months. Functional tests improved considerably in all patients at 1-year follow up (e.g. 6-minutes walking test increased from 497 (434-560) to 621 (560-688) meters). Range of motion of the ankle was slightly decreased after 6 months due to stiffness of the ankle, but regained at 12 months in all patients.

MRI revealed a decrease in volume of subchondral cysts and bone edema, and slight improvement of the joint space width.

Summary/Conclusion: This first prospective study investigating the efficacy of joint distraction in haemophilic ankle arthropathy, showed clear clinical and structural improvement in all patients at 1 yr follow-up. Although preliminary, these data indicate that joint distraction may be a promising treatment postponing more rigorous surgery like ankle arthrodesis in those patients not benefitting from conservative therapy.

Bleeding
ECTH-431
Board No. 88: Immune tolerance induction in patients with haemophilia A and inhibitors – a single centre experience
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Background: The immune tolerance induction (ITI) is considered the preferred method for the eradication of factor VIII (FVIII) inhibitor in patients with hemophilia A. Though, its effectiveness remains approx. 70-80%. Factors affecting the outcome of ITI include the inhibitor titer (IT) and *historical* peak *IT during ITI*. However, the risk mutations of FVIII gene, age of patient at the beginning of ITI, period from the inhibitor formation, type of FVIII concentrate, inflammatory complications during ITI and disruption of ITI can also be risk factors for the outcome of ITI. Modifications of ITI, e.g. the use of plasma FVIII concentrate rich in von Willebrand factor (pdFVIII/vWF) and repeated administration of intravenous immunoglobulins (IVIG) can improve the outcome of ITI.
Aims: Presentation of three ITI in patients with hemophilia A and unfavourable prognosis or previous failure of ITI.
Methods: Three patients with hemophilia A and inhibitor (3, 6 and 16-year-old boys, all high-responders > 5 BU) undergoing ITI are presented. One patient underwent repeated ITI, other two patients completed their first ITI. Prognosis of ITI for two patients was unfavourable due to the presence of the above-mentioned risk factors. The same ITI protocol using high-dose FVIII (100 IU/kg/b.i.d. i.v.) and repeated administration of IVIG was applied. All patients received pdFVIII/vWF. Recombinant activated FVII (rFVIIa) or high doses of FVIII were used to control bleeding episodes and prevent perioperative bleeding during ITI.
Results: The eradication of inhibitor was achieved during period of 13-32 months in all three patients. There were not recorded any complications related to the applied ITI medicaments.
Summary/Conclusion: According to our experience, the ITI using IVIG seems to be a safe and effective treatment in patients with hemophilia A and unfavourable prognosis or previous failure of ITI.
The work was supported by projects APVV-0222-11 and Vega 1/0168/16.

Bleeding
ECTH-440
Board No. 89: Bleeding disorders of haemostasis and thrombosis
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Background: Despite the natural haemorrhagic tendency of the bleeding disorders, these diseases do not prevent the afflicted patients from the development of the thrombosis. The major contributing factors to the thrombosis in these cases are the traditional risk factors, such as arterial hypertension, hyperlipidaemia, smoking, immobilization, advanced age, surgery or pregnancy, and also the coexistence of the thrombophilia, substitution treatment and the use of by-passing agents.
Aims: To discuss the topic of the coincidence of bleeding and thrombosis.
Methods: We present the case reports of the patients with bleeding coagulopathy who developed thrombosis and report on the successful clinical management of their challenging clinical situations.
Results: In all described cases, the antithrombotic treatment was administered without any complications.
Summary/Conclusion: Older patients with bleeding disorders of haemostasis have the prothrombotic risk factors, which are comparable with that of general population. In the concomitant existence of several prothrombotic risk factors, the risk of the development of thrombosis is increased. Generally, there is a lack of large studies supporting the universal guidelines for such situations. Therefore, the combination of the substitution and antithrombotic treatment should be individualized.
The project complies with the Declaration of Helsinki and the informed consent in each patient is obtained.
Acknowledgements: we would like to thank the support of projects of Scientific Grant Agency Vega 1/0168/16 and Agency for the Support of Research and Development APVV 0222-11.

Bleeding

ECTH-515

Board No. 90: Establishment of a premarital diagnosis schedule as part of a prophylaxis program of factor xiii deficiency in the sistan and baluchestan province

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Background: Congenital factor XIII deficiency (FXIIID) is an extraordinarily rare bleeding disorder (RBD) which is inherited in an autosomal recessive conduct.

Sistan and Baluchestan Province located in the southeast of Iran has the most comprehensive frequency of FXIIID due to the high rate of consanguinity,which is associated with significant morbidity and mortality, mainly caused by central nervous system bleeding.

Aims: Noteworthy to add dramatic increase in FXIIID registered cases from 45 cases to 410 since 2006 and significant increase in neurologic morbidity of cases emphasis on the importance of the screening premarital programs which can reduce the affected cases.

Methods: In order to conduct our premarital screening program candidates were molecularly checked for Trp187Arg mutation to confirm the carrier state

Inclusion criteria was :

1)couples resident in high prevalence cities such as Saravan, Khash and Zahedan

2)premarital couples with history of an affected patient with FXIIID in family members

3)couples with the history of any bleeding disorder in family

4) any candidate with the history of sudden death in neonates of family

5) any candidate with the history of menorrhagia ,

umbilical cord bleeding or usage of blood products

6) candidates with mental retardation any neurologic deficit which can be caused by CNS bleeding .

Results: We found 34 couples in premarital or married status which were in one of the 6 mentioned danger groups hence we checked them for Trp187Arg mutation which is the only casuative mutation for FXIIID in Sistan province .

In 15 couples (%44) none mutation was detected.

6 couples (%16) both were heterozygote for the mutation which in the situation of pregnancy they were provided with consultations about chorionic villus sampling .

In 13 couples (%38) only one of the couples had the mutation.

Evaluations demonstrated that %55 of candidates were positive for 4 categories of inclusion criteria .

2 patients had the history of cryoprecipitate usage.

Summary/Conclusion: Screening and evaluation for rare bleeding disorders specifically for FXIIID in high frequency zones may reduce the burden of morbidity and economical and social consequences .

Bleeding
ECTH-148
Board No. 91: Major surgery in haemophilia A patients with inhibitors
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Background: The development of inhibitors to FVIII is considered the most serious complication of haemophilia A treatment, occurring in 30% of patients with severe haemophilia A. Despite the improvement in control of bleeding with the availability of bypassing agents, surgery continues to be a major challenge in these patients.
Aims: Evaluate the safety and efficacy of recombinant activated FVII (rFVIIa) and/or activated prothrombin complex concentrate (APCC) in haemophilia A patients with inhibitors undergoing major surgery in our hospital.
Methods: Retrospective evaluation of 5 adult patients, with a median age of 47.4 years (range 38-53), with severe haemophilia A and inhibitors to FVIII, high responders, who underwent 4 elective and 2 emergency surgeries. In 2 of the surgeries (a colecistectomy and a total nephrectomy) the patients were treated with rFVIIa; one ileo-colic resection was treated with rFVIIa and APCC; the other 3 surgeries (one apendicectomy and 2 limb amputations) were treated with APCC. EACA and tranexamic acid (TXA) were associated in all but one of the surgeries, the ileo-colic resection. rFVIIa was administered in a dose of 90 – 100 µg Kg -1 every 2 – 6 hours during and after surgery; APCC was administered in a dose of 50 - 70 U Kg -1 every 8 – 12 hours during and after surgery. EACA and TXA were given according the recommendations of product characteristics.
Results: In our patients, haemostasis was considered excellent in 4 cases for not having noticed bleeding problems. In the remaining 2 we considered that haemostasis achieved was good. In fact, the patient who underwent the urgent apendicectomy and treated only with APCC, bleeding complications were not immediate, but 5 days after surgery there was a small hematoma on the surgical loca which fortunately resolved spontaneously without changing the therapeutic protocol. The patient who underwent the ileo-colic resection was treated initially with rFVIIa and EACA without complications however, after 5 days of surgery, hemorrhagic complaints appeared and the treatment was changed to APCC with good results during 4 days only; treatment with rFVIIa was reintroduced and bleeding disappeared having discharged without complications. The median hospital length stay was 16.1 days (range 8-21), with some of the patients having treatment at home afterwards for some more days until complete healing. No thromboembolic or other adverse events were observed.
Summary/Conclusion: The response to APCC and to rFVIIa was considered excellent in all but two surgeries, where the response was considered good after changing the bypassing agent. None of the two bypassing agents was considered better than the other. The use of tranexamic acid with APCC proved to be efficacious without thromboembolic or other risks. Nevertheless, the good results obtained since the availability of bypassing agents, surgery in this group of patients must still be considered a major challenge and should be done only in hospitals with expertise and experience in their treatment.

Bleeding
ECTH-394
Board No. 92: Can we predict haemorrhagic early death in acute promyelocytic leukaemia?
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Background: Hemorrhagic early death (HED), with reported rates between 5 and 26.52%, is a major impediment in the managing of acute promyelocytic leukemia (APL). Although previous studies identified several prognostic factors for HED such as poor performance status (PS), high white blood cell (WBC) count, high peripheral blast count, serum lactate dehydrogenase (LDH), low fibrinogen level, platelet count and prolonged prothrombin time (PT), a predictive model for HED has not yet been elaborated.
Aims: To identify factors predictive of HED and to develop prognostic scoring system
Methods: We analyzed data on HED in 85 newly diagnosed PML-RARA positive APL patients (median age 45 years, range 18-78; 36/49 female/male ratio) managed in the Clinic of Hematology from 2004 to 2014 with all-trans retinoic acid combined with anthracyclines. Central nervous system (CNS), retinal, pulmonary or gastrointestinal haemorrhages were considered a severe bleeding event. HED was defined as death from hemorrhage from the first day of hospitalization up to the end of the induction treatment. The following parameters were evaluated as risk factors for HED: age, ECOG PS, patient's (time from the first symptoms to seeking medical care), medical (time from the first medical contact to ATRA initiation) and treatment delay (time from hospitalization to ATRA initiation), severe bleeding, WBC count, peripheral blast count, platelet count, fibrinogen level, PT, D dimer, ISTH DIC score, morphological disease type, CD15 and CD56 positivity, additional cytogenetic abnormalities, PML-RARA isoforms and FLT3-ITD mutation. Pearson chi test and Fisher test were used to analyze differences for categorical data. For multivariate analysis a binary logistic regression model was constructed for EHD adjusting for 6 variables. A backwards elimination procedure was used to exclude redundant or unnecessary variables. Integer weights for the risk score were derived from logistic regression model. Prognostic score was validated via 10-fold cross validation

Results: HED occured in 12/85 (14.12%) patients. Predictors of HED in univariate analysis were: ECOG PS ≥ 2 (P=0.010), severe bleeding at diagnosis (P=0.023), WBC >20x10^9/L (P=0.015), fibrinogen level <2 g/L (P=0.05), PT <50% (P=0.005), and ISTH DIC score ≥7 (P=0.031) as the most statistically significant cutoff points. Independent risk factors in multivariate analysis were: severe bleeding at diagnosis (P=0.041), WBC >20x10^9/L (P=0.018) and ISTH DIC score ≥7 (P=0.024). A clinical prognostic scoring system was then developed: absence of severe bleeding at diagnosis, WBC <20x109/L, ISTH DIC score <7 = 0, WBC ≥ 20x10^9/L = 1 point and ITSH DIC score ≥7 = 2 points. Accordingly patients were classified in three groups: low risk = 0 points, intermediate = 1-2 points and high risk ≥ 3 points. The HED rate between these groups (low 2.4%, intermediate 26.1% and high 100%) was significantly different (p<0.001).
Summary/Conclusion: The prognostic model developed in this study allows for the identification of the patients at high risk for HED, needing more aggressive supportive measures. Further large population-based studies for testing new score systems and refinements therapeutic approaches for APL patients in high HED risk are needed.

Bleeding

ECTH-511

Board No 93: a comparison of clinical manifestation between combined deficiency of factor v and viii (f5 f8 d) and isolated factor viii deficiency

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Background: Congenital F5 F8 D is an autosomal recessive double-gene disorder estimated to be extremely rare (1: 1000 , 000) in the general population . This disease affecting males and females in equel numbers .Mutation in LMAN1 or MCFD2 cause reduce function in the transport of facV/VIII from the endoplasmic reticulum to the golgi.Isolated fac VIII deficiency or hemophilia A is X-linked disorder estimated to be 1:5000 male

Aims: .In addition reported that F5 F8 D is usually associated with fewer symptoms than hemophilia A because the concomitant presence of two coagulation defects does not enhance the hemorrhagic tendency that was observed in each defect separately . Therefore I encouraged That research these reports in my patients .

Methods: . In general there are 119 patients of hemophilia and Rare bleeding disorders in my center . 4 patients have F5 F8 D . (2 males and 2 females) In one female factor V = 25 % and factor VIII = 15 %.In another female Factor V = 12 % and factor VIII = 10 %.In one male factor V = 9.2 % and Factor VIII = 15 %.In another male Factore V = 8 % and Factor VIII = 9.5 %.4 males with hemophilia A and the same factor VIII levels enrolled.All patients evaluated for bleeding diathesis from June 2008 to June 2016.

Results: The mean of bleeding diathesis inF5F8D and hemophilia A were 0.1 in year and 3in year respectively.There is significant statistical difference between two groups

Summary/Conclusion: symptoms in F5F8D is fewer than F8D.Symtoms of F5F8Dwas repoted to be easy bruising . epistaxes and gum bleeding are not uncommon in affected individuals .Sever symptoms including haemarthrosis is rare . F5 F8 D Bleeding episodes are usually treated on demand and do not requlr prophylaxis . This finding is according with our results.

Bleeding

ECTH-231

Board No. 94: Lessons from first case reports using idarucizumab and spontaneous adverse reports of dabigatran in the French pharmacovigilance database

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Background: Dabigatran directly inhibits thrombin and has been the first non-VKA oral anticoagulant (NOACs) available. A drawback during these first years of using NOACs is the absence of a specific antidote in case of major bleeding or emergency surgery. In this context, an important clinical advance was the development of idarucizumab: the first specific antidote available to reverse dabigatran anticoagulant activity. The RE-VERSE AD clinical trial was designed to examine the efficacy and safety of idarucizumab in patients who presented a serious bleeding or who required urgent surgery or intervention.

Aims: To compare relevance of clinical outcomes related to dabigatran reversal in the real life.

Methods: First, we report the first 3 cases in our practice of dabigatran reversal after injection of 5 g of idarucizumab in patients with severe bleeding. In a second time, we have also analyzed cases of bleeding from dabigatran safety monitoring with National Pharmacovigilance Surveillance Plan in France from November 2009 to February 2015 and compared mortality observed in bleeding events related to dabigatran treatment in the real life to REVERSE-AD.

Results: In the 3 present cases of major bleeding related to dabigatran treatment (2 cases of gastro-intestinal bleeding and 1 of intracranial bleeding), we observed a full and immediate normalization of clotting times upon idarucizumab administration. We found a lengthening of clotting times after 24 hours with a clear increase dabigatran concentration and its anticoagulant effect for 2 patients. In all cases, no re-administration of idarucizumab was necessary, due to a favorable clinical course. Indeed, despite idarucizumab use in these cases, co-administration of non-specific hemostatic agents was decided (tranexamic acid and prothrombin complex concentrates) in cases 1 and 3, respectively). The French pharmacovigilance network was notified of 1548 cases of bleeding related to use of dabigatran, of which 1051 were considered as major bleeding according to ISTH criteria. Patient's general characteristics were close to those of the RE-VERSE AD trial population and reflected the actual use of dabigatran, mainly in elderly patients with atrial fibrillation and multiple comorbidities. Among the cases of major bleedings on dabigatran reported to the National pharmacovigilance network, a 16.7% (95%CI 14.4 to 19%) fatality rate (176/1051) was found, similar to that reported in the RE-VERSE AD trial (17.6*% [7.1; 28.1])*. We also analyzed the subgroup of patients treated with any PCC activated or not (18/97) and a similar fatality rate of 18.6% (95%CI 10.9 to 26.3%) was found.

Summary/Conclusion: These 3 first cases and analysis of French pharmacovigilance database confirm that a close follow up of idarucizumab treated patient is necessary to understand how to manage residual dabigatran level, thrombotic risk, bleeding management and idarucizumab potential re-injection, with a patient-centered outcome study and a biological follow-up longer than 24 hours.

Bleeding
ECTH-325
Board No. 95: Interferences in coagulation testing: what is new ?
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Background: Detection of hemolysis, icterus and lipemia is a critical step of the whole testing process. That samples' feature is inherently present throughout the preanalytical phase (specimen collection, transport and handling) and the analytical phase (physiopathological conditions of the patients). Owing to the peculiar sensitivity of the assay methodology and the potentially adverse clinical consequences of test results, standardization of each phase of the coagulation testing process, especially preanalytical steps, cannot be overlooked.
Aims: The present investigation evaluates the influence of those interferences on coagulation tests to define reliable threshold depending on groups of patients and interfering substances. Moreover, the interfering substances concentration threshold of each reagent is assessed.
Methods: PT, aPTT, fibrinogen, factor II and VIII and chromogenic anti-Xa assay were performed in triplicate on four pooled plasmas using an ACL TOP 500 (IL, Paris, France): one without abnormality, one spiked with lithium heparinate (aPPT ratio from 2.5 to 3.0), two from patients treated by vitamin K antagonists (INR=2 and 4). Pooled plasma samples were spiked with differents concentrations of interfering substances including hemoglobin, Intralipid® and bilirubin and were compared to a control without interfering substances.
Results: In presence of free hemoglobin, a significant decrease of aPTT and anti-Xa assay is solely observed on the pool spiked with lithium heparinate from 250 (p=0.009) and 150 mg/dL (p=0.008) respectively; those concentrations are below the reagent's threshold (500 and 300 mg/dL, respectively). Other tests results are not affected. In presence of triglyceride, a significant decrease of fibrinogen is observed for concentrations over 6 g/L (p<0.003); for PT and aPTT, no quantitative impact can be observed but the analyser fails to provide results over 6.5 g/L, concentration below the reagent's threshold (7.5, 10 and 10 g/L respectively); other tests results are not affected. Analytically significant interferences are noticed at bilirubin concentration of 160 mg for PT (shortening, p<0.001), for aPTT (prolongation, p<0,009) and for factor VIII (decrease, p<0.003), concentrations below the reagent's threshold (300, 260 and 260 mg/L respectively); other tests results are not affected.
Summary/Conclusion: Regardless of analytical considerations, the type of interference encountered with hemolysis, icterus, and lipemia is substantially different, and thus requires distinctive approaches. It is advisable to routinely assess the hemolysis, lipemia and icterus index in these samples, to clearly establish the degree of potential interference, and to decide the most suitable actions. Therefore, we suggest a visual chart for the three tested interfering substances, based on our results, to enhance the standardization of the interference screening process.

Bleeding

ECTH-518

Board No. 96: Primary hemostasis alterations induced by ibrutinib. Bleeding side effects of the treatment.

Rosa Vidal*[1], Raúl Cordoba[2], Aránzazu García Raso[3], Tamara Castaño[4], Marian Perez[2], Elham Askari[2], Pilar Llamas[4]

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Background: Ibrutinib is a covalent Tyrosine Kinase Bruton (TKB) inhibitor of 1st generation, approved for the treatment of Mantle Cell Lymphoma (MCL), Chronic Lymphocytic Leukemia (CLL) and Waldenstrom's macroglobulinemia (WM). TKB is expressed on platelets and is believed to have a central role in platelet activation through GPIb and GPIV pathway. However the clinical significance of inhibition of TKB is unclear in terms of bleeding and it is known that patients with congenitally deficient TKB do not exhibit increased bleeding.

Aims: To identify possible alterations in hemostasis caused by ibrutinib in patients who start taking the drug. Baseline hemostasis will be analyzed and studies will be repeat after a period of treatment with the drug.

 To monitor the development of haemorrhagic adverse effects in patients who start taking ibrutinib.

Methods: Prospective study included 12 patients diagnosed with MCL, CLL or WM treated in 2nd line or more, with ibrutinib as single-drug therapy.

 The following parameters were analyzed on day 0, 10 and 28 after starting treatment with ibrutinib: APTT, PT, platelet count, PFA-100, aggregometry impedance in whole blood by multiplate assay with AA, ADP, Ristocetin and TRAP as agonists. Von Willebrand factor antigen (FvW Ag), Ristocetin cofactor (Risto-co) and coagulant factor VIII were also analyzed.

 By reviewing electronic medical records bleeding complications during treatment with ibrutinib were collected.

Results: None of the subjects developed severe bleeding complications, and 3 had minor bleeding complications. Of these, 1 patient was under anticoagulant treatment with intermediate dose of enoxaparin, and another patient has low levels of FvW Ag and Risto-co, suggesting a possible an unknown acquired von Willebrand disease.

Summary/Conclusion: -

> 50% of patients show an altered haemostasis base-line study. The biology of the disease appears to cause in subjects primary hemostasis changes that result in a basal bleeding risk regardless of the treatment used.

-

Only moderate bleeding complications were observed under ibrutinib treatment, two of the three patients who developed bleeding complications have other bleeding risk factor associated.

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PFA-100, COL/EPI, is prolonged in almost the 50% of patients after ibrutinib is started compared with base-line study.

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There is an interindividual variability in alterations of hemostasis and hemorrhagic clinical signs.

Bleeding

ECTH-151

Board No. 97: Kinetic and haemostatic properties of the new specific plasmin inhibitor

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Background: The damage to parenchymatous organs in case of trauma, extensive burns, surgery, etc. leads to high intensity of bleeding and significant blood loss. Antifibrinolytics - ε-aminocaproic acid (ACA) and tranexamic acid (TXA) are used to stop the bleeding. These amino acids suppress the plasminogen activation by its activators and has a direct inhibitory effect on plasmin and other enzymes (kinases, kallikrein, trypsin, etc).

Aims: Comparison of the kinetic and hemostatic properties of a new specific inhibitor of plasmin Ac-Ala-Phe-Lys-Pip·AcOH (AFK) with the properties of ACA and TXA.

Methods: Inhibitory effect of the antifibrinolytics on amidolytic activities of plasmin, urokinase (Uk) and tissue plasminogen activator (tPA) was studied using their specific substrates AFK-pNA, S-2444 and S-2288, respectively. Hemostatic activity of AFK, ACA and TXA was compared in a model of liver parenchymal bleeding of anesthetized rats. The swipes of sterile bandage soaked in 5% solution of antifibrinolytic or in saline solution in a control group were applied on the wound surface (4 groups of 6 animals). Haemostatic activity of drugs was estimated by time to stop bleeding and by blood loss weight. The differences between groups were determined by OneWay-ANOVA for several groups (test, Holm-Sidak). P <0.05 was considered significant.

Results: Specific tripeptide inhibitor of plasmin AFK much more inhibited the amidolytic activity of plasmin (K_i 0.25±0.02 mM), than amino acids ACA (K_i 58±3 mM) and TXA (K_i 23±2 mM). AFK inhibited amidolytic activity of Uk (K_i 0.206±0.007 mM), but not tPA. In contrast to the ACA and TXA, AFK had no effect on the rate of plasminogen activation induced by Uk or tPA. However, it inhibited the conversion of single-chain pro-urokinase into two-chain Uk under the action of low concentrations of plasmin. The time before bleeding arrest was 217±25, 221±41, 216±25 and 303±24 s for TXA, ACA, AFK and saline solution, respectively. The blood loss weight was 1.81±0.3, 1.93±0.28, 1.91±0.11 and 2.17±0.46 g for TXA, ACA, AFK and saline solution, respectively. Comparative toxicity of TXA, ACA and AFK (LD_{50}) in mice and rats was 1.3, 3.3 and 6.5 g/kg, respectively.

Summary/Conclusion: Mechanism of haemostatic action of ACA and TXA is based mainly on the binding to lysine-binding sites (LBS) of the heavy chain of plasminogen and, to a lesser extent, to the active center of plasmin. Blocking LBS of plasminogen prevents its activation to plasmin on the fibrin surface and leads to inhibition of fibrinolysis and hemostasis. The AFK, which has no affinity for the LBS of plasminogen has no effect on its activation to plasmin. Hemostatic action of AFK is associated with the direct blocking of activity of plasmin and Uk due to the binding of lysine ε-amino group of AFK with the carboxyl group located at the end of the hydrophobic pocket of the active center of both enzymes. The results show that the toxicity of the specific tripeptide inhibitor of plasmin is lower in 2-5 times, and its hemostatic efficacy is comparable to the effectiveness of ACA and TXA, used in the clinic.

Bleeding
ECTH-392
Board No. 98: Aprotinin but not tranexamic acid improves in vitro platelet function in blood samples from ticagrelor-treated patients
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Background: Dual antiplatelet therapy with aspirin and the ADP- receptor platelet inhibitor ticagrelor reduces the risk for thrombotic events in patients with acute coronary syndrome (ACS) but markedly increases the risk of perioperative bleeding complications. As there is no clinically available antidote to ticagrelor and platelet transfusion only has minor effect in ticagrelor-treated patients, there is a need for other strategies to reduce the risk of bleeding complications in ticagrelor-treated cardiac surgery patients. Anti-fibrinolytics are used to reduce bleeding during cardiac surgery but their effect in patients on antiplatelet medication is not completely understood.
Aims: This study aimed to compare the *in vitro* effect of the anti-fibrinolytics aprotinin and tranexamic acid, alone or in combination with platelet concentrates, on ADP- and AA-dependent platelet aggregability in blood samples from ticagrelor-treated patients.
Methods: Whole blood samples were collected from thirty ACS patients with ongoing treatment with acetylsalicylic acid and ticagrelor. The samples were supplemented with aprotinin (low dose 125 KIU/ml; high dose 250 KIU/ml) or tranexamic acid (low dose 0.2 mg/ml; high dose 0.4 mg/ml). In a subset of samples (n=20), apheresis platelet concentrate (120 $\times 10^6$/ml) were also added. Impedance aggregometry was used to evaluate ADP- and AA-induced aggregation before and after supplementation. The study was approved by the Regional Research Ethics Committee and was conducted in agreement with the Declaration of Helsinki. Informed consent was obtained from all patients.
Results: Aprotinin increased ADP-induced aggregation compared to baseline; low dose +20.4±6.0% (mean ± SEM), from 13.4±0.9 to 15.8±1.2 units, (p=0.004), high dose +22.6±5.4%, from 13.4±0.9 to 16.0±1.1 units, (p<0.001). In contrast, addition of tranexamic acid did not influence ADP-induced aggregation with any dose; low dose +3.2±7.5% (p=0.55), high dose −5.3±6.3%, (p=0.50). AA-induced aggregation was not significantly different after addition of aprotinin; low dose +44.6±22.4% (p=0.07), high dose +30.2±17.5% (p=0.32). In contrast, addition of tranexamic acid decreased the AA-induced aggregation; low dose −4.7±12.6% (p=0.01), high dose −18.6±10.3% (p=0.002).
Addition of platelet concentrate only did not significantly improve ADP-induced aggregation. The combination of aprotinin and platelet concentrate increased ADP-induced aggregation significantly while the combination of tranexamic acid and platelet concentrate did not have any significant effect. Addition of platelet concentrate with or without anti-fibrinolytic agent increased AA-induced aggregation significantly compared to baseline (all p<0.001).

Summary/Conclusion: Aprotinin, but not tranexamic acid, improves platelet function in blood samples from ticagrelor-treated patients. Platelet concentrate in combination with aprotinin improves ADP-dependent platelet function compared to platelet concentrate only. The results support the use of aprotinin over tranexamic acid as anti-fibrinolytic agent in cardiac surgery patients with ongoing ticagrelor treatment.

Bleeding
ECTH-132
Board No. 99: Pharmacokinetics of a plasma-derived VWF/FVIII concentrate in adult/adolescent and paediatric subjects with severe von Willebrand disease
Toshko Lissitchkov[1], Evgeny Buevich[2], Kazimierz Kuliczkowski[3], Olexandra Stasyshyn[4], Monica Hermida Cerqueira[5], Anna Klukowska[6], Guenter Auerswald[7], Claudia Djambas Khayat[8], Genadi Iosova[9], Irina Ramasheuskaya[10], Marta Julia Lopez Ruano[11], Christine Joch[12], Wilfried Seifert[12], Tobias Rogosch[*12]
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Background: Voncento® is a plasma-derived, high-concentration, low-volume, high-purity concentrate, which contains a high level of high-molecular-weight multimers and a VWF:FVIII ratio of ~2.4:1. The SWIFT ("**S**tudies with von **Wi**llebrand factor/**F**ac**t**or VIII") program is evaluating Voncento® in haemophilia A and VWD patients in accordance with the European clinical and paediatric guidelines.
Aims: One aim of the two open-label multicentre studies SWIFT-VWD and SWIFTLY-VWD was to compare the pharmacokinetic (PK) parameters in paediatric subjects aged <12 years with adult/adolescent subjects.
Methods: Subjects with severe VWD received Voncento® as a single bolus infusion of 80 IU VWF:RCo/kg body weight on Day 1 and 180 (n=14: paediatrics, n=12: adults/adolescents). PK parameters for VWF and FVIII were derived from plasma concentration values collected prior to dosing and at 0.5, 4, 8, 24, and 48 h after infusion. PK parameters comprised Incremental recovery (IR), Half-life ($t_{1/2}$), AUC, C_{max}, t_{max}, Mean residence time (MRT), Clearance (CL), and Volume of distribution at steady state (V_{ss}). Individual informed consent was obtained prior to enrolment.
Results: In overall paediatric subjects showed a comparable but slightly lower VWF:RCo exposure than adults/adolescents as indicated by a lower IR (median: 0.015 versus 0.017 (IU/mL)/(IU/kg)), slightly shorter $t_{1/2}$ (11.4 versus 11.5 h), faster CL (7.26 versus 6.16 mL/(h*kg)) and higher V_{ss} (93.1 versus 68.1 mL/kg). VWF:Ag, VWF:CB, and FVIII:C showed similar trends with FVIII:C showing longer $t_{1/2}$ (19.0 versus 23.7 h) and a slower clearance (2.96 versus 1.28 mL/(h*kg)) compared to the VWF markers due to a plateau effect that may represent the net effect of decreasing levels of exogenous FVIII, combined with increasing endogenous FVIII levels. PK parameters for VWF markers from the repeat PK were similar to those from initial PK.
Summary/Conclusion: Although in general comparable to adults/adolescents, paediatric patients under 12 years of age had higher volumes of distribution and faster clearance for VWF markers as known for almost all factor concentrates used for replacement therapy. Thus, for prophylaxis treatment in patients under 12 years of age a higher dose range of 40-80 IU VWF:RCo/kg body weight (adults/adolescents: 25-40 IU VWF:RCo/kg body weight) 1-3 times a week should be considered to compensate for faster elimination and greater volume of distribution in paediatric subjects.

Bleeding

ECTH-133

Board No. 100: Efficacy and safety of a plasma-derived VWF/FVIII concentrate in paediatric subjects <12 years with von Willebrand disease

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Background: Voncento® is a plasma-derived, high-concentration, low-volume, high-purity concentrate, which contains a high level of high-molecular-weight multimers and a VWF:FVIII ratio of ~2.4:1. The SWIFT ("**S**tudies with von **Wi**llebrand factor/**F**ac**t**or VIII") program is evaluating Voncento® in haemophilia A and VWD patients in accordance with the European clinical and paediatric guidelines.
Aims: The aim of the open-label multicentre study SWIFTLY-VWD was to evaluate the on-demand and prophylactic efficacy and safety of Voncento® in paediatric subjects <12 years with severe VWD.
Methods: Thirteen subjects received Voncento® as on-demand therapy (n=6: <6 years; n=7: 6-12 years; n=6: type 2A; n=7: type 3 VWD) and 4 subjects as prophylaxis therapy with Voncento® infusions 1 to 3 times per week (n=3: <6 years of age; n=1: 8 years old; n=3: type 3 VWD; n=1: type 2A VWD) for 12 months. Individual informed consent was obtained prior to enrolment.
Results: The subjects who were treated on-demand experienced a total of 96 non-surgical bleeds (NSB) (median 5.5, range 1-22), 80 of which required treatment. A total of 26 NSB events were considered major, including 11 joint NSB events. The mean annualized bleeding rate (ABR) was 7.5 bleeding events per year (5.5 minor, 2.0 major bleeds per year). In the prophylaxis arm the subjects reported 91 NSB (median 23.5, range 9-35) including 3 major joint bleeds, 73 of which required additional treatment. The ABR was 22.0 minor bleeds per year and 0.7 major bleed per year, respectively. The ABR reported by the four subjects of the prophylaxis arm was still high although they were treated on prophylaxis. This may be the result of the fact that the allocation to a treatment regimen was not randomized but based on the severity of the VWD. Only very severe patients with a high bleeding rate - according to the investigator - were treated on a prophylaxis regimen.
Excellent or good efficacy was reported by investigators for prophylaxis and all bleeding events in the prophylaxis and on-demand arm without relevant differences between subgroups by age. The median total Voncento® dose received by the four subjects in the prophylaxis arm was 8,062 IU VWF:RCo/kg body weight (b.w.) (range: 3,244-13,642 IU VWF:RCo/kg b.w.) at 129 exposure days (range: 55-197 days), and 536 IU VWF:RCo/kg b.w. (range: 80-2,080 IU VWF:RCo /kg b.w.) at 8 exposure days (range: 1-36 days) by the 13 subjects in the on-demand arm. Voncento® was well tolerated and the adverse events seen were mild-moderate and consistent with the safety profile for this product in adults. There were no cases of anaphylactic reactions and angioedema, development of VWF/FVIII inhibitors, thromboembolic events, or viral infections.
Summary/Conclusion: This contemporary comprehensive development program evaluating Voncento® demonstrates safety and efficacy for prophylaxis and treatment of bleeds in paediatric subjects <12 years with severe VWD without affecting the benefit-risk profile. Noteworthily, the incidence of major and joint bleeding events in the prophylaxis arm was very low.

Bleeding
ECTH-330
Board No. 101: Histones and thrombin generation after intracranial haemorrhage
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Background: Intracranial haemorrhage is a potentially fatal condition with limited treatment options. Recent animal studies point to histone proteins as a contributing factor to haemostatic disturbances after intracranial haemorrhage. Performing thrombin generation analyses with the addition of anti-histone antibodies may contribute to evaluating the impact of histones on coagulation.
Aims: We aimed to quantify histone levels and thrombin generation with and without added anti-histone antibodies in blood samples obtained from patients diagnosed with an intracranial haemorrhage.
Methods: We have included 77 patients. Blood samples were obtained at time of arrival to hospital as well as 6 and 24 hours after symptom onset. Histone levels were quantified with Cell Death Detection ELISA^{PLUS}. Thrombin generation was quantified with Calibrated Automated Thrombogram® assay; lagtime, time to peak, peak and endogenous thrombin potential (ETP) were recorded.
A mouse IgG2a kappa isotype control antibody from StemCell Technologies was purchased as control antibody. Anti-histone antibodies MHIS 1952 and MHIS 1992 were kindly supplied by Charles T. Esmon, Oklahoma Medical Research Foundation, Oklahoma City, USA.
Informed consent was obtained from patients if possible. Otherwise, consent was given from patients' next of kin and general practitioner.
Results: All results are reported as median (interquartile range).
Histone level peaked at time of admission with 11.78 arbitrary units (AU) (6.85-25.43) compared to 3.44 AU (1.54-8.02) 24 hours later ($p < 0.0001$).
Thrombin generation parameters all changed significantly between the admission sample and the sample taken 24 hours later, indicating a decrease in thrombin generation from admission to 24 hours after symptom onset. Lagtime increased from admission = 3.07 min (2.67-3.33) to 3.33 min (3.00-3.93) ($p < 0.0001$) at 24 hours, and time to peak increased from 5.94 min (5.11-6.97) to 6.38 min (5.67-7.56) ($p = 0.003$). Peak thrombin decreased from 262 nM (207-306) to 211 nM (153-249) ($p = 0.0001$) and the ETP decreased from 1391 nM x min (1188-1525) to 1211 nM x min (1072-1385) ($p = 0.0001$) at 24 hours after symptom onset.
We found that the thrombin generation was significantly decreased after addition of control antibody (all p-values < 0.0001). MHIS 1952 and 1992 also suppressed thrombin generation but to a lesser extent than the control antibody.
Summary/Conclusion: The present study showed higher levels of histones and evidence of increased coagulation measured by thrombin generation in the acute phase after an intracranial haemorrhage compared to 24 hours later. Both ex vivo addition of anti-histone antibodies and of control antibody suppressed thrombin generation in intracranial haemorrhage patients.

Bleeding

ECTH-474

Board No. 102: Whole exome sequencing in haemostatic diseases, a new diagnostic approach

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Background: Hemorrhages and thrombosis may occur due to genetic defects in one or more components of the hemostatic balance. At least 100 genes/loci are known making a pre-selection based on the laboratory phenotype crucial. New technologies, may overcome this hurdle as they allow multiple gene analysis.

Aims: To test the usefulness of Whole Exome Sequencing (WES) as a genotypic approach to unravel the pathophysiology of patients with a idiotypic increased bleeding tendency.

Methods: WES was performed on a series of approximately 20 patients with an increased bleeding score (Male BS > 3, Female BS > 5 according to Tossetto) using a predefined hemostasis gene panel. A hemostatic gene panel we designed containing 93 OMIM disease related genes proven to be involved in hemostatic disease, including genes involved in primary hemostasis (platelet and vessel wall) and secondary hemostasis (coagulation and fibrinolysis). This WES panel was first tested in all patients. If the diagnostic panel revealed a negative result, analysis of a second set of 73 candidate genes was done as part of the "open exome strategy", where all approximately 20,000 genes were analysed for genetic variants. Prior to this diagnostic approach, patients were counselled by a clinical geneticist and gave informed consent for the first set of analysis only (panel), or for the complete analyses (panel and exome wide).

Results: Up to april 2016, 11 patients gave informed consent to use the first panel. This panel does not allow haplotype detection nor screening of common polymorphisms. Of the three patients analysed so far only two were heterozygous for an OMIM confirmed hemostatic abnormality. About half of the patients gave informed consent for the exome wide analyses including haplotype detection and detection of common pathological polymorphisms. The results will be presented.

Summary/Conclusion: Since the overall yield to detect (likely) pathogenic mutations in general is only approximately 30% with the conventional targeted gene analysis approach, we implemented the WES test as a first tier diagnostic test for hemostatic abnormalities. WES allows simultaneous analysis of a large panel of genes in one single test making the test a very time and cost efficient approach. More data are expected to become available within 4 months and will be presented.

Bleeding

ECTH-131

Board No. 103: Pharmacokinetics of a plasma derived FVIII/VWF concentrate in children, adolescents and adults with severe haemophilia A

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Background: Voncento® is a plasma-derived, high-concentration, low-volume, high-purity concentrate, which contains a high level of high-molecular-weight multimers and a VWF:FVIII ratio of ~2.4:1. The SWIFT ("**S**tudies with von **Wi**llebrand factor/**F**ac**t**or VIII") program is evaluating this product in hemophilia A and VWD patients in accordance with the European clinical and pediatric guidelines.
Aims: Within this program the pharmacokinetics (PK) of pretreated children < 12 years of age, adolescents (12-18 years) and adult patients with severe haemophilia A (FVIII:C < 1%) was studied.
Methods: Subjects with severe hemophilia A received Voncento® as a single bolus infusion of 50 IU FVIII/kg body weight (n=31: children < 12 years of age; n=16: adults/adolescents). PK parameters for VWF and FVIII were derived from plasma concentration values collected prior to dosing and at 0.5, 4, 8, 24, and 48 h after infusion. PK parameters comprised Incremental recovery (IR), Half-life ($t_{1/2}$), AUC, C_{max}, t_{max}, Mean residence time (MRT), Clearance (CL), Volume of distribution at steady state (V_{ss}).
Results: Concentration-time curves showed similar profiles for the adult/adolescents PK population and subjects aged < 12 years. The results for PK parameters for Factor VIII in pediatric population (≤ 12 yrs) are within the range of those in patients ≥ 12 years old. The median IR of FVIII in the pediatric population was somewhat lower (0.015 [IU/mL]/[IU/kg], range 0.009-0.026) compared to adult/adolescents hemophilia A subjects (0.021 [IU/mL]/[IU/kg], range 0.011-0.031), the median $t_{1/2}$ was shorter (10.7 h (range 7.8 -18.2) vs. 13.4 h (range 8.8 – 18.5)) and the median CL was higher (6.02 mL/h/kg (range 2.54 – 11.34) vs. 3.82 mL/h/kg (range 2.30 – 7.11)). The small differences in PK parameters observed between the pediatric age group and the adult/adolescent group is not expected to be clinically relevant.
Summary/Conclusion: The results for PK parameters for Factor VIII in pediatric population (≤ 12 yrs) are within the range of those in patients ≥ 12 years old, thus considered as comparable with the adolescents and adults. A contemporary comprehensive development program evaluating Voncento® across all age groups in hemophilia A is now available.

Bleeding
ECTH-146
Board No. 104: Clinical efficacy and safety results of a multicentre study with a plasma-derived factor VIII/VWF concentrate in paediatric haemophilia A patients below the age of 12 years
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Background: Voncento® is a plasma-derived, high-concentration, low-volume, high-purity concentrate, which contains a high level of high-molecular-weight multimers and a VWF:FVIII ratio of ~2.4:1. The SWIFT ("Studies with von Willebrand factor/Factor VIII") program is evaluating this product in haemophilia A and VWD patients in accordance with the European clinical and paediatric guidelines.
Aims: This was a Phase III study to investigate the efficacy and safety in paediatric subjects aged 0 to < 12 years with severe haemophilia A (FVIII:C < 1%) who had received previous FVIII treatment for a minimum of 20 exposure days. The pharmacokinetic data are presented in abstract ECTH-131.
Methods: Thirty-five paediatric subjects received Voncento® as on-demand therapy (n=17: 12 subjects were < 6 years including 4 subjects < 2 years) or as prophylactic therapy (n=18: 4 subjects were < 6 years). All subjects were analysed for haemostatic efficacy and safety; The subjects were treated up to 100 exposure days with a maximum study duration of 12 months.
Results: The 17 on-demand subjects reported 320 non-surgical bleeds (NSB) and received a median number of 29.0 infusions (median dose of 34.2 IU FVIII/kg).The haemostatic efficacy was assessed by the investigator as excellent/good in all cases (24%/76%). The 18 subjects in the prophylaxis arm (10 subjects were previously naïve to prophylaxis treatment) experienced 173 NSB events (97 NSBs (56%) were reported by 3 subjects). Five subjects (28%) had no NSBs. All received a median number of 92 infusions (median dose 30.6 IU FVIII/kg). The majority of the bleeds (92%) needed only 1 infusion. Haemostatic efficacy was assessed by the investigator as excellent (86%) or as good (14%). Inhibitors occurred in 3 subjects (2 low-, one high titre inhibitor) of which 1 disappeared (low titre) and 2 persisted. These 3 subjects had a median of 22 previous exposure days.
Summary/Conclusion: This study showed excellent or good haemostatic efficacy ratings in 100% of the bleeds. Voncento® was well tolerated, the AEs seen were only mild-moderate with inhibitor rates observed to be within the expected incidence range of minimally pre-treated paediatric subjects. No other safety findings were observed. This comprehensive program evaluating Voncento® across all ages demonstrates excellent safety and efficacy for treatment and prevention of bleeds in children with severe haemophilia A.

Bleeding

ECTH-215

Board No. 105: Population pharmacokinetics of recombinant fusion protein linking coagulation factor IX with recombinant albumin (rIX-FP) in adult and paediatric patients with severe haemophilia B

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Background: IDELVION®, a recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP), has recently been approved in the US and Canada for prophylaxis and on-demand treatment of children and adults with hemophilia B. IDELVION® has an improved pharmacokinetic profile, which allows dosing every 7–14 days.

Aims: A PPK model was developed to characterize rIX-FP PK, to describe and identify demographic and clinical covariates of rIX-FP PK variability and to simulate FIX activity-time profiles for various dosing regimens.

Methods: Blood PK samples from 104 patients were collected to determine the plasma FIX activity using a validated one-stage clotting assay. PPK modeling was performed using NONMEM 7, including the assessment of potential covariates on rIX-FP PK. Visual predictive check (VPC) was used for model evaluation.

Results: A 2-compartmental model appropriately described the rIX-FP PK. Body weight was a significant covariate on clearance and both central and peripheral volumes of distribution, and weight-adjusted dose was a significant covariate on central volume. The VPC results confirmed model stability, and the PK parameters were estimated with good precision. For the respective age groups of ≥12 y, 6 to <12 y and <6 y, simulations based on the final PPK model predicted a median trough activity of 8.0, 4 and 2% after 75 IU/kg rIX-FP once every 14 days, 12, 7, and 4% after 35 IU/kg once weekly and 18, 10, and 7% after 50 IU/kg once weekly. Time to 1% after a single dose was also estimated.

Summary/Conclusion: The PPK model adequately characterized rIX-FP PK. The PK parameters estimated by the PPK model were consistent with those by the non-compartmental approach, and results of the prediction support FIX activity measured in clinical studies. This model can be utilized as a tool to simulate FIX activity-time profiles for various dosing scenarios of rIX-FP.

Clotting
ECTH-320
Board No. 106: Blood coagulation contact pathway activation by bacterial endotoxins
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Background: The intrinsic pathway of blood plasma coagulation initiates from a contact with a foreign surface by activation of the coagulation contact pathway which consists of the proteolytic cleavages of plasma proteins factor XII (FXII) and prekallikrein (PK) promoted by the surface and requiring a cofactor, high molecular weight kininogen (HMWK). Animal studies suggest a potential pivotal role of contact pathway for thrombosis while it is dispensable in normal hemostasis. Yet, physiological activation mechanisms of FXII remain unclear. One of the frequently occurring events of contact pathway activation is bacterial infection. The FXII and PK seem to be activated by components of the outer leaflet of gram-negative bacteria outer membrane, lipopolysaccharides (LPS) or endotoxins. *In vitro* contact pathway activation by LPS assessed by kallikrein activity has an optimum around concentrations of LPS equaling 100 μg/ml suggesting surface-depending mechanisms of contact pathway activation by endotoxins.
Aims: Experimental investigation and computational analysis of the molecular mechanisms governing contact pathway activation on the bacterial endotoxins.
Methods: Platelet-free plasma (PFP) coagulation was monitored with spectrophotometry. The clotting time was determined as the time of half-maximum absorbance at 405 nm. The series of computational models were constructed to describe experimental data from (Morisson and Cochrane, J Exper Med (1974) 140:797-811; Kalter, van Dijk et al., J Infect Dis (1983) 148(4): 682-691) for purified protein systems and obtained in the current study data for PFP. The series of models consisted of 6-22 ODE describing interactions of proteins governed by mass action or Michaelis-Menten kinetics. The system was integrated by IDA/CVODE method in VCell (http://www.nrcam.uchc.edu/).
Results: The phenomenon of apparent contact activation inhibition by high LPS concentrations and the existence of optimal LPS concentration were experimentally observed in human plasma. The computational models described well experimental data for pure systems and PFP. The computational analysis suggested that, when LPS concentration is higher than the optimal one, surface concentrations of contact pathway factors decrease because of activating surface excess, while increase in the LPS concentration is not sufficient to keep the total velocity of activation reactions as high as it is at the optimal LPS concentration. In the case when LPS concentrations are smaller than the optimal one, activating surface deficiency is the reason for time endpoint concentrations of active factors to be smaller. If the velocity of spontaneous FXII activation is considered to be linearly dependent on surface FXII concentration only, FXII autoactivation contribution to the total velocity (at least in systems without HMWK or together with contribution of activation by kallikrein in other cases) is much greater than spontaneous activation contribution during contact pathway activation by LPS.
Summary/Conclusion: The contact activation of blood plasma coagulation on endotoxins proceeds as a two-step process. First, the factors (FXII, PK and HMWK) bind to the surface of LPS micelles and then the activation of FXII occur on the surface. Additionally we have shown that the autoactivation of surface-bound FXII contributes to the total velocity much greater than its spontaneous activation by LPS.

The study was supported by RFBR grant 15-34-70009.

Clotting
ECTH-350
Board No. 107: Rate-limiting steps in coagulation Factor X activation by intrinsic tenase
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Background: Factor X (FX) activation by the membrane-assembled complex of intrinsic tenase is well-established reaction, known to be crucial for the amplification of blood plasma coagulation. Its regulation, in particular, the rate-controlling steps in the tenase reaction, remains poorly understood.
Aims: Here we aimed to perform computational analysis of the molecular mechanisms governing FX activation by the phospholipid membrane-assembled intrinsic tenase.
Methods: The model development and validation was based mainly on experimental data from Panteleev et. al. (FEBS Journal 273 (2006) 374–387), where binding of factors VIIIa, IXa, and X to 800 nm phospholipid vesicles was studied in parallel with FX activation. We developed a comprehensive computational two-compartmental (two-dimensional surface and tree-dimensional solution) model. The model accounted for factor binding to the vesicle, the tenase assembly and FX activation. The model was created and integrated in the VCell (http://www.nrcam.uchc.edu/)) software.
Results: The unknown model parameters were tuned to describe dependences of the rate of FX activation on phospholipid and FX concentrations. The model described the data only in the case of the membrane-bound substrate mechanism. Sensitivity analysis revealed that binding of factor IXa and, to the lesser extent, FX to the surface are the rate-limiting steps, while diffusion of the factors on the surface is rapid enough and could not limit the reaction rate. The internal turnover rate of the tenase itself also has much smaller influence. Inhibition of the tenase activity by the excess phospholipids observed in the experiments could be explained by the fX depletion from the solution due to its binding to the phospholipids.
Summary/Conclusion: The substrate of intrinsic tenase is the membrane-bound FX, which results in FX and FIXa binding to the surface becoming rate-limiting steps in the reaction.
The study was supported by Russian President Grants MD-6347.2015.4 and MK-5879.2016.4.

Clotting
ECTH-209
Board No. 108: Recurrent venous thromboembolism during pregnancy in women with quantitative antithrombin deficiency with or without antithrombotic prophylaxis
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Background: Background: Little is known on pregnancies after an episode of venous thromboembolism (VTE) in women with quantitative antithrombin (AT) deficiency.
Aims: Aims: To investigate the frequency of pregnancy-related recurrent VTE and pregnancy outcome in women with AT deficiency.
Methods: Methods: Women referred (1986-2014) to the Thrombosis Center for thrombophilia screening after an episode of VTE who remained pregnant afterward were considered if they carried a quantitative inherited AT deficiency (antigen levels <60%).
Results: Results: Out of 50 women, 17 remained pregnant after VTE for a total of 29 pregnancies. Antithrombotic prophylaxis with enoxaparin 4000IU or nadroparin 3800IU od was prescribed in 16 pregnancies (by us in 14) of 11 women, whereas 13 pregnancies in 6 women occurred before the access to the Center and were not prophylaxed. Recurrent VTE complicated 1 pregnancy with antithrombotic prophylaxis (left popliteo-femoral, 10th week) and 3 pregnancies without (left popliteo-femoral, 10th week; left popliteal, 15th week; left popliteal 3rd day after a caesarean section) (6.3% and 23.1%, Fisher's exact test p=0.299). Three other pregnancies without prophylaxis were complicated by superficial vein thrombosis. In women with and without prophylaxis the frequency of miscarriage and of late placenta-mediated obstetrical complications was similar (25 and 23%; 1.2 and 0%, respectively). The two groups of women with and without prophylaxis were similar in terms of median age at first VTE (24.0 and 22.4 y), parity (0.8 and 1.1), age at index pregnancy (33.7 and 32.3 y) and BMI (22.1 and 25 kg/m^2).
Summary/Conclusion: Conclusion: In women with quantitative AT deficiency, 4000 or 3800 IU od of low-molecular-weight heparin reduces by 75% the frequency of recurrent pregnancy related VTE. The residual 6.3% of recurrent VTE despite prophylaxis may suggest the need of higher heparin doses. Obstetrical complications were similar in the two groups and in line with those observed in the general population.

Clotting
ECTH-202
Board No. 109: Higher bag-to-bag variations in single donor fresh frozen plasma units versus pooled, prion cleared and solvent-detergent treated plasma results in overlapping haemostatic potentials
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Background: The solvent/detergent (S/D) treatment enables effective and robust inactivation of all lipid-enveloped viruses, but also inactivates partly sensitive plasma proteins like protein S.
Aims: The aim of this study was to compare the thrombin generation capacities of single donor fresh frozen plasma (FFP) and pooled, prion cleared & solvent/detergent treated plasma (SDP). Particular focus was added to the role and function of protein S in TGA and the impact of assay settings.
Methods: Sixteen SDP batches manufactured at Octapharma PPGmbH (Vienna, Austria) and Octapharma AG (Stockholm, Sweden) were investigated. Thirty-two units of single donor FFP were used for comparison. For protein S, both functional activity and free protein S antigen levels were measured. TGA was performed using two fluorogenic tests (i.e. Technothrombin TGA and CAT assay) with different triggers. Finally, ROTEM was performed after supplementing plasma with tissue factor and phospholipids.
Results: The solvent/detergent virus inactivation step reduced mean protein S levels in SDP. However, due to higher bag-to-bag variations in the FFP group, single levels of both protein S antigen and activity overlapped between the two plasma groups. Spiking studies with protein S depleted plasma, human purified protein S or antibodies against protein S confirmed a correlation of protein S with thrombin generation and clot formation capacity under specific assay conditions, especially in an assay with low tissue factor concentration. Using TGA from both manufacturer and with different triggers, overlapping thrombin generation capacities were found between SDP and FFP.
Summary/Conclusion: Correlation between protein S and TG capacity was demonstrated in the TGA. Due to higher variability in protein S content in the FFP group, overlapping haemostatic potentials of SDP and FFP were found.

Clotting
ECTH-445
Board No. 110: A procoagulant profile in small cell lung carcinoma patients is associated with extracellular vesicles
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Background: Small cell lung cancer (SCLC) is considered to be one of the most aggressive malignant diseases and patients with SCLC have an increased risk of venous thromboembolisms (VTE). Extracellular vesicles (EVs) have recently been proposed to play a role in the disease, as EVs may possess a procoagulant nature mediated by tissue factor and phosphatidyleserine embedded in their surface membrane. Thus, EVs have been proposed to provoke a hypercoagulable profile in cancer patients.
Aims: The aim of this project is to investigate the hypercoagulability associated with EVs in SCLC patients during disease progression and treatment.
Methods: In a study conducted at Aalborg university hospital (N-20140055) blood samples from 18 SCLC patients (6 limited disease (LD) and 12 extensive disease (ED)) and 15 age-related controls were collected. Samples were collected at baseline, during treatment and at follow-up 2 months after the end of chemotherapy. The coagulation activity was determined by calibrated automated thrombography (CAT) and a clot-based method was applied to quantitate the procoagulant phospholipids activity (PPL assay). To enumerate EV concentration and size, Nanoparticle tracking analysis (NTA) was used. The procoagulant nature of EVs was confirmed through '*in vitro* spiking' of isolated vesicles from patients and controls in both a CAT and PPL assay.
Results: Through both the CAT and PPL assay SCLC patient's displays a significantly higher thrombogenic profile compared to healthy controls. This was demonstrated in plasma as well as in spiking with isolated vesicles. There was no difference in the coagulation profile of patients with LD and ED in either of the analyses performed. The concentration of particles was not significantly different in patients compared to controls.
Summary/Conclusion: Isolated vesicles from SCLC patients shows to be more potent activators of coagulation compared to that of healthy controls, as shown in both CAT and PPL analysis.

Clotting
ECTH-241
Board No 111: Alternatively spliced tissue factor has no function in haemostasis
Betul Unlu*

Background: Venous thrombosis is a high burden on western society as it is the third most common cardiovascular disease. Full length Tissue factor (flTF) is a key player in thrombosis and hemostasis, but, an alternatively spliced tissue factor isoform (asTF), appears to have minimal coagulant activity. More interestingly, both flTF and asTF have been detected in thrombi, suggesting a role for asTF in thrombosis and hemostasis as well.
Aims: To investigate the interplay between flTF and asTF in endothelial cells on hemostasis.
Methods: Immortalized endothelial (ECRF) cells were transduced with adenoviruses leading to expression of asTF and/or flTF, after which FXa generation assays were performed on cells and microparticles (MPs) to assess TF activity. To study co-localization between flTF and asTF, confocal images were taken. Also the distribution of flTF was studied in the presence or absence of an escalating dosis of asTF.
Results: FXa generation demonstrated equal TF activity when both isoforms are co-expressed. No co-localization of asTF and flTF could be observed. Only upon drastic overexpression of asTF, lower flTF antigen levels and activity was observed. There was a shift of flTF from non-raft to lipid raft fractions with escalating doses of asTF. Proteasome inhibition resulted in restored asTF but not flTF antigen expression.
Summary/Conclusion: At relevant concentrations asTF has minimal coagulant activity and it does not affect coagulant activity of flTF on cells nor on MPs when co-expressed.

Clotting

ECTH-296

Board No. 112: Influence of overweight and obesity on recurrent venous thrombosis risk: results from the MEGA follow-up study

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Background: Excess body weight has been associated with a 2-fold increased risk of recurrent venous thrombosis (VT) in one study, but external validation of this finding in a large population-based study is currently lacking.

Aims: To estimate whether excess body weight increases the risk of recurrent venous thrombosis.

Methods: In the MEGA follow-up study, a cohort of 4731patients with a first venous thrombosis, aged 18-70 years, was followed for a median period of 7 years. In this study we analyzed 3889 of these patients, with available body mass index (BMI) data. Patients were stratified according to BMI values calculated in kg/m^2 in three categories (<25 - normal weight; 25.0-29.9 - overweight and ≥30 - obese), as well as according to *a-priori* defined percentile (pctl) cut-off levels in five categories (<10th pctl - (BMI <21.5 kg/m^2), 10th-25th pctl (BMI 21.5-23.7 kg/m^2), 25th-75th pctl (BMI 23.8-29.2 kg/m^2), 75th-90th pctl (BMI 29.3-32.9 kg/m^2), and >90th pctl (BMI >32.9 kg/m^2)). Incidence rates with 95% confidence intervals (CIs) of recurrent venous thrombosis were estimated as the number of events over the accumulated follow-up time in each BMI category. For the main analyses, follow-up was started at the moment of discontinuation of anticoagulant treatment, while for a sensitivity analysis, follow-up time was started at the date of venous thrombosis diagnosis. Cox proportional hazard regression models were used to estimate incidence rate ratios, and the hazard ratios (HRs) were adjusted for possible confounding effects of age, sex, smoking history, and sports activity.

Results: During follow-up 594 recurrent events occurred with an incidence rate of 3.3/100 patient-years. The risk of recurrent VT in overweight or obese patients was similar to that in patients with normal weight (HR 1.06; 95%CI, 0.87-1.30; HR 0.92; 95%CI, 0.71-1.19, respectively). Similarly, risks stratified by BMI categories did not differ, with the following HRs for recurrent VT: 0.82 (95%CI, 0.58-1.17) for the <10th pctl group, 0.88 (0.67-1.15), for the 10th-25th pctl group, 0.80 (0.61-1.05) for the 75th-90th pctl group and 0.98 (0.71-1.34) for the >90th pctl group, all compared with the 25th-75th pctl group as reference category. The results of the sensitivity analysis were in the same range, as well as results of sub-analyses in which patients were analyzed separately for provoked and unprovoked VT events, and for deep vein thrombosis and pulmonary embolism.

Summary/Conclusion: Our results failed to confirm that excessive body weight is associated with an increased risk of recurrent venous thrombosis.

Clotting
ECTH-351
Board No. 113: Management of atrial fibrillation including anticoagulation in primary care – study protocol of the cluster randomized controlled trial ALL-IN
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Background: Atrial fibrillation (AF) is the most common cardiac arrhythmia with an increased risk of stroke and mortality. It often involves frail, elderly patients, requiring adequate care for cardiac and non-cardiac comorbidities, lifestyle and tailored anticoagulation treatment. Given the expected increase in prevalence of AF, transition of care for AF patients from secondary care to primary care is desired. However, data on the safety and (cost)effectiveness are lacking.
Aims: This study evaluates if integral management of patients with AF by the practice nurse and general practitioner, including care for comorbidities and anticoagulation, is non-inferior to usual care.
Methods: The ALL-IN study is a cluster randomized trial that will be performed in approximately 60 primary care practices in the region of Zwolle, The Netherlands, with more than 1000 AF patients aged 65 years or over. Patients from primary care practices randomized to the intervention arm will receive integral AF management, consisting of a) visits to the practice nurse three times a year and once yearly to the general practitioner, b) INR measurements performed by the practice nurse, and c) easy access consultation from secondary care through the establishment of an Expert Center for Anticoagulation and an Expert Center Cardiology. Patients from practices randomized to the control arm will receive care as usual by the Dutch Thrombosis Service, cardiologist and/or general practitioner.
Results: The study has started in 2016 with a follow-up time of 24 months. Primary endpoint is all-cause mortality. Secondary endpoints are cardiovascular mortality, (non)cardiovascular hospitalization, Major Adverse Cardiac Events (MACE), stroke, major bleeding, quality of life and cost-effectiveness.
Summary/Conclusion: The ALL-IN trial is the scientific evaluation of a health care innovation that – due to the delegation of tasks to the practice nurse and the establishment of the Expert Centers for Anticoagulation and Cardiology– aims for sustainable and accessible care, close to the AF patient.

Clotting

ECTH-230

Board No. 114: Normal pregnancy is associated with an increase in thrombin generation from the early stages of the first trimester of pregnancy

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Background: Pregnancy is a hypercoagulable state, associated with an increased risk of venous thrombosis. Furthermore, in some women, this hypercoagulable response to pregnancy may result in the development of placental vessel thrombosis and subsequent poor pregnancy outcome.
Thrombin generation, a global coagulation assay, has been demonstrated to accurately reflect the thrombotic phenotype in a number of clinical situations. Previous data have shown that thrombin generation increases during normal pregnancy but it is unknown exactly how early in pregnancy this increase occurs.
Aims: To demonstrate whether thrombin generation parameters significantly change in the very early stages of pregnancy, compared to the pre pregnant state, in women who subsequently have normal pregnancy outcomes.

Methods: We assessed thrombin generation in 22 women undergoing natural cycle *in vitro* fertilization, the best physiological representation of a 'normal' pregnancy, outside a free living population, who subsequently gave birth at term following a normal pregnancy. Blood samples were taken just prior to conception, termed Day 0. Day 0 is equivalent to Day 14 from the last menstrual period in a naturally occurring pregnancy i.e. 2 weeks gestation. A further 5 samples were then taken from the same women very early in gestation, at Day 7, 10, 18, 29 and 45 in the study, with Day 45 being equivalent to Day 59 gestation.
Thrombin generation was measured by Calibrated Automated Thrombography under four different assay conditions; 1pM and 5pM tissue factor with and without the addition of thrombomodulin.
The TG parameters measured were; endogenous thrombin potential (ETP) (nM.min), Peak thrombin concentration (nM), time to reach peak height (ttPeak) (min), lag time (min) the time to reach 1/6th of peak height, and Velocity Index (VI) (nmol/min, slope between lag time and ttPeak).
To limit the effect of inter-assay variability, all samples from each woman were assessed concurrently, alongside a standard plasma. To account for inter-individual variability, the mean change in thrombin generation between the pre-pregnant and pregnant state was measured.
Results: There was a significant increase from baseline (pre-pregnancy) in peak thrombin, ETP and VI to Study Day 18 (the 5th gestational week) for all assay conditions, except 5pM + TM (when ETP, VI and peak thrombin began to significantly increase from study day 29). Increases ranged from 8-49% (p = 0.035 - <0.0001). This mean increase from baseline persisted into the 8th gestational week (Study Day 45) under all four assay conditions with increases ranging from 22-100% [p<0.0001 for all 3 parameters]).
Lag time and ttPeak did not significant change between the pre-pregnant and pregnant state.
Summary/Conclusion: We have demonstrated that thrombin generation increases significantly during the very early stages of normal pregnancy when compared to the pre-pregnancy state. This indicates that the increased risk of venous thrombosis likely begins very early in a woman's pregnancy, suggesting that women at high risk of venous thrombosis during pregnancy should be advised to start thromboprophylaxis as early as possible after a positive pregnancy test.
Furthermore, this characterization of thrombin generation in early normal pregnancy may enable thrombin generation to be investigated further as a potential predictor of poor pregnancy outcome.

Clotting

ECTH-161

Board No. 115: Fibrinogen may possess a second factor XIII cross-linking site for alpha2-antiplasmin

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Background: The fibrin clot is subject to mechanical and proteolytic challenges, such as shear stress from the blood flow, and dissolution by plasmin (fibrinolysis). a_2-Antiplasmin (a_2-AP), a key regulator of fibrinolysis, is cross-linked to the fibrin a-chain by activated Factor XIII (FXIII) at residue Lys303.
Aims: The aim of this study was to investigate the contribution of a_2-AP cross-linking to the inhibition of fibrinolysis by generating a fibrinogen mutant lacking the cross-linking site for a_2-AP.
Methods: The a-chain of fibrinogen was mutated by site-directed mutagenesis to replace Lysine303 by Arginine (aK303R). The mutated a-chain was transfected into CHO cells already containing b- and g-chains. Following expression by CHO cells, precipitation by ammonium sulphate, and purification by IF-1 affinity chromatography, protein integrity was examined by SDS-PAGE. The effect of the aK303R and WT fibrinogens on clot formation and lysis, in the absence and presence of a_2-AP, were investigated to understand the relevance of a_2-AP in inhibiting fibrinolysis. A plate-based fibrin incorporation assay was used to investigate the cross-linking of a_2-AP to aK303R and WT fibrinogens.
Results: SDS-PAGE showed that both WT and aK303R fibrinogens had fully intact a-, b- and g-chains and were purified to homogeneity. In the absence of a_2-AP, the turbidity and lysis profiles were similar for both aK303R and WT fibrinogens (MaxOD 0.696±0.022 and 0.713±0.024, $^1/_2$lysis 32.2±0.8min and 32.3±0.8min, respectively) when FXIII was also absent. Similarly there was no difference between the two variants (MaxOD 0.840±0.016 and 0.840±0.006, $^1/_2$lysis 36.7±1.5min and 37.8±0.5min, respectively) when FXIII was added (in the absence of a_2-AP), indicating that this mutation intrinsically does not affect fibrin polymerisation and lysis. When a_2-AP was added, the difference in time to half-lysis between presence and absence of FXIII was reduced significantly (-38.8%, $p<0.05$) for fibrinogen aK303R (33.5±4.5 min) compared to WT (54.7±3.3min). These data indicate that although the mutation of the a_2-AP cross-linking site to fibrin reduced the effect of a_2-AP on fibrinolysis, it did not completely abolish the effects of a_2-AP. In agreement with these effects on clot lysis, a_2-AP incorporation into fibrin using a functional plate-based assay was decreased by 42.7±4.8% ($p<0.05$) for fibrinogen aK303R compared to WT. These data indicate the possibility of the presence of a second cross-linking site for a_2-AP in fibrin.
Summary/Conclusion: The results from this study indicated that fibrinogen may possess a second FXIII cross-linking site for a_2-AP. Further studies are underway to identify this potential second site and investigate its potential physiological relevance.

Clotting
ECTH-411
Board No. 116: Side-by-side evaluation of clotting parameters in human, porcine, rabbit and rat plasma
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Background: A wide variety of existing animal models allow for the preclinical assessment of anticoagulant drugs, procoagulant agents and hemostatic bypassing agents. While the vertebrate coagulome is highly conserved, human and animal plasmas differ distinctly when evaluated in coagulation assays such as prothrombin time (PT), activated partial thromboplastin time (APTT) and calibrated automated thrombography (CAT). In addition, the efficacy of anticoagulant agents such as direct oral anticoagulants (DOACs) are either uncertain or unknown in these models.
Aims: Here we aim to provide a comprehensive reference framework for the evaluation of hemostatic agents in preclinical animal models.
Methods: To evaluate the interspecies differences in clotting parameters, we have performed a side-by-side comparison of PT, APTT and CAT parameters in plasma of rat, rabbit, porcine, and human origin. In addition, the anti-FXa activity was assessed in these plasmas by determining the rate of human FXa inhibition through chromogenic analysis.
Results: Following a 1:6 plasma dilution, PT and APTT analyses resulted in clotting times of rat plasma (PT: 17.0s, APTT: 35.1s) that were most similar to those of human plasma (PT: 17.9s, APTT: 66.6s). Interestingly, while the PT clotting times were shorter in rabbit (10.4s) and porcine (13.4s) plasma, the APTT of rabbit plasma was extensively prolonged (124.9s) and notably short (20.0s) in porcine plasma. To produce detectable and robust thrombin generation (TG) curves after triggering with low tissue factor (TF) concentrations, all plasmas were diluted (1:3) in buffered saline. Initiation of TG with either a low (2pM), medium (6pM), or high (20pM) concentration of TF resulted in a dose-dependent increase in thrombin peak height and endogenous thrombin potential (ETP). Side-by-side comparisons of these parameters plus time to peak (TTP) showed that rabbit plasma (peak: 103nM, TTP: 4.3min, ETP: 476nM.min) behaved most similar to human plasma (88nM, 5.4min, 678nM.min) at 6pM of TF. On the other hand, both porcine (84nM, 3.1min, 359nM.min) and rat (94, 2.5min, 443nM.min) plasmas generated shorter TTPs and lower ETPs compared to human plasma. Strikingly, while addition of a pharmacological concentration of the DOAC apixaban (2µM) completely abolished TG in human and rabbit plasma, TG was present in porcine plasma (15.7nM, 21.2min, 184nM.min) and maintained in rat plasma (59nM, 4.9min, 443nM.min). Finally, analysis of plasma anti-FXa activity revealed that rat (23.2mU/s) and rabbit (21.1mU/s) plasma inhibited FXa at significantly higher rates compared to human (9.5mU/s) and porcine (8.2mU/s) plasma.
Summary/Conclusion: In summary, rabbit and rat plasma were relatively unresponsive to stimulation of both the extrinsic (PT) and intrinsic (APTT) pathway, required dilution with buffered saline in order to produce detectable and robust CAT values at low TF and displayed high anti-FXa activities. On the other hand, porcine plasma responded relatively well in PT and APTT assays, did not require dilution in order to produce CAT values at low TF and displayed an anti-FXa activity comparable to human plasma. Overall, our dataset underscores the wide variations between species in response to various pro- and anticoagulant stimuli, which directly impacts the use and interpretation of animal models in research and preclinical assessment.

Clotting
ECTH-156
Board No. 117: A case-control association study in ~5,500 subjects to follow-up on the main findings of the INVENT consortium GWAS on venous thrombo-embolism
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Background: Through a meta-analysis of twelve Genome-wide Association Studies, the INVENT consortium recently identified two novel susceptibility loci for venous thromboembolism (VTE). This project has also generated other candidates that need to be further replicated.
Aims: To assess the association with VTE of common SNPs that demonstrated strong statistical, but not genome-wide, significance in the INVENT cohorts.
Methods: Eleven SNPs were genotyped and tested for association with VTE in three case-control studies totaling 3,019 patients and 2,605 healthy individuals.
Results: None of the tested SNPs showed evidence for association with VTE.
Summary/Conclusion: These results suggest that the additional VTE-associated variants that remain to be discovered are likely rare (allele frequency < 0.05) or common but with very modest influence on disease risk.

Clotting
ECTH-451
Board No. 118: Prevalence of short partial thromboplastin times
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Background: a shorte activated partial thromboplastin time (aPTT) is associated with increased venous thromboembolic events (VTE).
Aims: The aims of this studi was to evaluate the prevalence of aPTT shortening.
Methods: We conducted a retrospective analysis of aPTT results over a 4-month period and 350 controls.
Results: 2858 aPTT samples, were analyzed in groups: ratio <0.9, 0.9-1.2, and >1.2.
675 samples had aPTT<0.9 (23.62%), 1964 samples between 0.9-1.2 (68.72%), and 219 samples>1.2 (31.5%).
we found a significant difference withe low aPTT levels in 350 controls ($p<0.001$).
Summary/Conclusion: an increased number of low-aPTT studies remains unexplained in our population. This simple test should be considered in the evaluation of the risk of VTE.

Clotting
ECTH-279
Board No. 119: Bemiparin long-term treatment of venous thromboembolism in patients with cancer: Elebama study, preliminary results
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Background: Patients with cancer and venous thromboembolism (VTE) have a high risk of recurrence despite the anticoagulant treatment. The low molecular weight heparins (LMWH) have proved to be more effective than antivitamin k (AVK). Currently, international guidelines recommend LMWH treatment for 3-6 months. However, there is little evidence to support all heparins alike. Bemiparin still does not have specific studies in VTE treatment in cancer patients.

Aims: The objective of this prospective observational study is to evaluate the efficacy and safety of bemiparin adjusted to weight (115 anti-Xa IU / kg / 24 h) for 6 months in patients with cancer and venous thromboembolism (VTE) in a single center. The treatment and follow-up of the patients has been performed in accordance with usual clinical practice.

Methods: Seventy-seven patients with active cancer and acute VTE have been included; 18 deep vein thrombosis (DVT) of the lower limbs (23%) and 59 pulmonary embolism (PE) (77 %). In half of the cases the presentation was symptomatic, and in the other half it was incidental. Median age: 65 years (range 32-83); 54.5% women. 74 are solid tumors (96%) and 3 hematological malignances (4%). Location of the primary tumor was lung 23%, colorectal 19%, gynecological 13.5%, breast 13.5%, pancreatic 11%, naso-pharynx 4%, esophagus 4%, central nervous system 4%, kidney 2.7%, prostate 2.7%, germ cell tumor 1.3%, sarcoma 1.3 %. At the time of VTE diagnosis 55.8 % of the patients had metastatic disease. Most of the patients (69%) were receiving some oncospecific treatment (chemotherapy +/- radiotherapy +/- surgery).

Results: Sixty-five patients (84.4%) have completed the 6-month follow-up. 2 of 65 patients (3%) have presented VTE recurrence during treatment with bemiparin (1 case of asymptomatic PE and 1 case of symptomatic DVT). 2 of 65 patients (3 %) have had a major bleeding (an upper gastrointestinal bleeding and an intracranial bleeding in a patient with brain tumor) and 3 of 65 patients (4.6 %) have had a clinically relevant no major bleeding. There has been no case of fatal bleeding or fatal PE. During the study 12 patients have died (18.4%), in all cases because of disease progression.
Summary/Conclusion: Bemiparin, administered at therapeutic doses (115 anti-Xa IU/kg/24 h), for six months as long-term treatment for VTE in cancer patients is associated with a low incidence of recurrent VTE and bleeding in the usual clinical practice.

Clotting
ECTH-256
Board No. 120: Characterization of the annonaceous acetogenin, annonacinone, a natural product inhibitor of plasminogen activator inhibitor-1
Stephane Pautus[1], Mouad Alami[2], Frederic Adam[1], Guillaume Bernadat [2], Allan De Carvalho[1], Gilles Ferry[3], Alain Rupin[3], Abdallah Hamze[2], Pierre Champy[2], Natacha Bonneau[2], Jean Daniel Brion[2], Delphine Borgel[1], Elsa P. Bianchini[* 1]
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Background: Plasminogen activator inhibitor-1 (PAI-1) is the main inhibitor of the tissue type (tPA) and urokinase type (uPA) plasminogen activators. High levels of PAI-1 are correlated with an increased risk of thrombotic events and several other pathologies such as hypertension, cancer and diabetes. As PAI-1 appears to be a very promising target, many research groups focused on the development of PAI-1 inhibitors. Despite several compounds with *in vitro* activity being developed, none of them are currently in clinical use.
Aims: In this study, we evaluated a novel PAI-1 inhibitor, annonacinone, a natural product from the Annonaceous acetogenins group, already known to have biological properties such as anticancer, antimicrobial and antiviral properties.
Methods: Annonacinone was identified in a chromogenic screening assay measuring the inhibition rate of tPA-catalyzed spectrozyme®tPA hydrolysis in the presence of PAI-1 and was more potent than tiplaxtinin, the most studied PAI-1 inhibitor to date. Annonacinone was further characterized ex-vivo and in-vivo for its ability to inhibit PAI-I and in-vitro to elucidate its mechanism of action
Results: Annonacinone and tiplaxtinin inhibited PAI-1 with an IC_{50} of 9 ± 1 µM and 28 ± 1 µM respectively in the chromogenic assay. Annonacinone showed high potency on thromboelastography measured in plasma: addition of 50 µM annonacinone resulted in the total inhibition of PAI-I induced anti-fibrinolytic activity. Annonacinone was also able to potentiate the thrombolytic effect of tPA *in vivo* in a murine model of thrombolysis. While 28% of mice exhibited a partial restoration of blood flow in $FeCl_3$-occluded venules after local application of low dose of rtPA, the addition of annonacinone to rtPA increased to 71% the recanalization.
SDS-PAGE and native-PAGE showed that annonacinone inhibited formation of PAI-1/tPA complex via PAI-1 polymerization and enhancement of the substrate pathway. Mutagenesis and molecular dynamics allowed us to identify annonacinone binding site close to helix D and E and *β*-sheets 2A.
Summary/Conclusion: This work showed that annonacinone had an effect on fibrinolysis by preventing inhibition of tPA by PAI-1 *in vitro*, *ex vivo* and *in vivo*. It also shed light on annonacinone mechanism of action and binding site. Annonacinone appears thus as a very promising antithrombotic agents

Clotting

ECTH-246

Board No. 121: Low D-dimer measured at first-time venous thromboembolism diagnosis is associated with low risk of recurrence

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Background: After a first episode of venous thromboembolism (VTE), 30-40% experience a recurrent event within 10-years. Secondary prevention with anticoagulants is efficient but at the cost of major bleeding risk. Extended treatment should therefore be limited to high-risk individuals. Measurement of d-dimer is used in the clinical algorithm for diagnosis of VTE. Moreover, d-dimer levels measured after cessation of anticoagulant therapy can be used to assess the individual recurrence risk and aid decisions on treatment prolongation. However, whether d-dimer measured at the time of VTE diagnosis can be used to assess recurrence risk is scarcely investigated.

Aims: To investigate the association between d-dimer, measured at the time of VTE diagnosis, and risk of recurrent VTE. We hypothesized that a low d-dimer concentration at VTE diagnosis could identify subjects at low risk of recurrence.

Methods: Patients with an objectively confirmed first-lifetime diagnosis of VTE derived from the outpatient setting and without active cancer (n=454) were included and followed in the period 1994-2012. Information on clinical risk factors and laboratory markers, including d-dimer at VTE diagnosis, were extracted by review of medical records. Recurrent VTE events and deaths during the course of follow-up were recorded. Cox regression models were used to estimate hazard ratios (HR) of VTE recurrence across quartiles of d-dimer in analyses adjusted for age, sex and duration of anticoagulant treatment. Subgroup analyses were carried out for provoked and unprovoked VTE, as well as for DVT and PE. The study was approved by the regional committee for research ethics and the participants gave informed, written consent.

Results: During a median follow-up time of 3.9 years, 84 patients experienced a recurrent VTE. The crude incidence rate was 1.7 (95% CI: 1.0-2.9) per 100 person-years in the lower quartile (≤1.5 mg/L), and 4.9 (95% CI: 3.9-6.1) per 100 person-years in the upper three quartiles combined, yielding an absolute risk difference of 3.2 per 100 per year. Patients with d-dimer ≤1.5 mg/L had 54% lower risk of recurrence than patients with d-dimer >1.5 mg/L (HR: 0.46, 95% CI: 0.25-0.82). Subgroup analyses revealed that the association was particularly pronounced among unprovoked events, with a 66% reduced risk of recurrence among patients with d-dimer ≤1.5 mg/L (HR: 0.34, 95% CI: 0.15-0.74). Furthermore, patients with an incident DVT and d-dimer ≤1.5 mg/L had almost 70% lower risk of recurrence (HR: 0.32, 95% CI: 0.14-0.71) than patients with an incident DVT and d-dimer >1.5 mg/L.

Summary/Conclusion: A low d-dimer (≤1.5 mg/L) measured at the time of VTE diagnosis identified a quarter of the patients to have a low risk of recurrence. The association was particularly pronounced in patients with a first unprovoked event and in patients with DVT. Our findings suggest that d-dimer measured at VTE diagnosis may be used to identify VTE patients at low risk of recurrence, and may guide decisions of short-term anticoagulant treatment in these patients.

Clotting
ECTH-502
Board No. 122: Hemorheological factors for thrombosis in some oncohematological diseases
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Background: Venous thromboembolic events (VTE) are the second most common cause of death in cancer patients. High incidence of VTE despite standard antithrombotic prevention suggests the presence of non-hemocoagulation conditions for thrombosis developing in cancer patients.
Aims: The aim was to study the features of blood rheological behavior and their role for thrombosis in patients with some oncohematologic diseases.
Methods: Study's population consisted 48 children with acute lymphoblastic leukemia (ALL), and 26 adults with polycythemia vera (PV), and 14 adults with chronic myeloid leukemia (CML), and 14 adults with acute myeloid leukemia (AML), and 67 volunteers as the control group. All patients had not any symptomatic organs failures. Hematocrit (Hct), erythrocytes count and erythrocyte indices (MCV, MCH, MCHC), leukocyte count, and fibrinogen were analyzed. Moreover in all patients we we performed assays of B-type natriuretic peptide concentration (BNP). Whole blood viscosity (WBV) under shear rates range 5-300s^{-1}, plasma viscosity by shear rate 250s^{-1} were measured with using «cylinder - cylinder» rotational viscometer (AKR-2, Russia). Measurement was performed as decreasing of shear stress (from 300 to 5s^{-1}) following by an increasing (from 5 to 300s^{-1}). No sample extracting was from device till the analysis is performed. WBV values were adjusted to Hct=40%. Using WBV date we calculated erythrocyte aggregability index and erythrocyte deformability index. Statistical differences were calculated using Mann-Whitney test ($p<0,05$), besides regression analysis was performed. We used MedCalc ver. 14.8.1 (MedCalc Software, Belgium) for statistical analysis.
Results: Regardless of the age 18-20% of patients had elevated BNP assuming subclinical cardiac dysfunction which is an independent VTE risk factor. In all patients WBV differed from normal values. The highest WBV was found in patients with PV, the lowest in patients with AML. Besides hemorheological curves behavior were significantly different among patient groups and in comparison with volunteers. The main differences of the blood rheological behavior were caused by the ratio of erythrocyte aggregation/disaggregation processes and by the differences in the composition and hydrodynamic resistance of cell clumps at high shear rates. In donors differences of WBV values were found at the same shear rate in the depending on the direction of measurement under low and middle ranges of shear rates. In patients WBV differences showed up at all shear rates implying reversible blood structuring process is not full finished.
Summary/Conclusion: Erythrocytes aggregation/disaggregation significantly affects the blood rheological behavior in patients with some oncohematological malignancies. Differences of WBV value depending on the direction of measurement (from 300 to 5s^{-1} and from 5 to 300s^{-1}) are specific for oncohematological patients. Such patient has non-hemocoagulation, namely hemorheological risk factor for thrombosis development. We assume that the revealed hemorheological features in combination with the high concentration of BNP could be one trigger to start of VTE despite standard antithrombotic prevention.

Clotting

ECTH-129

Board No. 123: Impact of subtype of antithrombin deficiency on the risk of venous thromboembolism in hereditary antithrombin deficiency

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Background: Antithrombin (AT) deficiency is associated with a high incidence of venous thromboembolism (VTE). Whether the subtype of antithrombin deficiency (type I, type IIPE, type IIRS or type IIHBS) has impact on the risk of VTE has not been investigated outside of a proband setting and is therefore unknown.
Aims: To investigate whether the varying subtypes of AT deficiency are associated with a different risk of VTE or recurrent VTE.
Methods: We performed a retrospective family cohort study. Probands were excluded from analyses of first and recurrent VTE. The study was investigator initiated without external funding.
Results: After obtaining informed consent, we included 81 subjects from 21 families: 15 original probands, 37 AT deficient family members (29 with type I, 3 type IIHBS and 5 type IIPE), and 29 non-AT-deficient family members. Seventeen of 37 AT deficient family members had a VTE, of which 14 had type I and 3 had type IIPE AT deficiency. None of three type IIHBS family members had a VTE. The annual incidence of VTE in AT-deficient family members was 1.24% (95%CI 0.72-1.99%), with no differences between type I and type II AT deficiency (p=0.57). In 29 family members with type 1 AT deficiency 14 first VTE occurred in 1044 observation years, annual incidence 1.34% (95%CI 0.73-2.25%). No VTE occurred in 3 type IIHBS AT-deficient family members in 119 observation years. Three VTE occurred in family members with 5 type IIPE AT deficiency in 204 observation years, annual incidence 1.47% (95%CI 0.30-4.30). The annual risk of recurrence in the family members after limited-duration anticoagulation for first VTE was 12.1% (95%CI 3.31-31.1%), vs 0.76% (95%CI 0.02-4.25%) in those on long-term treatment. This difference in recurrence free survival was significant, p<0.05. We separately analyzed pregnancies in our cohort, as pregnancy is a known acquired risk factor for VTE. Fifteen pregnancies were without thrombosis prophylaxis, and 24 with thrombosis prophylaxis. In 15 pregnancies without thrombosis prophylaxis 3 first VTE events occurred (20%, 95%CI 4.1-58.4%). In one of these 3 patients 1 recurrence (in the same pregnancy as the first event) occurred. In 24 pregnancies with thrombosis prophylaxis 1 first VTE event occurred (4.2%, 95%CI 0.11-23.2%). Two pregnancies were on full therapeutic anticoagulation due to a previous VTE. One of these was complicated by recurrent VTE.
Summary/Conclusion: Our family study shows a high risk of VTE in non-probands with AT deficiency. AT subtype was not associated with VTE risk, although none of three type II HBS AT deficient family members developed VTE. Type IIHBS may confer a lower risk of VTE, but more research is needed to confirm this. AT subtype (type I vs IIPE) was not associated with recurrence free survival. The recurrence rate was very high after limited-duration anticoagulation and much lower in those with long-term treatment. Therefore, long term anticoagulation is warranted in AT deficient individuals with a positive family history and a first VTE. VTE risk in AT-deficient women without thrombosis prophylaxis in pregnancy or puerperium was very high. VTE occurred despite prophylactic and therapeutic anticoagulation. Although the number of pregnancies were small, these findings support the use of thrombosis prophylaxis in previously asymptomatic AT-deficient women in pregnancy and the puerperium.

Clotting

ECTH-189

Board No. 124: The functional effects of fibrinogen γ' at (patho)physiological plasma levels on clot structure

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Background: A fraction of fibrinogen called the γ′ chain differs from the γA chain at its C-terminus where it has a unique 20-amino acid extension due to alternative polyadenylation and splice variation. In vitro studies have shown that fibrinogen γ′ influences clot structure, producing thinner fibers, increased branching and reduced pore size. However, the effects of fibrinogen γ′ at (patho)physiological levels are poorly understood.

Aims: To investigate the effects of high and low fibrinogen γ' plasma levels on clot structure.

Methods: Fibrinogen and fibrinogen γ' levels were measured by Clauss method and specific ELISA respectively in plasma samples from patients with AAA(Scott, DJ et al., 2011, ATVB). 33 high γ' (median (range): 27.4% (22.2-40.0)) and 41 low γ' (3.9% (1.4-6.2)) patient samples were selected. Purified γA/γ' fibrinogen and γA/γA fibrinogen were combined to give a final concentration of 0.5 mg/ml total fibrinogen, at increasing γA/γ' ratios (5%, 10%, 40%), ratios observed in patient plasma, in the absence and presence of FXIII. Fibrinogen depleted plasma was repleted with the same fibrinogen mixtures to give a final concentration of 1.5 mg/ml total fibrinogen. Clots were formed from the patient samples and purified fibrinogen samples and analyzed by laser scanning confocal microscopy. Patient and repleted plasma samples were analyzed by turbidity and lysis assays.

Results: In patient plasma, high γ' (37%) resulted in plasma clots with increased areas of agglomeration and a higher fiber count (37.3 fibers/100μm) compared to low γ' (4%) (34.3 fibers/100 μm)(*P=0.0126*). This was in agreement with clots made with purified fibrinogens in the presence of FXIII, with increased areas of agglomeration and fiber count (10.3 fibers/100μm) in high γ'(40%) compared to low γ' (3%) (6.0 fibers/100μm)(*P=0.002*). Patient samples with high γ' (37%) presented with a lower Max OD (0.2390 ± 0.005) suggesting thinner fibers and a decreased rate of lysis compared to patients with low γ' (4%)(0.2802 ± 0.0086)(*P=0.0002*). This was reflected in the fibrinogen repleted plasma samples with high γ' (40%) having a lower Max OD (0.1784 ± 0.003) and slower lysis rate than the low γ' (3%)(0.2233 ± 0.009)(*P=0.0103*).

Summary/Conclusion: High γ' levels as observed in patient samples resulted in increased agglomerations and fiber count in plasma clots as well as decreased Max OD and lysis rate. The findings in patient samples were corroborated by findings in both purified systems and in plasma samples repleted with different ratios of purified fibrinogen variants. These data show that fibrinogen γ' influences clot structure and function at plasma levels as observed in patients with vascular diseases.

Clotting

ECTH-227

Board No. 125: Multi-faceted implementation strategy to increase use of a clinical guideline for the diagnosis of deep venous thrombosis in primary care

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Background: A clinical decision rule (CDR), combined with a negative D-dimer test can safely rule out deep venous thrombosis (DVT) in primary care patients. By using this strategy only 50% of the patients need referral to secondary care for ultrasonography, as opposed to referring all patients. This diagnostic strategy is therefore recommended by the primary care guideline in The Netherlands. Yet, uptake by general practitioners (GPs) is low.

Aims: To evaluate a multi-faceted implementation strategy aimed at increasing the use of the guideline recommended CDR plus D-dimer test in primary care patients with suspected DVT.

Methods: This multi-faceted implementation strategy consisted of educational outreach visits, a guaranteed financial reimbursement and periodical newsletters. 217 Dutch GPs received this strategy and included patients from October 2013 to June 2015. Effectiveness was assessed by means of both patient and implementation outcomes. The patient outcomes quantified were (1) 'efficiency' (the proportion of non-referred patients), (2) 'safety' (the proportion of missed DVT cases within this non-referred group), and (3) incorrect application of the guideline (e.g.: application of the CDR in a pregnant patient). The implementation outcomes 'acceptability', 'feasibility', 'fidelity' and 'sustainability' were evaluated with an online questionnaire, sent in December 2014. The patient outcomes 'efficiency' and 'safety' were compared with a small sample of patients included by a comparison group of GPs during the same period, providing information about usual care.

Results: 135 GPs included 619 analyzable patients; 336 (54%) were not referred, missing 6 (1.8% [95% CI 0.7% to 3.9%]) DVT cases. Incorrect guideline use was observed in 199 (32%) patients. Outcomes were similar in a very small sample of only 62 patients included by 32 GPs (referral rate 50%, missing no DVT cases). It turned out that half of these GPs had arranged their own training and financial reimbursement, therefore being 'contaminated' and not providing usual care anymore. Consequently, any comparison should be made with caution.

The questionnaire filled out by the GPs that had received our implementation strategy (89 GPs, response rate 43%) showed high acceptability and feasibility of the guideline. Self-reported guideline use increased from 42% (before the study) to an expected continuation of use of 91% (expected sustainability). The educational outreach visits showed highest fidelity.

Summary/Conclusion: This multi-faceted implementation strategy safely reduced patient referral to secondary care, which is consistent with previous research and despite incorrect application of the guideline in 32% of the patients. This reaffirms the use and application of the CDR and D-dimer test to safely exclude DVT in primary care patients. Unfortunately, a formal comparison with usual care cannot be made due to the small number of included patients by and contamination of the comparison group. The implementation strategy also resulted in high acceptability, feasibility and expected sustainability, further supporting the use of CDR and D-dimer test. Future implementation research should evaluate strategies to enhance correct use of the guideline.

Clotting

ECTH-234

Board No. 126: Relationship of the SAMe-TT2R2 score to anticoagulation quality in atrial fibrillation

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Background: The efficacy and safety of vitamin K antagonists (VKAs) therapy strongly depends upon the quality of anticoagulation control, as reflected by the average time spent in the therapeutic range (TTR) of international normalized ratio (INR). A simple clinical tool (the SAMe-TT_2R_2 score) for identifying patients with atrial fibrillation (AF) who would do well on VKAs has been proposed to help when choosing between VKAs and non-vitamin K oral anticoagulants.

Aims: The aim of this study was to determine the value of the SAMe-TT_2R_2 score (sex-female, age < 60 years, medical history [more than two comorbidities], treatment [interacting drugs, e.g., amiodarone], tobacco use [doubled], race [doubled]) for prediction of anticoagulation quality with VKA in patients with non-valvular AF.

Methods: In a cohort of 518 consecutive AF patients on VKA, seen in our anticoagulation clinic between September 2006 and September 2015, we retrospectively calculated the TTR (using the Rosendaal method) and the SAMe-TT_2R_2 score. The predictive value of the SAMe-TT_2R_2 score was evaluated using the TTR of 70% as a cut-off point. The C statistic, a measure of the area under the receiver operating characteristic (ROC) curve, quantified the predictive validity of the SAMe-TT_2R_2 score and tested the hypothesis that the score performs significantly better than chance (indicated by a C statistic > 0.5).

Results: Of 518 patients (mean age 71.97 ± 8.35 years), 55.78% were male. The median follow-up was 756 (112 – 42,241) days, and the mean TTR was 54.46% ± 17.57%. No significant differences in sex, age, clinical characteristics or CHA_2DS_2-VASc score values were found between the group with a TTR value of ≥ 70% (n = 97, 18.73%) and the group with a TTR < 70% (n = 421, 81.27%). Of patients who achieved a TTR ≥ 70%, 84.54% had a SAMe-TT_2R_2 score of ≤ 2. The score had a modest predictive value for a TTR of ≥ 70% (c-statistic 0.57; 95% CI, 0.51 - 0.63, p = 0.043).

Summary/Conclusion: Our results show a moderate predictive ability of the SAMe-TT_2R_2 score for identification of patients who would do well on VKAs in a cohort of AF patients with relatively poor overall quality of anticoagulation with VKAs.

Clotting

ECTH-127

Board No. 127: Neutrophil to lymphocyte ratio and future risk of venous thromboembolism and case-fatality: the Tromsø study

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Background: Acute and chronic inflammatory diseases are risk factors for venous thromboembolism (VTE). Low-grade inflammation over time has been suggested as a potential mechanism for development of VTE, but studies on inflammatory markers, like high sensitivity CRP, have shown conflicting results. Neutrophil to lymphocyte ratio (NLR), a general marker of inflammation, is associated with thromboembolic complications in atrial fibrillation, arterial cardiovascular disease (CVD) and CVD-related mortality, but its role in VTE is scarcely studied.
Aims: The aim of our study was to investigate the association between NLR and risk of incident VTE, VTE recurrence and case-fatality in a cohort recruited from a general population.
Methods: Baseline NLR was calculated in 25107 men and women, aged 25-96 years, attending the Tromsø Study in 1994-95. Study participants were followed-up to the first of the following: a VTE event, migration, death or to the end of the study period (Dec 31, 2012). Cox regression models were used to estimate age- and sex-adjusted and multivariable-adjusted hazard ratios (HR) with 95% confidence intervals (CI) of VTE. An extra cut-off point was established at the 95-percentile (NLR >3.46), and we additionally compared those with NLR above the 95-percentile to those with NLR in the lowest quartile. Since regression dilution effects could potentially influence the exposure-outcome association, all analyses were additionally performed with a shorter follow-up time of three years. For analyses of death and recurrence, subjects were followed from the date of the first VTE, and hazard ratios were estimated according to NLR using the same models as described above. The study was approved by the Regional Committee of Research Ethics, and all participants provided informed written consent.
Results: During 17.7 years of follow-up, 664 had a first-lifetime VTE, and of these 107 had a recurrence and 313 died. There was no association between baseline NLR and risk of VTE in analyses adjusted for age, sex, body mass index, smoking and diabetes (HR upper versus lower quartile: 1.07, 95% CI 0.86-1.33). Moreover, NLR was not associated with VTE in separate analyses of provoked and unprovoked events or in analyses of deep vein thrombosis and pulmonary embolism. During the first three years of follow-up, 74 of the study participants had a VTE event. Even with this shorter follow-up time, there was still no association between baseline NLR and risk of VTE when comparing the upper with the lowest quartile of NLR (multivariable adjusted HR 1.48, 95% CI 0.75-2.92). However, the group with NLR above the 95-percentile had a 2.4-fold higher risk of VTE compared with those in the lowest quartile (multivariable adjusted HR 2.36, 95% CI 0.96-5.82). There was no association between NLR and VTE recurrence in those with a first VTE, but the total mortality rate after VTE increased across quartiles of NLR (HR upper versus lower quartile 1.41, 95% CI 1.03-1.94).
Summary/Conclusion: NLR was not associated with risk of incident or recurrent VTE, but baseline NLR was associated with higher mortality among VTE cases. Our findings imply that longstanding low-grade inflammation is not involved in the development of VTE.

Clotting

ECTH-442

Board No. 128: Impact of external control programme in apixaban treatment adherence

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Background: Novel oral anticoagulants (NOACs) drugs are available as alternatives to vitamine K antagonists (VKAs). These agents do not require routine monitoring of coagulation parameters, however there are other considerations that must be taken into account, expected adherence to therapy is an important consideration in clinical decision because poor adherence can increase the risk of severe complications.

Here we analyzed if the adherence of the patient can get better with some type of external control.

Aims: To find out the impact of an adherence program to the treatment (ELIPAC program) defined as an outside telephonic control carried out by a nurse together with a digitally programmed alarm every 12 hours.

To determine the influence that age and low creatinine clearance may have in the risk of bleeding or thrombotic complications.

Methods: We studied 55 patients with non valvular atrial fibrillation under treatment with apixaban (5mg bd or 2,5mg bd) divided in two groups: with ELIPAC program (n=25) and without ELIPAC program (n=30).

All patients were follow-up for clinic controls, analysis, therapeutic education and complications. After six months of treatment, they completed the MORISKY-GREEN test, a validated self-reporting tool of adherence assessment. An evaluation of 1-4 points is used to evaluate the adherence of the treatment (1-minimun, 4-maximun).

CHADS2, CHA2DS2-VASc, HAS-BLED score and creatinine clearance were included into the analysis.

Results: A total of 55 patients were included (58%women) with a mean age of 77 years (range 60-90). A total of 25 patients (45%) (12 with 5mg bd and 13 with 2,5mg bd) were included in the ELIPAC program. The data were analyzed using statistical analysis software (SPSS). There were no statistically significant differences in the results of the MORISKY-GREEN test between the two groups, although a slight tendency showing a better adherence to the treatment was observed in the patients into the ELIPAC program and 2´5mg bd subgroup (p=0´08).

There were no major bleeding or stroke event in patients included.

Summary/Conclusion: In the adherence of treatment, the education can play an important role. Follow-up with a structured form of visits in the hospital, at the begining, at 6 months and 1 year, including elements as education and motivation it´s enough for an adecuate adherencia to the treatment.

Outside control (ELIPAC program) may improve motivation and strengthen adherence.

We must be strict with the adjustment of dosage taking into account the patient characteristics to avoid clinical complications.

Clotting

ECTH-212

Board No. 129: In vitro analysis of FII c.1787G>A (prothrombin Belgrade) mutation effect on prothrombin gene expression

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Background: The FII c.1787G>A (Prothrombin Belgrade) is a novel prothrombotic mutation which leads to impaired inhibition of thrombin by its natural inhibitor, antithrombin (antithrombin resistance). The presence of this mutation has been associated with the increased risk of thrombosis, but the mechanism of this variant has not been fully elucidated so far.

Aims: The aim of this study was to investigate the effect of FII c.1787G>A mutation on the prothrombin gene expression by functional *in vitro* analysis.

Methods: Expression profile of FII c.1787G>A mutation was examined by transient transfections of Cos-7 cell line with wild-type and c.1787A prothrombin pcDNA3.1(+) expression vectors. Real-Time PCR was used for relative quantification of prothrombin mRNA levels. The relative quantification of prothrombin amount was performed by Western blot analysis.

Results: No difference has been observed in the expression profile of c.1787A mutant compared to wild-type prothrombin expression (RQ 0.96 vs. RQ 1.00, respectfully). Relative quantification of protein level showed that wild-type (1.12±0.16) does not differ significantly (p=0.17) from mutant prothrombin (0.97±0.24).

Summary/Conclusion: This is the first study in which the mechanism of FII c.1787G>A mutation was examined *in vitro*. Our results show that FII c.1787G>A mutation does not affect the prothrombin mRNA and protein level. This suggests that the mechanism of FII c.1787G>A mutation does not alter prothrombin expression profile.

Clotting

ECTH-371

Board No. 130: Evaluation of a new, ready-to-use reagent for prothrombin time determination

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Background: The current reagent HemosIL® RecombiPlasTin 2G (Werfen) for prothrombin time (PT) measurement is formulated as a lyophilized powder and needs to be dissolved and rest for at least 20 minutes before usage. A new, ready-to-use PT reagent HemosIL® ReadiPlasTin (Werfen) was evaluated in this study. For reconstitution of this new reagent, a concentrated solution of recombinant human tissue factor and a diluent only need to be poored together and the reagent is then immediately ready to use.

Aims: The performance characteristics of the HemosIL® ReadiPlasTin (Werfen) were evaluated. International normalized ratio (INR) results were correlated to those with the current reagent and to results measured with the CoaguChek® XS Plus point-of-care (POC) device (Roche Diagnostics).

Methods: The RecombiPlasTin 2G and ReadiPlasTin reagents were reconstituted and calibrated as prescribed by the manufacturer. Measurements were performed on ACL TOP 500 (Werfen). The mean normal PT (MNPT) for INR calculations was determined as the geometric mean of the PT (s) measured in citrated plasma samples from 20 healthy volunteers. Imprecision of ReadiPlasTin was determined according to the CLSI EP05-A3 protocol, using HemosIL® Plasma Coagulation Control Level 1 and 2 (Werfen). Correlation of ReadiPlasTin to RecombiPlasTin 2G and CoaguChek® XS Plus was performed on citrated plasma samples or capillary whole blood from healthy volunteers and patients treated with vitamin K antagonists (VKA), after informed consent. The results were evaluated by linear regression and Bland-Altman analysis. Clinical correlation was evaluated by inter-rater agreement (kappa) analysis on INR results from VKA patients.

Results: MNPT was found to be 11.4s for ReadiPlasTin and 10.7s for RecombiPlasTin. Within-run, between-run and total imprecision for INR with ReadiPlasTin were respectively 0.84%, 1.49% and 2.40% for level 1, and 1.76%, 2.71% and 3.55% for level 2. Linear regression analysis revealed the following correlation between ReadiPlasTin (y) and RecombiPlasTin (x): $y=-0.068+1.05 \cdot x$, with R^2 equal to 0.996 and a relative bias of |2.79|% (n=40). Linear regression analysis of ReadiPlasTin (y) INR results *vs.* CoaguChek® (x) showed the following correlation: $y=-0.048+1.03 \cdot x$, with R^2 equal to 0.982 and a relative bias of |4.79|% (n=40). For the inter-rater agreement analysis, the target ranges used for evaluating the INR results (therapeutic *vs.* non-therapeutic) were interpreted by taking into account measurement uncertainty. The clinical correlation of ReadiPlasTin with RecombiPlasTin (n=20) resulted in a weighted kappa equal to 1.00, indicating a very good agreement. Also for the correlation of ReadiPlasTin with CoaguCheck® (n=20), the weighted kappa was found to be 1.00.

Summary/Conclusion: We concluded that the INR performance characteristics with ReadiPlasTin fulfilled the acceptance criteria and correlated well with RecombiPlasTin 2G and CoaguChek® XS Plus. The ready-to-use formulation of ReadiPlasTin with the simplified pre-analytical steps make that this reagent is an interesting alternative to RecombiPlasTin 2G.

Clotting

ECTH-506

Board No. 131: Monitoring of low-molecular-weight heparin in women with high risk pregnancy.

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Background: Low Molecular Weight Heparin (LMWH) is the preferred anticoagulant for the prevention and treatment of venous thromboembolism (VTE) during pregnancy in women with thrombophilic disorders. Monitoring of anti-Factor Xa activity in high risk pregnancies treated with LMWH remains controversial.

Aims: This observational study evaluates outcomes of 138 pregnant women on antithrombotic therapy with prophylactic or therapeutic doses of LMWH from June 2011 to May 2016.

Methods: Initial dosage, dose changes and type of LMWH during the follow-up were analyzed. Monitoring of LMWH according to anti-Xa level was done by automated chromogenic assay (Liquid Anti Xa Hemosil, Instrumentation Laboratory™) using automated coagulation analyser ACL TOP (Instrumentation Laboratory™). LMWH dose changes were made empirically by medical monitoring based on anti-Factor Xa results. We analyzed demographic parameters, congenital or acquired thrombophilia data, obstetric history, thrombotic episodes and obstetric complications of 153 high risk pregnancies.

Results: Median age of women was 35 years (range: 20-47 years). 98% of pregnancies (150) were given enoxaparin; of these, only three patients required therapeutic dose of enoxaparin by deep vein thrombosis in two cases and pulmonary thromboembolism by another. Prophylactic dose of enoxaparin used in most cases was 40 mg daily (133/147). Another LMWH used was Bemiparin, always prophylactic dose in three patients.

LMWH was mainly indicated by positive thrombophilia studies, in 74% of cases (Protein C deficiency (7), Protein S deficiency (17), V Leiden Factor mutation (10), antithrombin deficiency (2), MTHFR homozygosity mutation (11), antiphospholipid syndrome (39) and both, MTHFR gen heterozygosity mutation and thrombosis history in 27 cases); poor obstetrical history in 12 cases; previous VTE in 17 and other causes in 11 events (four transient ischemic attack, three in vitro fertilization and one case each of the following: sickle-cell anemia, Raynaud syndrome, obesity and ophthalmic vein thrombosis).

A median of 8 anti-Factor Xa determinations (range: 1-16) were obtained for each case. The mean anti-Factor Xa level was 0.24 UI/mL (range: 0.07-0.87 UI/ml). 52 patients (33.9%) increased the dose of LMWH and 14 required more than one dose change (both increase and decrease).

Among the gestational complications were observed: threatened abortion (6), intrauterine growth retardation (2), preterm birth threat (1), uterine atony (1) and postsurgical hematoma (1). Infratherapeutic anti-Factor Xa levels in 2 patients incurred in 1 VTE and 1 eclampsia fetal loss in the 37th gestation week.

Summary/Conclusion: In our experience dose changes of LMWH throughout pregnancy based on anti-Factor Xa activity were common. A significant increase in the LMWH dose requirements suggests that closer monitoring of anti-Factor Xa activity may be appropriate in pregnant patients to reach optimal anticoagulant levels. Monitoring of LMWH would be helpful in high risk pregnancies with insufficient anticoagulation response.

Clotting
ECTH-176
Board No. 132: Influence of factor VII on INR determined with human recombinant and tissue-extract thromboplastins
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Background: Previous studies have suggested differences in sensitivity to clotting factor (FVII) between recombinant human and tissue-extract thromboplastins used for International Normalized Ratio (INR) measurement, but experimental evidence is scarce. Differences in FVII sensitivity between thromboplastins are clinically relevant, since they affect measured INR stability during treatment with vitamin K antagonists (VKA), and can explain INR discrepancies between measurement methods.
Aims: The goal of our study was to determine how different thromboplastins react to a fixed increase in FVII. We measured the change in INR, after addition of various concentrations of human FVII to plasma of patients treated with VKA, using three commercial tissue-extract thromboplastins (Hepato Quick, Neoplastin C1+, Thromborel S) and three recombinant human thromboplastins (Innovin, Recombiplastin 2G, Coaguchek XS), that are frequently used in daily practice.
Methods: We added three doses of purified human FVII (0.006, 0.012, and 0.062 µg per mL plasma), or buffer (0.15M NaCl, 20 mM Tris.HCl, pH 7.4, 1% BSA) as control, to five certified pooled plasmas of patients on VKA (INR 1.5-3.5). Certified prothrombin times (PT) and INRs of these plasmas had been determined by three external laboratories using the international standards for rabbit thromboplastin (RBT/05) and for recombinant human thromboplastin (rTF/09). The certified values were used to calibrate the above-mentioned commercial thromboplastin reagents. Plasma PTs were determined with these reagents, in duplicate, in polystyrene tubes using a semi-automatic coagulometer according to Schnitger and Gross. Coaguchek XS measurements were performed separately using the same FVII concentrations. PT values were derived from the instrument and transformed to INR using the certified INR results. INRs obtained for plasmas spiked with Factor VII were compared with the same plasmas spiked with the same volume of buffer without Factor VII. Change in FVII activity was measured with a specific bioassay (Recombiplastin 2G). Relative INR changes in the pooled plasmas were compared between thromboplastin reagents using the Wilcoxon signed-rank test or Friedman test.
Results: After FVII addition, FVII activity in the pooled plasmas increased by 3.2, 6.4 and 32.0% respectively. Prothrombin times shortened and INR values decreased with increasing FVII concentrations in all five pooled plasmas, irrespective of the used thromboplastin reagent. The relative INR change, after FVII addition, significantly differed between the six used thromboplastin reagents ($p<0.001$). The INR changes were similar amongst the recombinant human ($p=0.085$) and tissue-extract ($p=0.575$) thromboplastins. Pooled results showed that the relative INR change was significantly greater when a recombinant human thromboplastin was used for INR measurement compared to a tissue-extract thromboplastin ($p<0.001$).
Summary/Conclusion: Our results indicate that, despite optimal calibration, significant differences regarding FVII sensitivity exist between different thromboplastin reagents used for monitoring patients on VKA. Furthermore, recombinant human thromboplastins are more sensitive to FVII than tissue-extract thromboplastins. These findings can explain INR discrepancies between methods reported in prior studies and observations of differences regarding stability of anticoagulation control between patients on VKAs monitored with thromboplastins from different sources.

Clotting
ECTH-155
Board No. 133: Complement and coagulation in a clinical setting: a review
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Background: Interactions between the complement and coagulation systems is a growing field of interest. The lectin pathway of the complement system has lately gained a particular focus. Several authors have demonstrated that mannose-binding lectin (MBL) and its associated serine proteases (MASPs) of the lectin pathway activate coagulation and impair fibrinolysis in vitro.
A procoagulant effect of the lectin pathway is of great interest with regards to developing new biomarkers for thrombosis risk and, in the long run, new treatment modalities. This could be relevant not only in in classical complement-mediated diseases but also in other conditions with increased complement activation, among which are sepsis, cancer, and also pregnancy and the pregnancy-related complication preeclampsia. In these conditions, an increased thrombosis risk is well recognized, and there is evidence of altered levels of lectin pathway proteins.
Aims: We aimed to systematically review the existing literature on associations between lectin pathway protein levels and haemostatic activation in patients with sepsis, cancer, and pregnancy with and without preeclampsia.
Methods: The systematic literature search in PubMed and Embase covered the period from 1.1.1985-20.4.2016. The following MeSH/Emtree terms were employed, “Complement System Proteins”, “Thrombosis”, “Blood Coagulation”/”Blood Clotting”, “Sepsis”, “Disseminated Intravascular Coagulation”, “Neoplasms”, “Pregnancy”, and “Pre-Eclampsia”. Besides, free text searches were conducted using “mannose-binding lectin” and “thrombosis”, “sepsis”, “disseminated intravascular coagulation”, “cancer”, “pregnancy” and “pre-eclampsia”. Inclusion criteria for publications were 1) original work, 2) human study population, 3) associations between lectin pathway proteins and haemostatic activation or clinical thrombosis as a major subject. Exclusion criteria were 1) not original work, 2) case report with less than five cases, 3) in vitro or animal study, 4) other language than English.
Results: Of 2,802 initial hits, 1,784 were excluded based on the exclusion criteria described above. Of the 1,018 remaining relevant publications, only two met all the inclusion criteria. Both examined MBL plasma levels and/or genotype in sepsis patients. In addition, six other publications were identified which dealt with lectin pathway protein levels and clinical thrombosis in cardiovascular disease (n=2), venous thromboembolism (n=2), portal vein thrombosis in liver transplantation patients (n=1), and systemic lupus erythematosus (n=1).
Summary/Conclusion: Only very few previous studies have investigated associations between lectin pathway proteins and haemostatic activity in vivo in human populations. Thus, the clinical significance of the interaction between the two systems in patients who are at increased risk for thrombotic events is still unclear. Further studies in this field are warranted to elucidate the role of the lectin pathway in patients with an increased thrombosis risk.

Clotting
ECTH-425
Board No. 134: Protein S deficiency in a patient treated with rivaroxaban
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Background: Protein S is an important part of natural anticoagulants.
Some diseases and conditions such as pregnancy and treatment with vitamin K antagonists lower plasma (p) protein S levels. Little is known about Rivaroxaban effect on p-protein S measurements.
A 58-year-old female patient was diagnosed with deep venous thrombosis in left v. illiaca communis. She was started standard Rivaroxaban therapy.
The patient had flown to Canada 14 days before hospitalization. She was using a connecting flight with three changes, and duration of every flight was less than 6 hours (h). She was immobile for 48 h due to influenza she got after she had returned home. Previously she was diagnosed with DVT in same leg during pregnancy 38 ago.
The patient was tested for thrombophilia under Rivaroxaban treatment. She had factor (F) V Leiden heterozygous mutation (ARG506GLN) and low p-protein free S antigen concentration 0.28 x 10^3 IU/l (reference range 0.57-1.47 x 10^3 IU/l). Free protein S antigen concentration was measured by immunological method.
Aims: To investigate if the patient had protein S deficiency.
Methods: Members of patient's family underwent laboratory testing for thrombophilia.
Results: Patient's 72-year-old half-sister was diagnosed with FV Leiden heterozygous mutation (ARG506GLN). P-protein free S antigen concentration was within normal range.
Patient's 39-year-old daughter was diagnosed with FV Leiden heterozygous mutation (ARG506GLN). She had low p-protein free S antigen concentration of 0.32 x 10^3 IU/l. P-protein S activity measurement with coagulation method revealed low activity of 0.27 arb. unit/l (reference range 0.70-1.40 arb. unit/l). She is under genetic testing. Daughter's children (two sons) were invited for thrombophilia testing.
11-year-old son had FV Leiden heterozygous mutation (ARG506GLN). P-protein free S antigen concentration was within normal range.
9-year-old son had FV Leiden heterozygous mutation (ARG506GLN) and p-protein free S antigen concentration of 0.60 x 10^3 IU/l. He is under further testing for protein S deficiency with p-protein S activity measurement.
Laboratory testing for thrombophilia of patient's 33-year-old son revealed no abnormalities. No family members tested for thrombophilia had previously been diagnosed with thrombosis.

Summary/Conclusion: Patient's low p-protein free S antigen concentration is rather a heritable state than an effect of treatment with Rivaroxaban. Laboratory testing of patient's family can be a useful tool to investigate for protein S deficiency in patients treated with anticoagulants when interference of anticoagulants on laboratory tests is expected.

Clotting

ECTH-457

Board No. 135: Coagulation factor VIII activity in blood donors is lower in freshly prepared plasma component than in blood sample

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Background: Coagulation factor (F) VIII is a labile factor quickly decreasing over time. FVIII activity measurement has been used as a marker of quality control for fresh frozen plasma and cryoprecipitate. No previous studies have explored FVIII activity in donors' blood samples versus freshly prepared plasma component (FPPC).

Aims: To compare F VIII activity in stabilized blood sample from blood donors before blood donation and in FPPC.

Methods: 40 randomly selected blood donors (20 blood type 0 and 20 blood type A) underwent sample taking procedure for FVIII activity measurement. The first blood sample was collected in a 3.2% natrium-citrate tube just before blood donation. This sample was centrifuged at 2000 g for 12 min and immediately frozen at -80˚C according to local laboratory guidelines. The second sample was taken from FPPC and immediately frozen at -30˚C according to the guidelines for preparing of fresh frozen plasma. 17 FPPC (8/blood type 0 and 9/blood type A) were produced same day, and 23 FPPC (12/blood type 0 and 11/blood type A) were produced next day. All whole blood portions were stored at room temperature until FPPC was produced. FVIII activity was measured as a batch in all collected samples by the same experienced laboratory technician.

Chromogenic method (Siemens cat. No B4238-40, CS2100i, Sysmex) was used for FVIII activity measurement. Measurement range 0.01 – 4.80 x 10^3 IU/l.

Results: Mean FVIII activity of blood type 0 in citrate plasma was 1.25 ± 0.08 x 10^3 IU/l and 0.97 ± 0.06 x 10^3 IU/l in FPPC, respectively. The mean difference of FVIII activity between citrate plasma and FPPC was 22.4± 7.4 %. Mean FVIII activity of blood type A in citrate plasma revealed a value of 1.48 ± 0.09 x 10^3 IU/l and 1.09 ± 0.06 x 10^3 IU/l for FPPC, respectively. The mean difference of FVIII activity between citrate plasma and FPPC was 34.0 ± 16.8 %.

FPPC produced same day (mean time of whole blood storage was 2 h 50 min ± 36 min) revealed 15.5 ± 4.6 % mean difference of FVIII activity between citrate plasma and FPPC for all blood types. FPPC produced next day (mean time of whole blood storage was 17 h 10 min ± 54 min) showed a statistically significant decrease of FVIII activity with a mean difference between citrate plasma and FPPC by 29.1 ± 5.4 %, $p<0.001$, compared to FPPC production of same day (all blood types).

Summary/Conclusion: Our experiment has shown a mean decrease of FVIII activity by 22.4 % for blood type 0 and 34.0 % for blood type A in FPPC compared to citrate plasma. FPPC produced next day has shown statistically significant decrease of FVIII activity ($p<0.001$) compared to same day production of FPPC.

Clotting
ECTH-322
Board No. 136: Effect of anticoagulants on lupus anticoagulant assays
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Background:
Anticoagulant treatment may affect Lupus anticoagulant (LA) testing. Therefore, it is generally recommended not to perform LA testing in patients on anticoagulation. However, this can be problematic for patients requiring long-term anticoagulation. For a possible adjustment of current policy more knowledge is required about the exact effects of anticoagulants on LA assays.

Aims:
In this study we analysed the effect of six anticoagulants on the LA result of the dilute Russell's Viper Venom time (dRVVT) and Silica clotting time (SCT) assay.

Methods: Normal pool plasma (NP), Lupus-positive pool plasma (LP, Cryopep), and Lupus-weak positive pool plasma (LWP, Cryopep) were spiked with different concentrations (50% and maximum concentration of therapeutic range) of unfractionated Heparin (Leo), Nadroparin (Fraxiparin), Danaparoïd (Orgaran), Enoxaparin (Clexane), Fondaparinux (Arixtra), or Tinzaparin (Innohep). LA testing in these samples was performed using the dRVVT and SCT assay. For the NP analysis, dRVVT screen and confirm reagents from LifeDiagnostics and Instrumentation Laboratory (IL) were used. For the LP and LWP analysis only dRVVT reagents from IL was used. SCT screen and confirm reagents were from IL.
Results: False-positive LA results in the dRVVT assay were observed when NP was spiked with 1.2 U/ml Heparin (using IL reagent), 2 U/ml Nadroparin, 1 and 2 U/ml Danaparoïd, 2 U/ml Enoxaparin, and 1 and 2 U/ml Tinzaparin. In the SCT assay no false-positive LA results were observed when NP was spiked with the different amounts of anticoagulants. However, the normalized LA ratio (screen ratio/confirm ratio) from the SCT assay decreased substantially when NP was spiked with the different anticoagulants except for Fondaparinux. This decrease in normalized LA ratio from the SCT assay was also observed with LP and LWP spiked with the different anticoagulants, resulting in false-negative LA results for 1.2 U/ml Heparin, 2 U/ml Nadroparin, and 2 U/ml Tinzaparin. For the dRVVT assay no false-negative LA results were observed when LP and LWP were spiked with the different amounts of anticoagulants.
Summary/Conclusion: The dRVVT and SCT assays were not affected by Fondaparinux, even at peak levels. This indicates LA testing can be reliably performed in material from patients receiving Fondaparinux therapy. For Heparin, Nadroparin, Danaparoïd, Enoxaparin, and Tinzaparin false-positive LA results with the dRVVT assay and false-negative LA results with the SCT assay can be observed. Therefore, caution is required for LA testing in patients receiving Heparin, Nadroparin, Enoxaparin, Danaparoïd or Tinzaparin. With these anticoagulants the laboratory should first determine the exact concentration of this anticoagulant in order to decide whether and with which LA assay (dRVVT or SCT assay) the LA evaluation can be performed reliably.

Clotting

ECTH-222

Board No. 137: Differences between dilute Russell's viper venom time reagents in lupus anticoagulant testing

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Background: Lupus anticoagulant (LAC) detection represents diagnostic challenges among which existence of several assay principles, multitude of available reagents and interference by antithrombotic treatment. One of the two advised tests is the dilute Russell's viper venom time (dRVVT). However, it is currently not clear whether all dRVVT reagents may be considered equivalent. In addition, guidelines are lacking on how to deal with the influence of direct oral anticoagulants (DOAC) on dRVVT LAC testing.
Aims: To evaluate the performance of two commercial dRVVT screen/confirm reagents, with special attention to the influence of anticoagulant therapy, in order to acquire more knowledge on the diagnostic performance and pitfalls in dRVVT from different manufacturers.
Methods: STA®-Staclot® dRVV Screen/Confirm (Stago, Asnières-sur-Seine, France) and dRVT-LS/dRVT-LR (Haematex, Hornsby, Australia) were evaluated on 443 patient samples: 358 consecutive patients with LAC request including 6 antiphospholipid syndrome (APS) patients, 18 non-consecutively selected APS patients and 37 vitamin K antagonists (VKA)-treated and 30 DOAC-treated non-APS patients. Screen, mix and confirm test results were expressed as normalized ratios by dividing the patient plasma (PP) clotting time (CT) by the pooled normal plasma (PNP) CT analysed in the same run. PNP was spiked with factor deficient plasma (FII, FV and FX, levels between 1-100% (n=33)) and DOAC calibrators (apixaban (API), dabigatran (DAB), rivaroxaban (RIV), concentrations between 0-525 ng/mL (n=21)) to evaluate sensitivity for factor deficiencies and false-positivity rates, respectively. Final LAC conclusions were interpret by the three-step interpretation (screen, screen mix and LAC ratio (screen/confirm)).
Results: A higher number of consecutive patient samples were defined as LAC positive by Stago versus Haematex (11.5% (41/358) vs. 3.63% (13/358)). Most discordances were observed in the VKA and DOAC group. Spiking experiments showed that Haematex screen and confirm was less prone to VKA-related factor deficiencies (FII < 1%, FX 24%/36%, respectively) compared to Stago screen and confirm (FII 48%/46%, FX 57%, respectively), which might explain the absence of false-positive LAC results in VKA-treated patients compared to Stago (10.8%). Both Stago and Haematex screen and confirm showed a concentration-dependent prolongation by API, DAB and RIV. However, we observed no false-positive LAC ratios with Haematex in DOAC-spiked PNP and a lower number in DOAC-treated non-APS patients. Importantly, the increased specificity of Haematex was at cost of a reduced sensitivity, shown by less positive APS patient samples compared to Stago (45.8% vs. 87.5%).
Summary/Conclusion: dRVVT reagents differ in sensitivity for LAC detection and for VKA and DOAC interference in both APS and non-APS patients, which may cause opposing LAC conclusions. Falsely positive LAC introduced by DOAC also depends on the type of DOAC and the DOAC concentration. We hypothesize that distinct phospholipid composition and concentration in dRVVT screen and confirm reagents might influence the CT differently and hence the LAC interpretation.

Clotting

ECTH-365

Board No. 138: Cardiovascular disease proteomics: novel biomarkers and possible mechanisms

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Background: Venous thromboembolism (VTE), the third most common cardiovascular disease (CVD), has an incidence of approximately 1-2 per 1000 individuals per year. VTE risk prediction remains a challenge, due to the lack of clinically useful biomarkers.

Aims: To identify novel VTE biomarkers in plasma using an affinity proteomics strategy.

Methods: In the VEREMA project, a first discovery screen was performed in samples from Swedish (VEBIOS), Spanish (RETROVE) and French (FARIVE) cohorts. The discovery sample set contained both patients with acute VTE and patients sampled after discontinuation of anticoagulant treatment following a first time thrombosis, together with matched healthy controls. 768 antibodies from the Human Protein Atlas project, targeting 408 proteins, were used to profile 360 plasma samples from each of the discovery cohorts, using multiplex suspension antibody bead arrays. Selected targets were verified by immunocapture mass-spectrometry (IC-MS) and dual-binder immunoassays.

Results: The screening phase identified a number of potential biomarker candidates significantly associated with VTE. By IC-MS, the verification of antibody-targets for various candidates, such as PDGFB, BLVRB and CD47, was achieved. IC-MS analysis also revealed protein-protein interactions between antibody-targeted candidates and other proteins in plasma, which now are further investigated by dual binder assays of indicated physical protein-protein interactions and IC-targeted-MS experiments. Furthermore, we are developing ELISA-sandwich assays for use in a clinical setting, to test candidates in prospective studies.

Summary/Conclusion: Affinity proteomics-based techniques provide a tool for protein profiling of plasma samples from large patient cohorts to discover specific VTE biomarkers. IC-MS plays an important role in verification of antibody targets before the validation in a clinical setting is undertaken.

Clotting

ECTH-429

Board No. 139: The assessment of changes in haemostasis in women with high-risk pregnancy

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Background: Along with the pre-existing risk factors, physiological prothrombotic changes during pregnancy and in the postpartum period increase the risk of venous thromboembolism (VTE) and pregnancy complications, which contribute to the significant maternal morbidity and mortality. Their common sign is the increased synthesis of thrombin, leading to the development of microthrombi in the site of the implantation, or later to the disordered uteroplacental perfusion. The Guidelines of the American Society of the Chest Physicians (ACCP) in high-risk pregnancy preferthe use of low-molecular-weight heparin (LMWH).

Aims: To monitor the changes of haemostasis in the course of pregnancy and postpartum period in women with high-risk pregnancy using conventional and innovative laboratory methods.

Methods: In pregnant patients with previous thrombotic episode, repeated fetal loss or another pregnancy complication on anticoagulant thromboprophylaxis, the changes of haemostasis were evaluated by the use of standard coagulation tests, markers of haemostasis activation (quantitative analysis of D-dimers), detection of the anticoagulant anti-Xa activity of LMWH, rotational thromboelastometry and flow cytometry in chosen 4 samplings of the venous blood during pregnancy and 1 blood collection after the postpartum period.

Results: Our results confirm the graduation of the hypercoagulant state in pregnancy despite the use of adequate anticoagulation.

Summary/Conclusion: The monitoring of the changes of haemostasis in the group of high-risk pregnant patients could play a key role in the individualization of anticoagulant thromboprophylaxis. The project complies with the Declaration of Helsinki and the informed consent in each patient is obtained.

Acknowledgements: we would like to thank the support of projects of Scientific Grant Agency Vega 1/0168/16 and Agency for the Support of Research and Development APVV 0222-11.

Clotting

ECTH-437

Board No. 140: Management of acute venous thromboembolism in women with high-risk pregnancy

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Background: Two thirds of the cases of deep venous thrombosis (DVT) are developed during pregnancy. On the contrary, 43%>60% of the cases of pulmonary embolism (PE) have been noticed 4-6 weeks after the delivery. According to the Guidelines of the American College of Chest Physicians (ACCP) and anticoagulation forum, in pregnant patients with acute episode of venous thromboembolism (VTE), the use of adjusted doses of low-molecular-weight heparin (LMWH) over the administration of the unfractionated heparin is preferred. Dosing of LMWH adjusted to the body weight is managed by the same regimen such as in non-pregnant patients. Thrombolytic therapy should be reserved for pregnant women with PE and serious cardiovascular decompensation or with life-threatening DVT. Anticoagulation treatment should last up to the end of the postpartum period (total minimal length 3 months).

Aims: To illustrate the use of the recommendations for the management of the acute VTE in patients with high-risk pregnancy in the clinical practice.

Methods: We present the unusual case report of the 30-year-old pregnant women who experienced the dyspnoea and suspect PE with moderate cardiovascular risk in the 20th gestational week.

Results: The use of adequate anticoagulant and antiplatelet therapy led to the rapid disappearance of the symptoms and no further thrombotic event was developed.

Summary/Conclusion: The most of the above-mentioned recommendations is prepared according to the results of the clinical studies of the non-pregnant patients. Moreover, the data from further prospective randomised studies, on the basis of which the personalized management of acute VTE could be performed, are needed.

The project complies with the Declaration of Helsinki and the informed consent in each patient is obtained.

Acknowledgements: we would like to thank the support of projects of Scientific Grant Agency Vega 1/0168/16 and Agency for the Support of Research and Development APVV 0222-11.

Clotting

ECTH-194

Board No. 141: Next-generation sequencing approach to study burden of rare variants in loci susceptible to cerebral vein thrombosis

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Background: Cerebral vein thrombosis (CVT) is a rare life-threatening disease affecting annually 4 adults/million. Transient risk factors for CVT include oral contraceptive use, pregnancy, trauma, brain tumors and local infections. Genetic risk factors are deficiencies of the natural anticoagulant proteins antithrombin, protein C, protein S, factor V Leiden and prothrombin 20210A mutation. In 20% of patients, the cause of CVT remains unknown.
Aims: To identify genetic loci with an increased burden of rare variants as putative risk factors for CVT.
Methods: As part of Milan-Leiden Sequencing (MILES) study, we investigated 171 Italian CVT patients and 298 healthy controls. Patients were selected using the following criteria: objective diagnosis of CVT, European ancestry, no active cancer. Targeted NGS sequencing of the protein-coding regions and 3' and 5' UTRs of 737 candidate genes related to hemostasis and inflammation, 150 ancestry informative markers and 28 thrombosis-associated variants with a final target size of approximately 4 Mb was performed on Illumina HiSeq2000 sequencing platform.
Results: Sequencing and data analysis revealed 13,160 rare variants (MAF<1%) in 733 genes, of which 13,107 single nucleotide variants (SNVs) and 54 insertions/deletions (indels). Gene-based association analysis of these rare variants using Burden model for genes with a total minor allele count (MAC) ≥40 revealed *ANO6* (*P*=0.002, Benjamini-Hochberg [BH] FDR 25%), *HABP2* (*P*=0.004, BH FDR 25%) and *COL16A1* (*P*=0.008, BH FDR 36%) as strongest putative risk factors for CVT. The Sequence Kernel Association Test (SKAT) with MAC ≥40 revealed *TYK2* (*P*=0.028), *NOS1* (*P*=0.034), *ADAMTS13* (*P*=0.040) and *ANO6* (*P*=0.045) as putative susceptibility loci for CVT. However, BH FDR for all these 4 loci exceeded 50%.
Summary/Conclusion: We have applied NGS-based analysis of 737 candidate hemostatic and pro-inflammatory genes in 469 Italian CVT patients and controls. Gene-based tests of association using Burden model and SKAT test did not reveal an excess of rare variants in loci related to hemostasis and inflammation associated with CVT.

Clotting

ECTH-218

Board No. 142: Conformational and clinical consequences of the pleiotropic p.met283val SERPINC1 mutation identified in a family with twins: relevance of high levels of latent antithrombin in plasma

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Background: Antithrombin (AT) is a key anticoagulant serpin whose deficiency, caused by different mechanisms triggered by *SERPINC1* mutations, significantly increases the risk of thrombosis. The study of cases with AT deficiency may help to identify new structural or functional domains of this serpin and clinical factors increasing the risk of thrombosis.

Aims: To identify the mechanism responsible for AT deficiency and the prothrombotic factors of a family with two affected twins that presented different clinical features.

Methods: Plasma and DNA from the studied family and healthy subjects was collected. AT activity was determined by chromogenic methods, antigen levels by ELISA and heparin affinity by crossed-immunoelectrophoresis. Exons and promoter of *SERPINC1* were sequenced. Plasma AT was studied by SDS-PAGE (under reducing and non-reducing conditions) and Native-PAGE (in the presence and absence of urea 6M) and western blotting. Recombinant ATs were generated by site-directed mutagenesis and expressed in HEK-EBNA cells. Secreted and intracellular recombinant ATs were studied by western blotting and by functional assays. Structural modeling was also performed.

Results: We studied four members of a family with type II AT deficiency (anti-FXa: 52%; Anti-IIa: 50%; Ag: 86%) including a premature child (with reduced activity: 25%) who was admitted at the intensive care unit, and two twins, one developing post-partum cerebral vein thrombosis that caused severe neurological sequelae. Up to 30% of plasma antithrombin had low heparin affinity, faster mobility on native gels and hyper-stability in urea gels corresponded to the latent conformation. No polymers or disulphide linked dimers were observed in basal or heated samples. *SERPINC1* sequencing revealed a heterozygous p.Met283Val mutation affecting a conserved residue in the serpin superfamily located in strand 3C. The two mutations described in the HGMD affecting this residue (p.Met283Val and p.Met283Ile) did not significantly impair the secretion to the conditioned medium in the recombinant model. The variants, which do not form disulphide-linked dimers, mostly were hyperstable in urea gels and had negligible anticoagulant activity although small traces of thrombin-AT complexes were observed when incubated with thrombin.

Clinically, despite the high proportions of latent AT, a potent antiangiogenic molecule, also in a premature carrier, no vascular defects were described. The triggering thrombotic factor in the affected carrier was the use of forceps during delivery.

Summary/Conclusion: Our study identifies strand 3C as a new domain of AT and potentially all serpins involved in their structural stability. Mutations in this domain may indirectly open the central A-sheet favoring the transition of the native conformation to the latent form, which should occur spontaneously at physiological conditions. Moreover, we described the first case with high levels of latent AT in plasma (50 µg/mL, 6-fold higher than healthy subjects), arguing against a physiological role for this strong antiangiogenic molecule. Finally, the development of cerebral sinothrombosis in a neonate with AT deficiency who required forceps, together with the high incidence of cerebral sinovenous thrombosis among neonates with a history of assisted delivery, strongly recommend avoiding the use of these devices when any parent, not only the mother, carries strong thrombophilic disorders.

Clotting
ECTH-427
Board No. 143: Uncovering the unique APC-resistant modifications of p. Textilis FV
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Background: The proteolytic inactivation of blood coagulation factor Va (FVa) by activated protein C (APC) downregulates the procoagulant response, which is an essential step in hemostasis. APC cleaves FVa at several positions throughout the A2-domain, with Arg306 and Arg506 as major cleavage sites. While cleavage at 506 results in partial loss of cofactor function, subsequent proteolysis at position 306 leads to a complete loss of FVa activity due to A2-domain dissociation. Interestingly, we previously reported that venom FV from the Australian snake *P.textilis* (ptFV) is functionally resistant to human APC following limited proteolysis. Our analysis revealed that ptFV is proteolyzed by APC in the A2-domain at the 506-like position (Lys507) and at the A2-B-domain junction (Arg742), while no 306-like cleavage was observed. Further sequence analysis revealed that the 306 cleavage site and surrounding sequence is not conserved in ptFV.
Aims: Here we aimed to uncover whether the functional APC-resistance of ptFV can be explained by the absence of an additional A2-domain cleavage site homologous to the human APC cleavage site Arg306.
Methods: To do so, the non-conserved ptFV region (GNPDTLT) was exchanged for the human Arg306 region (PKKTRNL), thereby generating ptFV-h306. The recombinant FV variants were purified using ion-exchange chromatography. Following human APC-treatment, products of APC-induced cleavage were determined employing SDS-PAGE analysis. Functional assessment was performed by a PT-based clotting assay in human plasma.
Results: Consistent with previous findings, APC-treatment of ptFV resulted in cleavage at positions 507 and 742. Upon introduction of the human 306 region into ptFV, APC was able to cleave ptFV-h306 at the Arg306 site. While full proteolysis of human FV (750 nM) was achieved following treatment with 20 nM of APC, an over 30-fold higher APC concentration (640 nM) was required to obtain fully proteolyzed ptFV-h306, similar to ptFV. Functional analysis in a human plasma system revealed that while FVa cofactor activity was fully absent in APC-cleaved human FV, APC-cleaved ptFV-h306 retained approximately 5% of FVa cofactor activity. Fully proteolyzed ptFV, on the other hand, maintained a residual FVa cofactor activity of approximately 20%.
Summary/Conclusion: Our findings indicate that, conversely to human FV, APC-dependent cleavage of ptFV at Arg306 does not fully abrogate the FVa cofactor function, but reduces its activity by approximately 4-fold when assayed in a human plasma system. This may suggest that even following APC cleavage at the positions homologous to human 306 and 506, the functional integrity of the ptFV A2-domain is stabilized such that it is able to form productive interactions. As such, ptFV provides a biological model to further study structure-function requirements that may contribute to an enhanced procoagulant FV activity.

Clotting

ECTH-443

Board No. 144: Detection of haemostatic disturbances at the presentation of acute lymphoblastic leukaemia in children with classic haemostatic tests and rotational thromboelastometry – single centre experience

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Background: Acute lymphoblastic leukemia (ALL) is the most frequent malignancy of childhood. With the improvements of combined chemo and radio therapy, ALL became curable in over 90% of cases, but the treatment is associated with number of complications, among which haemostatic disturbances are very frequent. Guidelines for identification of patients at risk of thrombosis and hemorrhages are not yet established. Rotational thromboelastometry (RoTEM) test is considered a valid tool to identify hemorrhagic risk in surgical patients. Recently, the new data were published regarding the use of RoTEM for identification of hypercoagulabile states in cancer patients.

Aims: Coagulation profiles were estimated in 7 children with newely diagnosed acute lymphoblastic leukemia using classic coagulation tests and RoTEM.

Methods: This is preliminary report of prospective study conducted at the Hematology division of Mother and Child Health Care Institute of Serbia. All newly-diagnosed ALL patients from neonates up to 18 year were eligible for the study. Patients' blood specimens were collected at the time of diagnosis for complete blood counts, haemostatic examinations (PT, APTT, TT, fibrinogen, antithrombin, D-dimers, plasminogen. α2 antiplasmin, FVIII and vWF) and RoTEM test, respectively.

Results: ALL was diagnosed in 7 children from November 2015 up to April 2016. The median age was 53.5 months (range from 35 up to 162 months). There were 5 boys and 2 girls. B-II (common) ALL was diagnosed in 4 patients and pre-B ALL in 3. Two patients were assigned in standard, 4 patients intermediary and one patient in high risk group. The median hemoglobin level and erythrocyte, leukocyte and platelet counts were 79.4 g/l (range 59.3–117.0 g/l), $2.9x10^{12}$/l (range 2.13-4.49 $x10^{12}$/l), 8.0 $x10^{9}$/l (range 2.9– $92.5x10^{9}$/l), and $51x10^{9}$/l (range 22-$124.8x10^{9}$/l), respectively. PT was decreased in one boy (54,7%), while APTT and TT levels were within normal ranges in all patients. Fibrinogen level was increased in one patient with median level of 3.9 g/l (range 2.8-5.4 g/l). Levels of plasminogen, α2-antiplasmin and antithrombin were in the normal ranges in all patients, while D-dimers were elevated in four patients with the median level of 528 ng/ml, (range 191-1454.2 ng/ml). FVIII and vWF were elevated in all patients, with the median levels of 234.8% and 235.4%, respectively.

ROTEM test using EXTEM, INTEM and FIBTEM was performed in each patient. Clotting time, clot formation time and α angle were normal in all patients in EXTEM and INTEM, while maximum clot firmness (MCF) was decreased in 4 patients in EXTEM (median 49 mm, range from 43 up to 69 mm) and in 5 in INTEM (median 48 mm, range from 41 mm up to 66 mm) which correlated with low platelets count in all patients (with the platelets less then $60x10^{9}$/l in 5 patients). MCF in FIBTEM was elevated in 5 patients (median 27 mm, range from 19 up to 41 mm) which correlated with the Clauss fibrinogen assay, but is additionally influenced by fibrin polymerization disorders which cannot reliably be detected with clotting tests.

Summary/Conclusion: Extensive analysis of coagulation parameters in children with ALL at the time of diagnosis showed elevated levels of D-dimers in 4 of 7 patients, elevated levels of FVIII and vWF and decreased platelets' count in all patients. Using RoTEM we can identify patients with significant abnormalities more closely than with classical methods. Further investigations will set standards for manage the coagulation disturbances in children with ALL.

Clotting
ECTH-505
Board No. 145: Structural features of fucosylated chondroitin sulfates influence on the level of anticoagulant and antithrombotic activities
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Background: Polysaccharides fucosylated chondroitinsulfates (FCS) isolated from different species of sea cucumbers vary in structural features, such as types and numbers of branches, as well as pattern of sulfation. These biopolymers demonstrated anticoagulant and antithrombotic activities.
Aims: Comparable study of FCS from different sea cucumbers and determination of structural elements important for anticoagulant and antithrombotic effects.
Methods: FCS were isolated from body walls of sea cucumbers. Purification of the polysaccharides was performed by ion-exchange chromatography followed by gel filtration. Structural characterization of the polymers was performed by chemical and physicochemical (NMR) methods. Anticoagulant and antithrombotic activities of FCS were studied in APTT and TT tests, as well as anti-IIa and anti-Xa activities were determined. Heparin and enoxaparin were used as standards in the experiments. Moreover, the effect of FCS on platelets aggregation was studied in the experiments with platelet rich plasma using ADP, ristocetin, and adrenaline as inducers.
Results: Backbone of all studied FCS was built up of the repeating disaccharide units →3)-β-d-GalNAc-(1→4)-β-d-GlcA-(1→. The polysaccharides were different in position of fucosyl branches (O-3 of GlcA or O-6 of GalNAc) and in pattern of sulfation of all monosaccharide units. FCS effectively inhibit platelets aggregation induced by ristocetin, but not ADP and adrenalin, and increase the time of fibrin clot formation in APTT and TT tests with different efficiency. In the experiments with purified proteins FCS exhibit anti-IIa and anti-Xa activities.
Summary/Conclusion: The levels of anticoagulant and antithrombotic effects of FCS from sea cucumbers depend on structural features of the polysaccharides. Fucosyl branches and sulfation of GalNAc both at O-4 and O-6 are important for effective inhibition of fibrin clot formation and platelet aggregation. Therefore, FCS demonstrated high anticoagulant and antithrombotic activities could be considered as a base for new drug development.
Acknowledgment: This work was supported by the Russian Science Foundation (Grant 14-13-01325).

Clotting
ECTH-191
Board No. 146: Functional assays to selectively quantify the APC- and TFPI- cofactor activities of protein S in plasma
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Background: Protein S is an important anticoagulant in the down-regulation of blood coagulation by acting as cofactor for two main regulators of coagulation. Protein S acts as a cofactor for activated protein C (APC) in the inactivation of the procoagulants FVa and FVIIIa and for TFPI in the formation of the TFPI-FXa encounter complex that subsequently inactivates TF-FVIIa. Several studies have indicated that protein S deficiency is associated with increased risks of venous thrombosis.
Aims: Current protein S functional assays are influenced by plasma determinants such as FVLeiden. . The aim of the study was to develop thrombin generation based assays that enable quantification of both the APC- and TFPI-cofactor activities of protein S in plasma whereas the outcome of the assays should not be affected by the presence of FVLeiden or other plasma variables.
Methods: APC- and TFPI**-**cofactor activities of protein S in plasma were measured using calibrated automated thrombography (CAT) in protein S-depleted plasma supplemented with a small amount of sample plasma either in the presence of anti-TFPI antibodies and APC (APC-cofactor activity) or at excess full-length TFPI without APC (TFPI-cofactor activity). Total and free protein S levels in plasma were measured by ELISA's.
Results: Average APC-cofactor activities of protein S were 113%, 122%, and 87% in plasma from normal individuals (n=10), FV Leiden heterozygotes (n=4), and FV Leiden homozygotes (n=2), respectively, while the average APC-cofactor activity of protein S in plasma from heterozygous protein S-deficient individuals (n=21) was significantly lower (55%). Similar trends were observed for the TFPI-cofactor activity of protein S, with averages of 108%, 112%, and 97% in plasma from individuals with normal protein S levels and different FV Leiden genotypes, and 64% in plasma from protein S-deficient patients. APC-cofactor activities of protein S correlated significantly with free and total protein S antigen levels, while TFPI-cofactor activities correlated less with protein S antigen levels.
Summary/Conclusion: We have developed functional protein S assays that measure both the TFPI- and APC-cofactor activities of protein S in plasma and that are not affected by the FV Leiden mutation or other plasma influences.

Clotting

ECTH-456

Board No. 147: Difference in inflammation markers and coagulation factors between young and old patients with myocardial infarction

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Background: Inflammation represents a link between atherosclerosis and arterial thrombosis playing an important role in the progression of atherosclerosis, plaque vulnerability and thrombus formation, thus constituting an important pathogenetic factor in the development of myocardial infarction.

Aims: To determine the difference in the values inflammation markers and coagulation factors (fibrinogen, CRP, FVII , FVIII , FXII) in patients with myocardial infarction younger than 45 years, the group of patients with myocardial infarction older than 60 years and the control group.

Methods: In a prospective study, 143 patients were examined , i.e. 80 patients with myocardial infarction younger than 45 years and 63 patients with myocardial infarction older than 65 years.The value of inflammation markers and coagulation factors was analysed using standard biochemical and coagulometer tests. The Student T - test, $\chi 2$ test, Mann - Whitney U test and Spearman's correlation test were used for the statistical analysis.

Results: In the group of patients younger than 45 years there was the significantly higher (p = 0.038) number of males (69.6 % men and 30.4 % women) than in the group of patients with myocardial infarction older than 65 years (55.9 % of men and 44.1 % women). Patients with myocardial infarction of older age group compared with younger patients had statistically higher values of fibrinogen (p = 0.000) (5.0 ± 1.9 vs 3.8 ± 1.6 g / L) and statistically significant (p = 0.005) higher CRP concentration (32.8 ± 52.2 vs 15.0 ± 25.2 mg / L).

Coagulation factor VII activity was statistically insignificantly higher (p = 0.061) in younger patients with myocardial infarction than in older patients (109.3 ± 21.8 vs 101 ± 22.8%). Compared with younger patients with myocardial infarction, older patients had a statistically significantly higher (p = 0.004) levels of FVIII activity (198.2 ± 92.4 vs 129.1 ± 89.8%) and statistically significantly higher (p = 0.000) values of vWF activity (183.7 ± 86.5 vs 111.9 ± 47.1%). The Spearman's correlation test determined that the fibrinogen level, similarly to CRP, positively correlates with an increased number of leukocytes, elevated levels of factor VIII factor and vWF. However, there is also a negative correlation between CRP and factor XII found here.

Summary/Conclusion: The stated results seem to indicate the existence of different pathogenetic mechanisms in young and elderly patients with myocardial infarction as well as a potentially significant correlation between acute phase reactants and factors of the intrinsic coagulation pathway, FVIII and vWF in myocardial infarction development in older age groups. This result may suggest the importance of implementing more inflammation-focused approaches in prophylaxis of myocardial infarction development and prevention of complications in patients with myocardial infarction, predominantly in the elderly population. Further research involving a larger number of myocardial infarction patients is required to yield more reliable results.

Clotting
ECTH-149
Board No. 148: Transient desialylation in combination with a novel antithrombin deficiency causes severe and recurrent thrombosis despite anticoagulation therapy
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Background: Congenital and acquired factors may interact to increase the risk of thrombosis as well as to define the localization and the severity of the thromboembolic event. The number and types of congenital risk factors is extense, being antithrombin (AT) deficiency the strongest one. However, the knowledge of acquired risk factors is more limited. A deep analysis of exceptional cases may help to identify new factors involved in thrombosis.
Aims: To analyze the hemostatic system in an exceptional case with severe and recurrent thrombosis in order to identify novel thrombosis risk factors.
Methods: Analysis of plasma proteins (AT, prothrombin, FXII, FXI, fibrinogen, TFPI, α1-antitrypsin and transferrin) was performed by western blot, HPLC and/or functional methods. Glycomic studies were also performed in some plasma proteins by neuraminidase and PNGaseF treatment. *SERPINC1*, the gene encoding AT, was analyzed by SANGER sequencing. Finally, recombinant AT expression was studied in a cell model (HEK-EBNA).
Results: We report on a woman with early (first thrombosis when she was 30-year-old), severe and recurrent venous and arterial thrombosis despite anticoagulation therapy, who died after an ischemic stroke. No antiphospholipid antibodies were detected. Thrombophilic analysis only revealed a type I AT deficiency with anti-FXa activity ranging from 36 to 50% and similar antigen levels. *SERPINC1* sequencing revealed a heterozygous novel deletion of 3 nucleotides (c.651-653delCAT) that eliminates an isoleucine 218, a highly conserved residue in serpins located in helix F. The relevance of this mutation in the folding or secretion of the molecule was confirmed in the recombinant model, as only traces of a variant were secreted to the conditioned medium, with presence of disulphide linked polymers.
Interestingly, all residual plasma AT in the patient (theoretically from the normal allele) displayed faster electrophoretic mobility in SDS-PAGE accordingly to Western blot assays, which may only be explained by an impaired post-translational modification.
This detection was not specific for AT since all liver (FXII, FXI, prothrombin, fibrinogen, α1-antitrypsin) and non-liver studied proteins (TFPI) had faster electrophoretic mobility than that of control subjects. Treatment with PNGaseF and neuraminidase suggested a nearly full desialylation, which was confirmed by HPLC and Q-TOF analysis of transferrin glycoforms.
The analysis of samples collected at different time points revealed a partial desialylation in one and a normal electrophoretic pattern in another moment. Interestingly, desialylation was evident in samples collected after arterial thrombotic events and associated with reduced anti-FXa activity and lower platelet counts. No microbiological agent was detected in samples with desialylation.
Neither AT deficiency nor desialylation were identified in relatives (siblings and children) who have no history of thrombosis.
Summary/Conclusion: To our knowledge, this is the first description of a significant and transient desialylation of all plasma proteins associated to the development of thromboembolic diseases. The absence of the strong electronegative residue terminating branches of N-glycans and O-glycans, may modulate protein-protein interactions of hemostatic elements, which in combination with a strong prothrombotic situation, as AT deficiency identified in this patient, could increase the risk of thrombosis, particularly arterial thrombosis.

Clotting
ECTH-423
Board No. 149: Thrombophilic and cardiovascular risk factors in retinal vein occlusion
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Background: Retinal vein occlusion (RVO) is the second most common cause of vision loss after diabetic rethinopathy. It may involve the central retinal vein or a branch retinal vein. The role of inherited and acquired thrombophilia and cardiovascular risk factors in different manifestations of RVO (i.e., central or branch RVO) and at different ages is still debated.
Aims: 1) To evaluate the association between thrombophilic and cardiovascular risk factors and the risk of RVO, both overall and separately for central and branch RVO; 2) to assess whether these risks vary according to different age categories (≤50 vs >50 yrs).
Methods: A case-control study was carried out on 313 patients with a first episode of objectively-confirmed RVO (52% females, median age 54 yrs [IQR:41-63], 216 central and 97 branch RVO) consecutively referred at our Thrombosis Center for a thrombophilia work-up (antithrombin, protein C or protein S deficiency, factor V Leiden, G20210A prothrombin gene mutation, antiphospholipid antibodies, hyperhomocysteinemia, high factor VIII levels), and 415 healthy controls (71% females, median age 41 yrs [IQR:32-52]). Cardiovascular risk factors (hypertension, hyperlipidemia, diabetes and cigarette smoking) were also recorded. In a multivariable logistic regression model, odds ratios (OR) and 95% confidence intervals (CI) were calculated as an estimate of the risk of RVO in carriers relative to non-carriers of a particular risk factor, adjusting for the confounding effect of age, sex and the other risk factors.
Results: Deficiencies of antithrombin, protein C or protein S taken together, hyperhomocysteinemia, high factor VIII, factor V Leiden and the presence of at least one cardiovascular risk factor were all independently associated with the risk of RVO. The association was stronger for branch RVO (adjusted OR [95%CI]: 15.60 [2.01-121] for deficiencies of antithrombin, protein C or S, 3.22 [1.38-7.49] for hyperhomocysteinemia, 3.08 [1.20-7.89] for high factor VIII, 2.93 [0.97-8.86] for factor V Leiden, and 1.79 [1.00-3.23] for cardiovascular risk factors) than for central RVO, for which only hyperhomocysteinemia (2.15 [1.09-4.24]) and high factor VIII (1.99 [0.90-4.42]) showed an association. No association was found with the remaining thrombophilia markers. Hyperhomocysteinemia, high factor VIII and cardiovascular risk factors were associated with the risk of RVO more at age >50 yrs (adjusted OR [95%CI]: 3.41 [1.29-8.99], 2.57 [1.00-6.68] and 2.03 [1.16-3.56], respectively) than at age ≤50 yrs (adjusted OR [95%CI]: 1.93 [0.85-4.36], 1.67 [0.54-5.12] and 1.22 [0.73-2.03], respectively), while classic inherited thrombophilia (i.e., the presence of either antithrombin, protein C or S deficiency, factor V Leiden or G20210A prothrombin gene mutation) was more prevalent at age ≤50 yrs (adjusted OR 1.62 [95%CI:0.76-3.45]) than at age >50 yrs (adjusted OR 1.11 [95%CI:0.44-2.79]). There was no interaction between thrombophilic and cardiovascular risk factors on the risk of RVO.
Summary/Conclusion: Thrombophilic and cardiovascular risk factors are more associated with branch RVO than with central RVO. Only hyperhomocysteinemia and high factor VIII appear to be related to central RVO. The risk of RVO associated with hyperhomocysteinemia, high FVIII and cardiovascular risk factors is higher at an older age.

Clotting

ECTH-238

Board No 150: Anti-Xa oral anticoagulants inhibit in vivo platelet activation by modulating glycoprotein VI shedding

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Background: Urinary excretion of Thromboxane B_2 (TxB_2) is enhanced in patients with atrial fibrillation (AF) and is predictive of cardiovascular events. The effect of anti Xa non-vitamin k oral anticoagulant (anti Xa NOACs) on TxB_2 has never been investigated.

Aims: To compare the effect of anti Xa NOACs, namely Apixaban and Rivaroxaban, to Warfarin, on the excretion of urinary 11-dehydro-TxB_2, and to investigate the potential underlying mechanism by evaluating soluble glycoprotein GPVI (sGPVI), a protein involved in platelet activation.

Methods: We performed a cross-sectional including AF patients treated with Warfarin (n=30), or Apixaban 10 mg/day (n=40), or Rivaroxaban 20 mg/day (n=40). Patients were balanced for sex, age and cardiovascular risk factors. Urine and blood samples were collected at baseline and after 3 months of treatment.

Results: Baseline TxB_2 value was 155.2±42.7 ng/mg creatinine. The 3 months-variation of urinary excretion of TxB_2 was -6.5% with Warfarin ($p=0.197$), -29% with Apixaban ($p<0.001$) and -31% with Rivaroxaban ($p<0.001$). Use of anti Xa NOACs was independently associated to the variation of urinary TxB_2 (B: -0.469, $p<0.001$), after adjustment for clinical characteristics. We found that sGPVI was significantly lower in patients treated with NOACs at 3 months ($p<0.001$), while only a trend for the Warfarin group ($p=0.116$) was observed. The variation of sGPVI was correlated with that of TxB_2 in the NOAC groups (Rs:0.527, $p<0.001$).

Summary/Conclusion: The study provides the first evidence that anti Xa oral anticoagulants significantly inhibit urinary TxB_2 production compared to Warfarin, suggesting that NOACs possess antiplatelet property.

Clotting
ECTH-223
Board No. 151: Laboratory testing for lupus anticoagulant: impact of the new CLSI guideline versus the previous ISTH guideline
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Background: Lupus anticoagulant is still a challenge to diagnose. According to the guidelines there is a critical role for laboratory testing together with the presentation of typical thrombotic events. However, the definition for the perfect test remains highly debatable and is recently updated by the Clinical and Laboratory Standards Institute (CLSI).
Aims: We compare the new CLSI with the International Society on Haemostasis and Thrombosis (ISTH) guideline with its impact on laboratory testing. We especially focus on differences in the calculation of normalised ratios, the establishment of reference intervals, and the order of screen-confirm-mixing tests
Methods: The dRVVT test was performed using the HemosIL assay and the aPTT-based test was performed using the Silica Clotting Time (SCT) assay (ACLTOP analyser, Werfen). Citrated plasma (3.2%) was available from clinical patients in which lupus was suspicious (n=46, after exclusion of patients with missing data). Patient results eventually ranged from lupus negative to positive as based on our current clinical assays: dRVVT using the LA1/LA2 assay (Siemens) and aPTT using the PTT-LA (Roche) and Actin FS (Siemens) assays (CS2100i analyser, Sysmex). Citrated plasma of healthy individuals (n=40) and a normal pooled plasma (n=85) were prepared at our laboratory. For all individuals aPTT, thrombin time, and international normalised ratio were available. Normalised ratios and reference limits were established as prescribed by the guidelines, the latter either at the 97.5th or 99th percentile. Normal pooled plasma was run with every batch in duplo.
Results: For the dRVVT test, 9 and 11 patients were lupus positive and 35 and 31 patients were lupus negative according to the ISTH and CLSI guideline, respectively. Moreover, 2 and 3 patients using oral anticoagulants were indeterminate, and 0 and 1 patients were indeterminate because of unknown reasons. For the SCT test, according to the ISTH and CLSI guideline, 0 and 0 patients were lupus positive, 36 and 32 patients were lupus negative, 2 and 3 patients using oral anticoagulants were indeterminate, and 8 and 11 patients were indeterminate because of unknown reasons, respectively. In total, 80 dRVVT and 79 SCT tests were performed when following the ISTH guideline, while 83 and 86 tests were performed when following the CLSI guideline. In more detail, according to the ISTH guideline, 46 screen tests were followed for the dRVVT test by 20 mixing and 14 confirm tests and for the SCT test by 23 mixing and 10 confirm tests. According to the CLSI guideline, the 46 screen tests were followed for the dRVVT test by 20 mixing and 17 confirm tests, respectively, and for the SCT by 26 and 14 tests, respectively.
Summary/Conclusion: The new CLSI guideline, in comparison to the ISTH guideline, is more sensitive in detecting lupus anticoagulant or seems indeterminate in an increased number of patients, suggesting the presence of other inhibitors. Moreover, changing to the order of screen-confirm-mixing tests results in a small increase in number of tests. Future research has to reveal whether this is also true for other lupus anticoagulant tests that are available on the market.

Clotting
ECTH-138
Board No. 152: Risk profile and disease management of patients with venous thromboembolism attended in Spanish emergency departments: Espheria register
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Background: In most cases in which venous thromboembolism (VTE) is developed in the outpatient setting, the Emergency Departments (EDs) is where the disease is actually diagnosed. Surprisingly, however, few studies have analyzed the characteristics of patients with VTE and the disease itself from the perspective of the ED. This is relevant for several reasons. Firstly, little is known about the epidemiology of VTE in the ED and the risk factors most frequently presented by patients diagnosed with an episode of VTE in the ED. Secondly, few studies have evaluated the most adequate management of patients with VTE by emergency physicians or whether this managment is carried out according to the recommendations of the clinical practice guidelines (CPG), and if this has any repercussion on the final outcome of the patient. Lastly, little is known about the treatment performed in the ED and how this may influence the outcome of the patient.
Aims: The aim of this study was to determine the clinical presentation of VTE and the main risk factors involved in patients diagnosed in Spanish EDs as well as evaluate the management of these patients and adherence to clinical practice guidelines by emergency physicians.
Methods: We performed a prospective cohort study in 53 Spanish EDs, consecutively including patients diagnosed with VTE in the ED. The following data were evaluated: demographic, comorbidities, risk factors for the development of VTE, index event, hemorrhagic risk factors, prognostic factors [pulmonary embolism (PE)] and in-hospital mortality. To evaluate health care quality we determined the percentage of patients registered with clinical probability of PE, requests for D-dimer concentrations according to clinical probability, administration of treatment prior to confirmation of diagnosis based on clinical probability and records of risk of bleeding and prognosis of the patients with VTE.
Results: Of 549,840 ED visits made over a mean period of 40 days, 905 patients were diagnosed with VTE (impact 1.6/1000 visits). Of these, 801 patients were included in the analysis, 49.8% of whom had PE with or without deep venous trhrombosis (DVT). The most frequent risk factors for VTE were: age (≥70 years), obesity, new immobility, previous VTE and active cancer. In the ED medical reports a scale of clinical probability, the prognosis or the risk of bleeding were only described in 7.6%, 7.5% and 1%, respectively of the cases. Of the patients with PE and high clinical probability, D-dimer was determined in 87.2%, and treatment was initiated prior to confirmation in 35.9%. Regarding risk, 31.3% of the patients with PE presented low risk, 59.1% intermediate-low risk, 6% intermediate-high risk and 3.5% high risk. Of the patients with PE, 98.7% were hospitalized while 50.2% of the VTE (without PE) were admitted. The in-hospital mortality of the patients with PE was 3.8%.
Summary/Conclusion: VTE has an appreciable impact on Spanish EDs. Risk profile for the VTE development in patients diagnosed in ED being similar to previous studies. Adherence to CPG recommendations needs to significantly improve.

Clotting

ECTH-301

Board No. 153: Protein S Heerlen is associated with the risk of venous thrombosis in unselected individuals independenly from PS plasma levels

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Background: Hereditary Protein S (PS) deficiency is a rare coagulation disorder associated with an increased risk of venous thrombosis (VT). The PS Heerlen (rs121918472) is a S501P mutation that was initially considered as a neutral polymorphism. However, it has been later shown that PS Heerlen has a reduced half-life in vivo which may explain the association of PS Heerlen heterozygosity with mildly reduced levels of plasma free PS (FPS). Whether the risk of VT is increased in PS Heerlen heterozygous subjects remain doubtful.

Aims: we aimed to systematically analyze the association of PS Heerlen with VT using 4 French case-control collections totalling 4,267 patients and 5,982 healthy individuals.

Methods: Participants of the 4 studies (French GWAS, MARTHA12, FARIVE and EDITH) were European-ancestry individuals. In all studies, VT was objectively diagnosed by physicians. In French GWAS and MARTHA, participants were typed for the HumanExome Beadchip 12v1-2_A array (Illumina, Inc., Sand Diego, CA). In FARIVE and EDITH studies, the PS Heerlen variation was genotyped by allele specific PCR. Quantitative determination of FPS levels was performed in a subsample of 1257 VT patients by ELISA using the Asserachrom FPS assay (Diagnostica Stago).

Results: No homozygous carriers of the rare rs121918472-G allele was observed. In the combined samples, the AG genotype was more frequent in VT patients than in healthy controls, 2.2% vs 0.4% , and was then associated with an increased risk of VT of 6.61 [4.04 - 10.81] ($p = 4.89\ 10^{-14}$). This pattern of association holds in EDITH (2.4% vs 0.4%), in MARTHA12 (2.5% vs 0.6%) and the French GWAS (2.4% vs 0%) samples, but not in the FARIVE study (1.1% vs 1.8%). In VT patients, even if by design PS deficiency was excluded (ie FPS levels <55 IU/dL^{-1}), plasma FPS levels were significantly lower in individuals with PS Heerlen when compared to those without (mean +SD for PS Heerlen carriers (n=21) or not (n=1236) respectively: 72 + 13 vs 91 + 21 UI/dL; $p= 1.86x10^{-6}$).

Summary/Conclusion: We provide here evidence that the PS Heerlen is associated with an increased risk of VT in unselected individuals. Further investigations are needed to better understand the surprisingly high prevalence of PS Heerlen mutation in apparently healthy individuals from the FARIVE study. New aspects of the potential influence of PS Heerlen on the risk of VT should also be investigated in functional studies. These results also raised the question of genotyping PS Heerlen irrespective of plasma PS levels in thrombophilia screening.

Clotting

ECTH-315

Board No. 154: Perioperative thromboprophylaxis in severely obese patients undergoing bariatric surgery: insights from a French national survey

Pierre-Antoine Moulin[1], Anne Dutour[2, 3], Patricia Ancel[2, 3], Pierre Morange[* 1], Thierry Bège[3, 4], Olivier Ziegler[5], Stéphane Berdah[3, 4], Corinne Frère[1], Bénédicte Gaborit[2, 3]

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Background: Venous thromboembolism (VTE) is a leading cause of death in obese patients undergoing bariatric surgery, but there is yet neither consensus nor high-level guidelines on VTE prophylaxis in this specific population.

Aims: We aimed to evaluate patterns of bariatric surgery perioperative thromboprophylaxis practices in French obesity specialised care centers (centre spécialisé de l'obésité, CSO), which are tertiary – care referral hospitals for the management of the most severe or complex cases of obesity, as identified by the French Obesity Plan (2010-2013).

Methods: A detailed questionnaire survey including 11 opened and 15 closed questions and investigating their prophylactic schemes of anticoagulation (molecule, dose, weight adjustment, duration, associated measures, follow-up) was sent to the 37 CSO.

Results: Completion rate was 92%. Over 90% of respondents indicated using low molecular weight heparin (LMWH). Enoxaparin was the most commonly used molecule (89%), twice daily (71%), started mostly 6 hours after bariatric surgery (74%), whereas fondaparinux (9%), dalteparin (6%) and tinzaparin (6%) were less often prescribed. Dosing varied importantly according to the centers (from 4000 to 12 000IU once daily, the most commonly used dose being 8000IU once daily, 83%), as well as treatment duration (one week, 9%; three weeks, 47%). Half CSO adjusted LMWH dose to body weight. Biological monitoring was performed in 88%. Only one center followed systematically anti-Xa activity. Associated measures such as intermittent pneumatic compression were used in 40% of CSO.

Summary/Conclusion: This study clearly demonstrates significant discrepancies in thromboprophylaxis practices in obese patients undergoing BS, particularly with respect to treatment duration and dose adjustment, highlighting the urgent need for improved implementation of existing clinical practice guidelines in this VTE high-risk population.

Clotting

ECTH-224

Board No. 155: Air pollution particles influence endothelial cell biology, creating a prothrombotic phenotype leading to dense clots with increased resistance to lysis

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Background: Air pollution ranked ninth among the modifiable disease risk factors. Air pollution consists of both gaseous pollutants and particulate matter (PM). PM has been associated with increased risks of cardiovascular diseases in several epidemiological studies. Different cardiovascular diseases and diseases of both arterial and venous thrombosis have been associated with alterations in fibrin clot structure with more compact arranged fibre networks and prolonged lysis times.

Aims: The aim of this study was to investigate whether and how air particulate matter affects fibrin clot structure and endothelial behaviour.

Methods: Four particles purchased from NIST, which represented urban air pollution, were used in this study, PM_{10}, $PM_{0.2}$, total diesel particles and filtered diesel particles. To investigate the effects of these particles on fibrin clot structure, turbidity assay, turbidity lysis assay and laser scanning confocal microscopy were used with normal pooled plasma and purified fibrinogen. Human umbilical vein endothelial cell (HUVEC) play an important role in modulating thrombosis in blood vessels. As small sized particles are able to translocate to different organs by crossing into the circulatory and/or lymphatic systems, HUVEC may interact with particles directly. The effects of PM exposure on endothelial cells were investigated by MTT cytotoxicity test, laser scanning confocal microscopy (LSCM), Real-Time PCR analysis of tissue factor (TF) and thrombomodulin (TM), ELISA of vWF and PAI-1, and plasmid strand break assay.

Results: Turbidity lysis showed that as the concentrations of particles increased, the fibres formed from plasma were less sensitive to fibrinolysis and time to 50% lysis was significantly longer at 50 µg/ml of the particles compared to control. There were no apparent differences in clot structure when clots were made in the presence of particles from plasma or purified proteins compared to those produced without particles.

Next we incubated HUVECs with the particles and investigated the behaviour of the cells after exposure to the particles. 50 µg/ml of PM did not induce significant cell death after 24 hours exposure. However, fibrin clots formed from pooled plasma on top of the treated cells were significantly altered compared to control cells. When clots were formed from purified fibrinogen samples, there were no significant differences in clot structure between treated and untreated cells. Changes in expression of TF mRNA, thrombomodulin mRNA, vWf and PAI-1 following PM exposure of HUVECs were consistent with changes observed in clot structure in that the levels of TF mRNA, vWf and PAI-1 were all significantly increased after exposure to the particles, while TM mRNA was decreased, adding evidence for an effect of PM on risk of thrombosis via the activation of endothelial cells.

Summary/Conclusion: In conclusion, PM induce changes to clot structure and function, by changing TF and TM gene expression and influencing vWF and PAI-1 release in the endothelial cell. These biological mechanisms may contribute to prothrombotic state in response to PM exposure.

Clotting

ECTH-119

Board No. 156: Application of basic and special coagulation tests for measuring rivaroxaban activity

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Background: Rivaroxaban (Xarelto®) is an oral anticoagulant, direct and selective factor Xa inhibitor, which has a predictable pharmacokinetic. It is administered at fixed doses, with maximum anticoagulant effect at 2h-4h after intake. Coagulation studies will be needed in cases of hemorrhagic or thrombotic complication, urgent or planned surgery, and certain specific situations (extreme weight, renal insuficiecia, drug interactions).

Aims: Evaluate the biological effect of rivaroxaban in a group of patients treated with this anticoagulant using two techniques:

Analyze the correlation between these two techniques and see if you can apply a correction factor to estimate rivaroxaban plasma concentration (ng / ml) from the determination of anti-Xa activity in IU / ml

Methods: Multicenter study included 51 adult patients with VTE and AF treated with rivaroxaban followed in the University Hospital Fundación Jimenez , UH Gregorio Maranon and UH Clínico San Carlos. Exclusion criteria: patients taking rivaroxaban as primary prophylaxis in orthopedic surgery. The following data were collected: age, sex, weight, indication of rivaroxaban, thrombotic and hemorrhagic history, creatinine, glomerular filtration rate, concomitant diseases, concomitant medication, bleeding risk factors. Coagulation study includes: prothrombin time, cephalin time, fibrinogen. Levels of anti-Xa activity were determined using HemosIL Liquid Heparin kit (Instrumentation Laboratory). The concentration of rivaroxaban was tested by a chromogenic assay using specific calibrators TECHNOVIEW Rivaroxaban High Set Cal. All determinations were performed at 2 and 24 hours after ingestion of the drug.

Results: The mean age was 65.37±16.37 years (28-89), 45% (n=23) of patients were female and 55% (n=28) were men. 18% received a dose of 15 mg/24h, while the remaining 82% received 20 mg/24h. The average weight was 73.0±11.7 kg. Figures 2 and 3 show the correlation between levels of rivaroxaban and anti-Xa activity and PT. Mean concentration was 239.70 and 29.2 ng/ml at 2 and 24 hours after dosing. There is an interindividual variability in the concentration of rivaroxaban in patients older (289.5 ng /ml) and under 80 (225 ng/ml); which coincides with lower glomerular filtration rate (59.9 vs 78/1 ml/min).

Summary/Conclusion: Determination of the rivaroxaban plasma concentration is useful in some clinical circumstances. The anti Xa chromogenic assay with calibrators for rivaroxaban is a quantitative method, easy to perform, allowing us to determine its plasma level. Applying a correction factor rivaroxaban plasma concentration (ng/ml) can be estimated from determination of the anti-Xa activity with a calibrated for heparin (IU/ml) test.

Clotting
ECTH-494
Board No. 157: Benefits of treatment with low molecular weight heparins in women with obstetric complications
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Background: There is increasing interest in the study of LMWH therapy in women with poor obstetrical history seeking pregnancy and not always present a clear thrombotic risk. This is because many pleiotropic effects of LMWH which could favor a successful pregnancy and evolution of the same have been described. Non-anticoagulant effects of heparin are involved in the implementation and trophoblastic development also can improve endometrial receptivity and decidualization of endometrial stromal as well as adhesion and invasion of trophoblast (←TFPI, ←ILGF, inhibiting P-selectin and E-cadherin, ↓VEGF, ↓TGF, ↓TNF, matrix metalloproteinases and cytokines interaction).
Aims: Analyze reasons why the women in fertile age are referred from obstetric consultations to Thrombosis Unit, therapeutic actions in each case and evolution of pregnancy.
Methods: 198 women with a mean age 35.88 ± 4.05 years (22-45) referred to the Thrombosis Unit for evaluation because of problems related pregnancy (Figure 1A) were included in the study. Age, obstetrical history, number and moment of miscarriage, personal and family history of thrombosis, hereditary and acquired thrombophilia, anticoagulant treatment received, course of pregnancy and birth data were collected from each woman included in the study.
Results: 64.6% (n = 128) of women were older than 35 years and 63.1% (n = 125) had suffered at least one miscarriage: 43% (n = 86) had suffered two and 27% (n = 56) three or more. Patients referred by a previous thrombosis without other obstetric complications (n = 38) were excluded from the study (all of these patients were treated with therapeutic doses of LMWH). Of the remaining 160 patients 118 (74%) were treated with LMWH during pregnancy. In 90% of cases (n = 145) term pregnancy with the birth of a healthy child was achieved. Only in 15 cases pregnancy did not progress. 20 patients (12.5%) developed complications, none of them related to LMWH treatment.
Summary/Conclusion: Increasingly more often women with gestational problems are referred to Hematology consultations with several diagnostic: sterility, repeated miscarriages, implantation failure... In most of them cause or origin of these alterations is unknown. Probably current lifestyle, seeking pregnancy at a later age, is involved in the development of a greater number of complications. Therapy with LMWH at prophylactic dose seems to improve the rate of successful pregnancies (in our study we have obtained 90% of births) and the risk of associated complications are really low. Even so the level of evidence in this population remains low, so more research is needed.

Clotting
ECTH-128
Board No. 158: Apixaban in a paediatric patient with thrombosis and homozygous antithrombin deficiency, type 2 heparin binding site, Budapest variant
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Background: Antithrombin III deficiency is a severe thrombophilic risk factor and warrants long-term prophylaxis after a thromboembolic event. So far Apixaban has not been used for this purpose in the pediatric population.
Aims: We want to show the successful imlementation of long-term prophylaxis with Apixaban in a 11 yr old boy with Antithrombin III deficiency and a severe thromboembolic event.
Methods: A 11 yr old boy with adiposity (90 kgs) suffered bilateral pelvic- and leg- vein thrombosis and pulmonary embolism in January 2015. The mother hails from Romania, she is illiterate. A diagnosis of homozygous Antithrombin III deficiency , Type II; affecting the heparin binding site, Budapest variant was made by sequencing the SERPINC1- Gen, in exon 2 the following mutation was found:c.(391C>T);(391C>T) p.(Leu131Phe). After treatment with low- molecular weight heparin we switched to Apixaban after informed consent and after the insurance company had approved the off-label use. We gave 10 mg daily for treatment and then 2x 5mg per day, after 6 mths we initiated long-term prophylaxis with 2 x 2,5 mg per day. The levels are monitored regularly and always within the refernce ranges. Rehabilitations for weight reduction were undertaken twice. Patient had recanalization of the thrombosis, but problems with PTS begin to emerge.
Results: We showed that in a pediatric patient with severe thrombophilia and a serious thromboembolic event who needs long-term prophylaxis to prevent the recurrence of a possibly deadly event Apixaban can be used safely and effectively.
Summary/Conclusion: We feel that the benefits of the NOACs should not be withheld from the pediatric population. Apixaban should be monotired regularly, we suggest clinic visits every 4 weeks to monitor the level and adherence and to gain knowledge about the pharmacokinetics in the pediatric population.

Clotting
ECTH-367
Board No. 159: Retinal artery occlusion in two adolescents due to hypercoagulability
Tugba Alarcon Martinez[1], Selin Aytac [*2], Ilhan Altan[2], Hatice Inci Yaman Bajin[2], Sule Unal[2], Mualla Cetin[2], Murat Tuncer[2], Fatma Gumruk[2]
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Background: Retinal artery occlusion (RAO) is extremely rare occurrence in children that usually presents with painless, sudden onset vision loss. In most of these pediatric cases, RAO is generally associated with coagulopathies, hematologic malignancies, and autoimmune diseases.
Aims: Herein, we report two cases of retinal artery occlusion due to hypercoagulability.
Methods: *Case 1* A 16-year-old boy, who has been on prednisone treatment for diagnosis of isolated glucocorticoid deficiency since 2 years old, presented with sudden vision loss in left eye. In his hypercoagulability workup, he was found to be homozygous for the MTHFR C677T polymorphism and have high levels of Factor VIII. The visual field loss became smaller with acetylsalicylic acid (ASA) treatment and found to be not interfering with his quality of vision during his follow-up. *Case 2* A previously healthy 17-year-old girl, who has no history suggestive of any systemic comorbidity, presented with sudden painless loss of superotemporal visual field in her right eye due to branch RAO. Her thrombophilia screen demonstrated heterozygosity in Factor V Leiden, MTHFR C677T and MTHFR A1298C polymorphisms. The rest of the cardiac and rheumatologic workups were normal. She received ASA treatment. At the 12th month examination, her visual acuity remained unchanged.
Results: Although these cases had no family history suggestive of thromboembolic phenomena, they were found to have hypercoagulable states causing thrombosis.
Summary/Conclusion: These cases highlight the importance of thrombophilia workup in pediatric RAO cases.

Clotting

ECTH-372

Board No. 160: Two cases of Nicolau syndrome: a rare complication following benzathine penicilline injection

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Background: Nicolau syndrome (embolia cutis medicamentosa) is a rare complication of intramuscular (IM) injection, which is characterized by pain, edema, and livedoid discoloration of the skin immediately after injection. Its pathogenesis is not well understood, being inflammation, embolism, thrombosis and vasospasm various postulated causes.

Aims: Herein, we reported two cases of Nicolau syndrome following an intramuscular injection of benzathine penicillin.

Methods: *Case 1:* A 5 years old girl presented with skin discoloration in her left limb immediately after receiving IM benzathine penicillin and, a physical examination revealed patchy ecchymosis. She was treated with tissue plasminogen activator (tPA) and heparin. In addition, hyperbaric oxygen therapy was used early in the treatment with complete healing. *Case 2:* A 3.5 years old girl experienced a severe pain and swelling in her left limb after IM benzathine penicillin injection. On the third post injection day, she was referred because of a rapid development of vascular insufficiency in her left leg. She had been treated with heparin in previous center and received tPA and hyperbaric oxygen therapy after administration to our hospital. Unfortunately, since the ecchymosis on the left leg persisted and necrosis was also observed, she had amputation from her lower left leg.

Results: In Nicolau syndrome, treatment is generally supportive and symptomatic, ranging from local care to surgical intervention.

Summary/Conclusion: Early administration of anticoagulants and hyperbaric oxygen therapy play important role in preventing amputations.

Clotting

ECTH-159

Board No. 161: Investigation and management of suspected deep vein thrombosis: a multidisciplinary re-audit with development of a novel improvement initiative

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Background: Prompt diagnosis and treatment of deep vein thrombosis (DVT) is essential to prevent potentially life-threatening complications, including pulmonary embolism.
A multidisciplinary re-audit, using standards from NICE Clinical Guideline 144, was performed to establish local practice, compliance with guidelines and impact of previous audit interventions. A novel decision-support pathway is in development in 'app' format with preliminary feedback to assess user-ability and user-acceptance.
Aims: To assess current clinical practice, compliance with NICE clinical guidelines for DVT diagnosis and management, and Trust's DVT guidelines.
To ensure all patients experience same care of timely investigation, diagnosis, advice, treatment (if indicated) and continuity of care, with appropriate documentation for effective communication
Methods: A retrospective electronic search identified all patients with a lower limb ultrasound Doppler scan between 1st September and 31st October 2014 (excluding: non-DVT scans, pregnancy and primary care patients) (n=82).
Patient electronic records were reviewed for patient information; pathology and radiology examination results, communication notes and discharge summary.
Data was coded and analysed.
Qualitative DVT 'app' feedback was collected with a focus group.
Results: 82 patients were included in the re-audit.

- 89% patients had a general medical history and physical examination documented
- 23% patients had a documented Two-level DVT Wells score
- 100% patients with DVT unlikely Wells score had a D-dimer blood test requested
- 38% patients with DVT likely Wells score did not have a D-dimer blood test requested
- 100% patients with DVT unlikely Wells score and raised D-dimer result had imaging
- 100% patients with DVT likely Wells score had imaging requested
- 100% patients with no DVT detected were offered a repeat scan within one week if their symptoms persist or worsen
- 62% patients were given interim anticoagulation, unless contraindicated, until imaging
- 100% patients with a DVT were initiated on appropriate anticoagulation therapy
- 67% patients with a DVT and initiated on anticoagulation therapy had counselling
- 100% patients with DVT were appropriately referred for follow up if applicable
- 94% patients had documentation of investigation and management of suspected DVT at discharge for effective communication

Qualitative 'app' feedback:

- "Nursing staff have an active role, freeing medical staff time"
- "Comprehensive and full pathway"
- "?Deskilling clinicians"
- "Relies on clinicians entering data e.g. blood results and can lead to risk"

Summary/Conclusion: Compliance with diagnostic protocol is poor: 23% patients had a Well's score documented; 62% patients with a 'likely' score had a D-dimer incorrectly requested; 38% patients received no interim anticoagulation until imaging and documentation of counselling was low.
The 'app' demonstration received generally positive feedback. The 'app' guides clinicians step-by-step through the diagnostic and management pathway, with a comprehensive discharge summary. A further re-audit will be undertaken post 'app' pilot to determine compliance with standards.
Further improvements include:

- Educational meetings to clinicians highlighting areas where clinical improvement is needed
- Development of a patient educational aid on enoxaparin e.g. patients with DVT and with active cancer/cancer treatment

- Distribution of anticoagulation information booklets
- Pharmacy staff to ensure patients are counselled on anticoagulation agent

Clotting
ECTH-356
Board No. 162: Comparison of protocols and RNA carriers for plasma miRNA isolation: unraveling RNA carrier influence on miRNA isolation
Luis A. Ramon Núñez[1], Laura Martos[1], Alvaro Fernández-Pardo[1], Julia Oto[1], Pilar Medina[1], Francisco España[1], Silvia Navarro[*1]
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Background: microRNAs are promising biomarkers in biological fluids in several diseases. Different plasma RNA isolation protocols and carriers are available, but their efficiencies have not been comparatively studied. Here we compare different isolation protocols and the influence of different RNA carriers depending on the miRNA GC-content and thermodynamic stability.
Aims: The aim of the current study was to determine the most suitable RNA isolation method for plasma.
Methods: Plasma microRNAs were isolated using modified phenol and column-based isolation and column-based procedures with and without two RNA carriers (yeast RNA and MS2 RNA). We evaluated the presence of PCR inhibitors and the relative abundance of certain microRNAs by qRT-PCR. Furthermore, we analyzed the association between different isolation protocols, the GC-content and the free energy of microRNAs.
Results: In all microRNAs analyzed, the addition of yeast RNA as a carrier in the different isolation protocols used gave lower raw Cq values, indicating higher miRNA recovery. There was an increase in microRNAs recovery using yeast RNA as carrier, which was free energy-dependent. Moreover, the normalization of microRNA levels by an endogenous microRNA provided an advantage against normalization by plasma volume, as it reduced the difference in microRNA levels between the isolation protocols used.
Summary/Conclusion: Yeast RNA carrier improves microRNA isolation in both protocols studied, and this increased isolation efficiency is free energy-dependent. The use of an internal microRNA as normalizer gave the most consistent results between different protocols. Therefore, a standardization of pre- and analytical conditions is necessary to obtain reproducible inter-laboratory results in plasma microRNA studies.
ISCIII (PI12/00027, RD12/0042/0029, PI14/00512, PI14/00079, FI14/00269, CP09/00065), FEDER, Generalitat Valenciana (PROMETEOII/2015/017), IIS La Fe (2012/0221, 2014/0421, 2014/0718).

Clotting

ECTH-395

Board No. 163: Angioedema presence in patients with urticaria is associated with an increase in thrombin generation peak

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Background: Chronic urticaria (CU) is a widespread and recurrent disease of the skin characterized by the appearance of wheals and in many cases are accompanied by oedema in deeper layers, called angioedema (AE). These symptoms occur when immunologic, physical or infectious stimuli trigger the release of inflammatory mediators. UC patients show increased activation of coagulation and fibrinolysis. In fact, oral anticoagulants appear to be effective in the treatment of UC. The thrombin generation test (TGT) is a global coagulation test which quantifies *in vitro* the ability of plasma to generate thrombin.
Aims: To determine the thrombin generation to characterize patients with UC and AE, evaluating the association between coagulation and pathology.
Methods: We have analyzed thrombin generation in 45 patients with active UC, 23 of them with a history of AE. Plasma from patients was incubated with a mixture of tissue factor (4 pM), fluorogenic substrate and CaCl2. Lag time (LT, min), endogenous thrombin potential (ETP, nM.min), peak height (PK, nM), time to peak (ttPK, min) and the pending of thrombin formation or speed index (SI): 5 parameters were analyzed. The severity of the UC was assessed using the questionnaire Urticaria Severity Score (USS). TGT parameters were related to the CU severity, the presence of AE and other clinical parameters.
Results: The 23 UC patients with AE showed a significant increased in the PK (177 ± 49 nM) compared with the 22 UC patients without AE (142 ± 55 nM) (*P*=0.017). The ETP and SI were also higher in UC patients with AE (1380 ± 302 nM □ min and 43.32 ± 20.94 nM/min, respectively) than in those without AE (1283 ± 298 nM □ min and 31.92 ± 21, 48 nM / min), although the difference was not significant (*P*=0.200 and P=0.050). Both the LT and the ttPK were lower in UC patients with AE (5.61 ± 1.02 and 10.75 ± 1.94 min, respectively) than in those without AE (6.05 ± 1.59 and 11.59 ± 2 86 min), although the differences were not significant (*P*=0.658 and P=0.454). We observed no relationship between the different TGT parameters distributed according to the severity index. We also found no correlation between the different TGT parameters of EGT and clinical parameters associated with UC.
Summary/Conclusion: Patients with UC and a history of AE showed increased thrombin generation than UC patients without AE, indicating the presence of a hypercoagulation state in these patients. However, the clinical relevance of these findings is still under investigation. These results further suggest that the TGT can be useful to evaluate the role of the coagulation cascade in diseases with inflammatory component. ISCIII (PI12/00027, RD12/0042/0029, PI14/00512, PI14/00079, FI14/00269, CP09/00065), FEDER, Generalitat Valenciana (PROMETEOII/2015/017), IIS La Fe (2012/0221, 2014/0421, 2014/0718).

Clotting
ECTH-404
Board No. 164: Identification of a plasma metabolite profile associated to venous thromboembolism using untargeted UHPLC-QqTOF-MS/MS analyses
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Background: The blood clotting system comprises pro- and anticoagulant factors that, under normal conditions, keep the blood flowing. Qualitative and quantitative changes in this balance may result in bleeding or thrombosis. The current laboratory techniques do not identify all individuals at high risk of thrombosis, thus there must be unknown thrombotic risk factors that may interact with those already known. In addition, little is known about environmental factors that trigger venous thromboembolism (VTE). In addition, the association between exposure to environmental pollutants and the incidence of VTE is controversial.
Aims: To develop an advanced metabolomic method that allows us to establish a characteristic metabolomic profile in patients diagnosed with VTE.
Methods: We selected citrated plasma samples from 10 healthy volunteers, 10 patients with a history of provoked VTE and 10 patients with unprovoked VTE age and sex-matched. Informed consent was obtained from all subjects. Ultra-high performance liquid chromatography mass spectrometry (UHPLC-QqTOF-MS/MS) offers high separation power and high sensitivity for metabolomic research. The utility of metabolomic screens largely depends on the number of metabolites identified and links to their biological interpretation. To achieve this phase, different methods of treatment involving plasma protein precipitation were analyzed. These processes included extraction with methanol, methanol acidified with 1% formic acid, acetonitrile and acetonitrile as acidified methanol. Often the challenge step occurs during the identification of these metabolites. Therefore, we developed a homemade extracted ion chromatograms (XIC) manager and a MS/MS Library to improve the identification of these metabolites.
Results: The number of metabolites identified depends on the extraction procedure used. To this end, several methods based on acetonitrile protein precipitation, were compared to establish which of them were able to extract the higher number of metabolites in plasma samples of healthy individuals. The best results were obtained with the acetonitrile acidified with 1% formic acid extraction method. Then the untargeted metabolomic UHPLC-QqTOF-MS/MS analyses were performed in all samples. The results were analyzed using different statistical tests, in order to reduce the study only for those variables that have the discriminant information between the study groups, and were carried out using the specifically homemade XIC manager and MS/MS Library. The results showed that various metabolites, such as L-carnitine, are responsible for distinguishing samples from the study groups. These results are consistent with previous results in which plasma acylcarnitines were associated with the risk for VTE. The next step would be to expand the study to include a larger number of individuals and to corroborate the results of this study and the implications of this and other metabolites in the blood clotting system.
Summary/Conclusion: These results demonstrate the usefulness of untargeted metabolomics analyses, and have implications for the future approach of basic/clinical studies for the identification of biomarkers associated with pro- or anticoagulant functions, and the identification of important molecules in patients diagnosed with VTE due to environmental causes. ISCIII (PI12/00027, RD12/0042/0029, PI14/00512, PI14/00079, FI14/00269, CP09/00065), FEDER, Generalitat Valenciana (PROMETEOII/2015/017), IIS La Fe (2012/0221, 2014/0421, 2014/0718).

Clotting
ECTH-389
Board No. 165: The relation between pulmonary embolism severity and haemostasis parameters at presentation in patients with acute pulmonary embolism
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Background: The relationship between haemostasis parameters and the severity of pulmonary thromboembolism (PTE) is not well characterized.
Aims: The aim of this study is to determine relationship between hamostasis parameters and the severity of pulmonary trhomboembolism.
Methods: One hundred and thirty three consecutive patients with acute pulmonary embolism treated during period of six years in the single university center were enrolled in this study. According to European severity score (ESS) patients were divided into low, intermediate and high-risk group (45, 70 and 18 patients respectively). According to simplified pulmonary embolism severity index (sPESI) patients were also divided into low-risk (score 0) intermediate-risk (score 1 and 2) and high-risk (3 or more)(45, 62 and 26 patients respectively). Plasma activity of coagulation factors II, VII, VIII, fibrinogen and anticoagulation factors antithrombin and protein C were determined by the coagulation assays at admission.
Results: Patients with high-risk ESS had lower activity of factor II (1.00±0.33 vs 1.23±0.30 vs 1.15±0.32 IU/L, p=0.025) and VII (0.73±0.23 vs 0.93±0.27 vs 0.90±0.27 IU/L, p=0.045). Patients with high-risk sPESI had significantly lower factor VII (0.74±0.26 vs 0.90±0.28 vs 0.96±0.23 IU/L, p=0.008) and antithrombin activity (0.74±0.12 vs 0.87±0.15 vs 0.85±0.16 IU/L, p=0.001) and higher factor VIII activity (3.57±1.50 vs 2.81±1.22 vs 2.48±0.86 IU/L, p=0.003) compared to other to groups.
Summary/Conclusion: High-risk PTE patients have significant consumption of factor II, VII and antithrombin as well as up-regulation of factor VIII activity.

Clotting
ECTH-229
Board No. 166: Pulmonary embolism in a patient with COPD–bronchiectasis overlap syndrome and subacute invasive pulmonary aspergillosis
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Background: Chronic pulmonary aspergillosis (CPA) is an uncommon and problematic pulmonary disease, complicating many other respiratory diseases, assumed to affect 240,000 people in Europe. Subacute invasive pulmonary aspergillossis (SAIA), one form of CPA, is a more rapidly progressive infection (< 3 months) usually found in moderately immunocompromised patients. Immunocompromising conditions include diabetes mellitus, chronic obstructive lung disease, bronchiectasis, malnutrition, alcoholism, advanced age. A chronic infection may be a risk factor for pulmonary embolism.
Aims: We report a case of a female patient who has for a year been treated for chronic obstructive pulmonary disease and bronchiectasis (BCOS), verified by CT imaging. She also had arterial hypertension and diabetes as accompanying diseases. The patient developed current symptoms (dyspnea, blood expectoration, chest pains) three days prior to admission. Due to these symptoms, in addition to elevated D-dimer (8500ng/ml), partial respiratory failure, and a paracardial infiltration on the left presented on the chest X-ray, pulmonary embolism was suspected.
Methods: The ECG finding was presented with the right heart overload signs. The acute right heart overload was confirmed by US of the heart. Computerized tomography of the chest with pulmonary angiography verified a large filling defect in the left pulmonary artery lumen, extending into the right pulmonary artery lumen in the form of "a riding thrombus"; a smaller defect was seen in the lobar branch for the upper bronchus on the left, while the branch for the lower bronchus remained obscured; a defect was also seen in the region of the right pulmonary artery bifurcation, as well as in the lobar branches for the central and lower bronchi (this finding suggested pulmonary embolism). The basal parenchyma was bilaterally, predominantly to the left, presented with collections of inflamed cystic bronchiestases. The mycological blood testing to Aspergillus provided positive IgM antibody and Galactomannan test findings, and sputum bacteriology established Aspergillus species.
Results: The patient was treated with oxygenation, non-fractionated heparin in treatment doses, and Aspergillus-specific therapy (amp. Voriconazole), and symptomatic drugs. She responded to the applied treatment with a radiologic and clinical improvement. The patient was discharged, given recommendations for oral anticoagulant and antimycotic treatment. Under the applied treatment, the symptoms gradually disappeared, and the control chest X-ray showed a radiological remission. Control mycological blood assays provided a negative Galactomannan test finding and decreasing IgM antibodies to Aspergilus. The antimycotic treatment was discontinued after five months, while oral anticoagulant treatment continued.
Summary/Conclusion: This is a case in which chronic obstructive pulmonary disease with bronchiectasis contributed to the onset of subacute invasive pulmonary aspergillosis, and the chronic infection to pulmonary embolism.

Clotting

ECTH-259

Board No. 167: Construction of fibrin cross-linking tissue plasminogen activator to reduce bleeding risk associated with stroke treatment

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Background: Clinically approved drugs can be modified to improve their mode of action and decrease their side effects. Thrombolytic drugs dissolve blood clots by activating plasminogen to plasmin that subsequently cleaves fibrin clots into soluble components. Tissue plasminogen activator (tPA) is a serine protease (527 aa) that is used to treat acute events such as myocardial infarction and cerebral stroke, but is associated with bleeding side effects. A modified version of recombinant tissue plasminogen activator (rtPA) could improve efficacy, thus requiring lower doses of tPA, leading to a decrease in bleeding side effects.
Aims: Development of a tPA variant that can covalently crosslink to fibrin can result in improved specific activity thereby requiring lower doses and reducing bleeding risks associated with tPA treatment.
Methods: The *N*-terminus of α_2-antiplasmin (α_2-AP) is covalently linked to fibrin by FXIIIa during clot formation through glutamine residue-3 in α_2-AP. A chimera of rtPA and the *N*-terminus of α_2-AP (A14) can thus covalently attach rtPA to the fibrin clot. The chimera was synthesized by oxidation of the *N*-terminal serine of rtPA, followed by oxime ligation with A14 peptide to result in the chimera protein A14-rtPA. The reaction was followed by MALDI mass spectrometry and western blot analysis. After obtaining the A14-rtPA constructs, the covalent attachment of A14-rtPA to fibrin was evaluated by incubation of A14-rtPA with fibrinogen, FXIII and thrombin. Covalent attachment of A14-rtPA to fibrin was assessed using western blot analysis. Subsequently, the activity of the constructs was monitored in a plasma clot lysis assay in static conditions as well as a clot lysis assay using whole blood under flow.
Results: Chimera proteins combining A14 with rtPA were successfully synthesized and modification only occurred at the desired *N*-terminal position. The chimeras all contained a biotin moiety which made detection possible using western blot analysis. We verified that A14-rtPA was covalently linked to fibrin by FXIIIa, whereas a negative control that does not contain the critical glutamine-3 residue showed no fibrin linkage. The activity of the tissue plasminogen activators in a static plasma clot lysis assay showed no difference when comparing A14-rtPA to rtPA. In order to mimic an occluded artery more realistically, clot lysis was studied under flow conditions. In this assay, however, also no difference was observed between rtPA and A14-rtPA.
Summary/Conclusion: We have successfully synthesized chimera proteins combining the crosslinking activity of the *N*-terminus of α_2-AP with the plasminogen activation activity of tPA. Moreover, we could prove that this chimera protein is crosslinked to fibrin by FXIIIa. The clot lysis assays, however, showed that the activity of rtPA was not affected by cross linking to fibrin. This can be partly explained by restricting A14-rtPA in accessing its target cleavage sites on fibrin. Currently, variants of A14-rtPA are prepared with increased linker length between A14 and rtPA to improve activity.

Clotting
ECTH-335
Board No. 168: Substrate delivery mechanism and the role of membrane curvature in factor X activation by extrinsic tenase: a computational study
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Background: Extrinsic tenase is an enzyme complex catalyzing factor X (FX) activation in the tissue factor pathway of blood coagulation. The functioning of serine protease factor VIIa in this complex depends on the availability of its cofactor, the protein called tissue factor (TF). Additionally, interaction of FX and FXa with negatively-charged phospholipid membranes plays an essential role in FX activation by extrinsic tenase. Still, some of the mechanisms and the underlying molecular events remain unclear.
Aims: Computational analysis of the molecular mechanisms governing FX activation by extrinsic tenase assembled on phospholipid vesicles.
Methods: We developed a comprehensive computational model accounting for protein-protein and protein-lipid interactions during FX activation by extrinsic tenase. The model comprised ten ordinary differential equations integrated in MATLAB(2012) with standard solver ode45. The unknown parameters of the model were tuned to describe experimental data (Hathcock et al. Biochemistry, 44, 8187-97, 2005).
Results: The predicted dependence of FXa formation rate on phospholipid concentration had markedly different forms for different mechanisms of substrate delivery, reaching a reasonable agreement with the experimental data only when membrane-bound FX served as a substrate. To achieve better description of the reported dependencies of reaction rate on phospholipid concentration and of the kinetic parameters on the vesicle radii, it was necessary to introduce the dependence of equilibrium dissociation constant for interaction of FX/FXa with phospholipid membrane and of the number of phospholipid molecules per bound FX (FXa) on the vesicle radius (membrane curvature). In this assumption, binding of FX to the surface was best for either very small vesicles (10 nm) or flat surfaces with the poorest binding at the range of 100 nm. This could be explained by the collisional limit on the one hand and by phosphatidylserine clustering around FX on the other.
Summary/Conclusion: The substrate of the extrinsic tenase is the membrane-bound FX. The best description of experimental data could be achieved if the dependence of FX (FXa) binding parameters on the curvature of the surface is taken into account.

Clotting

ECTH-455

Board No. 169: The potential role of procoagulant extracellular vesicles on thrombosis in patients with multiple myeloma and the precursor monoclonal gammopathy of undetermined significance

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Background: Both multiple myeloma (MM) and its precursor monoclonal gammopathy of undetermined significance (MGUS) have increased risk of developing venous thromboembolism. It is believed that high levels of extracellular vesicles (EVs) play a role in both thrombotic diseases and cancer. Moreover, EVs may carry procoagulant factors, such as tissue factor (TF) and phosphatidylserine, that may contribute to this VTE-risk.

Aims: We investigated for increased coagulation and elevated EV-levels in blood from MM and MGUS patients. Furthermore, we aimed to relate coagulation to EV-phenotype.

Methods: Plasma was collected from 20 MM and 14 MGUS patients which were compared against plasma of 15 age- and sex-related control persons. Prior to inclusion all test subjects gave their written consent. The study was approved by the local ethics committee and complies with the Declaration of Helsinki. Particle concentrations were examined by nanoparticle tracking analysis (NTA). EV-presence were confirmed through immunotransmission electron microscopy (IEM) and Western blotting (WB). As a measure of coagulation, tissue factor triggered calibrated automated thrombogram (CAT) and analysis of procoagulant phospholipids (PPL) were conducted. In order to measure the procoagulant effect of EVs, i.e.TF and PPL, a 'spiking'-assay were applied for both the CAT- and PPL-analyses with isolated EVs as spiking-medium. Furthermore, an EV-TF activity assay was performed.

Results: NTA showed a higher particle count in both patient groups and the presence of EVs was confirmed via several EV-specific markers in IEM and WB. In CAT-analyses both MM and MGUS patients exhibited both shorter lag time and time-to-peak. Furthermore, we found a markedly increased peak thrombin and ETP for the patient groups. Analysis of PPL revealed increased PPL activity in both MGUS and MM patients, with the most for the latter, compared to controls. Additionally spiking of both CAT- and PPL-analyses as well as the EV-TF activity assay revealed that EVs play a role in contributing to the procoagulant state observed in these patients.

Summary/Conclusion: Our results suggest a likely link between EV-phenotype and increased coagulation not only in patients with MM, but also in the precursor stage, MGUS. These findings may prove valuable in the search for biomarkers capable of identifying single patients at high risk of developing thrombosis and allow for an early treatment intervention.

Clotting
ECTH-375

Board No. 170: Monitoring of thromboprophylaxis with low dose dabigatran in elderly patients – first results

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Background: There is increasing evidence that dabigatran (DBG) exerts variable, pharmacokinetic-dependent effects on routine coagulation tests such as the prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays. Fortunately, due to its mode of action, the influence of DBG on such coagulation assays is predictable and transient. Nevertheless, in urgent situations, such as emergency surgery or cases of stroke, it is not possible to warn patients to stop taking DBG. The results of several laboratory studies suggest that it is feasible to measure the anticoagulant response of DBG; and to determine the exact concentrations of DBG in the patient if needed.
Aims: To verify whether the diluted thrombin time (dTT) can be useful test in clinical practice for monitoring of the "low dose" DBG plasma concentrations in long-term thromboprophylaxis in elderly patients with atrial fibrilation (AF) and comorbidities.
Methods: Thirteen patients (8 men, 5 women, mean age 73,6±6,0 years) with non-valvular AF and comorbidities treated with "low dose" DBG (110 mg twice a day) were included in this preliminary prospective observational study. dTT (®Hemoclot assay) was used for monitoring of DBG plasma concentrations.
Results: The mean trough DBG concentration in patients treated with 110 mg twice daily was 55.1±35.0 ng/mL, while the mean peak DBG concentration reached 120.0±68.2 ng/mL. Routine coagulation assays (PT, aPTT) didn´t correlate with DBG concentrations.
Summary/Conclusion: Results of this study confirmed that dTT is useful test also for the monitoring of "low dose" DBG plasma levels in long-term thromboprophylaxis in elderly patients with AF. According to this test, more tailored management of DBG might be ensured. The study was supported by projects Martin Center of Biomedicine (BioMed Martin, ITMS 26220220187) and VEGA 1/0168/16.

Clotting

ECTH-333

Board No. 171: A different clinical profile of direct oral anticoagulants-treated patients: results from the real life cohort study

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Background: The use of DOAC is increasing for stroke prevention in patients with non-valvular atrial fibrillation (NVAF). Regarding to thrombotic complications, the rate of stroke is described around 1.11%>1.7%. However, clinical trials patients are carefully selected and some risk factors could result in lower rates of therapeutic failure.

Aims: Determine the percentage of stroke in “real life” population treated with DOACs, and try to characterize the clinical profile of the patient with stroke despite correct anticoagulation.

Methods: We included consecutively from June 2010 to November 2015 patients with DOAC treatment. Clinical features and risk factors for stroke were registred. We used CHA2DS2-VASc, HASBLED scores and renal function was calculated using the Cockroft-Gault method.

Results: A total of 563 patients were recruited and 87.9 % (n= 495) had indication for NVAF. Mean age was 74.9 years (range: 40-93) and 53.5% were female. Among them, 158 (31.92%) were under dabigatran, 229 (46.26%) rivaroxaban, and 108 (21.82%) apixaban. Mean CHADSVASC score was 4.39, with HASBLED of 2.89. We found 144 patients with a glomerular filtration rate < 50 ml/min.

Thirteen patients (2.6%) suffered a stroke and the mean time since the start treatment to event onset was 12.7 months. They all shared a high thrombotic risk. The mean CHA2DS2- VASC score was 5.69 with female predominance (11:2), mean age was 70.5, all of them had hypertension and only 2 severe renal failure. Notably, 11 patients were receiving DOAC as secondary prophylaxis.

Patients under dabigatran had a mean follow up of 36.65 months, rivaroxaban 19.07 and apixaban 12.79 months . Stroke occurred in 2 patients with dabigatran 75mg, 4 dabigatran 110mg, 2 dabigatran 150mg, and 5 rivaroxaban at correct doses. We did not observe any ischemic event in apixaban patients. We confirmed good adherence in all patients.

Regarding the subsequent management, 4 patients under rivaroxaban changed to dabigatran and only 1 to apixaban; patients with dabigatran 75mg changed to AVK, patients with dabigatran 110mg changed to higher doses of 150mg.

Summary/Conclusion: In daily clinical practice we described a low rate of stroke in patients treated with DOACs, further studies in real population are necessary in order to identify very high-risk patients with special emphasis in those with high CHA2DS2-VASC score, previous stroke and a correct anticoagulation.

Clotting

ECTH-334

Board No. 172: Is the dilute Russell's viper venom time (DRVVT) a useful assay for all direct oral anticoagulants?

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Background: DOACs occasionally may require measurement of anticoagulation intensity in certain clinical situations. Routine coagulation screening assays do not quantify anticoagulation intensity. Therefore, it is necessary a rapid, simple, and quantitative test that correlates with these drugs concentrations. Dilute Russell's Viper Venom Time (DRVV-T) contains a potent activator of factor X and catalyzes the transformation from prothrombin to thrombin, the presence of factor IIa or factor Xa inhibitors may increase the coagulation time. It is an universal and standard assessment, that could play a role in the DOACs daily practice.

Aims: To analyze the correlation between DRVV-T and direct oral anticoagulants plasma levels.

Methods: Levels of dabigatran and apixaban were tested in 45 blood samples from 26 patients. Rivaroxaban samples are being processed but results are not definitive yet. To determine the highest and the lowest blood levels of the drugs by obtaining a blood sample just before the morning dose then sampling the blood again 2 hours after of the administered dose.

DOACs plasma concentrations were measured using the Direct Thrombin Inhibitor Assay from HemosIL (Europe and International Launch) for dabigatran and HemosIL Testing Solution (UE/International Launch) for apixaban. Calibraton curves were obtained from 100 healthy patients.

We performed DRVV-T and normal ratio was established by measuring 35 normal samples.

Results: We observed that DRVV-T was prolonged in a concentration-dependent fashion at higher DOACs levels. There was a linear correlation between apixaban levels (ranging between 49.74 ng/mL – 423.47 ng/mL) and ratios for DRVV-T test, Pearson´s correlation value was 0.84 with bilateral significance p value< 0.01. The same results with direct thrombin inhibitor, dabigatran (43.79 ng/mL - 250 ng/mL), with a Pearson´s value of 0.69 (p <0.01). When we analyzed both together, Pearson´s correlation value was 0.62 (p value <0.01).

A cut-off level of 1.2 for DRVVT ratio, corresponds to the presence of more than 30 ng/mL DOAC concentration.

Summary/Conclusion: DRVV-T showed a close linear correlation between its ratio and plasma drug concentrations. Although this method requires further investigations and standardization.

Clotting

ECTH-369

Board No. 173: Self-testing and self-management of oral anticoagulation therapy in children with congenital heart disease: from the bedside to home, a pilot study in Spain

Laura Lopez Andreoni, Dimpna Albert[1], Veronica Pons[* 2], María Jose Cabanas[3], Miren Gabilondo[4], Pavel Olivera[4], Juan Jose Diaz[4], Rosario Soria[4], Rosa Estevez[4], Francesc Bosch[4], Amparo Santamaria[4]
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Background: OAT self-control has shown an improvement in the time in therapeutic range *(TRT)* with a significant reduction in thromboembolic and bleeding events. Children and adolescents on OAT, present also other special challenges in terms of rapid fluctuations in International Normalised Ratio (INR) values and interruption in daily life due to frequent hospital visits. Limited data are available on the safety and efficacy of this modality of anticoagulation control in children with CHD

Aims: During 2015, a pilot study on coumarin self-control was initiated. This single centre prospective clinical study was designed to evaluate the safety, efficacy and quality of life of a home OAT monitoring with a CoaguChek XProÒ system in paediatric population with CHD, mostly mechanic heart valves (*MHV).*

Methods: The programme development was structured in three parts: cardiology department doctors and nurses education, families and children´s training and patients clinical follow up. The education program for parents and adolescents consists on general OAT theoretical information and practical sessions on point of care device management, dosage algorithms and problem solving guidance. The patient´s follow up was made by hospital visits (month 1-3-6 and 12) or by phone if necessary. New technology support (*e-mail and WhatsApp*) was used for brief doubts resolution and the all families has access to a web-application (TAO-NetÒ) to introduce the INR results. The self-control programme was offered and started in ambulatory patients already on OAT or in hospital post-surgical children.

Results: Out of the 20 patients screened, 15 were eligible and accepted to enrol in the study, 47,7% were girls and 53,3% boys. The median age was 8 years (range: 8 months-17 y). 13 patients were anticoagulated for: 8 mitral and 4 aortic *MHV*, 2 for other *CHD* and 1 child for recurrent venous thromboembolism. Cases were vitamin K antagonist naïve. At six months of follow up, adherence was good, TRT is superior to 70%. There were not thrombotic or major haemorrhagic events, and all the families and children were satisfied with the improvement of quality of life.

Summary/Conclusion: The primary results of this study suggest that self-control of OAT shows a net benefit clinical outcome as first option of coumarin-management. Also the reduction of outpatient visits showed a high level of parents and children´s satisfaction and an improvement in their quality of life.

Clotting

ECTH-313

Board No. 174: Do physicians accurately evaluate the quality of anticoagulation control in their patients with atrial fibrillation receiving vitamin K antagonists? Results of the Argos registry

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Background: High-quality anticoagulation control (AC) is required to keep narrow therapeutic index vitamin K antagonists (VKA) as effective and safe as possible. Time spent in therapeutic range (TTR) is known to accurately reflect the quality of anticoagulation. However, little is known on the physician's and patient's perception of AC.

Aims: i/To compare the physicians perception of AC in their own patients receiving VKA with calculated TTR; ii/ to identify factors explaining discrepancy between physician's perception of AC and effective TTR; iii/ to compare patients' satisfaction with their AC and physicians' satisfaction.

Methods: We conducted an observational, prospective, national study in a set of French cardiologists or GPs managing AF patients in a usual care setting. Physicians had to include 5 consecutive patients with AF receiving VKA for at least 6 months. An E-CRF questionnaire was completed, recording patient socio-demographic, clinical and biological data including INRs, and the physician's perception of the AC. TTR was calculated using Rosendaal's method. Patients completed the Anti-Clot Treatment Scale questionnaire to evaluate their perception of VKA treatment.

Results: During the study period (January 2014 – May 2015), 123 physicians (82 GPs, 41 cardiologists) from 8 French regions included 833 patients (75 ±11.5 years; males: 58.5%; mean body-weight 77 ± 16 kg). At least one comorbid condition was recorded in 82% of patients (creatinine clearance-Cockcroft ≤ 50 mL/min in 29.0%), history of cardiovascular or surgical event in 51.5%, thromboembolic event in 23.0% and bleeding event in 10.0%. The mean duration of VKA treatment was 6.2 (± 5.1) years. The mean CHA_2DS_2-VASc score was 3.4 (±1.8) and the mean HAS-BLED score 2.5 (±1.2).

Overall, mean TTR was 76.9% (±28.4). Mean TTR was of 82% (±25.4) when physicians were satisfied/very satisfied with patient's AC versus 59.5% (±31.2) when they were dissatisfied/very dissatisfied. When physicians were satisfied/very satisfied, 72.4% of patients had TTR >70%; when they were dissatisfied/very dissatisfied, 61.7% of patients had TTR < 70%, indicating a strong relationship between TTR and physician's perception ($p<0.0001$), both in GPs and cardiologists (Cochran-Mantel-Haenszel test). However, a discrepancy was observed between physician's perception and TTR level (> 70% or ≤ 70%) in 30% of patients, which was observed more frequently when the physician was dissatisfied (38.3%) than when he was satisfied (27.6%). Factors associated with a discrepancy between physicians' perception and effective TTR were HAS-bled score ≥ 3 ($p<0.001$) and NSAI intake ($p=0.026$) (multivariate analysis). Regarding patients' perception of their AC, one third were dissatisfied whereas their physician was satisfied; half of them were satisfied whereas their physician was dissatisfied.

Summary/Conclusion: This registry is a snapshot of the present population receiving VKA in France, with most patients with high-quality of AC. Interestingly, we showed that physicians have an accurate perception of their patients' AC when compared with calculated TTR. Discrepancy between accurate perception and TTR may be due to the physician's fear of patient's bleeding.

Clotting
ECTH-435

Board No. 175: Antepartum prophylactic anticoagulation treatment in pregnant women with thrombophilia

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Background: Thrombophilias are associated with early and late pregnancy loss.
Aims: The question: whether pregnant women with thrombophilia and history of recurrent (3 and more pregnancy losses) should receive antepartum thromboprophylaxis?
Methods: We have studied 111 pregnant women (mean age 31±6 years). Inclusion criteria was pregnancy (gestational age < 4-5 weeks) confirmed by positive serum or urine βhCG. One or more of the following thrombophilias were identified: PAI-1 in 89% of patients (pts), genetic mutations in folate-related enzyme genes – in 69% of pts, FGβ – 43% of pts, fV Leiden – 6% of pts, prothrombin G20210A – 5% of pts. Blood recalcification time (in our modification: patent of RF # 2015515) was determined by thrombelastometry (TEM) (ROTEM): recorded parameter was the reaction time – r (normal range = 640 – 960 s). Fibrinolysis (F) (in our modification: patent of RF # 2358657) was investigated by adding plasmin to kaolin-activated blood sample: the difference between R kaolin TEM and R1 plasmin+kaolin TEM was calculated as percentage of R1 prolongation (normal range = 70-130%). We tested the blood by TEM repeatedly in the 1st, 2nd, 3rd trimester of pregnancy. Treatment with dalteparin was started when r was < 460 s and F < 35%. Blood analysis was taken after 3-4 hours of dalteparin injection.
Results: There were no TEM indications to dalteparin treatment up to 5-6 weeks' gestation. There were only 11 pts (10%) requiring dalteparin prophilaxis (2500 IU once daily) from 6 weeks until 12 weeks gestational age (hypofibrinolysis). Up to 24 weeks' gestation 9 pts (8%) received deltaparin 2500 IU once daily and 28 pts (25%) – 2500 IU twice daily (hypofibrinolysis). From 24 weeks until at least 37 weeks gestational age 46 pts (41%) received dalteparin: 11 pts (24%) – 2500 IU once daily, 30 pts (65%) – 2500 IU twice daily and 5 pts (11%) – trice a day (hypofibrinolysis). At the beginnig of the study there were three unexplained pregnancy losses at or more than 24 weeks' gestation in the group of pts receiving dalteparin.
Summary/Conclusion: There is a big group of pregnant women with thrombophilia and burdened obstetrical history, who does not need any pharmacological antithrombotic prophylaxis. TEM can provide useful individual information on the risk of pregnancy loss and the necessity of antepartum prophylactic anticoagulation treatment.

Clotting
ECTH-476

Board No. 176: Brain natriuretic peptide is decreased in saliva of patients treated with vitamin K antagonists and direct oral anticoagulants

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Background: Prevalence of anticoagulation treatment for various medical conditions to prevent thromboembolism reaches 5% in the elderly population. Several treatment options exist such as vitamin K antagonists and direct oral anticoagulants (DOAC). The advantage of DOAC is that this category of drugs can be used safely without frequent monitoring. The disadvantage, however, is the lack of possibilities to monitor compliance and drug accumulation. Previously we have shown in a small proof of concept study the usefulness of saliva to monitor vitamin K antagonists. Saliva, an ultra filtrate of blood, is an attractive specimen as it can be collected non-invasively at any time without special requirements. One of the markers previously identified to correlate with INR in saliva was Brain Natriuretic Peptide.
Aims: To determine whether DOACs also effect BNP levels in saliva as previously observed in saliva of patients treated with vitamin K antagonists.
Methods: BNP was measured by ELISA in saliva of 49 patients treated with vitamin K antagonists, 6 patients with dabigatran, 12 with rivaroxaban and 21 with apixaban. The levels were either compared to BNP saliva levels in controls and correlated to the INR or DOAC concentrations Finally, BNP saliva levels were correlated to NT-proBNP plasma levels.
Results: In controls the BNP levels were the highest of all groups (282 pg/mL; SD 71 pg/mL). The levels were 156 pg/mL (SD 74 pg/mL) for patients treated with vitamin K antagonists; 184 pg/mL (SD 56 pg/mL) for the dabigatran group, 146 pg/mL (SD 67 pg/mL) for rivaroxaban and 144 pg/mL (SD 57 pg/mL) for apixaban. In the total population the lowest levels were found in patients treated with vitamin K antagonists and apixaban. For all groups there was a negative correlation with either the INR or the specific DOAC concentration. The saliva did not correlate with BNP plasma levels.
Summary/Conclusion: Patients treated with vitamin K antagonists have lower BNP saliva levels as compared to controls thereby confirming our proof of concept results. However, the effect seems not to be provoked by vitamin K antagonists only as a similar effect is seen in patients treated with DOAC. The mechanism for this decreased level of BNP- a heart failure marker- is so far not understood.

Clotting

ECTH-152

Board No. 177: Mild antithrombin deficiency and the risk of first venous thrombosis: results from a population based case-control study

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Background: Family studies revealed that individuals with inherited antithrombin deficiency have a high risk of first and recurrent venous thrombosis. The relevance of findings from these family studies within normal populations remains to be established.
Aims: We aimed to determine if low levels of antithrombin were associated with an increased risk of venous thrombosis in a population based study.
Methods: In a large population-based case-control study we studied if low levels of antithrombin were associated with an increased risk of first venous thrombosis. Several cut-off points for establishing antithrombin deficiency were applied. All relative risk estimates were adjusted for age and gender by means of logistic regression.
Results: There were 2377 venous thrombosis patients (mean age 49 years) and 2940 controls (mean age 46 years) tested for antithrombin. Individuals with lower antithrombin levels (<2.5th percentile, <84U/dL) had an adjusted odds ratio of 1.14 (95% CI, 0.80-1.63) as compared with the ≥2.5th percentile. Next, we broke down the ≥2.5th percentile into more specific percentiles to observe any changes towards the lowest percentile. This led to an odds ratio of of 1.19 (95% CI, 0.82-1.11) when comparing the <2.5th percentile with the reference category (75th to 97.5th percentile). When clinical cut off antithrombin values were used odds ratios were 0.99 (95% CI, 0.84-1.16), 0.77 (95% CI, 0.58-1.01), 0.97 (95% CI, 0.48-1.94) and 0.67 (95% CI, 0.28-1.94) for levels of 100-90%, 90-80%, 80-70% and <70%, as compared with levels > 100%, respectively.
Summary/Conclusion: In this population based case-control study, individuals mild antithrombin deficiency were not prone to an increased risk of venous thrombosis.

Clotting
ECTH-179

Board No. 178: Varying incidences of chronic thromboembolic pulmonary hypertension after acute pulmonary embolism: the final thread that links them all

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Background: The reported incidence of chronic thromboembolic pulmonary hypertension (CTEPH) after pulmonary embolism (PE) ranges from 0.1% to 11.8%. This wide spectrum complicates the formulation of recommendations regarding intensity and duration of follow-up after PE.
Aims: We performed a systematic review and meta-analysis aimed at gaining more accurate insight in the CTEPH incidence by predefining relevant subpopulations of PE patients.
Methods: High quality original studies reporting on dedicated follow-up for CTEPH after PE diagnosed by right heart catheterisation (RHC) were selected. Study cohorts were categorized in '*all comers*' (all consecutive patients with PE), '*survivors*' (consecutive PE patients alive after six months) or '*survivors without major comorbidities*' (survivors without known major cardiopulmonary and/or oncological comorbidity). The incidences among the three subcategories were calculated using random effects models.
Results: Sixteen studies totalling 4047 consecutive PE patients were selected, with a 2-year follow-up period in most studies. Patients were subjected to either echocardiography or lung scintigraphy, followed by RHC in case of abnormal result of initial screening test. In 1186 '*all comers*', the incidence of CTEPH was 0.56% (95%CI 0.13-0.98). In 999 '*survivors*' and 1775 '*survivors without major comorbidities*' the incidence of CTEPH was 3.22% (2.01-4.43) and 2.79% (1.46-4.11) respectively. One study in survivors of recurrent PE only reported an incidence of 5.75% (2.48-12.76). Both unprovoked PE and recurrent venous thromboembolism were significantly associated with a higher risk of CTEPH, for Odds Ratios of 4.13 (2.08-8.22) and 3.17 (1.70-5.91). Twelve studies that based the CTEPH diagnosis on other examinations than RHC reported a higher pooled incidence of 6.28% (4.14-8.42).
Summary/Conclusion: Although the risk of CTEPH after PE in general is low, ~3% of patients who visit the outpatient clinic after the first months of treatment will develop CTEPH, independent of comorbidities. Studies that did not confirm CTEPH by RHC provide overestimated CTEPH incidences.

Clotting

ECTH-121

Board No. 179: Deep venous thrombosis and immune thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder with a low platelet count characterized by premature platelet destruction and suppression of platelet production mediated by autoantibodies, which may predispose to bleeding. Patients with ITP have increased risks for thrombosis and atherosclerosis associated with haemostatic factors, endothelial damage and the negative effects of steroid and immunoglobulin therapies.

Aims: There is no general agreement on the role of platelets in venous thrombosis (VT), it was significantly associated in a recent study with increased platelet activation as measured by three platelet indices (mean platelet volume, mean platelet mass and mean platelet component).

Here, the authors present the case of a middle-aged woman with ITP who developed a DVT a month after starting steroid treatment.

Methods: A 42-year-old woman, cook by profession, presented in Casualty having been remitted with haematemesis, petechiae of the lower limbs and severe thrombocytopenia (4,000 x $10x^9$/L).

History: Smoker 20 cigarettes a day. Primary hypothyroidism.

Physical examination: Some isolated petechiae on the soft palate and internal canthus of the sclera of the left eye.

Analyses: Blood count: Haemoglobin 10.8 g/dl, Haematocrit 31.4%, reticulocytes 63,000, leucocytes 6,800 (82% segmented neutróphils) and platelets 4,000 observed in peripheral blood smear.

Immunohaematology: DAT negative, Anti-A1 positive. Basic coagulation study: normal. Antiphospholipid antibody: negative.

Kidney and liver function tests, LDH, lipid profile, TSH normal. ANA: Positive 1/320 with speckled pattern y ENA: Positive for anti-SSA.

Virus serology: Negative

Results: From these data ITP was diagnosed, the patient was admitted to hospital and started on a treatment of steroids at a dose of 1.5 mg/ kg, gammaglobulins at a dose of 1 g/kg for 48 hours. D. Complete remission was achieved, reaching a platelet count of 241,000, continuing with steroid treatment under outpatient supervision.

At 34 days after initial diagnosis with ITP, whilst still undergoing steroid treatment , the patient presented in Casualty with oedema in the right lower limb, diagnosed by Doppler ultrasound as due to a poorly recanalized femoro-popliteal venous thrombosis in the right lower limb. At that point the platelet count was 82,000. Treatment with LMWH at therapeutic doses produced a good response and so oral anticoagulant treatment was begun with acenocoumarol.

Thrombophilia study was carried out, finding a level of Factor VIII:C of 200%. As a result, the patient was maintained on anticoagulant treatment 12 months.

Summary/Conclusion: There are many possible explanations of this tendency of patients with ITP to have thrombosis; the best known is the role of platelet microparticles (PMPs),larger and more adhesive platelets and direct endothelial damage,circulating platelet-leukocyte-monocyte aggregates, endothelium-activating antibodies, the activation of the complement system and low serum levels of a disintegrin and metalloproteinase with a thrombospondin type I motif, member 13.

The indications for the management of thrombosis in patients with thrombocytopenia have not been established yet, however, the overall tendency is to treat them as other thrombosis patients, even though every attempt has to be made to raise platelet number.In our case, as the patient maintained normal platelet levels, she received conventional anticoagulant treatment without bleeding complications at any time.

Clotting
ECTH-122
Board No. 180: Cerebral thrombosis and von Willebrand disease
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Background: Congenital coagulopathies are conditions that first manifest themselves with, and are characterised by a clinical picture of haemorrhage but ever more frequently we are presented with a paradox: the presence of thrombotic phenomena in these patients, which represents both a diagnostic and therapeutic challenge.
Aims: The epidemiology of thrombotic pathology in the coagulopathies is unknown and that is the reason for the present effort to report case series and begin registers to better understand the physiopathology of the thromboses. While levels of factor VIII above 150% seem to predict an increased risk of venous thrombosis, and lower levels act as a protecting factor, the studies involving von Willebrand factor (FvW) provide contradictory results. Most of the authors postulate that the existence of associated prothrombotic factors can generate a haemostatic imbalance which favours thrombotic events in these patients, but that even when these risk factors are absent, venous or arterial thromboses occur in subjects with coagulopathies.
Methods: Female 5-year-old patient. The patient presented in Casualty with odynophagia, high temperature and cephalea. Physical examination: Haematomas on the lower limbs of varying degrees of development. ENT examination: bulging of the soft palate and hypertrophy of the tonsils. Patient was admitted to hospital with a diagnosis of acute tonsillopharyngitis and left otitis media, given the deterioration of the general state. The haemogram showed leukocytosis with deviation to the right, a level of D-dimer: 1000 mg/dl. Twenty four hours after admission the patient presented with a clinical picture of stupor and a low level of consciousness with no neurological focus and so it was decided to carry out an urgent cranial CAT scan, with the following results: there was complete occupation of the mastoid air cells and the left mastoid antrum with thrombosis of the sigmoid sinus, the transverse sinus and the proximal part of the ipsilateral jugular vein.
Results: The patient was treated initially with enoxaparin and subsequently treatment continued with warfarin.
At this point a thrombophilia study was carried out without finding any prothrombotic factors. Nonetheless, the determination of FVIII (19% chromogenic - 22% coagulometric) led to the investigation of the factor VIII complex (Factor VIII: C: 22.8%, Factor VIII:Cro 24,6%, Factor vW Ag : 25.1%, Factor vW Rco: 22.1%, Factor vW:CB: 25%, Ratio FVIII:Cro/FvW:Ag 2.2, Ratio FvW:CB/FvW:Ag 1, Ratio FvW:Rco/FvW:Ag: 0.9). Platelet aggregation: normal. With this information, a diagnosis of mild type I von Willebrand's disease was made. The family study confirmed the presence of the disease in the patient's mother and twin sister.
It was decided to maintain anticoagulant treatment for 6 months longer, when another cerebral venous NMR was done, indicating complete canalization of all the venous system.
Summary/Conclusion: To reach a diagnosis of this pathology after a study of thrombophilia motivated by a thrombotic event is remarkable and demonstrates the clinical heterogeneity of this disease. Another aspect of this case that is unusual is that the patient should be a girl of less than 10 years of age who suffered a thrombotic event in the brain with a background of local infection, although having underlying vWD. This contrasts with the cases described, since these thrombotic events tend to present in vWD patients who reach middle or old age with additional risk factors for thrombosis.

Platelets
ECTH-472

Board No. 181: The involvement of alpha 2B beta3 integrin on the release of microvesicles and the chemokines CXCL4 and CCL5 by platelets

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Background: Platelets play a significant role in hemostasis, however platelets receive increasingly more recognition as inflammatory cells. Platelets release microvesicles upon activation as well as spontaneously during prolonged storage. Platelets represent the main source of microvesicles and they have been recognized to be involved in inflammatory diseases. Stimulation of platelets result in a transition of αIIbβ3 integrin to the activated form, which then can bind to fibrinogen or von Willebrand factor. Binding to this integrin may subsequently result in outside-in signaling causing spreading or aggregation of platelets. Previous studies have shown that αIIbβ3 integrin is also involved in microvesicle release. Besides the release of microvesicles, platelets are also a rich source of chemokines i.e. platelet factor 4 (CXCL4) and RANTES (CCL5) which are involved in the inflammatory recruitment of leukocytes. The exact mechanism behind the involvement of αIIbβ3 integrin in the release of microvesicles and chemokines is currently unknown.
Aims: We aim to identify the mechanism underlying the release of microvesicles and of the chemokines CXCL4 and CCL5 by activated platelets and the involvement of integrin αIIbβ3 in this process.
Methods: Platelets were isolated from healthy volunteers and activated with the agonists convulxin, TRAP-6 and ionomycin. The release of microvesicles and chemokines (CXCL4 and CCL5) was determined by a prothrombinase based assay and by ELISA, respectively. The involvement of αIIbβ3 was investigated using the inhibitor integrilin.
Results: Convulxin- and ionomycin-activated platelets increased the release of microvesicles compared to non-activated platelets. Chemokine CXCL4 and CCL5, levels were increased upon convulxin, TRAP-6 and ionomycin platelet activation. Pretreatment with the αIIbβ3 inhibitor integrilin reduced the level of microvesicle release triggered by convulxin. However, integrilin did not affect the release of microvesicles by ionomycin-triggered platelets. Remarkably, integrilin decreased CCL5 release by convulxin-triggered platelets but not upon activation with TRAP-6 or ionomycin. Interestingly, CXCL4 release was not affected by integrilin pretreatment of platelets.
Summary/Conclusion: Platelet stimulation by TRAP-6 did not increase microvesicle release by platelets, however the release of chemokines CXCL4 and CCL5 was induced. Inhibition of αIIbβ3 integrin resulted in decreased microvesicle and CCL5 release but did not influence the release of CXCL4. These data conclude that the αIIbβ3 integrin is involved in the release of microvesicles and the chemokine CCL5 and that release of CXCL4 does not depend on the actions of αIIbβ3 integrin.

Platelets
ECTH-321

Board No. 182: Computational systems biology analysis of platelet CLEC2 signaling

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Background: CLEC2 is a recently discovered platelet receptor for podoplanin, a cell surface ligand known to be exposed on tumor cells. Podoplanin-CLEC2 interaction is also important during ontogenesis. It is possible that podoplanin-induced platelet activation has its share in tumor-associated thrombophilia; CLEC2-mediated platelet activation leading to trombocytopenia, consumptive coagulopathy and purpura are associated with Kasabach-Merritt Phenomenon.
Aims: Development of a comprehensive computational systems biology model of CLEC2-mediated platelet activation to investigate its regulation mechanisms and identify possible pharmacological targets.
Methods: The model describes behavior of CLEC2, phosphorylated CLEC2 and its complex with Syk kinase in platelet membrane and lipid raft. Reactions involving Src, Btk and PI3K kinases are also included. The final activation event incorporated in the model is PLC-gamma-mediated rise in cytosolic calcium. The final set of ODE was integrated by LSODA method in COPASI software (COPASI.org).
Results: The constructed model described well experimental data of platelets activation and cytosolic calcium rise in platelet suspensions from (*Jillian L. Astarita et al.,* Frontiers in Immunology, vol. 3, Article 238). Interestingly, the model predicted that CLEC2-dependent signaling pathway can induce intracellular calcium oscillations in individual platelets. The experimentally observed lag times of CLEC2-mediated platelet activation could be explained by the time required for the receptor-ligand complex diffusion into the lipid raft. Addition of a PI3K inhibitor in the model abolished platelet CLEC2-medited activation completely, while a Btk kinase inhibitor ibrutinib was predicted to decrease the amplitude of platelet activation two-fold.
Summary/Conclusion: A first comprehensive multicompartmental model of platelet CLEC2 signaling is developed and validated leading to generation of several predictions for basic research and pharmacology.
The study was supported by RFBR grants 15-51-15008, 14-04-00670, 15-34-70009.

Platelets
ECTH-419
Board No. 183: Different modulation of neutrophil activities by platelets
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Background: During inflammatory reactions, neutrophils are one of the first cells of the immune system recruited at the reaction site. All steps of neutrophil activation - mobilization, infiltration, effector functions- need to be finely tuned to ensure optimal effectiveness while limiting collateral tissue damage. In various models of inflammation, platelets have been shown to promote neutrophil recruitment, to modulate neutrophil histotoxic activites, and to repair vascular damage associated with neutrophil trafficking. Depending on the experimental model, platelets were shown to either stimulate or inhibit neutrophil histotoxic activities. However, the basic mechanisms governing this regulation are still poorly understood.
Aims: We investigated the determinants of the platelet action towards neutrophil histotoxic activities.
Methods: In a first set of experiments, isolated human neutrophils were incubated with washed platelets. These experiments were performed in the absence or presence of TNF-alpha and with or without culture insert to prevent contact between the two cell types. In some experiments collagen or thrombin was added to activate platelets. In a second time, the same experiments were performed in the presence of endothelial cells (EC) cultured on cell culture inserts. Neutrophil activation markers such as elastase, myeloperoxidase activity and ROS production were measured in each experiment. In vivo, immune-complex (IC) mediated inflammation was elicited by i.v injection of BSA combined with either i.v or i.p injection of anti-BSA IgG in wild-type or thrombocytopenic mice.

Results: In vitro, platelets co-incubated with control or TNF-alpha-stimulated neutrophils reduced neutrophil elastase and myeloperoxydase activity. This inhibition was not modified by the presence of platelet agonists and was abolished when the contact between platelets and neutrophils was prevented. In the presence of EC cultured, the same inhibitory effects were observed except when neutrophils were pre-incubated with EC prior to platelet addition. In vivo, in a model of IC-mediated inflammation, we observed that platelets exerted opposite effects towards neutrophil histotoxic activities depending on whether the reaction was induced systemically in the bloodstream or locally, in the peritoneal cavity.
Summary/Conclusion: Together, our preliminary results suggest that platelets inhibit the histotoxic activities of unstimulated and stimulated neutrophils through contact-dependent mechanisms unless neutrophils are engaged and primed by interactions with EC. Thus, our results designate neutrophil/EC interactions as a crucial determinant of the stimulatory effect of platelets on neutrophil histotoxic activities.

Platelets

ECTH-436

Board No. 184: he importance of reticulated platelets and the platelet receptor CD61 in laboratory haematology

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Background: Reticulated platelets are the youngest form of circulating platelets characterized by a residual amount of RNA. It has been suggested that the reticulated platelet count, providing an estimate of thrombopoiesis. The platelet receptor CD61 is a receptor for fibrinogen, fibronectin, prothrombin, thrombospondin, vitronectin and von Willebrand factor. Flow-cytometric immunological determination of platelets number is proposed by the International Council for Standardization in Hematology (ICSH) as reference. The method is developed using monoclonal antibodies against platelets membrane antigens. The antibody directed against platelet receptor CD61 is the most used.

Aims: To determine significance of reticular platelets and platelet receptor CD61 testing in laboratorical diagnosis of platelets diseases.

Methods: A group of patients with thrombocytopenia in the Clinical Center of Serbia is examined. The experimental group 1 consisted of 28 patients (12 men and 16 women) with chronic thrombocytopenia, which are divided into two subgroups: with hypoplastic thrombocytopenia (12 patients) and destructive thrombocytopenia with hyperactive platelet lineage (16 patients).

The experimental group 2 consisted of 30 patients (18 men, 12 women) with acute thrombocytopenia with platelets less than 50 x 10^9/l, which are divided into two subgroups: with hypoplastic thrombocytopenia (18 patients) and destructive thrombocytopenia with hyperactive platelet lineage (12 patients).

The control group consisted of 20 volunteers (9 men and 11 women), in which the platelet parameters (the number and medium platelets volume - MPV) were within the normal limits and therefore they did not take any treatment.

The blood samples are collected in aliquots of 3 ml by venipuncture in Becton Dickinson's vacuum tubes with K-2 EDTA anticoagulant. The number and medium platelets' volume - MPV (are determined by specific optical method) and the percentage of reticular platelets - rP% (is determined by flow-cytometric method) which are examined by hematological parameters. In the second part of the test, platelets' counting using monoclonal antibodies is directed to platelet receptor CD61 analyzer "Cell Dyn Sapphire" (Abbott Diagnostics, USA) is added.

Results: There was a statistically significant difference between the percentage of reticular platelets between the control group and the patients with thrombocytopenia (13,31% against 1,78%). There was also a statistically significant difference against between reticular platelets from the patients with destructive thrombocytopenia (18,78%) and hypoplastic thrombocytopenia (6,02%). There was no statistically significant difference (P = 0.9431) between the number and and medium platelet volume (MPV) between levels of platelets and platelet receptor CD61, which is determined by PLTo method. The platelets values are determined by specific PLTi method which is statisticaly higher than the PLTo value (P = 0.0071) and platelet receptor CD61 values (P = 0.0049).

Summary/Conclusion: Differential diagnostic methods are limited and the biopsy of bone marrow is considered as the "gold standard" for diagnosis of thrombocytopenia. Flow-cytometric analysis of reticulted platelets are additional, non-invasive diagnostic method. The task of biochemical laboratory is to encourage this type of test, in order to obtain more complete information of the diagnosis, treatment and thrombocytopenia' prognosis.

Platelets

ECTH-500

Board No. 185: Falls of platelets hemostatic activity in stored platelet concentrate

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Background: Apheresis and storage of platelet concentrates (PCs) affected by the platelets activation and total functional capacity of these cells. We assume that after transfusion the prevalence of platelets with changed activity lead to worse quality of blood clot in vivo.

Aims: The aim was the in vitro study of platelet-dependent clot properties as a function of storage time.

Methods: Fifty single-donor apheresis PCs were divided in two groups: group 1 - platelets were remained in autologous plasma (PCs-P; n=26); group 2 – platelets were resuspended in PAS (SSP+; Macopharma, France) which substituted up to 70 vol% of autoplasma (PCs-PAS; n=24). Storage conditions were equal. PCs samples were analyzed by modified thromboelastography, and by aggregometry, and for platelets count, pH, lactate, glucose, and other platelets parameters. The testing were carried out in the day of proceeding, after 24 hours, and at 3rd and 5th days of storage. Dates were present as median (95% CI). Statistical differences were calculated using Mann-Whitney test ($p<0,05$), besides regression analysis was performed. We used MedCalc ver. 14.8.1 (MedCalc Software, Belgium) for statistical analysis.

Results: Between PCs-P and PCs-PAS no significantly differences had for platelets count. From the day of producing to the 5th days of storage glucose decreased in PCs-P from 18,3 mmol/L to 9,4 mmol/L (-48.6%), and in PSc-PAS from 5,2 mmol/L to 1,3 mmol/L (-52%), and lactate concentration had the increase in PCs-P from 2,7 mmol/L to 16,4 mmol/L (6-fold up), and in PCs-PAS from 1,4 mmol/L to 9,6 mmol/L (6,9-fold up). However pH was almost unchanged that indicated buffer conditions were good in both types of PCs. During the storage platelets aggregability and adhesion had worsened independently PCs type. Platelet aggregation decreased in PCs-P from the day of producing to the 5th days of storage ADP-induced by 44%, collagen-induced by 29,5%, ristomycin-induced by 40,4%. In PCs-PAS platelet aggregation decrease in response to ADP, collagen, ristomycin was 44%, 30%, 26%, respectively. We found that clot demonstrated gradual reduction of elasticity and deformability in both PCs groups (in PCs-P: Angle -30%, MA -9%, G -24%; in PCs-PAS: Angle -19%, MA -13%, G -29%). According to regression analysis in PCs-P platelets lost their meaning for clot properties from the third storage day, in PCs-PAS activated platelets had no impact to clot properties during full storage time.

Summary/Conclusion: Irrespective of the proceeding method platelets viability was saved during the first five days of storage. Platelets apheresis and storage are accompanied by aggregation-and- adhesion activity depression. It could be speculated that storage impairs platelets granules secretion and thromboxane A2 synthesis, and cell-cell interaction. We found total decline of clot quality including low elasticity and impaired deformability during of storage time. We assume that clot properties are forming at the day of proceeding. Therefore we suppose that effect PCs transfusion is related to successful of platelets activity recovery in vivo.

Platelets

ECTH-195

Board No. 186: Hypersensitivity reactions to enoxaparin and unfractionated heparin in pregnant woman with gestational thrombocytopenia

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Background: The risk of venous thromboembolism (VTE) and arterial thrombosis (stroke and heart attack) increases in women during pregnancy. Enoxaparin (a low molecular weight heparin) and unfractionated heparin are extensively used in the VTE prophylaxis and therapeutic doses.

Aims: We retrospectively describe an apparent relationship between enoxaparin and unfractionated heparin (UFH) in a woman with gestational thrombocytopenia developing a hypersensitivity reaction

Methods: we report a clinical scenario of a 43 years old pregnant women (gravida 18, para 12 and living 6) with co-morbid obesity (BMI 47.9) and well known to have a gestational thrombocytopenia during the last two pregnancies. She was administered enoxaparin 0.4ml (40 IU) daily for VTE prophylaxis. After three days, the patient came with hypersensitivity reactions like erythema, pain, swelling, irritation at the site of injection and rash all over the body (lower limbs, abdomen and neck) appeared after the first dose of enoxaparin. Hypersensitivity skin reaction to enoxaparin was suspected and immediately drug was withdrawn. The performed UFH skin test was negative, and then she was prescribed UFH 5000 units subcutaneous every 12 hours. The patient had similar reaction but a severe form and UFH was discontinued.

Results: Fondaparinux was the only nonheparin anticoagulant available and was prescribed for this patient

Summary/Conclusion: The patient had an allergic drug reaction and provides an evidence of cross-allergenicity between enoxaparin and UFH. Nonheparin anticoagulant improves both rashes and gestational thrombocytopenia

Platelets
ECTH-450
Board No. 187: Gestational thrombocytopenia and platelet transfusions
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Background: Thrombocytopenia occurs in 7-8%of all pregnancies.Women diagnosed with platelet disorders during pregnancy are detected by screening of automated laboratory analysis of blood counts.Thrombocytopenia can result from a wide range of disorders and several of them are related to the pregnancy.
Aims: To review the use of concentrated platelet transfusions at gestational thrombocytopenia of pregnant women hospitalized in the Clinic of Gynaecology and Obstetrics "Narodni front"".
Methods: The study included 100 pregnant women and mothers (25-40 years old), who were according to the degree of thrombocytopenia divided into three groups:
Group 1-mild thrombocytopenia with a platelet count 100-150x10^9/l
Group 2-moderate to severe thrombocytopenia with a platelet count 50-100x10^9/l
Group 3-severe thrombocytopenia with a platelet count below 50x10^9/l.
Results: During the period of several months in 2015 (March-December), 100 pregnant women diagnosed with gestational thrombocytopenia were followed.Platelet count was determined by an automatic hematology analyzer confirmed by a manual method with an ammonium oxalate counting chamber.The lowest value of the number of platelets in research was 9x10^9/l.The highest value was 116x10^9/l.During pregnancy and after delivery the platelet count was determined at least four times.In the research 10 of 100 pregnant women were tested by the platelet multiple function analyzer and results were regular.In 13 0f 100 pregnant women during and after cesarean section, there was an indication of the use of concentrated platelets.Normalization of the platelet count was observed 48 hours after cesarean section or vaginal delivery.
Summary/Conclusion: Gestational thrombocytopenia may recur in the next pregnancy.The risk of recurrance is unknown.In gestational thrombocytopenia a periodic monitoring of platelet count is very important.Therapeutic treatment is not usually necessary and platelet transfusion is justified only in cases of severe bleeding and life-threatening.Invasive approaches of a fetal monitoring in pregnant women with gestational thrombocytopenia is not recommended.

Platelets

ECTH-173

Board No. 188: Wiskott-Aldrich syndrome: description of two new cases masquerading primary immune thrombocytopenia with divergent platelet size

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Background: Primary immune thrombocytopenia (ITP) is the most common cause of thrombocytopenia in childhood. Its diagnosis is one of exclusion and other disorders, in particular inherited thrombocytopenias (ITs), are often mistaken for ITP. A cause of ITs in boys from which ITP must be differentiated is Wiskott-Aldrich syndrome (WAS). Classically, WAS is distinguished from other forms of thrombocytopenia by virtue of small platelet size.

Aims: The goal of this study was to establish the accurate diagnosis of WAS in 2 cases with different clinical, laboratory findings, and severity, that had been initially diagnosed and treated as ITP.

Methods: The severe form of WAS is represented by one child who at 2 years of age, presented with petechiae and bruising. Platelet counts were $4.1x10^9$/L and MPV showed to be increased (20.5fl). Peripheral blood smear showed giant platelets and bone marrow aspirate evidenced megakaryocytic thrombopoiesis. A diagnosis of ITP was established, and the patient showed no response to 6 different lines of ITP treatment. The milder case of WAS is represented by a 4-year old boy who exhibited fluctuating platelet counts with decreased size (usually $40\text{-}80x10^9$/L, 7.9 fl) since birth, but during viral infections platelets tended to decrease ($<20x10^9$/L), suffering from petechiae and epistaxis. Bone marrow examination suggested peripheral thrombocytopenia. Administration of corticosteroids and IVIG for a diagnosis of ITP resulted in a moderate 2-3 fold increase in platelet counts.

Results: We have characterized two patients on opposite ends of the WAS clinical spectrum, both previously misdiagnosed as ITP. DNA was analyzed by a panel of 71 candidates genes related to Inherited Platelet Disorders (IlluminaMiSeq). The first patient bears a novel hemizygous 1 bp deletion (p.Arg268Glyfs*40) in *WAS* and represents a classical form of WAS syndrome, exhibiting severe refractory thrombocytopenia but with increased MPV. Sequencing of parents' DNA confirmed that mother was a carrier. Flow cytometry (FCM) analysis revealed absent WAS protein (WASP) expression on T-cells from the patient and heterogeneous (bimodal) WASP expression on the mother. Four months after diagnosis he underwent hematopoietic progenitor cell transplantation from an unrelated donor. At present, the platelet count is in the normal range and donor engraftment is 100%. The second patient, carrying a splice site mutation (IVS6+5 g>a) in *WAS* and a rs145139708 (c.5323A>G; p.Lys1775Glu) in *MYH9* represents a milder form of WAS (X-linked thrombocytopenia) displaying microcytic thrombocytopenia with thrombopoietic responses to ITP therapies. WASP expression in non-erythroid hematopoietic cell populations in the patient was decreased, while his mother exhibited a normal WASP intensity.

Summary/Conclusion: A high index suspicion of WAS or XLT is required for boys with chronic thrombocytopenia even in the setting of normal or increased sized platelets, including patients that show not only failure, but also partial responses to treatments that are effective for ITP. In WAS, it is expected that in the future knowledge of the actual molecular effect of the mutation will help to link genotype with both platelet volume and the consumptive process by macrophages.

Platelets

ECTH-509

Board No. 189: Decrease of aggregation measured by platelet impedance aggregometry (multiplate) in patients treated with dabigatran

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Background: Dabigatran is a direct oral anticoagulant (DOAC) that has been proved effective and safe in preventing thromboembolic events experienced by patients with non-valvular atrial fibrillation (AF). This drug acts directly inhibiting thrombin and particularly affects the intrinsic and the final coagulation pathways. Thrombin is a platelet agonist therefore dabigatran could be involved in altering the aggregation. However, it is not well known whether they modify the platelet function.

Aims: We conducted an initial study in our hospital to assess platelet aggregation in patients treated with dabigatran.

Methods: An observational study was conducted in 17 randomly selected patients who had started dabigatran in the last year in our center. The results were compared with 8 healthy platelet donors randomly selected from our database. Platelet aggregation was quantified in patients and controls using MEA (Multiplate, Roche Diagnostics, Switzerland). The indication and dosing of dabigatran was performed as clinically indicated. The main variables investigated were: aggregation time with four agonists: thrombin, ADP, collagen, ristocetin, APTT, INR addition, D Dimer, kidney and liver function and the usual demographic parameters. Statistical analysis were performed using SPSS 21.0 (SPSS Inc., Chicago, IL).

Results: All patients had AF as an indication for treatment with dabigatran. Dabigatran doses were 110 mg in 7 patients (41%) and 150 mg in 10 patients (58%). No patient was on any antiplatelet agent. Platelet aggregation induced by TRAP measured by MEA was not significantly different in patients compared to controls: 121 RI (94-127) vs. RI 122 (108-137) units ($p = 0.711$). Aggregation using collagen and ristocetin as agonists was significantly decreased compared to that of controls: RI 48 (32-63) vs. 83 RI (69-110) ($p = 0.05$) and 76 RI (71-32) vs. 120 RI (97-142) units ($p < 0.01$). There were no differences in the aggregation depending on the dose of dabigatran.

Summary/Conclusion: Despite the limitations of the study we observed a change in the aggregation of patients treated with dabigatran measured by MEA, compared with healthy controls. Contrary to what one might expect, it was not dependent on the thrombin pathway unless the ristocetin and collagen agonists. These findings must be confirmed in more powerful studies designed for this purpose because others studies, with the same characteristics than our work, have shown TRAP-induced platelet aggregation is enhanced in patients treated with dabigatran.[1]

1 TRAP-induced platelet aggregation is enhanced in cardiovascular patients receiving dabigatran. Christoph B. Olivier et al. Thrombosis Research Thromb Res. 2016 Feb;138:63-8

Platelets

ECTH-206

Board No. 190: Does radiotherapy affect coagulation?

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Background: Solid evidence exists that cancer is associated with an increased risk of thromboembolic disease and the risk is further increased by chemotherapy. Thromboembolic disease further augments morbidity and mortality in cancer patients. Today, only sparse knowledge exists about the influence of radiotherapy on the thromboembolic risk in cancer patients.
How radiotherapy affects coagulation is difficult to investigate because coagulation is also influenced by the cancer itself and chemotherapy. In the present study we want to investigate platelet aggregation and more general coagulation factors in women receiving adjuvant radiotherapy for breast cancer after local excision and ending chemotherapy. This group of patients have few competing thrombosis risk factors, providing an opportunity to isolate the effects of radiotherapy on coagulation.
Aims: We aim to investigate platelet aggregation and coagulation acitivity during radiotherapy.
Methods: We plan to include 32 patients receiving adjuvant radiotherapy. All patients have completed surgery for breast cancer and finish their chemotherapy.
Blood samples will be obtained before and immediately after the first, the middle and the last radiotherapy treatment. The results will be compared with a self-controlling base-line-measurement in the assessment of coagulation function.
Platelet aggregation is measured by a Multiplate® Analyzer using adenosine diphosphate (ADP test, 6.5 µM), collagen (COL test, 3.2 µg/ml), arachidonic acid (ASPI test, 0.5 mM) and a thrombin receptor activator peptide (TRAP test, 32 µM) as agonists.
The coagulation will be analyzed through factor VIII, von Willebrand factor, thrombin generation, P-selectin, vascular endothelial growth factor, INR, APTT and fibrinogen.
Other analyzes are hemoglobin, leukocyte number, thrombocyte number, immature platelet count, immature platelet fraction, and C-reactive protein.
Results: Patients will be included from March 2016. The platelet aggregation measured before, during radiotherapy, will be ready for presentation at the congress.
Summary/Conclusion: The present study will establish if radiotherapy affects the platelet aggregation and the coagulation in general. If this can be shown, the study will give reason to further clinical studies investigating prophylactic antithrombotic treatment of cancer patients receiving radiotherapy.

Platelets
ECTH-414

Board No. 191: Investigating platelet functional heterogeneity using droplet microfluidics

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Background: Platelets have long since been known to be heterogeneous in size, volume and density. Functional heterogeneity has been suggested in several studies, however, there are no methods currently available that can study platelets on a single cell level. Research on a single cell level is needed to study pure intrinsic heterogeneity without the influence of adjacent cells. Such a method should provide a high throughput way of studying platelets in isolation, to be able to detect potentially rare phenotypes, without interfering with normal platelet function. To this effect, this study adapts a droplet microfluidics approach to study single platelet functionality.
Aims: This study aimed to develop a method suitable for the investigation of single platelet function.
Methods: The response of single platelets to an agonist is investigated by adding the agonist in excess during the encapsulation in droplets. After an incubation period within the droplets, the platelets are extracted from the droplets into fixative and followed by flow cytometry analysis. The PAC-1 and anti-CD62P antibodies are used to measure activation of platelets, while anti-CD42b is used separate platelets from noise. The outcomes of the droplet microfluidics are compared with a conventional flow cytometry set up to study the effects of paracrine signalling during activation and serve as a control. All platelets used in this study were donated by healthy volunteers who gave informed consent.
Results: Platelets are individually encapsulated in monodisperse (CV of 1-4%) water-in-oil droplets with a mean diameter of 30 µm. Droplets are produced with a throughput of 4 kHz, with droplets containing a single platelet produced at a rate of 0.25 kHz (following a Poisson distribution). No platelet activation is observed that originates from the flowing through the device, but can be observed with sufficiently high concentrations of convulxin. With this method an intrinsic variation in the platelet response to convulxin is observed, that is unrelated to the size of the platelet.
Summary/Conclusion: This study developed a method capable of studying purely intrinsic variation in platelet response to an agonist.

This study is kindly sponsored by the British Heart Foundation and a Marie Curie research fund.

Platelets

ECTH-349

Board No. 192: Quantitative mass spectrometry identifies alpha-granule release and alterations in membrane composition as early events during platelet storage

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Background: Platelet transfusions are widely used to manage bleeding in patients with thrombocytopenia or platelet dysfunction. Platelet concentrates are routinely stored at room temperature for up to 7 days under continuous agitation. Shelf life of platelet concentrates is limited due to the risk of bacterial contamination and the loss of platelet functionality during prolonged storage. It has been shown that during storage, platelets lose their shape, get activated and lose receptors crucial for platelet functionality, commonly referred to as the platelet storage lesion. So far, our knowledge on the molecular events contributing to the development of the platelet storage lesion is incomplete.

Aims: To evaluate early and late changes of platelets during storage, we monitored changes of the platelet proteome during standard platelet storage using quantitative mass spectrometry.

Methods: Platelet concentrates in plasma were stored under standard blood banking conditions for 16 days and samples were taken at day 1, 2, 5, 7, 9, 13 and 16. Using label free quantitative mass spectrometry, proteins levels were determined for each time point and proteins which changed significantly over time were assigned. GO term annotation and analysis was performed using CytoScape to study global changes during platelet storage. These findings were complemented by an extensive characterization of platelet function as well as metabolic profile and surface expression of the glycoprotein Ib-IX-V complex.

Results: A total number of 2501 proteins was identified in the samples analyzed. Limited changes in platelet proteome were observed between day 1 and day 2 of storage. On day 5, 7 and 9 more pronounced changes were observed; a decline in the level of alpha-granule proteins like von Willebrand Factor and SPARC, was observed at these time points. This observation provides evidence for spontaneous release of alpha-granules during platelet storage. Also changes in membrane composition were first identified at these time points. Distinct patterns were observed for the platelet proteome at day 13 and 16 of storage. A more sustained decline in levels of alpha-granules was observed at these time points. Interestingly, an increase in the level of alpha-2-macroglobulin, IgM and glycogenin-1 was observed suggesting that ageing platelets can recruit proteins from the circulation. Alpha-2-macroglobulin which was particularly abundant at day 16. Western blot analysis confirmed that the alpha-2-macroglobulin content of platelets increased upon storage.

Summary/Conclusion: Overall, our findings highlight changes in the platelet proteome during storage. Previous studies have suggested that platelet functionality is maintained for up to 5-7 days of storage. Our data suggests that development of the platelet storage lesion precedes loss of platelet function and is primarily linked to sustained release of platelet granules.

Platelets

ECTH-514

Board No. 193: Exposure smartphone call is led to abolition adp-induced platelet aggregation in vitro in mpn patients, but not in healthy volunteers.

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Background: Significant concerns are raised about the safety of excessive mobile phone use are actual. Significant effects of 30 minutes of exposure to a smartphone call on in vitro platelet function in recently publication has been shown (G. Lippi e.a., Blood Transfus DOI 10.2450/2016.0327-15). Investigators were using the PFA-100 and prolongation of collagen-epinephrine aggregation but not collagen-adp aggregation in healthy volunteers has been registred.

Aims: Reproduction effect smartphone call when it exposed in vitro on blood samples of healthy and patients with myeloproliferative neoplasms (MPN) using impedance aggregometry in whole blood.

Methods: Two sequential citrated blood samples were collected from 4 healthy volunteers and 12 MPN pateints. The first sample was placed in a plastic rack, 1 cm distant from a commercial smartphone receiving a 30-min call and emitting 900 MHz radiofrequency waves. The second sample was placed in another plastic rack, isolated from radiofrequency wave sources, for the same period. Platelet aggregation was evaluated using the impedance analyser Chronolog-700.

Results: A 30-min exposure of smartphone radiofrequency waves led to full abolish adp- platelet aggregation in 10 MPN pateints but not in healthy volunteers.

Any blood cells count and mean platelet volume remained unchanged in both group.

Summary/Conclusion: This study demonstrates that smartphone radiofrequency waves induce perturbation of platelet function preferably in MPN patients. The detection method and inducers of aggregation are influence to the measurement of expression of the smartphone call effect . The mechanism of this action requires study. Possible concerns are also associated with the distortion of the test results if laboratory staff used of their smartphones.

Platelets

ECTH-311

Board No. 194: Real-world on-treatment platelet reactivity in the era of new ADP-receptor blockers

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Background: New ADP receptor blockers (ADPRB), such as prasugrel and ticagrelor, provide more potent and more consistent platelet inhibition compared to clopidogrel. Nevertheless, there are several reports about high on-treatment platelet reactivity (HTPR) in prasugrel- and ticagrelor-treated patients.
Aims: To evaluate the prevalence of HTPR among acute ST-elevation myocardial infarction (STEMI) patients treated with new ADPRB in real-world settings.
Methods: A single-centre prospective study enrolling 38 acute previously ADPRB naive STEMI patients (25 men, 13 women) undergoing primary percutaneous coronary intervention (pPCI) was performed. Among the studied population 23 patients received prasugrel and 15 patients received ticagrelor. Antiplatelet response was tested with light transmission aggregometry (LTA) and vasodilator-stimulated phosphoprotein phosphorylation (VASP-P) flow cytometry assay. Samples were taken prior to coronary angiography (sample 1) and on the day after this procedure (sample 2).
Results: The mean ADP-induced platelet aggregation was 50.8 ± 25.5% in sample 1 and 26.2 ± 21.7% in sample 2. VASP-P showed a mean platelet reactivity index of 56.4 ± 26.8% in sample 1 and 25.5 ± 25.3% in sample 2, respectively. The study identified 13.2% of patients in sample 2 as ADPRB non-responders. No significant differences were found between prasugrel-treated to ticagrelor-treated patients.
Summary/Conclusion: This preliminary study demonstrated HTPR among acute STEMI patients treated with newer ADPRB.

Acknowledgments: This study was supported by the APVV project (Slovak Research and Development Agency) 0222-11 and by research project 2012-2015 of the Slovak Society of Cardiology.

Platelets

ECTH-391

Board No. 195: Predictive factors associated with long-term effects of splenectomy for idiopathic thrombocytopenic purpura

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Background: Splenectomy leads to a good response in 60-80% of adult patients with corticosteroid refractory idiopathic thrombocytopenic purpura (ITP). However, data on the long-term efficacy are controversial. Furthermore, it is not clear whether there are pre or post-operative parameters able to predict the therapy failure.
Aims: To evalute long-term efficacy and to determine the preoperative and postoperative factors that predict a successful splenectomy outcome.
Methods: We retrospectively analyzed the data on 120 patients (median age 43 years, range 19-66; 82/38 female/male ratio; median follow-up from diagnosis 124 months, range 26-321 median follow-up from splenectomy 34 months, range 12-1312), who underwent splenectomy (laparotomic 76, laparascopic 44) for ITP. Platelet kinetic study with Indium-111 was performed in 40 patients before splenectomy. Patients with platelet count <30×109/L or steroid-dependent were splenectomized. Complete response (CR) and partial response (PR) were defined as platelet count >150×109/L and 50×109/L one month after surgery. Refractoriness was diagnosed when the platelet count remained <30×109/L. Relapse was defined as a drop in the platelet count <150×109/L or 50×109/L after CR or PR. Refractory or relapsed patients with a platelet count <30×109/L or bleeding were treated with steroids, azathioprine, danazol, vinca alkaloids and TPO-agonist.
Results: The median time from diagnosis to splenectomy was 6 months (range 2-132). The median pre-operative platelet count was 99×109/L (range 20-320×109/L). 95 of 93 patients (79%) achieved CR and 10/120 (8.5%) PR. Remaining 15/120 (12.5%) were refractory. All refractory patients were treated with good response in 8/15 (53%) cases. Fifteen of the 105 (15%) responsive patients relapsed with a median time to relapse of 6 months (range 3-168). Eleven of these 15 patients (73%) were treated with a good response in 9/11 (82%) cases.
Patients with good response (CR+PR) to splenectomy were previously treated with only one drug (79% vs.43% p=0.003) or had lower median number of pre-splenectomy therapies (1 (range 1-3) vs. 2 (range 1-5), p=0.028), had a higher platelet count at the time of splenectomy (91×109/L vs. 45×109/L, p=0.021) and higher platelet count seven days after splenectomy (425×259/L vs. 25×109/L, p=0.0032) than refractory patients. Mixed (the liver and the spleen) platelet destruction site was frequent in refractory patients (4/4 (100%) vs. 5/39 (13%) p=0.0025). By multivariate analysis, only the number of former therapies (p=0.045) and higher peak post-splenectomy platelet count (p=0.0089) were predictive of a favorable response to splenectomy.
Relapsed patients had significantly lower platelet count seven days (213×109/L vs 387×109/L, p=0.048) and three months (55×109/L vs 346×109/L, p=0.007) after splenectomy. The same parameters retained statistical significance (p=0.026, p=0.0013) by multivariate analysis.

Summary/Conclusion: Splenectomy is effective in approximately two thirds of patients with ITP. Number of pre-splenectomy treatments and the platelet count seven days after surgery were predictive of refractoriness. Platelet count seven days and three months after surgery was predictive of relapse. Further large population based studies for testing a cut-off platelet count that could predict the refractoriness or relapse are needed.

Platelets
ECTH-410
Board No. 196: Statins modulate platelet function
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Background: Statins are potent inhibitors of HMGCoA reductase in the mevalonate pathway that is necessary for cholesterol synthesis. Inhibition of this pathway by statins also impairs prenylation of proteins by depleting cells of the lipid geranylgeranyl diphosphate (GGPP). Prenylation involves post-translational modification of a protein by addition of a lipid tag GGPP, enabling their association with target membranes. One such protein is Rab27b, a GTPase involved in regulating the secretion of platelet dense granules. Dense granules contain molecules, such as ADP, serotonin and polyphosphate, which mediate platelet aggregation and coagulation.
Aims: Here we investigate whether statins directly interfere with prenylation of Rab27b in platelets and whether they modulate platelet function.
Methods: Coagulation time, clot formation time and clot firmness were analysed by thromboelastography in whole blood pre-treated with atorvastatin (ATV) (0, 10, 20, 40 μM) for 24 h. Washed platelets were prepared from fresh blood obtained from healthy donors or were kindly provided by the Scottish National Blood Transfusion service. Light transmission aggregometry was used to investigate platelet aggregation in platelets pre-treated with ATV in response to 0.125 U/ml thrombin. Dense granule secretion was analysed using an ADP release assay. Fibrinogen binding to thrombin stimulated platelets and P-selectin (CD62P) were analysed by flow cytometry. Unprenylated Rab27b was detected in platelet lysates by Western blot.
Results: Incubation of whole blood with increasing concentrations of ATV for 24 h significantly increased the coagulation time at 40 μM ($p<0.005$) , and clot formation time at 20 and 40 μM ($p<0.005$) and ($p<0.001$) respectively. Clot firmness was also significantly reduced at 20 mM (39.6 ± 3.91 mm) ($p<0.005$) and at 40 μM (21.4 ± 4.39 mm) ($p<0.001$) compared to in the absence of statins (51.8 ± 3.83 mm). The effect on clot firmness indicates platelet dysfunction and/or a change in fibrinogen concentration and function; the latter was ruled out using a Fibtem test. Pre-treatment of washed platelets with ATV for 24 h significantly reduced binding of exogenous fibrinogen to thrombin stimulated platelets ($p<0.05$) as quantified by flow cytometry. ATV also impeded α-granules release in thrombin activated platelets ($p<0.05$), visualised as impaired accumulation of P-selectin on the activated membrane. Defective clot retraction was observed in platelet-rich plasma clots pre-treated with 20 mM and 40 mM ATV, consistent with the reduction in fibrinogen binding. Furthermore, treatment of platelets for 24 h with ATV induced a dose-dependent accumulation of unprenylated Rab27b in the aqueous fractions by Western blotting. ATV (1-10 μM) significantly inhibited thrombin-induced platelet aggregation ($p<0.005$) and ADP release ($p<0.005$). Addition of GGPP rescued prenylation of Rab27b and restored ADP release in platelets treated with ATV, but surprisingly did not rescue the effect of ATV on platelet aggregation.
Summary/Conclusion: Statins modulate a number of platelet functions including fibrinogen binding, clot retraction, a-granule and dense granule release and platelet aggregation *in vitro*, suggesting that these drugs may directly interfere with platelet function *in vivo*. Attenuated dense granule release is most likely accounted for by inhibition of Rab27b prenylation as this can be rescued by addition of GGPP.

Platelets

ECTH-235

Board No. 197: Protein arginine deiminase 4 (PAD4) is not required for effective immunity in gram-negative pneumonia derived sepsis

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Background: Neutrophil extracellular traps (NETs) have been identified as a function of neutrophils to capture and kill bacteria. Protein arginine deiminase 4 (PAD4) is important for NETs formation. Several studies have investigated the contribution of NETs and PAD4 in host defense against bacteria using different methods, with contradictory results.
Aims: We aimed to investigate the contribution of NETs formation and PAD4 to the host response during pneumonia derived sepsis.
Methods: Patients on the Intensive care unit with either pneumosepsis or non-infectious critical illness without lung pathology were subjected to a non-directed bronchoalveolar lavage. Lavage fluid was assessed for formation of NETs by citrullinated Histon 3 (CitH3) western blots. Human isolated neutrophils were stimulated with phorbol myristate acetate (PMA), *Klebsiella (K.) pneumoniae*, *Pseudomonas(P.) aeruginosa* or Lipopolysaccharide (LPS) and NET formation in supernatants was assessed by CitH3 western blots. PAD4 deficient and wild-type mice were infected with the common human sepsis pathogens *K. pneumoniae* and *P. aeruginosa* via the airways.

Results: Patients with pneumosepsis, but not patients with non-infectious disease, showed NET formation in the airways. *K. pneumoniae*, *P. aeruginosa* and LPS all induced NET formation by human neutrophils, albeit to a lesser extent as PMA. PAD4 deficient and wild-type mice had similar levels of bacterial outgrowth during respiratory tract infection with *K. pneumoniae* and *P. aeruginossa.* PAD4 deficiency reduced platelet activation (reduced P-selectin and CD63 expression), but did not prevent sepsis induced thrombocytopenia. PAD4 deficiency did not influence activation of coagulation, but was associated with increased endothelial cell activation.
Summary/Conclusion: These results suggest that NETs are present in the airways of critically ill pneumosepsis patients, and that this may be a direct effect of causative pathogens. However, PAD4 mediated NETs formation does not play a role in antibacterial defense during gram-negative pneumonia, suggesting that other immune functions are sufficient to maintain host defense when NET formation is inhibited.

Platelets
ECTH-260
Board No. 198: Evaluation of platelets inhibition pathways in newborns
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Background: Platelet reactivity is dependent on a complex balance of inhibitory and activatory pathways. Among inhibitory signals released from the endothelium, prostacyclin (PGI2) and nitric oxide (NO) are the most important. Prostaglandin E1 (PGE1) and sodium nitroprusside (SNP) are platelet inhibitors that stimulate cAMP and cGMP production, respectively. Cyclic nucleotides activate protein kinase A (PKA) and protein kinase C (PKC) to phosphorylate proteins such as VASP. Newborn platelets are hyporeactive to most platelet agonists. Despite this hyporeactivity, full-term neonates show no major bleeding disorders due to the presence of neonatal blood factors that may enhance platelet-vessel wall interactions (higher hematocrit, higher vWF levels and predominance of longer vWF polymers). To date, the involvement of inhibitory pathways in the hyporeactivity of the neonatal platelets remains to be clarified**.**
Aims: To evaluate cAMP/PKA and cGMP/PKC-dependent signaling in full-term newborn and adult platelets.
Methods: Washed platelets and platelet-rich plasma obtained from adult and umbilical cord blood (CB) (n≥9/group) were pre-incubated with increasing doses of SNP (0-1µM) or PGE1 (0-1µM) respectively, and we assessed: 1) Platelet aggregation with 25µM TRAP, 5µM ADP, 2.5µM U46619, 0.5U/mL thrombin and 5 µg/mL collagen; 2) P-Selectin expression by flow cytometry after stimulation with 25µM TRAP or 5µM ADP upon increasing PGE1 doses; 3) Quantification of PKC, PKA, and VASP protein levels and VASP phosphorylation using specific antibodies (Ser157-VASP and Ser239-VASP) by immunoblotting; 4) the cAMP levels by ELISA before and after platelets pre-incubation with increasing PGE1 doses (0-0.5 µM).
Results: Upon PGE1 incubation, the inhibition of ADP- and collagen-induced aggregation was significantly decreased in neonates (IC_{50}: 16 ± 7 *vs*. 142 ± 56 nM, $p<0.01$ for collagen; IC_{50}: 109 ± 60 *vs.* 424 ± 159 nM, $p<0.01$ for ADP) but not with TRAP. In case of SNP, only the inhibition of thrombin induced aggregation was lower in neonates (IC_{50}: 0.004 ± 0.002 *vs*. 0.18 ± 0.3 nM, p<0.004). However, the reduction of P-Selectin expression respect basal levels was similar in both groups. The expression levels of all tested proteins (VASP, PKG. PKA) were lower in neonatal than in adult platelets, reaching significant differences for PKG and PKA (64 ± 31%, $p<0.04$ for PKA; 44 ± 33 %, $p<0.05$ for PKG) but not for VASP. VASP phosphorylation at Ser239 and Ser157 was lower in neonates after the treatment with both platelet inhibitors, being the differences statistically significant after PGE1 treatment. Additionally, both groups showed an increase in cAMP levels upon PGE1 stimulation, in a dose-dependent manner. cAMP levels were not significantly different between neonates and adults, although net cAMP increase after inhibition was slightly lower in neonatal samples.
Summary/Conclusion: Our study reveals that neonatal platelets are hyporeactive towards both activator and inhibitor agents. Proteins of the inhibitory pathway (PKG/PKA) showed lower expression in neonates. Additionally, VASP phosphorylation and cAMP increase induced by platelet inhibitors tend to be lower in neonates. Overall, our results contribute to explain the proper neonatal hemostasis despite of their well known platelet hyporeactivity. Not only the medium, but also intrinsic platelet mechanisms balance this hyporeactivity.
ISCIII (PI14/01956) and CIBERER (CB15/00055)

Platelets
ECTH-192
Board No. 199: B1-tubulin differential expression may contribute to the reduced adhesive functionality of neonatal platelets
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Background: Neonatal platelets exhibit greater adhesive ability than adult platelets, as a consequence, among other factors, of higher levels of vWF multimers in the neonatal plasma. Recently it has been shown that, as compared to adult, neonatal platelets present longer PFA-100 occlusion times regardless of the circulating milieu (neonatal platelets depleted blood vs. adult), suggesting intrinsic differences in platelet adhesive capacity during development. β1-Tubulin is the main constituent of the marginal band and its partial deficiency, associated to the Q43P polymorphism, determines a decrease in adhesion, impaired platelet reactivity and defective secretion.
Aims: To evaluate possible differences in platelet adhesion throughout the development, and whether changes in the expression of β1-tubulin exist.
Methods: We conducted adhesion experiments on poly-L-lysine (PLL) at different times (0-30 min at intervals of 5 min) of washed platelets from adult peripheral blood (PB) or umbilical cord blood (UCB) (N=6/group). We evaluated the following parameters by fluorescence microscopy: a) percentage of area occupied by fluorescence (AOF, defined as the percentage of fluorescence with respect to the total area); b) mean platelet area (MPA, a measure of platelet spreading); c) number and phenotype of adhered platelets [non extended (0-15µm2); partially extended (15-40µm2) and totally extended (>40 µm2)]. Furthermore, levels of β1-tubulin mRNA (*TUBB1*) and protein from leukocyte-depleted PB or UCB platelets (N=15/group) were quantified.
Results: On initial seeding (0 min), AOF and MPA of spread platelets were similar in neonatal and adult-platelets. Over time, and at all time points thereafter (ranging from 5 to 30 min), both variables were reduced in neonatal compared with adult platelets. The differences became significant during the late-time period (20-30 min). Moreover, in this interval, the number of totally extended platelets was significantly lower in the neonatal group. In contrast, neonatal-platelets showed a larger percent of non extended platelets at the late-time period (15-30 min, $p<0.05$), compared to adult-platelets. In general, for all the evaluated times, the number of adhered platelets to PLL was lower in neonatal than in adult samples, although the differences were not statistically significant. Neonatal platelets displayed less *TUBB1* mRNA expression (41%, $p<0.01$) and β1-tubulin protein (53%, $p<0.01$) than adult platelets, and this difference was not due to the presence of the *TUBB1* Q43P SNP.

Summary/Conclusion: Our study demonstrated delayed and decreased adhesion and spreading of neonatal platelets compared with adult platelets. These findings are consistent with the previous observation that neonatal platelets display longer PFA-100 closure times than adult platelets, regardless of the whole blood milieu in which they are resuspended (neonatal vs. adult thrombocytopenic blood), and further support the concept of an intrinsically defective adhesive capacity of neonatal platelets.
FSENECA, ISCIII and CIBERER (19841/FPI/15, PI14/01956 and CB15/00055)

Vessel wall
ECTH-380
Board No. 200: Antiphospholipid antibodies activate vascular smooth muscle cells and increase thrombin generation
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Background: Activation of platelets, monocytes and endothelial cells antiphospholipid antibodies (aPL) has been proposed as one of the pathogenic mechanisms contributing to the antiphospholipid syndrome (APS).
Aims: We determined effects of aPL on characteristic functions of human vascular smooth muscle cells (hVSMCs).
Methods: Total IgG were purified from sera of 10 patients with APS by chromatography on protein G. Thrombin generation was monitored using calibrated automated thrombography (CAT) at the surface of adherent cultured normal hVSMCs incubated with IgG (250 µg/ml) from APS patients or healthy controls, and with or without activated protein C (APC). Proliferation of hVSMCs in response to stimulation by IgG from patients or controls was studied in medium without fetal bovine serum (FBS). Negatively-charged phospholipids were quantified in the cell supernatant. hVSMC microparticles (MPs) release was measured by flow cytometry in medium without FBS.
Results: Thrombin generation was markedly increased after incubation with IgG from all patients irrespective of the aPL concentrations in sera compared to control IgG or to medium without IgG. The increase ranged from 2-fold to 4-fold for triple positive patients (anti-β_2GPI antibodies, anticardiolipin and lupus anticoagulant). In addition, IgG from the majority of APS patients induced a resistance to APC. For all patients, procoagulant activity increase without FBS in parallel with increased apoptosis (75%) compared with unstimulated cells or cells stimulated with IgG control. IgG of all patients induced significant increase of MPs released from hVSMCs (from 200% to 300%).
Summary/Conclusion: The results show the activating effects of aPL on hVSMCs: thrombin generation increased and this hypercoagulability depends on aPL antibody profiles. The aPL-induced release of MPs and apoptotic cells induction may represent crucial mechanisms driving increased thrombin generation.

Vessel wall
ECTH-272
Board No. 201: Whole proteome analysis of Hermansky-Pudlak syndrome type 2 BOECs reveals depletion of the entire AP-3 complex
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Background: Weibel-Palade bodies (WPBs) play a crucial role in hemostasis, angiogenesis and wound healing, as being the secretory compartment of endothelial cells. They are categorized as lysosome-related organelles (LROs), and apart from von Willebrand factor (VWF), they also contain CD63 and P-selectin. CD63 stabilizes the leucocyte receptor P-selectin, enabling adhesion of leucocytes to the activated endothelium. Adaptor protein (AP) 3 complex has been identified to be the key regulator of CD63 trafficking on the WPBs membrane during their maturation. Hermansky- Pudlak syndrome type 2 (HPS-2), a rare genetic disorder, is the consequence of a mutation on *AP3B1* gene encoding for part of AP-3 complex (AP3β3). HPS-2 results in severe effects on the maturation of LROs, including the melanosomes and platelet dense granules, however, it has not been established whether the biogenesis or exocytosis of WPBs is impaired in patients with HPS-2.
Aims: The aim of this research is to further investigate the role of the AP-3 complex in the maturation of WPBs and examine how a genetic mutation in the AP3β3 subunit affects the functionality and exocytosis of WPBs.
Methods: We have isolated blood outgrowth endothelial cells (BOECs) from a homozygous HPS-2 patient. To examine possible differences of the full proteome of wild type (WT) and HPS-2 endothelial cells, we have employed mass spectrometry (MS) and the data were analyzed by means of MaxQuant and Perseus. The CRISPR/Cas9 system was used for generating AP3β3-deficient endothelial cells and the efficiency was tested by western blot. Finally, the morphology of WT and HPS-2 endothelial cells was analyzed by confocal microscopy.
Results: The overall morphology of HPS-2 BOECs was similar to that of control BOECs. Staining for VWF revealed the presence of elongated WPBs that were morphologically similar to that observed in WT BOECs. Full proteome analysis showed that the β3 subunit of the AP-3 complex was not present in HPS-2 BOECs. Significantly reduced levels of AP3δ1 and AP3μ1 that are both subunits of the AP-3 complex were observed in HPS-2 BOECs when compared to controls. No AP3σ1 was detected in either HPS-2 or WT BOECs. Western blot analysis confirmed the absence of AP3β3 in HPS-2 BOECs and also revealed that AP3μ1 was absent. Similar results were observed upon CRISPR/Cas9 mediated editing of the *AP3B1* gene (encoding AP3β3). In general, the proteomic profile of HPS-2 BOEC was similar to that of WT BOECs. Nonetheless, apart from AP3δ1 and AP3μ1 a number of other proteins were down-regulated in HPS-2 BOECs. These proteins are now under investigation as possible interacting partners of components of the AP-3 complex.
Summary/Conclusion: Taken together, we have established a model system to study the role of the AP-3 complex in endothelial cells that allows for the investigation of the impact of this complex on the functionality of WPBs.

Vessel wall

ECTH-498

Board No. 202: Modulation of endothelial activation by omega-3 (n-3) fatty acids in sickle cell disease

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Background: Vasocclusive crises are the devastating hallmark of Sickle Cell Disease. The paradigm shift in understanding this chronic inflammatory status revealed the central role of adhesiveness of Sickle reticulocytes and leukocytes in triggering crises. Omega-3(n-3) polyunsaturated fatty acids have well established anti-inflammatory and anti-adhesive roles. Interestingly, N-3atty acids are profoundly reduced in membranes of Sickle blood cells proportionally to the degree of anaemia.

Aims: This study aimed to investigate the potential anti-adhesive properties of N-3 fatty acids in ameliorating SCD crises. Endothelial adhesion was evaluated by measuring Vascular Adhesion Molecule (VCAM -1), which demonstarated singificant increment in SCD during both steady state and cirses and correlate with HB level inversely.

Methods: Thirty SCD patients (HB SS) were supplemented with two to three capsules of N-3 fatty acids (277.8 mg docosahexaenoic (DHA) and 39.0mg eicosapentaenoic (EPA)) according to age for one year. N-3 fatty acids-treated patients were matched by age, gender and socioeconomic status to placebo-supplemented SCD patients and Twenty-four healthy controls(HB AA) from their siblings. Serum levels of soluble VCAM-1 were analysed by Quantitative Indirect ELISA in duplicates.

Results: The ameliorative effect of N-3 fatty acids on frequency of vasocclusive crises and need for blood transfusion was re-observed. Haemoglobin level improved significantly by N-3 fatty acid supplemenation. Mann - Whitney U test showed significant reduction in the level of sVCAM-1 in SCD patients treated with N-3 fatty acid compared to unsupplemented patients with P value of 0.001. Summary/Conclusion: N-3 fatty acids are promising dietary modifiers of adhesiveness and infl

Summary/Conclusion: N-3 fatty acids are promising dietary modifiers of adhesiveness and inflammation in SCD. Further studies on gene expression and membrane levels of adhesion molecules to be carried out.

Vessel wall
ECTH-374
Board No. 203: SERPINE2 expression and function in neutrophils
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Background: Inflammation and coagulation play pivotal roles in the pathogenesis of vascular disease ranging from arterial disease to arthritis and severe septic infections. Increasing evidence points to extensive cross-talk between these two systems, whereby inflammation leads to activation of coagulation, and coagulation further augments inflammation. Thrombin, the end product of the coagulation cascade, is the main driver of this cross-talk. Thrombin activation and activity are tightly regulated by proteins belonging to the serpin superfamily (serine protease inhibitor).
Recently, **protease nexin-1** (**PN-1**, encoded by *SerpinE2*) has emerged as the most potent endogenous inhibitor of thrombin. PN-1 can regulate all stages of the host response to trauma, including clotting / coagulation, inflammation, cell migration and proliferation, and tissue remodelling. PN-1 is an inhibitory serpin barely detectable in plasma but expressed by vascular cells, platelets and inflammatory cells. However the potential role of PN1 in regulating inflammatory processes is not known. PN-1 has been detected in monocytes/macrophages, but no data are available concerning its expression and potential function in neutrophils.
Aims: That's why we investigated whether PN-1 is expressed by neutrophils and plays a role in their histotoxic functions.
Methods: Neutrophils are isolated from human blood or from Wild Type or PN-1 deficient mice bone marrow. They are activated by the addition of different neutrophils agonists (LPS or PMA). Following these incubations, expression of PN-1 is quantified and analysed by western blot and flow cytometry. The activity of myeloperoxidase (MPO) and the production of reactive oxygen species (ROS) by murin neutrophils were quantified. Vascular recruitment of neutrophils are analysed in vivo by intravitale microscopy in mesenteric vessel stimulated with LTB4 and in a model of LPS-induced peritonitis.
Results: We have demonstrated by immunoblot and flow cytometry the presence of PN-1 in human and murine neutrophils, and localized PN-1 in the different human neutrophil subcellular compartments (specific and azurophil granules, and cytosol).PN-1 is secreted after neutrophil activation by LPS and is detected on neutrophil surface.
Measurements of MPO activity and of the generation of ROS from mouse neutrophils isolated from PN-1-KO and WT bone marrow, showed that PN-1-deficiency leads to a significant decrease of neutrophil activities.
Neutrophil vascular recruitment induced by topical application of LTB4 on mesenteric veins was analysed by intravital microscopy. Neutrophil recruitment is much less important in PN-1-KO mice compared with WT. Neutrophil recruitment is also less important in irradiated WT mice grafted with bone marrow cells obtained from PN-1-KO mice than in irradiated WT mice grafted with WT cells. This lower neutrophil recruitment was also observed in the acute inflammatory response to intraperitoneal administration of LPS. Indeed, much less neutrophils were detected in the intraperitoneal lavages of PN-1-KO compared with lavages of WT mice.
Summary/Conclusion: Altogether, our data show that PN-1 is a serine protease inhibitor able to regulate positively neutrophil functions.

Vessel wall
ECTH-269
Board No. 204: The role of an endothelial specific G-protein coupled receptor in thrombosis
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Background: Venous thromboembolism (VTE) is the third most common cardiovascular disease and affects roughly 1 in 1000 individuals annually. The role of the endothelium in VTE is understudied. We recently identified an endothelial enriched orphan g-protein coupled receptor (GPCR) that is expressed throughout the adult human vasculature. In a genome wide association study of VTE patients 'MARTHA' we found an association between this gene locus and the development of VTE. Using expression quantitative trait loci analysis we found that this VTE associated SNP was linked to reduced transcription of the GPCR in human endothelial cells (EC).
Aims: The objective of this study was to investigate the possible role of this endothelial enriched orphan GPCR in VTE. Specifically, how this GPCR is regulated and if it modulates the expression of EC proteins important in the regulation of coagulation, cell signalling and blood clotting.
Methods: We used siRNA to deplete the GPCR in human umbilical vein endothelial cells (HUVEC). Effects of the siRNA-mediated depletion on the expression of a panel of EC coagulation and inflammation related proteins were assessed by real-time pcr, protein immunoblotting and quantitative mass spectrometry. We used calibrated automated thrombography and thromboelastrometry (ROTEM), specifically adapted to incorporate EC, to study the effects of GPCR depletion on thrombin generation in plasma and whole blood clotting.
Results: siRNA-mediated depletion of the orphan GPCR in HUVEC resulted in a significant increase in tissue factor (*F3*) transcription. The induction of *F3* expression by tumour necrosis factor treatment of HUVEC was also significantly amplified following depletion. Using a calibrated automated thrombogram assay we also observed increased thrombin generation in plasma following depletion, an effect that was abolished when an inhibitory antibody against *F3* was added.
Summary/Conclusion: Our results suggest that this GPCR could play a role in VTE development though the regulation of tissue factor expression.

Vessel wall

ECTH-381

Board No. 205: SMC integrin Avβ3 and thrombin generation in thoracic and abdominal aneurisms

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Background: The cause of aortic aneurisms is abdominal atherosclerosis (AAA) and thoracic non-inflammatory matrix (AAT). The role of smooth muscle cell (SMC) integrin $\alpha_v\beta_3$ in the generation of thrombin at their surface and in the differential development of the 2 types of aneurisms is not known.
Aims: To study thrombin generation *in vitro* and its dependence with respect to SMC integrin $\alpha_v\beta_3$ in AAA *vs* AAT at a late clinical stage in man.
Methods: SMC primary cell cultures seeded on different supports (collagen 1, fibronectin or nothing) were prepared from human biopsies of AAT (n=2) and AAA (n=5) as well as for healthy vessels (n=3). Thrombin generation and inhibition was monitored by thrombography in the presence of healthy plasma or plasmas deficient for coagulation factors synthesised by the SMCs. The effects of an integrin specific peptide inhibitor (cRGDpV) was also analysed.
Results: Proliferation of health and pathologic SMCs was maximum in the presence of collagen 1. Differentiation marker expression (smoothelin, SM-MHC and α actin) was lower in aneurism-derived SMCs compared to controls, with the lowest from AAA SMCs. AAT SMC α_v sub-unit expression was increased with respect to the 2 other groups.
Thrombin generation was increased at the surface of SMCs from the 2 types of aortic aneurisms at quantities required for procoagulant activities in the presence of normal plasma (between 10 and 100 nM). In the presence of prothrombin deficient plasma SMCs produced amounts of thrombin known to enhance cell proliferation and migration (in the order of nM). Deficiencies of factors VII or X supressed the response. The addition of the inhibitor peptide (cRGDpV) decreased by a factor of 2 thrombin generation in all conditions.
Summary/Conclusion: These results demonstrate aneurism SMC capacity to generate thrombin in the absence of exogenous prothrombin, at concentrations needed to exert proliferative effects. In the presence of plasma aneurism SMCs are more thrombogenic than control SMCs. These responses appear dependent on integrin $\alpha_v\beta_3$ and its environmental matrix. The thoracic or abdominal location appears to influence the SMC differentiation state and their procoagulant properties.

Vessel wall
ECTH-124
Board No. 206: Haemostatic changes occur in vivo already in the early non-hepatosplenic phase of schistosomiasis
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Background: Schistosomiasis, caused by parasites of the *Schistosoma* genus, is the second major parasitic disease after malaria. These blood-dwelling worms affect around 200 million individuals worldwide, with 500 million being at risk. Schistosomiasis is estimated to cause 280.000 deaths yearly in Sub-Saharan Africa alone. Haemostatic abnormalities are observed in the hepatosplenic phase of the disease, such as thrombocytopenia, increased von Willebrand Factor antigen (VWF:ag) levels, decreased levels of coagulation factors, and increased fibrinolysis. In contrast, in the non-hepatosplenic phase of the disease, which is the early phase of disease, platelet counts are normal. However, it is unclear whether other haemostatic changes observed in the hepatosplenic form of the disease are also absent in early schistosomiasis.
Aims: The aim of this study is to investigate the occurrence of haemostatic changes in patients suffering from the non-hepatosplenic phase of schistosomiasis haematobium.
Methods: Informed consent was obtained from all study participants included in the study. Citrate plasma was obtained from ten individuals with non-hepatosplenic schistosomiasis haematobium and four healthy controls recruited from the Lambaréné area in Gabon. *Schistosoma* urine egg count and circulating anodic antigen (CAA) levels were used to confirm infection. Levels of VWF:ag, active VWF, ADAMTS13 antigen (ADAMTS13:ag), osteoprotegerin (OPG), thrombin-antithrombin III (TAT), and D-dimers were measured in plasma with ELISA. ADAMTS13 activity was determined with FRETS-VWF73 substrate and ristocetin co-factor activity (VWF:RCo) was assessed with the BC VWF reagent.
Results: VWF:ag and active VWF levels were elevated in individuals with non-hepatosplenic schistosomiasis haematobium compared to healthy controls (p=0.002 and p=0.004, respectively). The percentage of active VWF was slightly lowered in patients compared to controls (p=0.024). No defects in the VWF degrading protease ADAMTS13 were observed, since ADAMTS13:ag and ADAMTS13 activity were normal in both patients and healthy controls. VWF:RCo was similar between patients and healthy controls and platelet counts were normal in all individuals. Increased VWF:ag levels could be caused by inflammation-mediated endothelial activation as OPG levels, a marker of inflammation-mediated endothelial activation, were elevated in patients versus healthy controls (p=0.036). TAT and D-dimer levels were similar between schistosomiasis patients and healthy individuals (p=0.4116 and p=0.7524, respectively), indicating that non-hepatosplenic schistosomiasis is not associated with a hypercoagulable state.
Summary/Conclusion: In plasma of patients in the early non-hepatosplenic phase of schistosomiasis haematobium VWF:ag, active VWF, and OPG levels are elevated, whereas levels of TAT, D-dimer, ADAMTS:13 ag, VWF:RCo and platelet counts are not affected. This indicates that already early in infection, inflammation-mediated endothelial activation occurs while clot formation and fibrin degradation do not occur yet.

Vessel wall
ECTH-289
Board No. 207: Platelet microvesicles induce a pro-inflammatory smooth muscle cell phenotype
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Background: Microvesicles are gathering increasing attention as mediators of cell communication and as integral effectors of disease. Platelets present a major source of microvesicles and release these microvesicles either spontaneously or upon activation. Platelet microvesicles (PMVs) retain many features of their parent cells and have been shown to exert modulatory effects on vascular and immune cells.
Aims: We hypothesize that PMVs interact with vascular smooth muscle cells (SMCs) and modulate their function in the context of vascular remodeling.
Methods: PMVs were isolated from aging human platelet concentrates by serial centrifugation steps. PMVs were quantified and characterized by flow cytometry using annexin A5/phosphatidylserine and antibodies against CD41a/GP_{IIb}. Size calibrated micro beads were used to quantify the absolute amount of PMVs/mL. Cell migration experiments were performed using a boyden chemotaxis chamber. Platelet receptors implicated in PMV-SMC interaction were identified by blocking antibodies. Proliferation of SMCs was measured by the BrdU-cell proliferation kit. Adhesion of monocytic cells to SMCs was determined by a flow adhesion assay. Relative quantification of gene expression was determined by real time and quantitative PCR.
Results: PMVs increased SMCs migration to a similar extent as platelet factor 4 (CXCL4) and platelet derived growth factor (PDGF). PMV-induced migration could be attenuated by the addition of heparin but not by a neutralizing PDGF antibody. Under resting conditions, the PMV binding to SMCs was specifically abrogated by the integrin $\alpha_{IIb}\beta_3$ inhibitor (integrilin) indicating an integrin-dependent mechanism of interaction. A proliferative effect on SMCs was measured 48 hours after incubation with PMVs and this proliferation relied on interactions via CD40 and P-selectin. The firm adhesion of monocytic cells to PMV-stimulated SMCs under flow conditions was significantly increased compared to untreated, resting SMCs. The adhesion mainly depended on the integrin $\alpha_{IIb}\beta_3$ and P-selectin but also CD40 and the transmembane chemokine fractalkine. Culture of SMC with PMVs decreased gene expression of contractile proteins, i.e. αSMA and calponin.
Summary/Conclusion: Isolated PMVs have shown to exert an immunomodulatory activity on various cell types. The present data indicate a role of PMVs in inducing a phenotypic switch towards a pro-inflammatory SMC phenotype, thus contributing to vascular remodeling processes, e.g. atherosclerosis or neointima formation.

Vessel wall
ECTH-424
Board No. 208: Changes in the lectin pathway of the complement system after out-of-hospital cardiac arrest
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Background: Out-of-hospital cardiac arrest strikes more than 400.000 European citizens every year. Only around 8 % survives, and the majority of survivors get serious brain injuries. Recent studies suggest that complement system proteins might influence brain injury after out-of-hospital cardiac arrest.
Aims: The aim was to investigate levels of complement system proteins in patients resuscitated after out-of-hospital cardiac arrest compared to healthy individuals.
Methods: We included 82 comatose patients resuscitated after out-of-hospital cardiac arrest from February 2013 through May 2015 at Aarhus University Hospital, Denmark. Upon admission, treatment with targeted temperature management at 33 ± 1 °C was initiated and a blood sample was obtained 22 hours after the target temperature was reached. Informed consent was collected from relatives, the general practitioner and the patient if they became capable of giving informed consent. Data from 300 healthy individuals were obtained from a cohort of blood donors.

Levels of complement system proteins (mannan-binding-lectin, ficolin M, ficolin H, CL-L1, Masp1, Masp2, Masp3, Map19 and Map44) in EDTA-plasma will be measured by Time Resolved Immuno Fluorometric Assays (TRIFMA®).
Results: Time Resolved Immuno Fluorometric Assays (TRIFMA®) is currently being performed, and continues through May 2016. Data from patients resuscitated after out-of-hospital cardiac arrest will be compared with complement system protein levels in healthy individuals to test whether plasma levels of complement system proteins are higher in patients resuscitated after out-of-hospital cardiac arrest than in healthy individuals. Data will be ready for presentation at the congress in September 2016.
Summary/Conclusion: This study will enable us to investigate levels of complement system proteins in patients resuscitated after out-of-hospital cardiac arrest compared with healthy individuals. The study provides new knowledge about future possibilities for better prevention and treatment of serious injuries in patients resuscitated after out-of-hospital cardiac arrest.

Vessel wall

ECTH-280

Board No. 209: Role of routine laboratory tests in assessing risk of recurrent venous thrombosis

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Background: Prediction of recurrent venous thrombosis remains a challenge in the clinic, partly because markers used as predictors of recurrence have practical drawbacks, such as influence of anticoagulant treatment on the measurements, or non-availability in laboratories. Glucose, kidney function, defined as estimated glomerular filtration rate (eGFR), and hematologic variables are easily obtainable tests, not influenced by anticoagulation, and available as they have generally been routinely measured for diagnostic workup at time of first venous thrombosis. Nevertheless, their role in the risk of recurrence is largely unknown.

Aims: To assess the predictive value of glucose, eGFR and hematologic variables (hemoglobin, hematocrit, erythrocytes, red cell indices and monocyte count) to identify patients at increased risk of recurrent venous thrombosis.

Methods: Patients aged 18-70 years with a first venous thrombosis, and without cancer at time of first event, were included between March 1999 and September 2004, and followed from discontinuation of anticoagulant treatment (MEGA follow-up study). For logistic reasons, patients provided blood samples until May 31, 2002. For patients included after this date (about 50%), the missing data of laboratory tests were imputed. Cutoff points were *a-priori* established in controls (subjects without venous thrombosis) from the MEGA study (eGFR: 2.5th, 5th, 10th, 50th percentiles; glucose: 50th, 90th, 95th, 97.5th percentiles; hematologic variables: 1st, 5th, 95th, 97.5th, 99th percentiles). Incidence rates of recurrence were estimated as the number of events over the accumulated follow-up time. Cox proportional hazards models were used to evaluate risk between groups. Consent and ethical approval were obtained for this study.

Results: Of 3750 patients followed for a median of 5.7 years, 600 developed a recurrent thrombosis (incidence rate 3.1/100 patient-years; 95%CI, 2.9-3.4). In age-and sex-adjusted models, the hazard ratio was 1.3 (95%CI 0.9-1.9) for eGFR below the lowest percentile category (<59mL/min/1.73m^2) vs. the reference (≥50th percentile, ≥86mL/min/1.73m^2). Glucose levels above the highest percentile category (≥6.6mmol/L) vs. the reference (50th-90th percentiles, 4.8-5.6mmol/L) resulted in a hazard ratio adjusted for age, sex and body mass index of 1.1 (95%CI 0.6-1.7). Hemoglobin levels below the lowest or above the highest percentile categories vs. the reference (5th-95th percentiles) were not associated with recurrence in either sex, with hazard ratios adjusted for age ranging from 1.0 (95%CI 0.5-1.9) to 1.3 (95%CI 0.7-2.6). Likewise, the other hematologic variables were not associated with recurrence. On the whole, data without imputation yielded similar results with slightly larger confidence intervals.

Summary/Conclusion: High glucose levels and abnormal values of hematologic variables were not associated with an increased risk of recurrent venous thrombosis, and an association between renal dysfunction and recurrence appeared to be slight at most. Notably, diabetes, renal failure and anaemia have been reported as risk factors for bleeding during anticoagulant treatment. Our findings, considered in light of these reports, do not support the decision to prolong anticoagulant treatment in patients with first venous thrombosis who have these comorbidities.

Vessel wall
ECTH-399
Board No. 210: 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. Studies from our lab have shown that thrombi from abdominal aortic aneurysm patients are highly enriched in leukocytes and in bacteria, eg. *Porphyromonas gingivalis (Pg)*. The continuous release of neutrophil-derived proteases from these thrombi prevents vascular healing. This protease-rich thrombus is considered as 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. To study the role of platelets in the aneurysmal thrombus formation and its leukocyte-derived deleterious activities, we have developed a new mouse model of AAA with intramural thrombus formation.
Aims: The overall objective is to identify mechanisms by which platelets (i) contribute to aneurysmal thrombus formation and (ii) evaluate these mechanisms in the initiation and progression of AAA.
Methods: AAA was induced by applying an elastase-soaked filter paper on the infrarenal abdominal aorta of wild-type mice (WT). Mice were then injected or not with *Porphyromonas gingivalis* once a week for 2 weeks. Platelet adhesion and leukocyte recruitment to the vessel wall were analyzed by intravital microscopy and the presence of thrombi was quantified by immunohistology at early and late time points.
Results: In elastase-treated WT mice, we observed, by intravital microscopy, an early recruitment of platelets and leukocytes to the damaged vessel wall. At 14 days, abdominal aorta aneurysm was found in all treated mice. The diameter of the aorta was increased two-fold compared to sham mice and histological analysis did not reveal thrombus formation. In contrast, in elastase-treated WT mice injected with *Pg*, the dilatation was three-fold compared to untreated mice and importantly, immunohistology showed large thrombi in the dilated vessel wall which were enriched with platelets and leukocytes.
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.

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