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Coagulation Standards Standing Committee

09 July 2019
12:15 - 13:45

Chairman: Craig Thelwell, NIBSC, Potters Bar, United Kingdom

Review of ISTH/SSC Secondary Coagulation Standard Lot #4 (C Thelwell)

Dispatch of Lot #4: Between the beginning of June 2018 and the end of May 2019 there were a total of 55 orders from 23 different manufacturers (16 Europe, 3 USA, 2 Canada, 2 Japan) and one external quality assurance scheme (College of American Pathologists). A total of 12,060 vials were issued. Lot #4 was also included in an extension to the multi-centre study for the value assignment of VWF:GPIb-binding methods to Lot #5 (80 vials) and an NIBSC study on commercial calibrators (80 vials). A request was also approved to supply 400 vials to Freeline therapeutics, for use in an AAV gene therapy haemophilia B study. Remaining stock at the end of May 2017 was around 2,000 vials and is expected to be depleted within a few weeks.

Introduction of Secondary Coagulation Standard Lot #5 (C Thelwell)

Additional laboratories were recruited for the calibration of Lot #5 for VWF activity by ristocetin cofactor (VWF:RCo) and the GPIb binding methods (VWF:GPIbR and VWF:GPIbM). Calibration for VWF:GPIbR and VWF:GPIbM methods were carried out relative to the values assigned to the WHO 6th IS FVIII/VWF plasma, which was established as the WHO Reference Reagent for VWF:GPIbR and VWF:GPIbM by the Expert Committee on Biological Standardization of the WHO in October 2018. The study was successful and the assigned values (Table 1) were approved by the participants.

Stability testing based on an accelerated degradation protocol indicated mean loss per year at -20°C of approx. 0.01% for four analytes (Factor V, Factor VII, Factor VIII, Antithrombin) - this indicates Lot #5 is very stable. Based on the upper 95% confidence limit of % loss, Lot #5 is predicted to retain more than 99.5% activity following 10 years of storage at -20°C, which supports assigning an expiry date of ‘end of December 2029’.

Approval for the assigned values has been obtained from the study participants for the 24 analytes in Table 1. The calibration and expiry date have now been approved by the Executive Board of the Standing Committee, SSC Subcommittee chairs and co-chairs and the ISTH Executive Committee. Lot #5 has now been introduced into the NIBSC catalogue alongside the remaining vials of Lot #4.

A study is planned to assign a value for FXII to SSC Lot #5. Other potential analytes include ADAMTS13, FXI antigen and FIX antigen. A customer survey on the use of the SSC Standard is planned to determine which values are used (and frequency of use) and which analytes not assigned would be useful. This information will help to inform future calibration studies to assign values to Lot #5.

The traceability of the IU from SSC Lot #4 to Lot #5 using commercial plasma calibrators (H Wilmot)

Commercial plasma calibrators are used in clinical laboratories and EQA schemes have identified some reagent/calibrator bias. A study was organised to assess the performance of plasma calibrators from seven different manufacturers for FIX and FVIII activity against the WHO International Standards and SSC Plasma Lot #4 and Lot #5. Different activated partial thromboplastin time (APTT) reagents were used (SynthASil, Actin-FS and Pathromtin SL) and different sources of deficient plasmas. There was good agreement of FIX chromogenic assays with one-stage clotting assays and no within-method (reagent) bias was found. There was good traceability of the IU for Lot #4 and Lot #5 for both FIX and FVIII and the change to Lot #5 should not present any issues for these analytes.

Experience of EQA schemes with Lot #4 UK NEQAS (S Kitchen)

The use of Lot #4 for troubleshooting was reviewed. Between 2008 and 2018 there were approx. 70 vials issued for this purpose (covering factors II, V, VIII, IX, XI, VWF, fibrinogen, Antithrombin, Protein C, Protein S). In the last 12 months vials were issued for troubleshooting problems in 2 centres associated with UK NEQAS and for 4 centres for the WFH IEQAS scheme. There was an update on survey results for factor IX. A normal plasma was sent out as a test sample and a significant difference was found (17% higher reported value) for one of the three commercial calibrators used. The new SSC Lot #5 will be requested to be included in future UK NEQAS surveys on VWF activity methods, once the new analytes for GPIbR and GPIbM assigned to Lot #5 are being used for commercial calibrators, and for other analytes (including FIX, FII).

College of American Pathologists (JD Olson)

SSC Lot #4 was issued in two surveys in 2018; one for thrombophilia testing and a special survey for VWF and Factor VIII (one-stage clotting assay). Analytes are tested at the discretion of participating laboratories. Results are presented as mean for 10 or more participants and only as median for less than 10 participants. Results for Antithrombin activity were close to the assigned value for Lot #4 whereas the median from one antigen method was ≈10% higher. Mean estimates for Protein C activity by chromogenic methods were close to the assigned value whereas one clotting end-point method gave much higher results (112.8 vs 92 IU/dl). Mean results for Protein S activity, total and free antigen were within 10% of the assigned values. Mean results for factor VIII activity was variable demonstrating bias of 6.1%, 13.6% and 18.6% with three methods. The bias for VWF:RCo was -12% while the bias for, VWF:GPIb was +10%. The VWF:antigen agreed very closely with the assigned value. Overall, the means of VWF:Ag, VWF:RCo, Antithrombin activity and antigen, Protein S activity and antigens (free and total) and Protein C activity and antigen generally agreed with ISTH assigned values. External quality assurance programs can use the ISTH standard to identify biases among assays.

There were 18 attendees.

Table 1 Values assigned to SSC Standard Lot #5 (July 2019)

Analyte	Value (IU/vial)	Inter-lab variability (GCV%)	n
Fibrinogen	3.19 mg/vial	4.5	18
Factor II:C	0.95	4.2	16
Factor V:C	0.87	3.7	34
antigen	0.98	1.6	5
Factor VII:C	1.00	2.7	17
Factor VIII:C	0.82	5.5	33
Factor IX:C	1.09	7.5	26
Factor X:C	0.97	4.3	18
Factor XI:C	0.87	5.8	20
Factor XIII			
function	0.77	6.6	14
antigen	0.73	6.2	9
von Willebrand Factor			
antigen	1.14	8.7	18
collagen binding	1.02	9.9	17
propeptide	1.03	4.9	12
ristocetin co-factor	0.82	11.4	13
GPIbR	0.95	7.0	14
GPIbM	0.80	7.6	17
Protein C			
function	0.97	4.5	31
antigen	0.89	6.4	11
Protein S			
function	0.78	9.0	17
free antigen	0.98	4.2	16
total antigen	0.96	6.3	9
Antithrombin			
function	0.95	4.1	26
antigen	0.94	5.3	13

Biorheology

6 July 2019
14:30 – 16:30

Chairman: Pierre Mangin

Co-Chairs: Elizabeth Gardiner, Wilbur Lam, Warwick Nesbitt, Mikhail Panteleev, Steve Kerrigan, and Netanel Korin

Talk 1: Update of a novel SSC project: Haemodynamics: "WSR/blood flow values in human and mouse vessels" (Mikhail Panteleev, Moscow University, Russia).

It is presently recognized that blood flow has profound effects on both platelet adhesion and blood coagulation. Various microfluidic flow devices represent remarkable tools to mimic the rheological conditions found in various physiological and pathological settings in vivo. Their use has been tremendously increased recently. However, what should be the correct conditions used in these devices? The main objective of the project is to provide a repertoire of blood flow values found in various human and mouse vessels under physiological and pathological conditions. This will help researchers using blood flow assays to choose relevant hemodynamic conditions to perform their experiments.

Q/A: 1) **Comment from Pr. Goto** (Japan) who has underlined that this new project is important to the field as we are lacking of a repertoire of shear values in most vessels. Pr. Goto suggested that it would be of great interest to extend this project to measures of blood flow values not only in healthy vessels, but also in diseased arteries.

Talk 2: Update on an ongoing SSC project: "Multicenter study on flow-based assays" (Keith Neeves, University of Colorado, USA):

Keith Neeves provided an update on the SSC Project on Multicenter Study on Flow Chambers. The objectives of the study were reviewed, followed by a description of the alpha version of micropatterning and microfluidic devices for conduction platelet adhesion studies under flow. Over the last year, technical issues were identified with these devices and potential solutions were presented. The major issue was related to leakage during flow assays. Potential solution included permanent (plasma) bonding of flow chambers, reversible vacuum bonding, or a clamp for the device and glass slide. Data was presented from initial studies among Committee co-chairs on platelet adhesion to fibrinogen and collagen. Goals for Year 3 of the project were discussed.

Session on visualizing and characterizing thrombosis and hemostasis in real-time:

Chairs: Elizabeth Gardiner and Warwick Nesbitt.

Talk 3: "Blood Flow and the Adhesion of Kinetics of Platelet Mimetic Nano- Carriers". Netanel Korin (Israel):

Hemodynamics plays a key role in the cardiovascular system under normal and diseased conditions, as well as in the development of cardiovascular drug carriers for targeted drug

delivery. Inspired by circulating platelets' natural ability to target vascular injuries, two platelet mimetic approaches were presented by Netanel Korin: 1). Shear responsive drug carrier that leverages the high shear stress in regions of artery stenosis to locally deliver drugs at these disease sites. 2). Functionalized nanocarriers decorated with ligands, mimicking platelet receptors, that are designed to selectively target sites of arterial thrombosis. In order to rationally engineer such drug carriers, *in silico*, *in vitro* microfluidic models and *macro-fluidic* models were utilized. By applying these models, the adhesion kinetics of platelet mimetic drug carriers under relevant hydrodynamic conditions were investigated. The results emphasize the importance of accurately recapitulating blood flow conditions in studying vascular targeting drug carriers.

Q/A:

1) **Question**: What is tha half-life of the nanoparticules (aggregates)? **Answer**: the lifetime is very short (5 min) but appears sufficient to induce a lysis of a thrombus.

3) **Question** from Pr Goto (Japan): we do not want NP to adhere to healthy endothelial cells. **Answer**: NK indicted that he used GPVI-Fc which does not bind to endothelial cells but collagen and thrombi, so no risk to bind healthy endothelial cells.

4) **Question** from Keith Neeves: What is known concerning the margination of NP in the stenosed region of the channel? **Answer**: We have a clear view on margination in small vessels, but still very unclear in large vessel and this is currently under investigation.

5) **Question** from Liz Gardiner: Have you used pulsatile flow on the NP? **Answer**: Piston is used because the regular flow pump are not accurate/rapid enough to generate relevant pulsatile flow

Talk 4: Josie Carberry (Monash University, Melbourne): New tools to quantify shear stress on the surface of a forming thrombus.

There is an established relationship between flow induced forces (shear) and platelet function. Measuring and understanding the shears that flowing and adherent platelets experience is very challenging. Two different approaches to this problem were presented. The first is a simple way of measuring the response of platelets to a flow pulse without needing to experimentally measure a time varying flow. The second is a combined experimental and CFD approach which allows to measure the shear history of platelets adhering on a growing thrombus.

Q/A:

1) **Question** from "Arnold" (Sydney Uni): Comment on the size of thrombi with regards to WSR. **Answer**: the speaker confirmed that they observed bigger thrombi when increasing the shear, but indicated that they did not investigate why the thrombi were bigger.

Talk 5: Dalton Harvie (University of Melbourne): Numerical models of Blood flows and Thrombosis

Dalton Harvie's interdisciplinary team is developing a Computational Fluid Dynamics (CFD) model of blood coagulation, with the overarching goal of being able to predict the formation and growth of either haemostatic or thrombotic clots, resolved in both space and time. In this talk he detailed progress and current challenges within three critical modules of this ambitious project: a) the

coagulation cascade (factor biochemistry) in the presence of surfaces, diffusion and flow; b) multifluid modelling of cell movement and margination during blood flow; and c) simulating shear induced platelet binding.

Q/A: no question because of time

Talk 6: Steve Lee (ANU): New optical modalities to image Thrombus formation in vitro and in vivo.

Steve Lee's group aims to better understand blood cells and their interaction with a myriad network of living vessels holds key to the health of circulatory system. Modern optical engineering techniques has transformed light into an indispensable tool to investigate and monitor the health of the blood circulatory system. In this talk, he presented high-speed quantitative volumetric microscopy imaging approaches that present new in-vitro and in-vivo opportunities to study dynamic thrombus formation.

Q/A: no question

Control of Anticoagulation

7 July 2019
16:30 – 18:30

Chairman: Mark Crowther

Co-Chairs: Julia Anderson, Chatree Chai-Adisaksopha, Adam Cuker, Jonathan Douxfils, Ravi Sarode, and Jovan Antovic

Session Moderators: Jovan P. Antovic, Sweden, Jonathan Douxfils France

Bleeding and Thrombotic Events After Resumption of Oral Anticoagulants Following Gastrointestinal Bleeding

Speaker: Walter Ageno, Italy

WA presented on behalf of Dr. Marcello Di Nisio an update on this ongoing project. This international, observational, retrospective cohort study aims to evaluate clinical, laboratory, and endoscopic characteristics of patients who experience a GI bleeding while on oral anticoagulant treatment; to evaluate whether these patients are restarted on DOACs at the same or reduced dose, VKAs, or are switched to another anticoagulant agent; to assess the incidence of recurrent GI bleeding, major bleeding at other sites, thromboembolic events, and all-cause mortality during follow-up; and to determine the optimal timing of anticoagulation resumption defined as the lowest incidence of the combined outcome of recurrent bleeding and thromboembolic events.

Five centers (2 in Italy, 2 in Canada, 1 in the Netherlands) are participating and, as of June 2019 997 patients have been enrolled.

International Registry on the Use of the Direct Oral Anticoagulants for the Treatment of Unusual Site Venous Thromboembolism

Speaker: Walter Ageno, Italy

WA presented an update on this international, observational, prospective cohort study that aims to enroll patients with venous thrombosis at unusual sites treated with direct oral anticoagulants. Aims of this study are to evaluate the rationale for the use of these drugs for these patients and to assess the safety and effectiveness of this approach in real life clinical practice. All adult patients presenting with any unusual site venous thrombosis (not involving the limbs and the pulmonary arteries) will be eligible for inclusion, unless they are participating in interventional studies. After a 12-month follow up, study outcomes include thrombotic events (arterial or venous), major bleeding events, mortality, and vessel recanalization. There are currently 40 centers from 19 countries that will participate, but more centers are welcome. Colleagues who are interested in this study can contact the Principal Investigator Dr. Nicoletta Riva (nico.riva@hotmail.it). The study protocol has been finalized and registered at clinicaltrials.gov; the eCRF will be available on RedCap provided by the ISTH, and the protocol has been submitted for ethics review in some countries.

Treatment of unusual VTE: a physician's survey

WA also briefly presented a recently completed survey of physicians on the treatment of unusual site VTE carried out through the ISTH, Thrombosis Canada, and the Italian Society for the Study of Haemostasis and Thrombosis. A total of 397 colleagues from all continents have kindly participated. Questions on the management of 4 different clinical scenarios were presented. The results will be submitted for publication soon.

Management of Cancer-Associated Thrombosis in Patients with Thrombocytopenia: Guidance from the SSC of the ISTH

Speaker: Marc Carrier, Canada

The management of anticoagulation in cancer patient with thrombocytopenia (platelet count is <50 × 109/L) is challenging. Proposed management strategies include therapeutic anticoagulation with platelet transfusion support and dose-modified anticoagulation. Dr. Carrier reported a systematic review reporting the rates of recurrent venous thromboembolism (VTE) and major bleeding complications among patients treated with both of the two management strategies. The systematic review did not support one management strategy over another. Based on these finding, the SSC developed a Guidance document to help clinicians managing these patients. The SSC is suggesting considering the severity of the thrombocytopenia along with the time elapsed since the index (and extend) thrombotic event in order to tailor anticoagulation in this patient population. The full Guidance document was published in the Journal of Thrombosis and Haemostaiss in June 2018 (https://www.ncbi.nlm.nih.gov/pubmed/29737593).

An International Registry of the Outcomes of Stents in Patients with Venous Thrombosis

Speaker: James Douketis, Canada

An International Registry of the Outcomes of Stents in Patients with Venous Thrombosis supported by the ISTH, aims to gather information about management practices and clinical outcomes in patients with extensive venous thrombosis who have undergone catheter-directed thrombolysis and venous stent implantation. This study is a first step aimed at identifying the scope of the problem, rates of clinical with a view to developing hypotheses that can be addressed in well-designed clinical trials.

Survey on Anticoagulated Patients – Register (Start Scientific and Standardization Commitee - the International Society on Thrombosis and hemostasis

Speaker: Walter Ageno, Italy

WA presented an update on this ongoing registry that aims to collect information on patients treated with direct oral anticoagulants experiencing a major bleeding event or a thrombotic event during active treatment. This observational, international, prospective study with a 6-month follow up is actively enrolling in Italy, Germany, Switzerland, Belgium, US, Thailand, and

Brazil. Baseline characteristics, management strategies, and clinical outcomes during follow-up are recorded. There are currently 187 patients enrolled after major bleeding, of whom 82 with intracranial bleeding, and 72 patients with thrombotic events. The following analyses are planned: timing of anticoagulation resumption in patients with major bleeding, incidence of thromboembolic events in patients with major bleeding, anticoagulant management of thromboembolic events occurred during DOACs treatment.

Ad Hoc Working Party to Guide the Laboratory Measurement of the ODIs

Speaker: Ismail Elalamy, France

Dr Elalamy did not submit a report for the minutes but did present on the work of this working party. Further information will follow in the published reports of the working group. A vigorous discussion did occur as a component of this presentation around current best practices concerning laboratory measurement and reporting; this area could represent an interesting area for the committee to concentration in future communications.

Post-Bleed Management of Antithrombotic Therapy (PANTHER): A Mixed-Methods Study of Healthcare Providers

Speaker: Deborah M. Siegal, Canada

Gastrointestinal bleeding (GI) accounts for ~40% of major oral anticoagulant (OAC) related bleeding. OACs are permanently discontinued in up to 50% of patients. Resuming OAC after GI bleeding is associated with a reduced risk of thromboembolism and death, but with an increased risk of recurrent GI bleeding. The main clinical challenges are whether, when and how to restart OACs. There are no studies evaluating physician values and preferences in this setting. The objectives of this study are to (i) identify key factors that influence physician decision-making regarding resumption of OAC after GI bleeding, and (ii) determine the relative importance (utilities) of these factors.

Phase 1. We conducted focus group discussions with healthcare providers involved in the care of patients with OAC-related GI bleeding. Factors identified during the discussions were ranked through a dot voting exercise. Themes (factors) were identified by 2 independent reviewers. Coding was generated from the data as per the qualitative description method.

Phase 2. We developed a discrete choice experiments survey using the attributes derived from focus group discussions. The web-based survey was developed and analyzed using Sawtooth software platform. The survey was pilot tested and modified by iterative feedback (n=8). The survey is being distributed online through professional societies (International Society on Thrombosis and Haemostasis, Canadian Association of Gastroenterology, Thrombosis Canada, CanVECTOR Network, Canadian Society of Internal Medicine and the Anticoagulation Forum). Our target response rate is 150 respondents.

Preliminary Results: There were 4 focus group discussions involving 29 participants (mean age 44 years [±11], male 55%). Twelve factors were identified: thrombosis risk, re-bleeding risk, comorbidities, type of OAC, indication for OAC, index bleed severity, source of bleeding/treatment (i.e. type of lesion, endoscopic therapy), patient values/preferences,

physician comfort/experience, responsibility to prescribe, patient factors (i.e. supports, frailty), and resources/monitoring. The most highly ranked factors were (in descending order) thrombosis risk, indication for OAC, index bleed severity, re-bleeding risk, and patient factors. Barriers to resuming OAC included uncertainty about re-bleeding risk, lack of guidelines and information from specialists, and concerns about the impact of re-bleeding on the patient-physician relationship. Facilitators of resuming OACs included specialist advice and patient understanding of thrombotic complications. Thus far, 96 respondents have completed the discrete choice experiments survey. Preliminary results show that thrombosis (utility 26.72) risk and bleeding risk (utility 26.71) are similarly important when making decisions about OACs post-bleed. Although the majority of respondents indicated they would restart OACs within 1 month of the index bleed, there was significant variability depending on the thrombosis and rebleeding risks. However, 20% indicated they would not restart OACs in patients at low thrombotic risk and high rebleeding risk.

Conclusions. Our preliminary results suggest that thrombosis and rebleeding risks are similarly important to physicians when making decisions about resuming OACs after GI bleeding. Future analyses will assess the importance of factors for decision-making based on physician characteristics. Clinical uncertainty exists regarding the optimal timing of OAC resumption based on the risks of re-bleeding and thrombosis.

DIC

9 July 2019
16:30 – 18:30

Chairman: Toshiaki Iba

Co-Chairs: Alessandro Squizzato, Florea Lupu, Theodore Warkentin, Kazuma Yamakawa, Ecaterina Scarlatescu, and Carl-Erik Dempfle

Disseminated Intravascular coagulation in Non-human Primate Models of Sepsis

Speaker: Florea Lupu

DIC is a frequent complication of sepsis and a major contributor to organ failure and death. The presentation will summarize data on activation of coagulation and complement systems in non-human primate models of sepsis. Particular focus will be on differences between gram positive and gram negative bacteria and the coagulopathic responses induced by live versus dead bacteria or bacteria derived peptidoglycan. Further, data on novel therapeutic approaches targeting the complement and contact activation will be discussed.

What Vitamin C is purported to do? Purported Effects of Vitamin C in Sepsis and DIC

Speaker: Donald Brophy

There is now much interest in using intravenous Vitamin C as an adjunctive treatment for severe sepsis. This is because Vitamin C has pleiotropic mechanisms including those that counteract systemic inflammation, endothelial dysfunction, and disseminated coagulation. In both animal models of polymicrobial sepsis as well as clinical trials of human sepsis, Vitamin C is an effective modulator of inflammation and endothelial dysfunction, and coagulation, as well as NETosis. This presentation will discuss the pre-clinical and clinical data that have been collected using intravenous Vitamin C in severe sepsis.

Clot wave form analysis for DIC

Speaker: Hideo Wada

The usefulness of clot waveform analysis (CWA) including the activated partial thromboplastin time (APTT) waveform has been reported in hemophilia, acquired hemophilia and monitoring for anticoagulants. The changes of APTT waveform in patients with DIC have been attracted. The CWA was examined in patients suspected of having disseminated intravascular coagulation (DIC) to analyze its usefulness for the diagnosis of DIC or the prediction of the outcome or bleeding risk. DIC with fibrinogen < 2g/L was frequently associated with infectious diseases (43.3%). The heights of the first derivative peak (1stDP) and second DP (2ndDP) were extremely low in DIC, especially DIC with hypofibrinogenemia, but high in infectious patients without DIC. The peak time and width of the 1stDP and 2ndDP were prolonged in patients with DIC. The heights of the 1stDP and 2ndDP were markedly low in patients with a poor outcome or those with hemoglobin <8.0 g/dl. As bleeding type DIC was observed in infectious DIC, DIC

without hypofibrinogenemia might switch to DIC with hypofibrinogenemia by the progression of DIC. The height of the 1stDP and 2ndDP is useful for the diagnosis of DIC and prediction of the bleeding risk or outcome.

DIC in your Emergency Room

Speaker: Bernd Jilma

Overt DIC is a rare but underdiagnosed event in ED patients. Malignancies and cardiovascular disorders prevail over infectious diseases as underlying causes. Preventive (vaccination) strategies are discussed for the later. DIC presenting with a fibrinolytic phenotype is frequently found in cardiac arrest patients. The degree of hypofibrinogenaemia on admission strongly and linearly predicts early death

Recombinant thrombomodulin for sepsis-induced coagulopathy

Speaker: Kazuma Yamakawa

The reported Phase III trial of recombinant human soluble thrombomodulin in sepsis failed to show a 28-day all-cause mortality reduction. However, the big concern about the eligibility of patient selection possibly caused by a protocol amendment would be raised. In this talk, I will report our latest systematic review and meta-analysis of recombinant thrombomodulin including reasonable SCARLET trial results and discuss the clinical significance of this treatment.

Constructing the Japanese Guidelines for Sepsis Management 2020

Speaker: Yutaka Umemura

Despite the frequency and severity of disseminated intravascular coagulation (DIC) complicated with sepsis, Surviving Sepsis Campaign (SSC) Guideline has not sufficiently discussed about the management of sepsis-induced DIC. The Japanese Clinical Practice Guidelines for the Management of Sepsis and Septic Shock (J-SSCG) were constructed to cover such topics which were not mentioned in the SSC guideline. Prior to the next revision of J-SSCG planed in 2020, we will assess the current body of evidence regarding DIC management.

Assessment of fibrinolysis in sepsis

Speaker: Ecaterina Scarlatescu

Hypercoagulability and hypo-fibrinolysis correlate with organ dysfunctions and increased mortality in septic patients, however, the assessment of fibrinolytic activity in critically ill septic patients is still lacking in clinical practice. The assessment of fibrinolytic activity using plasmatic global tests or measurement of biomarkers levels is cumbersome, time-consuming and sometimes difficult to interpret. On the other hand, septic coagulopathy is an evolving process with dynamic changes of the fibrinolytic activity occurring in a short timeframe, making its assessment even more difficult.
Viscoelastic tests have proved useful for the real-time detection of overt hyperfibrinolysis

diagnosed by the decrease of clot firmness related to the maximum clot amplitude, however resistance to fibrinolysis is more difficult to diagnose using lysis parameters, because at baseline, normal healthy individuals demonstrate minor clot lysis which is also a common finding in septic patients. Also, as viscoelastic tests globally assess the hemostatic system by recording the clot amplitude over time, their fibrinolysis measure is not an isolated quantification of the fibrinolytic activation but the balance between clot formation and degradation. Thus, if clot formation is still maintained in the same time, normal or decreased clot lysis can actually stand for increased fibrinolysis, normal fibrinolysis or fibrinolytic shutdown. Modified viscoelastic tests with added plasminogen activators and measurements derived from clot formation velocity curve could enable a better identification of septic patients with low fibrinolytic activity.

FVIII, FIX and Rare Coagulation Disorders

8 July 2019
16:30 – 18:30

Chairman: Johnny Mahlangu

Co-Chairs: Peter Collins, Alfonso Iorio, Gili Kenet, Maria Mancuso, Guy Young, and Savita Rangarajan

SSC Project Updates
Moderators: Alfonso Iorio, *Canada*, Johnny Mahlangu, *South Africa*

The Chairman introduced the session and the first speaker.

Hemophilia Carrier Nomenclature

Speaker: Robert Sidonio, United States

Introduction and rationale of the project
- There is no formal terminology to apply to hemophilia A and B carriers regarding whether they have normal or reduced factor level in the mild, moderate and severe range. This can create confusion in academic discussion and conveying data to the consumer population.
- There are limited guidelines on approach and management

- The objective is to propose terminology and a conceptual framework for categorization of hemophilia A and B carriers accounting for personal bleeding history, genetic determinants and baseline factor level to improve communication between providers, researchers, payers and community members

Proposal
- The proponents have identified a possible terminology to be used and definitions. The terminology proposed is “hemophilia carrier” for patients > 40% and distinguish between asymptomatic and symptomatic hemophilia carriers. The term “hemophilia” is proposed to characterize all the others in accordance with the residual factor level (Woman/girl with mild/moderate/severe hemophilia)

Project progress
- July 2018: ISTH proposal presentation at ISTH SSC, literature review
- June 2018 to June 2019: presented concept proposal to Code Rouge, NHF/MASAC, ATHN, WFH and HFA; awaiting feedback and endorsement.
- 3rd to 4th quarter 2019: Gain approval from SSC; finalize proposal and submit to ISTH

[no comments/questions from the audience]

Gene Therapy Working Group

Speaker: Glenn Pierce (USA) on behalf of Alok Srivastava (India)

Introduction and aim of the project
- To help development of gene therapy for hemophilia and rare bleeding disorders through guidance on 'standardization' of requirements/options of methods for product development and clinical trials.
- To identify and review unresolved issues related to development of products and conduct of clinical trials for gene therapy of hemophilia/rare bleeding disorders.
- To provide guidance/recommendations for addressing these issues to help move research forward and promote harmonization of requirements and path to product development and market authorization.

Methodology
- Review existing data on these issues and seek opinions through a structured questionnaire
- Engage stakeholders – Academia, Industry, Regulators and Advocacy Organizations
- Analyze all information/draft guidance/recommendations and present in F2F discussions (meetings in Glasgow and Dublin).
- Revisit stakeholders through ISTH SSC website posting to seek opinions
- Finalize guidance/recommendations on behalf of the SSC of the ISTH

Project progress/next steps
- Further discussion of recommendations at GT TF session on 9th July
- Receive further comments by email until end of July, 2019.
- Prepare final recommendations and submit for comments by major regulators.
- Finalize recommendations and submit to SSC for approval for submission to JTH

[Questions from the audience:

***Q**. What is the definitions of ABR? **A**. will be the standard definitions, number of bleeds*

***Q**. What is the goal of standardization of AAV antibodies assay? **A**. The main objective is to capture actual seroprevalence]*

Definitions in Acquired Hemophilia

Speaker: Andreas Tiede, Germany

Introduction and aim of the project
Definitions in acquired hemophilia are not uniform and several components need to be defined. In particular, the group has focused on the following:

1. Disease definition
2. Remission
3. Recurrence
4. Mortality
5. Bleeding

6. Hemostatic response

A systematic review of the literature has been completed

Project status and next steps
- Definitions for clinically and scientifically relevant outcomes in AHA have been suggested
- Publication will be submitted after ISTH SSC endorsement

[Questions from the audience:

***Q**. Will the working group consider the new therapies available (Hemlibra, Obizur)? **A**. The definitions will take into account the fact that new therapies might be available.]*

Definition of Immune Tolerance Induction Response with Extended Half-Life FVIII Products Working Group

Speaker: Maria Elisa Mancuso, Italy

Introduction and aim of the project
- The PK profile of EHL-FVIII products is longer than SHL-FVIII products.
- The usual definition based on the observation of a half-life >6-7 hours is not precise enough
- Objective: To define ITI success in patients receiving ITI with EHL-FVIII products

Methods

- Review of the literature
- Direct inquiry to pharmaceutical companies to get on-file data
- Review of PK data from clinical trials and WAPPS-Hemo database

WG Proposal
- A pragmatic definition as already done for SHL products by the UKHCDO
- Based on available data a half-life of 11 hours could be considered for children age 0-11 and half-life of 14 hours for subjects > 11yrs
- Because of high inter-individual variability a proportion of half-life restoration rather than a fixed cut-off could be more adequate

Project status and next steps
- A report is under revision within the working group

[Questions/comments from the audience:

***Q**. What is the reason for the half-life criteria? **A**. Mostly because it's relevant for the clinical approach – an EHL product that needs to be used everyday would lose its value*

***C**. Comments from member: It is very important to know that a patient is partially or fully tolerant, definitions in the context are needed*

***C**. Comments from member: Need to define also an immunological tolerance, what is proof of immunological tolerance beyond the definitions used so far.*

***C**. Comments from member: Suggestion to leave half-life out of definitions and use instead the wash-out time.]*

SSC New Projects

Moderators: Maria Elisa Mancuso, *Italy*, Guy Young, *United States*

Novel Non Replacement Therapies for Hemostasis in Patients with Rare Bleeding Disorders, an Ex Vivo Thrombin Generation Guided Study

Speaker: Maria Elisa Mancuso (Italy)

Introduction and aim of the project
- A number of novel non-replacement therapies will be soon available, and not strictly for hemophilia
- Little is know about the effect on thrombin generation of these new therapies such as anti-TFPI, Fitusiran, Emicizumab
- Propose a systematic in vitro investigation of the effects of these new drugs

[no comments/questions from the audience]

International Multicenter Validation Study of a Standardization Mathematical Tool to Improve the Reproducibility of Factor IX Results in Patients with Hemophilia B treated with rFIXFc

Speaker: Felipe Guerrero, France

Introduction and aim of the project
- The measurement error of FIX in rFIXFc treated patients can be modelled using a reference material like standardized spiked samples.
- The modelling allows for the correction of the calibration bias observed with CK Pres reactant.
- The above noticed effect is not restricted to CK Pret and could improve overall agreement between methods.

Proposal for an observational, multicenter, prospective international study. A software application and an educational program for mathematical standardization will be shared with the participating centers.

[no comments/questions from the audience]

Standardization Issues in Novel Hemophilia Therapies - Guidance from Experts
Moderators: Maria Elisa Mancuso, *Italy*, Savita Rangarajan, *England*

Key Considerations in Choosing Outcome for Assessing Gene Therapy
Speaker: K. John Pasi, United Kingdom

Traditional methods of outcome assessment have limitations in the gene therapy field. Traditional endpoints are ABR - used to demonstrate clinical benefit - or factor levels as a surrogate endpoint for primary efficacy assessment under the accelerated approval pathway. ABR has a number of significant limitations such as sensitivity, subjectivity (with greater impact of bias at lower bleeds rates) and are affected by the presence of chronic arthropathy. Factor level, although the primary target, is a potential surrogate for a clinical outcome. However, it's difficult to assess additional value, when increasing the factor activity level from one value to another higher level. Centralization and field studies might be helpful in this context. Long term assessment is still a major requirement.
With gene therapy the ABR can be reduced to almost zero. Possible alternative measures when ABR is zero are: long-term outcomes (joint score, US changes, durability) and medium-term endpoints (functional measures, activity trackers).
Assessment of global benefit in this context is also important, in particular the patient relevant outcomes (using tools like PROBE). None of these aspects can be used in isolation but it should be kept in mind to use the totality of these instruments to evaluate the treatment efficacy.

[Questions/comments from the audience:

***Comment:** Researchers and clinicians should keep in mind that factors coming from gene therapy can behave differently from exogenous factor concentrates.]*

Key Considerations in Choosing Outcome for Assessing Non-Replacement Therapies

Speaker: Johannes Oldenburg, Germany

Speaker reviews the mode of action of non replacement therapies.
The Speaker highlights the fact that thrombotic risk is an important consideration with non-factor replacement therapies. Laboratory assessment is considered important in this context.
Main Outcome parameters to be considered are ABR and Joint status.
Clinical monitoring (HJHS, Gilbert-score): chronic synovitis can be clinically silent and only later clinically overt. Monitoring can be done with US and MRI before joint damage occurs. Imaging can be done with X-ray (Petterson) for late joint damage, MRI for bleed, synovitis, early and late joint damage, Ultrasound for bleed, synovitis, early joint damage but limited for late joint damage. Evaluation of joint outcome: ABR, Ultrasound, MRI, X-ray, HJHS. Laboratory assays for efficacy assessment can be thrombin generation, chromogenic (bovine) FVIII:C (Emicizumab). Laboratory tests suggested for thrombosis risk assessment: prothrombin F1+F2, D-dimer, APC. Also important to consider pain assessment, activity and quality of life.

Achievable Goals in Standardizing Factor Activity Level Measurement for Extended Half Life Products

Speaker: Steve Kitchen, United Kingdom

Standardizing measurement of EHL products is a relevant issue. We have many different versions of one stage and chromogenic FVIII and FIX assays can be used. Assays for EHL FVIII and FIX give different results. Patient management sometimes relies on assay results.

What is needed today?

1. Guidance on what level of difference can be tolerated.
2. Details of potency assignment assays in public domain.
3. Data on results obtained using different assays – available early in process pre-licensing.
4. Published product specific guidance on what assay should not be used.
5. Local product-specific certification of assay method before use.
6. Ongoing post marketing surveillance of assays effects since reagent change – proficiency-testing organization.

[Chairman concludes meeting, farewell.]

Factor XI and the Contact System

8 July 2019
16:30– 18:30

Chairman: Joost Meijers

Co-Chairs: Edward Feener, Heiko Herwald, Coen Maas, Owen McCarty, Stephanie Smith, and Helen Philippou

The Factor XI and the Contact System session on July 8 drew approximately 150-200 attendees.

Factor XI inhibitors, according to Jeffrey Weitz (Hamilton, Canada), have the potential to be at least as effective, and safer than the direct oral anticoagulants (DOACs). Although spontaneous bleeding is rare in patients with congenital factor XI deficiency, there can be bleeding with surgery or trauma. Therefore, as we have learned with the DOACs, reversal strategies for factor XI inhibitors are needed.

Most bleeding with factor XI deficiency is mucocutaneous, and anti-fibrinolytic agents such as tranexamic acid often controls it. If there is ongoing bleeding or for major trauma or surgery, factor XI replacement will reverse factor XI knockdown with the antisense oligonucleotide, whereas low-dose factor VIIa is likely to bypass factor XIa inhibition by antibodies or small molecules. Because repeated doses of factor VIIa may be needed for reversal of long-acting factor XI-directed antibodies, development of inhibitory anti-idiotype antibodies may be useful.

Helen Wilmot (Potters Bar, UK) gave an update on the Global Working Group activity for the measurement of procoagulant activity in immunoglobulins and a summary of the ongoing NIBSC work on using factor XIa chromogenic assays for measurement of FXIa in immunoglobulin preparations.

For 15 years, the ECAT foundation provides an external quality scheme assessment program for factor XI:C and factor XII:C assays. Piet Meijer (Leiden, the Netherlands) discussed that currently 340 laboratories from all over the world are participating in this program. Evaluation of the survey data for 2017 and 2018 demonstrated that for factor levels >40 IU/dL, the between-laboratory variation is 10-15%, which is similar to other APTT-based coagulation factors. For lower levels, a variation up to 30% is observed.

For both FXI and FXII small differences are observed in the mean values between different test systems. These differences are most likely caused by the calibrator that is used.

Steve Kitchen (Sheffield, UK) described the UK NEQAS experience with factor XI and factor XII assays. Approximately 250-300 centres (mainly UK and Europe) perform FXI and FXII assays in UK NEQAS surveys. All use one-stage APTT-based assays including more than 10 different types of APTT reagent, deficient plasma and calibration plasma. For both assays there is an inverse

relationship between level of activity and between-centre CV (ie higher CV at lower activity levels) - with CVs currently around 10-15% for normal levels. The adoption of the International Unit for Factor XI after 2005 led to an improvement in between lab CV from an average of 21% to an average of 14%.

Coen Maas (Utrecht, the Netherlands) introduced the COSYNE (COntact SYstem NEtwork) program, which is supported by the SSC. There are a multitude of emerging therapeutic strategies to block the contact system, but it remains challenging to directly demonstrate contact system activation in the plasma of (thrombosis) patients. This is for a large part attributable to the absence of generally available and standardized detection methods. Maas recently developed nanobody-based ELISA strategies to detect contact system activation in human plasma. The aim of the COSYNE project is to disseminate reagents and protocols of these assays for external validation and standardization.

In this session, he hopes to identify additional parties that are willing to contribute to the COSYNE initiative and to receive input on the approach of assay standardization.

Stephanie Smith (Ann Arbor, USA) discussed the use of polyphosphates, and the pitfalls in purifying DNA with silica-based columns that leach silica that has strong procoagulant effects. She provided practical tips on how to handle DNA and polyphosphates.

Paul Kim (Hamilton, Canada) reported on two commercially available methods that can be used to isolate DNA without the use of silica: 1) QIAGEN PAXgene (Mississauga, Canada), and 2) QuickGene DNA kit (Kurabo Industries, Osaka, Japan). The resulting DNA appears to be free of any silica or functionally detectable levels of polyphosphate contamination, and the procoagulant effect they exert can be ameliorated with DNase I. Use of these kits to isolate DNA from the whole blood are ideal for investigating the role of DNA in various biochemical processes.

Thomas Renné (Hamburg, Germany) discussed the inorganic polymer polyphosphate that has been recognized as important mediator of contact system´s and factor XI´s activities. PolyP triggers factor XII contact activation in vivo and propagates various other procoagulant pathways in vitro and in plasma systems. Furthermore, the polymer has the capacity to modulate immunologic and inflammatory reactions.

His presentation reviewed the literature, discusses tools and technology to analyze polyphosphate and standardize their activities, and identifies open questions in the emerging field of polyphosphate that require future research.

We had a lively business meeting with a lot of discussion and comments from the audience. Two potential projects came out of the discussions after the talks. Coen Maas will lead an initiative to come up with practical tools, standardization and the do’s and don’ts of dealing with

polyphosphates. He will form a working group with Thomas Renné and Stephanie Smith to come up with a proposal that can be discussed in next year's business meeting.

Joost Meijers will lead an initiative to investigate if it is feasible to standardize the coagulation assays for prekallikrein and high molecular weight kininogen. These assays are performed in relatively few laboratories in the world, and using the EQA surveys (Piet Meijer/Steve Kitchen) it should be established if sufficient participating laboratories can be identified that would like to join an initiative for standardization. In addition, Helen Wilmot and Craig Thelwell from NIBSC will be involved.

Factor XIII and Fibrinogen

8 July 2019
16:30 – 18:30

Chairman: Verena Schroeder

Co-Chairs: Zsuzsa Bagoly, Anetta Undas, Sanj Raut, Martin Guthold, Munira Borhany, and Alessandro Casini

The session was chaired by Verena Schroeder and Zsuzsa Bagoly.

The session was dedicated to the memory of Prof. László Lóránd, the co-discoverer of factor XIII (also referred to as Laki-Lorand factor).

Prof. Lóránd passed away most recently on December 6, 2018. He was 95 years old. He was an internationally recognized biochemist known for his landmark discoveries in the field of hemostasis. He authored more than 200 publications and he was the recipient of numerous awards and honorary degrees throughout his career. He was a highly respected educator and mentor, whose presence will be missed in our scientific community.

László Lóránd (1923-2018)

Standardization topics

Sanj Raut (NIBSC, Potters Bar, UK): Update on the collaborative study to additionally assign value for total factor XIII-B subunit to the WHO 1st IS for FXIII Plasma (02/206). (Presented by Verena Schroeder on behalf of Sanj Raut.)

A Collaborative Study to Additionally Assign Value for Total Factor XIII-B Subunit Antigen to the WHO 1st International Standard for Factor XIII Plasma, (02/206)

Sanj Raut[1], Éva Katona[2], Carmen Coxon[1], László Muszbek[2], Verena Schroeder[3] & Peter Rigsby[1]

[1]NIBSC, UK; [2]University of Debrecen, Hungary; [3]University of Bern, Switzerland

A collaborative study was undertaken to value assign the current WHO 1st International Standard (IS) Factor XIII (FXIII) Plasma for Total FXIII-B subunit, relative to locally collected normal plasma pools. Laboratories were instructed to use a validated method (specific ELISA antibodies provided) for assessment of Total FXIII-B subunit antigen potency. All laboratories used this method with one laboratory using an additional in-house method also. Nine (9) data sets were received from 7 laboratories (37 assays in total), which provided a total of 35 valid estimates for this new assignment. Total FXIII-B subunit estimates were calculated relative to locally collected normal plasma pools, using an arbitrary value of 1.00 unit of Total FXIII-B subunit per ml, for each pool. Combination of results produced an overall mean of 0.98 units/ml with an inter-laboratory variability (GCV%) of 18.3% [95% confidence interval: 0.86 - 1.11].

Proposal: It was proposed that the current WHO 1st International Standard (IS) Factor XIII (FXIII) Plasma (02/206) be additionally assigned with a Total FXIII-B subunit antigen potency of 0.98 IU/ml. This proposal was endorsed by the Participants, SSC Experts and by the SSC Board.

Matthew Locke (NIBSC, Potters Bar, UK): The WHO 3rd International Standard for Thrombin

The current 2nd IS for Thrombin (coded 01/580) was established in 2002 and unified the US “NIH” unit and International Unit (IU), which until that point were similar, but not identical. 01/580 is distributed worldwide by NIBSC on behalf of WHO and is used by manufacturers to calibrate thrombin reagents in a variety of applications, including fibrin sealant kits, clinical diagnostics, and hirudin-based anticoagulants. Stocks of 01/580 are now running low, and a replacement is required.

The proposed international collaborative study will calibrate the potency of a candidate human alpha-thrombin preparation donated by a manufacturer, relative to the 2nd IS. Potencies will be determined using chromogenic and/or clotting assays. Formulation and freeze-drying of the candidate will be based on the 2nd IS, which has an excellent long-term stability profile. An alternative candidate preparation from the 2002 study (coded 01/578), will also be included in the proposed study, and its potential use as a secondary working standard will be evaluated following

recalibration in this exercise. The study is currently recruiting participants, and laboratories with appropriate expertise were encouraged to contact NIBSC to register an interest in participating.

Scientific topics

Fraser Macrae (University of Leeds, UK): Fibrin films and their future implications

Haemostasis requires the conversion of fibrinogen to fibrin, resulting in the formation of a fibre network that interacts with blood cells, preventing blood loss. Extensive research has been carried out on the fibrin network, but the structural characteristics of the exterior face of the blood clot are currently unknown. Electron, confocal and atomic force microscopy, immunohistochemistry and Langmuir-Blodgett trough were used to investigate the blood-air interface of blood clots in-vitro. A murine dermal injury model was used to investigate in-vivo film formation and its role in preventing bacteria proliferation and migration. We uncovered a remarkable new aspect of blood clotting where fibrin transitioned to the blood-air interface, producing a hitherto undisclosed protective film covering the clot. Film formation occurred at the same rate as the fibre network, connecting to the underlying clot network through tethering fibres. It was digested by plasmin at a similar rate as fibrin fibres and formation was prevented with surfactants and oil. The film retained erythrocytes within the clot in-vitro and in-vivo. It also acted as a critical immediate host-protection mechanism, preventing bacterial invasion and proliferation in Boyden chambers and in murine dermal puncture wounds. Further research is required to understand if these films play a role in wound healing and thrombosis.

Laszlo Muszbek (University of Debrecen, HU): Factor XIII and platelet microparticles

We monitored the surface exposure of FXIII-A and phosphatidylserine (PS) on platelets undergoing receptor-, and non-receptor mediated activation and on the formed microparticles (MPs) using flow cytometry and scanning fluorescence microscopy. After stimulation of platelets with dual agonist (CVX+thrombin) 35% of platelets and more than half of MPs became PS positive as detected by Annexin V. Such receptor mediated activation also induced the transposition of cFXIII-A to the outer membrane surface in 66% of platelets and 65% of MPs. The overwhelming majority of PS-positive platelets and MPs also showed FXIII-A positivity. Non-receptor mediated activation triggered by Ca^{2+}-ionophore resulted Annexin V positivity of platelets in a concentration-dependent manner. Annnexin V positivity of MPs was even more considerable than that of activated cells. However, in case of Ca2+ ionophore activation neither the cells nor the formed MPs expressed FXIII-A on their surface to any significant extent. The increase of intracellular Ca^{2+} concentration was not sufficient to induce the surface exposure of cFXIII, hence other mechanisms induced by receptor mediated activation are also required. Our results indicate that two types of platelet MPs are produced, FXIII-A-positive MPs formed during platelets stimulation by CVX+thrombin and FXIII-A-negative MPs formed during stimulation by Ca^{2+}-ionophore.

Alessandro Porrello (University of North Carolina, Chapel Hill, US) Factor XIII-A in lung squamous cancer

Lung cancer is the leading cause of cancer-related deaths worldwide, and lung squamous carcinomas (LUSC) represent about 30% of these malignancies. Using computational and experimental methods, we found that there is a subset of LUSC tumors characterized by dense infiltration of inflammatory monocytes (IMs); these patients have particularly poor survival. We also generated the first murine immunocompetent metastatic model for LUSC, which was used to validate our computational results and to understand some steps of the mechanism used by IMs. Tumor cell derived CCL2-mediated recruitment of IMs was found to be necessary and sufficient for LUSC metastasis. The pharmacologic inhibition of IM recruitment has strong anti-metastatic effects and shows therapeutic potential. As for the biological mechanism, we determined that IMs express high levels of Factor XIIIA, which promotes fibrin cross-linking to create a scaffold that sustains LUSC cell invasion and metastasis. Consistent with this result, human LUSC samples containing extensive cross-linked fibrin in the tumor microenvironment have worse survival than samples for which this feature is less pronounced.

John Weisel (University of Pennsylvania, Philadelphia, US): Factor XIII structure: organization of B subunits and changes with activation studied with single-molecule atomic force microscopy

Factor XIII Topology: Organization of B Subunits and Changes with Activation Studied with Single-Molecule Atomic Force Microscopy

A. D. Protopopova, A. Ramirez, D. V. Klinov, R. I. Litvinov, J. W. Weisel

Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

Factor XIII (FXIII) is a precursor of the blood plasma transglutaminase (FXIIIa) that is generated by thrombin in the presence of Ca^{2+} and covalently cross-links fibrin to strengthen blood clots. Inactive plasma FXIII is a heterotetramer with two catalytic A subunits and two non-catalytic B subunits. Inactive A subunits have been characterized crystallographically, while the atomic structure of the entire FXIII and B subunits is unknown and the oligomerization state of activated A subunits remains controversial. Our goal was to characterize the (sub)molecular structure of inactive FXIII and changes upon activation. Plasma FXIII, non-activated or activated with thrombin and Ca^{2+}, was studied by single-molecule atomic force microscopy, in which all protein samples were adsorbed on a highly oriented pyrolytic graphite coated with an amphiphilic graphite modifier. Additionally, recombinant separate A and B subunits were visualized to compare with their conformations and dimensions in FXIII and FXIIIa. We showed that heterotetrameric FXIII forms a globule composed of two catalytic A subunits with two flexible strands comprising individual non-catalytic B subunits that protrude on one side of the globule. Each strand corresponds to 7-8 out of ten tandem repeats building each B subunit called sushi domains. The remainder were not seen, presumably because they were tightly bound to the globular A_2 dimer.

Some FXIII molecules had one or no visible strands, which is accounted for by the dissociation constant and partial wrapping of the B subunit around the globular core. After activation of FXIII with thrombin and Ca^{2+}, B subunits dissociated and formed B_2 homodimers, while the activated globular A subunits dissociated into 2 monomeric A subunits. These results characterize the molecular organization of FXIII and changes with activation.

Munira Borhany (National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, PK): Congenital factor XIII deficiency in Pakistan - challenges and chances

Factor XIII (FXIII) deficiency is a rare bleeding disorder (RBDs) with an incidence of about one in 1–2.5 million but its incidence is higher in populations with consanguineous marriages. As a result of these marriages, rare autosomal recessive disorders run in close families and tribes. At National Institute of Blood diseases and Bone Marrow Transplantation (NIBD) around 600 patients were diagnosed with inherited bleeding disorders among them 125 subjects had RBDs (20.8%). Among RBDs FXIII is the most common (30%). The aim of this study was to characterize patients and relatives from twenty nine families with suspected FXIII deficiency from Pakistan and to identify the clinical characteristics and underlying mutations. The patients' medical histories were recorded in a questionnaire. As the first indicator of FXIII deficiency, a 5M urea clot solubility test was used. Plasma FXIII A- and B-subunit antigen levels were determined by ELISA. FXIII activity was measured with an incorporation assay. Sequencing of all exons and intron/exon boundaries of F13A was performed. A total of 54 patients from 29 families were enrolled out of which 25 (46.3%) were females and 29 (53.7%) males. Age at first presentation ranged from birth to 18 years. Every patient had a history of consanguineous marriages except for one. 50% had significant family history of bleeding. 19 first-degree relatives with mean FXIII level 71.19 ±21.1 were asymptomatic. 35 patients had severe type FXIII level <5%, 17 patients of moderate type. Majority patients have grade II and grade III bleeding severity. Umbilical cord bleeding, intracranial bleed, gum bleed, hematoma, bruises, hemarthrosis, abortions and menorrhagia, circumcision were the main clinical manifestations. So far we have analyzed 18 families (n=43 patients) for the presence of FXIII deficiency and identified 22 mutations. This includes Missense mutations, Splicing mutations and Nonsense mutations with 7 novel mutations published. Fresh frozen plasma / cryoprecipitate were used in the management of most patients and for weekly prophylaxis in 3 patients with grade III bleeding. No FXIII concentrate is available in Pakistan.

In conclusion, we have analyzed a cohort of 43 individuals and identified 22 mutations leading to congenital FXIII deficiency. To our knowledge, this is the first largest data on FXIII-deficient patients from Pakistan. Diagnosis of FXIII deficiency should be made on time so that prophylaxis can be initiated immediately to prevent fatal bleeding and for genetic counseling.

Fibrinolysis

9 July 2019
16:30 – 18:30

Chairman: Paul Kim

Co-Chairs: Krasimir Kolev, Colin Longstaff, Victoria Ploplis, Guy Reed, Tetsumei Urano, and Tine Wyseure

Update and proposal of standardization projects

Matthew Locke, NIBSC, UK

Matthew Locke presented an update of WHO standardization projects from the National Institute of Biological Standards and Control (NIBSC).

Streptokinase: An overview of the completed international collaborative study to establish the 4th International Standard (IS) for SK was provided. The study included 15 laboratories (regulatory, industrial and academic), spanning nine countries. Participants were asked to perform 4 independent plasminogen activation assays, using either clot lysis or chromogenic methods. Nine laboratories performed clot lysis and eleven chromogenic, contributing 69 independent assays in total. The potencies of two candidate materials, 16/356 and 16/358 (native therapeutic SK products), were calculated relative to the 3rd IS (00/464) using parallel line analysis. The results showed low variability between laboratories (geometric coefficient of variation (GCV) <10%) and good agreement between assay methods, allowing potency estimates to be combined for all assay methods. Stability testing by accelerated degradation predicted excellent long-term stability of both candidates. Based on slightly lower GCVs and better agreement between assay methods, 16/358 was proposed as the 4th IS with a potency of 1013 IU/ampoule. This was agreed by all study participants and endorsed by a panel of SSC experts with no objections raised. The proposal will be submitted to WHO ECBS for establishment October 2019.

PAI-1: The current 1st IS for PAI-1 activity in plasma (92/654) requires replacing due to age and potential stability issues. While the IS is not sold in large numbers, it is nevertheless used by manufacturers of PAI-1 activity kits, which are broadly divided into bioimmunoassays (tPA or uPA capture) or chromogenic (tPA/uPA:PAI-1 titration). The IS consists of CHO cell expressed PAI-1 added to pooled plasma (15-20 % active, ~250 ng/ml), filled and freeze-dried in 1992. A small-scale study will examine the suitability of *E.coli* expressed PAI-1 (~ 70% activity) added to plasma as a replacement for the 1st IS, using formulations with and without excipients (HEPES/trehalose). Preliminary data suggests HEPES/trehalose results in better recovery of PAI-1 activity after freeze-drying. The study is currently recruiting participants, and laboratories with the necessary expertise were encouraged to register their interest with NIBSC. In addition, laboratories with expertise in isotope dilution mass spectrometry are being sort, to help with potential antigen assignment in the future.

D-dimer: The next phase of the D-dimer project will determine the suitability of heterogenous pool of D-dimer patient plasma (purchased from CliniSys Associates, USA) and synthetic D-dimer

added to normal pooled plasma, as IS. Formulations will include trehalose, which markedly improves D-dimer stability.

SSC Project Update: Validation of the t-AUCi Parameter Using ROTEM to Assess Fibrinolytic Resistance in Septic Patients

Ecaterina Scarlatescu, Institutul Clinic Fundeni, Romania

Detection of subtle changes in fibrinolysis may be important to the clinical management of critically-ill septic patients as increased resistance to fibrinolysis is associated with increased mortality. Viscoelastic tests (VET) have a limited sensitivity for fibrinolysis diagnosis, detecting overt fibrinolysis by the decrease of clot firmness related to the maximum clot amplitude. Resistance to fibrinolysis is more difficult to diagnose using clot lysis indices, as in most clinical situations characterized by low-grade fibrinolysis the decrease in clot firmness after reaching the maximum amplitude is not visible during the limited measurement time. However, coagulation and fibrinolysis are processes that overlap in time, and, as fibrinolysis begins before the clot reaches its maximal firmness, it should also be reflected by the kinetics of clot formation before reaching the point of maximal amplitude.

Based on this assumption, in our study we calculated a new early kinetic parameter (t-AUCi) represented by the portion of time required to reach maximal clot amplitude after maximal clot formation velocity has been reached. The main objective of the study is to evaluate the correlation between the degree of fibrinolytic activation and the newly calculated parameter (t-AUCi), by using standard and tPA spiked ROTEM analysis in patients with sepsis and in healthy controls. Preliminary results in a sample of healthy volunteers confirmed the decrease of t-AUCi value with increasing clot lysis triggered by incremental concentrations of tPA in spiked ROTEM traces. However, these results need to be confirmed in the entire study population by ROTEM analysis and by the measurement of plasmatic markers of fibrinolytic activity such as D-dimer, plasmin-α2-antiplasmin (PAP) complex, and plasminogen activator inhibitor 1 (PAI-1) levels.

Trauma Induced Coagulopathy: The Need for Nomenclature Standardization

Satoshi Gando, Sapporo Higashi Tokushukai Hospital, Japan

Trauma, sepsis, and cardiac arrest and resuscitation are three major insults in the field of acute and critical care medicine. To standardize the nomenclature of trauma-induced coagulopathy, we must first acknowledge that all three insults bring about the same non-specific body responses, known as innate immune inflammation, which is tightly coupled with coagulation and fibrinolysis. In other words, all three insults elicit the same changes in coagulation and fibrinolysis. After obtaining agreement on this principle concept, we can move on to next step, where the following points should be discussed in order to achieve nomenclature standardization for trauma-induced coagulopathy:

1. Understanding the differences between primary and secondary changes in coagulopathy. The essential discussion point is the pathomechanism of trauma-itself-induced primary coagulopathy.
2. Understanding the differences between physiologic local coagulation and pathologic dysregulated coagulation responses. Trauma-induced coagulopathy is a dysregulated coagulation response to severe trauma.
3. Understanding the differences in coagulation between inside and outside vessels. Hypercoagulation inside vessels and hypocoagulation outside vessels, namely at the site of injury, should be recognized.
4. Understanding the time courses in coagulation and fibrinolysis after insults. One-point blood sampling is not enough to elucidate the truth.
5. Understanding the differences between increased fibrinolysis and suppression of fibrinolysis. Recognizing the undeniable fact that increased coagulation always underlies changes in fibrinolysis.

For nomenclature standardization, several points should be also kept in mind. For more than two decades, we have recognized that thrombin plays a central role in thrombosis and hemostasis (Semin Thromb Hemost 2001; 27:619, Nat Rev Dis Prim 2016; 2:16037). Thrombin acts as both procoagulant/antifibrinolytic and anticoagulant/profibrinolytic factors that giving rise to thrombosis and bleeding, respectively. In addition to these discussion points, a brief historical perspective concerning trauma-induced coagulopathy will be reviewed in this presentation.

Finding Consensus in Defining Trauma Induced Coagulopathy: Part 1

Hunter Moore, University of Colorado, USA

Hunter Moore highlighted some key areas in which are crucial for achieving consensus in defining trauma induced coagulopathy from a goal-oriented treatment perspective. These views included the importance of being able to accurately diagnose the state of a patient using rapid measurement methods, namely viscoelastic hemostatic assay (VHA). He also addressed that tranexamic acid (TXA) should be given to all within the first 3 hours of injury, especially in countries of low socioeconomic status that may not have accessibility to a state-of-the-art trauma center. He focused on the need for identifying the underlying mechanism of why different patients respond differently to TXA.

Finding Consensus in Defining Trauma Induced Coagulopathy: Part 2

Beverley J. Hunt, King's College London, United Kingdom

Beverley Hunt discussed areas that require attention in order to achieve a consensus statement in defining trauma induced coagulopathy from the perspective in which TXA should be

administered to all trauma patients within 3 hours of injury, a widely accepted standard of care based on CRASH-2 randomized clinical trial. She highlighted the difficulties in utilizing VHA to diagnose the fibrinolytic state of patients due to the inherent properties associated with VHAs – largely not sensitive enough with questionable reproducibility. Therefore, it is important to keep in mind these apparent physical limitations of VHA when proposing to identify subgroups of patients based on these test results inferring their fibrinolytic state as a diagnostic tool.

Bridging the Gap to Find Consensus on Trauma Induced Coagulopathy Nomenclature: Updates from the Tranexamic Acid/Trauma Symposia

Dominik F. Draxler, University of Bern, Switzerland

In my presentation, I will summarize the main conclusions gained from the “TXA in trauma” symposium, held on the 5th of July prior to the ISTH Congress. In this meeting international experts in trauma surgery, anesthesia, intensive care, emergency medicine and hematology will discuss the open questions around the use of TXA in trauma patients. The exiting difference in guidelines around the globe will be addressed as well as considerations for specific trauma patient populations. Trials on TXA in indications other than trauma will also be presented to extract information, which may be relevant to the trauma patient population. Moreover, the role of fibrinolytic phenotypes trauma patients can present with, in the decision-making in trauma resuscitation will be discussed, with a particular focus on the recent research on “fibrinolysis shutdown” and “occult fibrinolysis” as well as the relevance of viscoelastic coagulation assays in guiding resuscitation measures. This meeting aims to facilitate a direct discussion of current discrepancies in the interpretation of available evidence, to identify any gaps in knowledge, which may be responsible for these differences, and how these can be filled through future research.

Genomics in Thrombosis and Hemostasis

7 July 2019
16:30 – 18:30

Chairman: Kathleen Freson

Co-Chairs: Daniel Bellissimo, Paul Bray, Kathleen Freson, Anne Goodeve, Michele Lambert, Pieter Reitsma, and Willem Ouwehand

Introduction

Kathleen Freson

A brief overview was given of previous and current projects performed by GinTH.

- Publication of a curated diagnostic grade gene list in JTH (Megy et al, Curated disease-causing genes for bleeding, thrombotic, and platelet disorders: Communication from the SSC of the ISTH. JTH 2019 or available online via **https://www.isth.org/page/GinTh_GeneLists**

- Ongoing: Building a RedCap-based Gold Variants database for curated gene variants

- Foreseen: Guidance document on Ethics and Consenting of NGS testing

This SSC session provides updates from rare versus complex genetics for bleeding and thrombosis. Both fields now also explore variants that have moderate impacts on bleeding and thrombosis. We expect that polygenic risk scores will be required to estimate the effect of variation on disease.

Part 1. Genetics for Rare Bleeding and Platelet Disorders

Discussion leader: Michele Lambert

Tier1 Genes and Gold Variants for Bleeding, Thrombotic and Platelet Disorders

Karyn Megy

Setup of a panel test by delivering a curated gene list of diagnostic grade genes (TIER1) using three criteria (level 1, 2, 3), mutation mechanism added to the gene as specific mutations can have different effects. A specific transcript was chosen for each gene (EBI-LRG collaboration). Gene and variant curation will be the main task of the ClinGen working group in the future but a fast implementation of a diagnostic grade gene list was immediately required.

The ThromboGenomics V2 paper showed that at least 50% of gene variants found are novel, requiring a database to submit these variants to inform the community about the scoring of these variants. A RedCap based Gold variant database is under development and will ask for a specific set of entries to take up each variant with their evaluation (PV, LPV, VUS and benign). Sharing of such curated variants to the community will assist future genetic diagnostic studies and their variant interpretation. We expect to present a first version of this database at the ISTH congress in Milan 2020.

Update TIER1 genes. IKZF5 was presented as cause for thrombocytopenia in 5 unrelated pedigrees with strong co-segregation data. Pathogenic variants are located in DNA-binding zinc fingers. A functional test that measures DNA binding of mutant IKZF5 zinc fingers can be used to validate variant pathogenicity. This gene will be added to **https://www.isth.org/page/GinTh_GeneLists**

The ClinGen clinical domain Working Group (CDWG) for Thrombosis and Hemostasis

Jorge Di Paola.

The ClinGen CDWG for thrombosis and hemostasis is chaired by Nigel Key, Jonathan Berg and Kristy Lee. An overview of the different WGs in ClinGen and their working scheme was presented. For more information see https://clinicalgenome.org/working-groups/clinical-domain/hemostasis-thrombosis/

The ClinGen platelet disorder WG started 1 year ago and consists of 22 members, 14 institutions, 5 countries. Progress of the variant curation work by the Platelet Disorder VCEP from June 2018 to 2019 was illustrated. They have tested 60 variants related to Glanzmann Thrombasthenia (GT) as proof of principle. This pilot study will finish in end of Summer to propose a curated gene variant list.

Some discussion on expert groups for gene and variant curation that should contain members with expertise and include:

- clinical phenotype group
- functional group
- computational group

Diagnostics of Inherited Thrombocytopenia: A Clinical Perspective

Patrizia Noris

Clinicians approach a thrombocytopenia (TP) patient via investigating the family history and performing a physical examination, followed by lab testing (PLT count, CBC, FACS, Aggregation, DNA testing). Important question is whether to test all genes or a restricted group of genes

(currently 26 genes for TP via Panel, WES or WGS). Consenting is a big issue because some genes for TP cause leukemia (ETV6, ANKRD26 and RUNX1).

When is it especially relevant to perform genetic testing? 1. When the variant changes the clinical management e.g. MYH9 where gene-phenotype correlation is well studied. 2. When a bone transplant is required and the genetic information of the donor is relevant to know e.g. RUNX1. 3. For TP patients at risk for (or already with) bone marrow failure (variants in GALE, MPL, MECOM) as such patients can be treated with bone marrow transplantation; conversely, a similar clinical picture related to THPO variants can be treated by TPO-receptor agonists. 4. Patients with severe WAS mutations that can be treated with bone marrow transplantation. 5. If ITP or MDS therapy fails, it is highly relevant to know the genetics. Example of a TP case that had intense treatment for ITP and MDS, while during recall for genetic testing it was discovered that he was member of a dominant TP family and the genetic test was positive for an ANKRD26 variant. 6. Parents can be very keen that genetic testing is performed for their child e.g. for the PTPRJ case.

Ethical Considerations When Applying Gene Panel Tests for Platelet Disorders

Andreas Greinacher

Pro's for genetic testing were mentioned (decreasing costs, improved quality, centralized labs) but NGS testing raised ethical concerns. These include: 1. Disconnect between patient/treating physician and reference center specialist. 2. Treating physician often has difficulties to interpret the findings. 3. Informed consent procedure is often lacking information or too complex (patients often do not understand what they are signing). 4.The reason for testing is often not clearly defined.

In contrast to coagulation disorders where the correct diagnosis is essential for selection of treatment, there are limited treatment options for platelet disorders. Confirmative NGS testing in most cases doesn't change clinical management for platelet disorders (except for Glanzmann thrombastenia, WAS, CAMT, and MYH9 disorders). One can also perform NGS testing to better understand the underlying clinical problem (this is part of research). This issue should be clearly discussed with the patient before performing the genetic test.

Concluding remark: This SSC could develop a "template informed consent document" for adults (and children), addressing also the diverse issues of performing NGS panels for bleeding and platelet disorders.

Ethical concerns for NGS testing include: 1. Over-interpretation of NGS results must be prevented. It is still not known how strong the leukemia risk is for variants in RUNX1, ETV6 and ANKRD26 (10-30%). Genotype-phenotype relations for these genes are not well known. It is disputable whether NGS testing in TP patients for these genes are always the best option. The confirmation of a variant in those genes doesn't change the clinical management. The genetic test is still costly and more important the results may cause distress. 2. What to do with the many variants of unknown significance? These are not reported for clinical teams. 3. Interpretation of

genetic findings requires good phenotype characterization but a bleeding phenotype and function test alterations can be rather unspecific.

Lively discussion followed the presentation with following topics:

1: If ITP treatment is ineffective, it's useful to test for TP as inappropriate treatment can harm.

2: Besides MYH9 and Glanzmann Thrombasthenia for which it's useful to know the causal variant, a differentially diagnosis for VWDtype2B or Platelet type VWD is useful as it changes therapy (providing VWF or platelets to patients, respectively).

3: Knowledge of predisposition to malignancy gene variants present in donors is very important when doing a bone marrow transplant. Answer can be that the genetic test itself can be included in the test for "matching" that is done anyway before any bone marrow transplant.

4: It was pointed out that knowing a variant in RUNX1 might help in management in later follow-up time points

5: NGS Panel test requests should be submitted by experts only and the SSC should guide who and when to order such test. Consent should clearly inform for what we test, and how it will change management.

Part 2. Genetics for Complex Bleeding and Platelet Disorders

Discussion leader: David Trégouet

French Genomics Initiatives for Rare and Common Venous Thrombosis

Pierre Morange

Venous Thrombosis (VT) is a complex & chronic disease but some families present with strong inheritance of VT caused by rare variants. Common variants (small impact size for VT) have been found in ABO, FGG, F11, PROCR while rare high impact variants have been detected in AT, PC, PS, FV Nara, FV Padua, MYH genes and moderate variants (in between common and rare) for VT are FVL and PT20210.

The Meta-GWAS INVENT study has identified multiple genes (7500 VT cases – 52600 controls) that are linked to VT and 9 genes (known loci: *ABO*, *F2*, *F5*, *F11*, *FGG*, *PROCR* and novel loci *TSPAN15, ZFPM2* and *SLC44A2*) that have reached genome wide significance (Germain et al. AJHG 2015). Meta-GWAS INVENT further increased the sample size (0,324 VT and 172,122 Controls) and 15 additional loci have been discovered for VT.

An introduction was given to the FAMILY project (INNOVTE) to detect rare variation causing VT. It was shown that rare and private mutations do also contribute to VT (rare VT forms that are familial) as now studied by WES instead of GWAS. If pedigree is informative, rare variant selection can be performed via sequencing more individuals. An example was given for a large pedigree

with a private MAST2 missense variant. Affected family members have decreased levels of tissue factor pathway inhibitor (TFPI) plasma levels while increased plasma levels of plasminogen activator inhibitor-1 (PAI-1).

Findings of the GenMed consortium were discussed (WGS of 200 unrelated patients from the MARTHA project with unprovoked VT and negative thrombophilia screening). WGS of VT at young age with selection of variants with MAF <1%. This study has identified 37 non-coding variants in known VT genes. In addition, variants were detected with a coding effect in a known VT gene but with an expected effect e.g. E227K variant in SERPINC1 in a patient with normal AT levels that indicatives that the lab test is not always perfect. This missense had an effect on AT glycosylation.

Whole Genome Sequencing Study for Platelet Function

Andrew Johnson

WGS was performed to identify genetic variation involved in PLT function phenotypes (with focus on PLT aggregation testing). In contrast to modifiers of platelet size and count (GWAS using millions of samples), large studies related to platelet function (few thousands of samples) didn't exist and are more difficult to perform. Hyper-reactive platelets result in an increased risk for CVD.

TOPMed data have increased the number of genotype variants from 2.2 to 22 M variants. More specifically 3,855 TOPMed individuals that underwent phenotyping for platelet aggregation were WGS and 16 loci have been identified (MAF<0.5). Blueprint data were used for non-coding discoveries. Data available from BioRiv see https://www.biorxiv.org/content/10.1101/621565v1

14 loci are novel and have no known role in platelets while for ADRA2A, RGS18, PEAR1 and SVEP1 some evidence was available.

RGS18 has a MAF of 45% and has an effect on epinephrine aggregation. RGS18 KO mice already exist and have TP and altered bleeding times.

SVEP1 (has VWF binding domain) and its variant is expected to be a gain of function variant that would be associated with higher protein levels. This gene was previous identified as coronary artery disease risk factor.

Complex Genetics for Platelet Traits

Willem Ouwehand

This domain clearly illustrates the fusion of the two genetic worlds being rare and common genetics. Discoveries led by Nicole Soranzo at the Wellcome Sanger Institute in Cambridge.

Data from the 0.5 million participants in the UK BioBank have been used to assess their predictive power in a combined polygenic score (being a predictive power of the cumulative genetic burden of associated variants) on blood cell indices. The ultimate goal is to achieve an evaluation of the contribution of polygenic variation (including both common and rare variants) in health and disease. Such initiative builds a bridge between the Mendelian monogenic approach and the complex disease polygenic approach. Because of the large sample size of this study, it was found that a rare pathogenic variant with an assumed autosomal recessive mode of inheritance in heterozygosity state can still cause a (mild) phenotype effect (these are the parental carriers of recessive diseases that have previously mostly been identified as having not phenotype). Examples given for TUBB1 and MPL where carriers of heterozygous variants have altered platelet distribution width and plateletcrit, respectively. These are typically low effect size variants on phenotypes.

Moreover, the integration of a polygenic risk score has to be integrated with the rare mutational events and this will determine your true phenotype. It could be expected that the polygenic background will determine the true impact of disease outcome and impact of a monogenic disease. This is highly relevant for medication use and its development. We should look at the polygenic load, then superimpose the rare variants, and assess phenotype.

Genetic Control of Platelet Gene Expression

Len Edelstein

eQTLs (expression Quantitative Trait Loci) are genomic loci that are associated with the expression level of mRNAs. eQTL are useful to determine the effect of non-coding variants on gene expression. A previous study has studied the platelet cis-eQTL landscape of 612 genes (Simon et al, AJHG, 2016). Platelet trans-eQTLs were detected for 113 gene using an MPRA (Massively Parallel Reporter Assay) assay where alleles get connected to a barcode and next cloned, transfected and analyzed by RNAseq of barcodes connected transcripts. Complex analysis has characterized functional variants in super-enhancers that are physically located in intronic regions. Functional platelet eQTLs (mostly represented by rare variation) are more likely to be in introns. An example was provided for GP6 SNPs that are associated with MPV and GP6 expression and collagen reactivity. A non-coding SNP in the first intron of GP6 was identified that altered ETV6 binding site and mediated a difference in GP6 expression.

Hemostasis and Malignancy

6 July 2019
14:30 – 16:30

Chairman: Marc Carrier

Co-Chairs: Cihan ay, Christophe Dubois, Guy Meyer, Casey O'Connell, Jeffrey Zwicker, and Tzu-Fei Wang

Minutes - SSC Hemostasis & Malignancy meeting

Introduction/Welcome

Dr. Carrier (Canada) started by welcoming the audience an introduced the outline of the meeting as well as the new chairman Dr. Jeff Zwicker.

Guidance documents

Atrial Fibrillation
Dr. Delluc (Canada) provided an update on the new guidance document from our committee on the management of anticoagulation in cancer patients with nonvalvular atrial fibrillation receiving chemotherapy. Dr. Delluc emphasized the incidence of atrial fibrillation in cancer patients and reviewed the data summarizing the efficacy, safety, and potential drug-to-drug interaction of the different anticoagulation with chemotherapy regiments. The SSC Provided the following guidance statement.

- We recommend individualized anticoagulation regimens after shared decision making with patients, based wherever possible on risk of stroke, bleeding, and patient values.
- In cancer patients with NVAF already on an anticoagulant regimen before starting chemotherapy, we recommend continuing the same anticoagulation regimen unless there are clinically relevant drug-drug interactions.
    a. In cancer patients on chemotherapies with clinically relevant VKA interactions, we suggest considering a DOAC if no additional drug-drug interactions with DOAC or close monitoring of VKA (target international normalized ratio between 2 and3).
    b. In cancer patients on chemotherapies unable to tolerate an oral route of administration (e.g. nausea and vomiting), we suggest the use of parenteral anticoagulation with therapeutic dosing of LMWH with resumption of oral anticoagulation as soon as possible.
- In cancer patients on chemotherapy with newly diagnosed NVAF, with the exception of patients with luminal gastrointestinal cancers with an intact primary or patients with active gastrointestinal mucosal abnormalities such as duodenal ulcers, gastritis, esophagitis, or colitis, we suggest the use of a DOAC over a VKA or LMWH as anticoagulant therapy if no clinically relevant drug-to- drug interactions are expected.

The manuscript was accepted for publication and published in the August17th issue of the society's Journal.

Primary thromboprophylaxis

Dr. Wang (United States) reviewed the recent randomized controlled trials assessing the role of oral anticoagulation for primary prevention among cancer patients receiving chemotherapy in the ambulatory setting. The SSC also provided the following guidance statement.

1) We suggest the use of DOACs as primary thromboprophylaxis in ambulatory cancer patients starting chemotherapy with Khorana score ≥ 2 in patients with no drug-drug interactions and not at high risk for bleeding (such as patients with gastro-esophageal cancers). Currently, apixaban and rivaroxaban are the only DOACs with evidence from RCTs. A final treatment decision should be made after considering the risk of both VTE and bleeding, as well as patients' preference and values.
2) We suggest that if DOACs were to be used for primary thromboprophylaxis in ambulatory cancer patients, it is administered for up to 6 months after the initiation of chemotherapy. We recommend monitoring of platelet counts and risk of bleeding complications while on anticoagulation.
3) In high-risk ambulatory cancer patients where primary thromboprophylaxis is planned but with concerns for safety of DOAC (such as in patients with concern of drug interaction or high risk of gastrointestinal bleeding), we suggest using LWMH.

The manuscript was accepted for publication and published (epub ahead of print) in the JTH.

Asparaginase

Dr. Zwicker summarized the literature on the prevention and management of VTE among ALL patient receiving chemotherapy. This was previously identified by the committee as an important knowledge gap to provide clinicians with clinical guidance. Based on currently best available evidence on the management of acute VTE in adults with ALL, Dr. Zwicker and the SSC formulated the following statements:

1) We suggest LMWH for the acute treatment of VTE related to asparaginase therapy if severe thrombocytopenia (i.e. platelet count < 50 x 109/L) is anticipated. Following resolution of severe thrombocytopenia, DOACs may be considered in the absence of other relative contraindications such drug-drug interactions (e.g. azole-antimycotics).
2) We recommend therapeutic dosing of LMWH. We suggest monitoring of anti-Xa levels due to increased variability in the setting of decreased plasma antithrombin concentrations.
3) For life-threatening VTE such as cerebral venous thrombosis or central PE, we suggest short term concurrent administration of antithrombin concentrate until therapeutic anticoagulation and clinical stability is established.
4) We recommend therapeutic anticoagulation for a catheter-related deep vein thrombosis and non-removal of a functioning catheter in accordance with prior ISTH guidance.
5) For high-risk thrombotic events such as cerebral venous or sinus thrombosis, central PE, proximal DVT, or arterial thrombosis we recommend holding asparaginase therapy, at least temporarily.
6) We suggest the consideration to resume asparaginase following successful stabilization of the acute VTE event (approximately 4 weeks). There is limited literature on the safety of resumption of asparaginase following a cerebral venous thrombosis and resumption should be considered on a case-by-case basis accounting for number of asparaginase doses missed, resolution of thrombosis and symptomatology, and ongoing VTE risk factors.
7) We recommend at least 6 months of therapeutic anticoagulation for treatment of VTE associated with asparaginase. Shorter duration may be considered on a case-by-case

basis with the minimum duration extending 4-6 weeks following completion of asparaginase therapy. In those patients who developed a life-threatening VTE such as cerebral venous thrombosis, central PE, proximal DVT, or arterial thrombosis and who are not otherwise considered at increased risk for hemorrhage, we suggest continuation of anticoagulation until completion of chemotherapy and achievement of complete remission.

Dr. Zwicker Received good feedback from the audience and based on the consensus, the guidance document was submitted for publication at the JTH

On-going projects and registries

Dr. Wang (United States) presented an update on her international registry to evaluate the outcomes of patients on concurrent targeted cancer therapies and DOACs. She emphasized that although DOAC is thought to have less drug interactions compared to vitamin K antagonists such as warfarin, they are substrates of P-glycoprotein (P-gp) and/or CPY3A4, so significant drug interactions could be a concern with other medications metabolized by P-gp and/or CPY3A4. Many targeted cancer therapies such as tyrosine kinase inhibitors are P-gp or CYP3A4 inducers or inhibitors. Unlike a vitamin K antagonist, laboratory testing to monitor DOAC level or therapeutic effects is not routinely available. Therefore, the effects of potential drug interactions on anticoagulation are largely unknown, and the efficacy and safety of concurrent use of these targeted cancer therapies and DOAC are unclear. Therefore, her registry is aiming at evaluating the outcomes of patients on concurrent targeted cancer therapies and DOACs.

Dr. Avi Leaders (The Netherlands) provided the rational and update on an on-going prospective cohort study assessing the management of anticoagulation among thrombocytopenic patients with cancer receiving chemotherapy. Enrollment is going well and additional sites are welcomed in order to achieve the sample size required to provide clinically meaningful results.

Future Projects:

Animal Model

Dr. Dubois proposed the development of an accurate mouse model to the study cancer-associated thrombosis. He elegantly provided a good background and emphasized that mouse models are a critical tool for investigating the mechanisms of cancer-associated thrombosis. New models that better match human cancer development may enhance understanding of the biology driving thrombosis in cancer.

Transplantation models are frequently used in cancer-associated thrombosis research, with mouse cells lines implanted into mice with an intact immune system or human cells lines/tissues implanted into immunodeficient mice. Cells/tissues can be implanted subcutaneously or into the equivalent organ site (orthotopically) to recapitulate aspects of the tumor microenvironment. Overall these models can be adopted in labs with appropriate surgical expertise, and, depending on the model, tumor development is largely reproducible. However, each transplant model iteration has advantages and disadvantages. With mouse cells lines, immune system function is preserved, but the model depends upon cells lines that may genetically drift. Human cells line models may also be affected by genetic drift and the absence of a fully functioning

immune system may mask contributions of immune cells and inflammatory processes to thrombosis.

Mouse models have developed rapidly with technology advancements (such as CRISPR), resulting in a plethora of genetically engineered mouse models (GEMMs) that better simulate human disease. Although the *de novo* tumor initiation and progression in GEMMs may present challenges with inconsistent time to cancer development and tumor variability, the close modeling of the biology of human cancers have made GEMMs strong model systems for basic cancer research studies. GEMMs are infrequently used in cancer-associated thrombosis research and approaches to implement well-characterized and established genetic models should be considered. The aim for this proposal is to develop a new *in vivo* model(s) of cancer and thrombosis, taking advantage of advances in cancer model development, to enhance scientific discovery in cancer-associated thrombosis.

Dr. Dubois identified many potential opportunities:

A. Partner with cancer investigators with well-developed, well-characterized GEMMs of cancers of elevated thrombosis risk (lung, GI, ovary). Changes in hemostatic pathways can be assessed throughout cancer progression. Alterations may be exploited to induce thrombosis or current models of thrombosis may be applied. This approach capitalizes on the expertise of both cancer and thrombosis investigators. Cancer investigators will contribute their expertise with the mouse models and thrombosis investigations will contribute their expertise in pathways of thrombosis and application of thrombosis models. This may allow for a better understanding of how genetic changes and cancer development promote thrombosis.

B. Recent advances in CRISPR/Cas9 systems facilitate gene editing approaches. Investigators can leverage genetic models of cancer, partnering with cancer investigators, and explore genetic changes to induce spontaneous thrombosis or predispose to thrombosis in the setting of cancer. This approach may be challenging as genetic drivers of cancer-associated thrombosis are largely unknown. Factors that predispose individuals to thrombosis such as factor V Leiden and prothrombin G20210A could be considered. Other approaches include tissue-targeted gene editing, such as modifications to the endothelium. Combining genetic changes to induce cancer and promote thrombosis provide a new model to study mechanisms and an opportunity to evaluate how cancer treatments may influence development of thrombosis. This approach applies cutting-edge science to study cancer-associated thrombosis.

NYMPHEA

Dr. Cristina Belizna (France) proposed a collaboration between two SSCs (Antiphospholipid and Malignancy). She proposed a prospective international multicenter 5 years register study of lymphoma associated with antiphospholipid antibodies (aPL). (NYMPHEA study). The study group population will include patients with all types of non-hodgkinian lymphoma (incident and prevalent cases). Dr. Belizna reviewed the data reporting elevated levels of aPL in various malignancies. The pathological significance in patients with solid cancer or hematological malignancy and aPL is still unclear and controversial.

The main aim of this registry will be to characterize the clinico-biological features of non-hodgkinian lymphoma (NHL) patients with associated aPL.

Secondary aims will be :

- To estimate the prevalence of the IgG, IgM and IgA anticardiolipin and antiB2GP1 antibodies and of lupus anticoagulant in this population.
- To evaluate whether the presence of antiphospholipid antibodies in different types of NHL lymphoma influences their response to treatment, their survival and their rate of thromboembolic complications,
- To establish correlations of aPL and circulating TF with the markers of unfavorable prognosis of lymphoma (such as score FLIPI, Bulky, International Prognostic Index (IPI) score, high LDH, high B2 microglobulin) ; with age, sex, lymphome type, stage and grade, bone marrow involvement, presence of extranodal disease, presence of various lymphoma symptoms, performance status, type of treatment, response to treatment, number of relapses, number of un-programmed hospitalizations, number of transfusions.
- To establish if the treatments for NHL lymphoma could influence aPL titers.

This research proposal focused on the association between the antiphospholipid antibodies and hematological malignancies has a major importance, as it has a potential impact on the identification of high-risk population and would allow formal conclusions if aPL could be a potential marker of prognosis and survival in this population. The registry in on-going hand 250 patients have already been included. These results will have potential clinical consequences with respect to the screening and therapeutic regimens in the high risk identified subgroups of patients. The proposal was well received by the committee and members accepted to collaborate and help on the research study

Closure

Dr. Carrier closed the meeting and invited the audience to provide feedback and join the next meeting in Milan in 2020.

Lupus Anticoagulant/Antiphospholipid Antibodies

6 July 2019
14:30 – 16:30

Chairman: Katrien Devreese

Co-Chairs: Cristina Belizna, Hannah Cohen, Doruk Erkan, Masahiro Ieko, Hilde Kelchtermans, and Stephane Zuily

An update on ongoing projects supported by the SSC antiphospholipid antibodies/lupus anticoagulant is presented.

Screening for the antiphospholipid syndrome: relevance of IgA anti-cardiolipin and anti-β2glycoprotein I antibodies

Walid Chayoua, *Synapse Research Institute, Cardiovascular Research Institute Maastricht, Maastricht University, the Netherlands*

Anti-cardiolipin (aCL) and anti-β2glycoprotein I (aβ2GPI) IgA antiphospholipid antibodies (aPL) were shown to associate with thrombosis and pregnancy morbidity. However, inclusion of IgA aPL in the current classification criteria of the antiphospholipid syndrome (APS) has been debated. We aimed to investigate the added value of aCL and aβ2GPI IgA aPL in the classification of APS patients.

From eight European medical centers 1068 patients were enrolled: 259 thrombotic APS patients, 122 obstetric APS patients, 204 non-APS thrombosis patients, 33 non-APS obstetric patients, 60 APS patients of which the local center could not specify their clinical manifestation, 196 patients with autoimmune diseases and 194 normal controls. To minimize inter-assay and inter-laboratory variation, aCL and aβ2GPI aPL were detected with four commercially available assays: Bioplex 2200®, ImmunoCap®EliA, ACL AcuStar® and QUANTA Lite ELISA® by a single technician. aCL and aβ2GPI IgA titers were divided in a low, medium and high titer interval by dividing the upper limit of detection in three equal parts. Manufacturer's cut-off values were used. LAC was determined by the local center.

Positivity for IgA aPL was detected in 17-26% of the patients with clinical manifestations of APS (depending on the assay) and in 6-8% of the patients with an autoimmune disease and controls. Both aCL and aβ2GPI IgA were significantly associated with thrombosis and pregnancy morbidity. However, the majority of these patients (82-98%) were also positive for LAC, IgG and/or IgM aPL. Isolated IgA positivity was rare in patients with clinical manifestations of APS (0.3-3%) and not associated with thrombosis and/or pregnancy morbidity. Addition of IgA to the current criteria panel did not result in higher odds ratios for thrombosis or pregnancy morbidity. Low aCL and aβ2GPI IgA titers are associated with thrombosis. However, the association of low aCL and aβ2GPI IgA titers was dependent on the solid phase assay used. High aCL and aβ2GPI IgA titers increased the association with thrombosis or pregnancy in three out of four solid phase assays.

aCL and aβ2GPI IgA are associated with clinical manifestations of APS. However, aCL and aβ2GPI IgA aPL are not helpful in screening for APS along with the current aPL panel as isolated positivity is rare and not significantly associated with thrombosis or pregnancy

morbidity. High aCL and aβ2GPI IgA titers could be helpful in risk stratification for thrombosis or pregnancy morbidity, dependent on the solid phase used to detect these antibodies.

Role of anti-domain I β2-glycoprotein I antibodies measured by different methods, in the diagnosis and risk stratification of antiphospholipid syndrome

Dongmei Yin, *Synapse Research Institute, Cardiovascular Research Institute Maastricht, Maastricht University, The Netherlands*

Antibodies against epitope G40-R43 on domain I (DI) of β2GPI proved to be pathogenic in APS. But a recently published review showed the results about role of anti-DI in classification of APS were inconsistent and depended on the assays used to detect the anti-DI antibodies. A multicenter study found the anti-DI measured by the only available commercial CIA hardly improved the classification of APS. Unpublished data showed no exposure of G40-R43 on DI coated on beads on this CIA by using two human-derived monoclonal anti-β2GPI antibodies.

Anti-DI measured by an in house developed two-step anti-DI ELISA demonstrated the higher OR of anti-DI compared to anti-B2GPI for clinical manifestations of APS. We will use these two monoclonal antibodies to technically verify the exposure of the pathogenic G40-R43 epitope in this in house two- step anti-DI ELISA. Further, we will clinically validate the role of anti-DI measured by the in-house ELISA in this multicenter study population, by comparing results with the commercial anti-DI CIA as well as the conventional laboratory criteria of APS. The possible added value of anti-domain I β2-glycoprotein I antibodies in the diagnosis and risk stratification of APS will be investigated.

Antiphospholipid antibodies and lymphoma and follow up of antiphospholipid antibody fluctuation in patients with clinical Sydney criteria for APS

Johanna Gebhart*, University of Vienna, Austria*

In patients with lymphoma, the presence of aPL has been reported as associated with an increased risk of thrombosis, although data are contradictory. Data are also controversial regarding the eventual role of aPL as markers of disease activity and progression in hematological malignancies. Nowadays, based on these data, on the limited number of patients and of limited type of hematological malignancies analyzed, no formal conclusions could be drawn with respect to the potential role of aPL as markers of worse prognosis in lymphoma and of the increased risk of thrombosis in this population. Therefore, we propose an international register in lymphoma patients with positive aPL. We have chosen to concentrate this study only in non-hodgkinian lymphoma patients for the homogeneity of the studied population. The main aim of this register will be to characterize the clinico-biological features of non-hodgkinian lymphoma (NHL) patients with associated aPL.

Data on changes in aPL titers are rare, but are recognized and may also be influenced by treatment. Fluctuation of titers during pregnancy and their influence on pregnancy outcomes are controversial. Few studies have reported serial aPL determinations through pregnancy. Some authors found no relationship between aPL fluctuations with adverse pregnancy outcomes, while others reported favorable pregnancy outcomes associated with falling titers of aPL. The value and optimal timing of repeated testing for aPL requires further definition. Repeat testing in all APS patients may not be of added value, especially not in triple aPL positive patients.

Preliminary observations in a French cohort suggest that aPL titers fluctuate during follow-up in obstetrical and thrombotic APS, but also that the optimal timing for aPL detection remains a major clinical need. Definition of the optimal timing of the first determination for aPL status and of LA positivity confirmation, to establish persistence of aPL, the correlations of aPL titer fluctuations and LA positivity with clinico-biological features and various treatments in APS would be expected to have a major impact in clinical practice and to answer to an unmet clinical need. Therefore, an international multicentre prospective study of follow up for 3 years of aPL titers and LA positivity in patients with clinical criteria of APS according to Sydney criteria will be set up.

Unraveling Difficulties in Laboratory Diagnosis of Antiphospholipid Syndrome by Evaluation of 'Real Life' Sample Results in External Quality Control Program

Piet Meijer, *ECAT Foundation, The Netherlands;* ***Katrien Devreese****, Ghent University Hospital, Belgium*

The laboratory diagnosis of APS remains a challenge. LAC tests, as well as solid phase assays for aCL and aβ2GPI show methodological shortcomings, and the methodology is not standardized. Guidelines for LAC detection and performance of solid phase assays were published by the Scientific Standardisation Subcommittee (SSC) of the International Society of Thrombosis and Haemostasis (ISTH) in 2009 and 2014, respectively. Despite the efforts over the years standardization has not been reached. Disagreement between different commercial assay kits and methods is observed particular in the lower range of antibody levels. External Quality Schemes illustrate that most laboratories are able to detect strong LAC, whereas the diagnosis of a weak LAC remains a problem. Large differences in antibody titre for aCL and aβ2GPI measured with different kits are still observed, even in the newest generation assays, a long standing problem. The ECAT (External quality Control of diagnostic Assays and Tests Foundation, Voorschoten, The Netherlands) sends laboratories freeze-dried patient plasmas on a regular basis for LAC and aPL analysis to diagnostics laboratories worldwide. EQA results reflect the real-world diagnostic practices. Active in the field for many years, ECAT has built up a database of results that may be a source to explore some unanswered questions.

Based on EQC data, inter-method variability, inter-lab variability, variation in titer and qualitative classification for aCL IgG, aCL IgM, aβ2GPI IgG, aβ2GPI IgM will be evaluated. For LAC, sensitivity of aPTT and dRVVT and inter-method variability within aPTT test system and dRVVT test system will be examined.

During the last years detecting weak LAC has ameliorated, as shown in the ECAT exercises. Preliminary results on aCL IgG are shown, illustrating the large inter-method and inter-lab variation. An international standard is urgently needed to produce more comparable results between methods. Also, the qualitative classification needs to be harmonized, since no clear rules are available how to do so.

Update of the Guidelines For Lupus Anticoagulant Detection and Interpretation

Hannah Cohen, *University College London Hospitals NHS Foundation Trust, UK*

The strategy for the update of the 2009 guidelines on lupus anticoagulant (LA) testing was discussed. There will be two separate, linked documents. The first will be a guidance document, which will focus on aspects where there was less agreement in the 2018 ISTH SSC survey on LA testing, which was presented at the 2018 ISTH SSC meeting. A manuscript on the results of this survey has been accepted for publication as an official communication of the ISTH SSC for LA/aPL, in the Journal of Thrombosis and Haemostasis. The second document will be on recommendations for LA testing in anticoagulated patients. This will be a brief, more technical document that will focus on the methodology of laboratory testing for LA in anticoagulated patients and also on interference by various anticoagulants in laboratory tests for LA. The aspects to be covered in the new guidance will include the following: timing of testing in relation to thrombosis or pregnancy, LA testing in anticoagulated patients from the clinical perspective, cut-off values; and calculation and interpretation of results. The literature and recommendations in current guidelines was discussed in relation to these aspects. The presentation also addressed some pertinent points regarding the choice of tests for LA in patients on various anticoagulants. The plan for development of guidance/recommendations is to aim for the "best fit", based on a literature review led by the ISTH SSC for LA/aPL, with the members of this SSC as a Sounding Board.

Standardization of thrombine generation assays

***Marisa Ninavaggi**, Synapse Research Institute, Cardiovascular Research Institute Maastricht, Maastricht University, The Netherlands*

The questionnaire about thrombin generation consisted of 51 questions and in total 240 questionnaires were started of which 45% were completed. 43% was a research lab and 48% a diagnostic lab. The number of samples measured per year varies among the laboratories, but 47% measures less than 100 samples a year and only 8% indicated to measure more than 1000% samples per year. If we look at the methods to measure TG, we saw that 56% indicated to use the CAT assay of Diagnostica Stago. 7% the technothrombin assay of technoclone, 4% the ceveron alpha of technoclone, 6% the ST Genesia of Diagnostica Stago, 9% the siemens ETP, 7% an in-house method and 11% another type of assay. Preanalytical variation should be reduced as much as possible. It is recommend to use commercial reagents according to the manufacturer. 81% of the laboratories uses commercial reagents and 16% a combination of commercial and home-made reagents. 92% said to use the reagents according to the manufacturer. PPP is the most common type of sample being used (95%), with only a minority measuring only PRP or WB. Blood should be drawn using a straight needle, without applying a tourniquet, after discarding the first tube of blood. According to the survey 39% uses a straight needle and 40% a butterfly needle. 71% of the labs discards the first tube of blood. The blood should be taken directly into citrate and addition of CTI to reduce contact activation is recommended e.g. when using a butterfly needle or when activating samples with a very low TF concentration. From the survey we observed that citrate is being used in 83% of the labs and citrate with CTI is being used in only 6% of the labs. Most labs, use a plastic blood tube less than 5ml to collect their blood samples.

The preparation of PPP should be done by centrifugating the sample twice at no less than 2500g for 10 minutes at room temperature. Most of the labs (64%) indicated to indeed do this 2-step centrifugation, however, the duration and force varied a lot. Ultracentrifugation is used in

only 8% of the labs. Samples should be processed within one hour of the blood drawing and kept at RT. When PPP is prepared, it should be frozen directly if not needed, otherwise within 4 hours. The max time the plasma is kept before using/freezing is indeed in most of the cases one hour or less. Only a minority (3%) keeps the plasma more than 3 hours before processing. PPP stored at -20°C is for a short-term period, while at -80°C it is for long-term storage. Ideally for a maximum of one year. 84% of the labs indicated to freeze their samples directly and 15% snap-freezes their samples. 85% of the labs keep their plasma samples at -80°C and 11% at -20°C. The sample storage time varies a lot, 20% keeps their samples up to 5 year. Thawing PPP should be done for 5 minutes at 37°C. Sample integrity may be compromised if samples are either not completely thawed or if maintained too long at 37℃. 90% indicated to thaw the samples in a warm water bath. Also regarding the thawing duration, 67% uses a fixed thawing time, although the duration varied a lot. When using an in-house method, many different triggers are being used (e.g. FIXa, FXIa, Kaolin, ellagic acid, drvv, etc.). Samples should be measured undiluted and in triplicate. 92% does not predilute the samples. The final plasma concentration is dependent of the method used to measure TG, 57% uses 67% plasma (as the CAT assay) and 11% used 40% plasma concentration (Technothrombin assay). The calibration method is done in 79% with a2M-IIa, in 10% with a fixed amount of IIa and in 2% with a fixed fluorophore concentration. The number of replicates done are duplicates in 49% and triplicates in 43% of the labs. Samples and reagents should be preheated for 10 minutes before the start and measure TG at 37°C as it is known that temperature affects TG kinetics. 96% of the labs indicated to indeed measure TG at 37°C, but only 72% preheats the samples and reagents of which 61% preheats the samples and reagents in de device itself. Reference values should be based on at least 120 healthy donors. 68% of the labs indicated the use of reference values, which are only in 7% of the labs based on more than 100 donors. TG parameters used are: 92% peak, 93% ETP, 90% lagtime, 77% TTP, only 48% VI (more recent parameter) and 11% only looks at the start tail. Normalizing TG data, e.g. in a multicenter study, should be done towards the same control plasma that is measured in the same run as the samples. 45% indicated to do this, while 3% normalizes towards a control plasma measured in another experiment. The type of reference plasma varied a lot, with a preference for an in-house frozen plasma sample.

An international, multi-centre study to validate the Taipan snake venom time as a lupus anticoagulant screening test with ecarin time as confirmatory test

***Gary Moore**, Viapath at St. Thomas' Hospital, London, UK*

Snake venom FII-activators were first proposed for LAC testing as they are unaffected by VKA anticoagulation, although limited reagent availability precluded widespread use. There has been a resurgence of interest in the assays because direct FII activation bypasses the in vitro effects of direct FXa inhibitor anticoagulants. A multi-centre study to validate TVST screening with ecarin time confirmation using the only available standardised reagents is registered with the SSC, involving seven centres from across Europe and the USA. Four centres have submitted data, which exhibit similar reference ranges/cut-offs, and low imprecision from a variety of platforms. The assays correctly identified the LAC-positive and -negative WHO reference plasmas. Residual plasmas from non-anticoagulated established APS patients have been difficult to source but 186 plasmas from VKA or DFXaI anticoagulated APS patients have been tested so far. The main interferences are dabigatran and argatroban. Excluding samples from

patients on DTIs, combined diagnostic performance data are sensitivity 73.3%, specificity 95.9%, PPV 91.3% and NPV 85.7%.

Update on the new APS classification criteria development efforts
***Stéphane Zuily**, University Hospital of Nancy, France*

An international multi-disciplinary effort, supported by American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR), is underway to develop rigorous, new consensus- and evidence-based classification criteria to identify patients with high likelihood of Antiphospholipid Syndrome (APS) for clinical research. The ACR/EULAR endorsed methodological approach includes four phases: item generation (I); item reduction (II); item weighting and threshold identification (III); and classification criteria refinement and validation (IV). This procedure has largely formed the basis of the recent development of classification criteria in a variety of rheumatic disease areas, such as the 2010 rheumatoid arthritis classification criteria, the 2013 systemic sclerosis criteria and more recently the new systemic lupus erythematosus classification criteria which will be published soon. To date, Phases I and II were performed: From 261 items generated in Phase I, 27 candidate criteria organized into six independent domains were identified. Results also demonstrated different weights for macrovascular domain outcomes with or without “provoking” thrombosis risk factors. In the next phase, these proposed candidate criteria will be used for real-world case collection and further refined, organized and weighted and a preliminary threshold for classification will be sought.

Models of Thrombosis and Hemostasis

6 July 2019
14:30 – 16:30

Chairman: Jose A. Diaz

Co-Chairs: Brian Cooley, Margarethe Geiger, Laura Gutierrez, Nobuo Nagal, Laura Sanchez Rivera, and Maxim Shaydakov

Session Program:

The program this year was based on presenting the SSC projects. Below you can see the project details.

1. Pre-clinical Models of Platelet Transfusion Related Adverse Events.
   Laura Gutierrez, Co-Chair.
2. Standardization on selection and conduct of murine bleeding models – Final Report.
   Jose Diaz, Chairman.
3. Cellular Models of Megakaryopoiesis and Platelet Production.
   Laura Gutierrez, Co-Chair.
4. The cremaster arteriole laser-injury model of thrombosis – standardization.
   Robert Flaumenhaft on Behalf of Steve Grover UNC.
5. Platelet Rich Plasma: Usage in Cell Culture.
   Laura Gutierrez, Co-Chair.
6. Pre-clinical Models: The need for Standardization.
   Jose Diaz, Chairman.

All speakers engaged the audience, and this was translated into many questions following the talks (all talks had at least 3 questions. Robert got 7 questions on his topic. We finalized on time at 16:29 and we had a ¾ full room initially and half-room full at the end. All projects received input and we are in the process of updating the projects and submitting them for ISTH grants.

Publications

Publications since last ISTH, Berlin 2017:

Consensus on animal models of venous thrombosis – Jose A. Diaz. American Venous Forum project that was endorsed by ISTH, based on previous conversation with Dr. Walter Ageno (chariman of SSC at that time) during ISTH in Berlin. **J Thomb Hemost, 2019, Apr;17(4):699-707.**

Publications in preparations:

Standardization on selection and conduct of murine bleeding models.

Brian Cooley. ISTH SSC grant. Dr. Cooley reported that the work has been completed and the manuscript is in preparation. Lacey Schmeidler will send an email reminder to Dr. Cooley notifying him about the next steps toward to a publication through our SSC. A progress report has been requested by Jose Diaz and is attached.

Future publications planned:

The cremaster arteriole laser-injury model of thrombosis – standardization. Dr. Steven Grover (expert in modeling from UNC) is working on a review of the Cremaster arteriole laser-injury model of thrombosis models and he expressed interest in working with the SSC co-chairs to make this an SSC project. A paper was sent to our SSC for evaluation by the committee. After revision, input from all co-chairs was collected and a response will be submitted back to Dr. Grover group during July/2019.

Current Projects

Current SSC Projects:

All projects were presented at ISTH 2019 in Melbourne. Below the project descriptions.

Bleeding time (Final Report)
Project awarded by ISTH. Dr. Cooley's work is at the end stage and his work. It was presented in Melbourne.

The cremaster arteriole laser-injury model of thrombosis – standardization
This project is led by Dr. Steve Grover, PhD, from UNC. Dr. Grover sent the manuscript to be reviewed by our SSC. An initial review of it with 26 points was evaluated by the Animal, Cellular and Molecular Models SSC with feedback to be considered by the authors.

We are supporting our members working on standardizations on techniques/subjects under the umbrella of our SSC and endorsement of manuscripts will depend first on our revision and then passing on our recommendation to the board.

Pre-clinical Models of Platelet Transfusion Related Adverse Events
In light of the adverse events that might occur upon platelet transfusion, and the need to study these events in vivo, we think that this is an important new aspect to cover from our SSC. The two principal adverse events that we aim to include in this study are:

1.- Alloimmunization

2.- TRALI

There are several models described when considering these two adverse events of platelet transfusion, and we aim at reaching a consensus on methodologies (as they are inducible), and the potential applications these models offer, considering now the plethora of platelet functions, including immunomodulation.

Character: Multicenter study.

SSC should still find relevant network partners for this study. One of our Co-Chairs proposed this study. In particular the Co-Chair is interested in studying treatments to prevent TRALI in vivo, using mouse models, and the Co-Chair would be willing to coordinate such a study, expanding to alloimmunization.

Cellular Models of Megakaryopoiesis and Platelet Production

There are several methods described in order to culture primary human or mouse megakaryocytes, that differentiate from hematopoietic progenitors present in blood or bone marrow. The study of megakaryopoiesis in vitro is a difficult task as primary cultures are heterogeneous. At the same time, production of platelets in vitro, or the possibility to study their function is complicated, as it is very difficult to isolate the platelet produce from other cellular debris from the culture. Several methodologies and approaches have been described in order to improve the cultures at different levels (increase the yield, the maturity of MKs, the amount of platelets produced, ways to isolate the platelets), but there is little consensus, and there are still limitations on the applications from these cultures, while they would be valuable tools, especially to study the physiology of certain pathologies.

There are also a number of human and mouse cell lines, however, each cell line has its properties and characteristics, and there should be an adequate description of each one.

At the same time, the development of TPO-RA which are used for in vitro studies, requires a description of their potentiation and function in vitro.

We propose to describe and study current methods of primary cell culture, depending on species and tissue of origin, and cell lines, at two different levels:

Level: TPO vs TPO-RAs

Level: Platelet production, platelet isolation from culture and study of platelet function

Level: flow cytometry of megakaryocyte differentiation in mouse and human

One of our Co-Chair proposed this study. In particular the Co-Chair is interested in studying further a culture method she has developed that can be applied to study both mouse and human Megakaryopoiesis in vitro, comparing the effects of TPO or TPO-RAs, and testing several methods in order to isolate the produced platelets and to evaluate the possibility to even study platelet aggregation from those isolated from cultures. This technical challenge would ease many research objectives to different labs and could be applied to iPS-based technologies.

The study would gain more meaning when becoming multicenter, and when other labs would contribute with their own preferred culture methods, so the different methods would be classified and described for a better usage by future researchers. The same applies to cell lines.

Platelet Rich Plasma: usage in cell culture

As recently suggested by a communication by the SSC Platelet Physiology, there is little consensus on the processing and production, and applications of platelet-rich plasma.

We propose a study to assess properties in culture.

- PRP Production on a closed system at the Blood Bank
- Analysis of PRP from 40 donors
- Measurement by Multiplex of around 40 compounds
- Study of regenerative capacity or synergic properties in various culture systems
- Correlation studies Multiplex results vs Culture results in order to define bioactive compounds of PRP

Platelet Rich Plasma: usage in the clinic - tendinitis

As recently suggested by a communication by the SSC Platelet Physiology, there is little consensus on the processing and production, and applications of platelet-rich plasma.

We propose a study to assess properties in a two-arm study with patients with a specific lesion (tendinitis), treated or not with PRP.

- Production on a closed system at the Blood Bank
- Two-arm study, same type of lesion, treated or untreated with PRP
- Study of platelet functional profile prior PRP preparation
- Measurement by Multiplex of around 40 compounds
- Study of regenerative capacity or synergic properties in various culture systems
- Correlation platelet function prior PRP preparation, multiplex and PRP effects in culture with clinical outcomes in terms of recovery in order to identify key parameters that could predict efficacy of PRP treatment in the clinic (at least in the lesion and intervention of study, but with potential application in further studies with other clinical indications)

Platelet Proteomics and Function Profile – identifying associations

We aim at profiling platelets from different pathologies and generating an algorithm that could combine platelet proteome data (massive) with platelet functional characteristics, to implement it in the study of platelets in different pathologies where platelet function is out of balance, as it may occur in hemophilia or diabetes.

We aim at studying platelet function, and to define platelet proteome of patients with hemophilia, with bleeding or non-bleeding symptomatology.

The same study will be performed in diabetic patients, that have had or not thrombotic episodes.

The functional profile will be performed comparing diagnostic-validated devices such as Verify or Multiplate, or a platelet function test able to discern single-receptor function (De Cuyper et al, 2013, Blood).

Pediatric and Neonatal Hemostasis and Thrombosis

7 July 2019
16:30 – 18:30

Chairman: Christoph Male

Co-Chairs: Fiona Newall, Manuela Albisetti, Sarah O'Brian, Leslie Raffini, Tina Biss, and Ayesha Zia

1. **Development of Appropriate and Necessary Care Guidelines for Adolescents with Heavy Menstrual Bleeding and Bleeding Disorders: Results from an International Expert Panel – Project report**

Speaker: Ayesha Zia (United States)
Working group and collaborators: S. Responsible co-chair: Ayesha Zia; Members: Revel-Vilk, Peter Koides, Maha Othman, Rochelle Winikoff, Susan Halimeh, Michelle Lavin, Rezan Abdul Kadir, Dvora Bauman

Phase I: Expert Workgroup Composition;
Phase II: Systematic Review, additional evidence document, standardized definitions;
Phase III: RANC/ExpertLens panel voting on appropriateness and necessity of HBM care by international panel of experts.
Results: 15 appropriate and necessary care statements have been generated; 6 statements judged to be uncertain will guide future research.
Further initiatives: Audits of care adolescents receive at multidisciplinary adolescent hematology programs to further refine the concept and improve care; Transform appropriateness criteria for HMB care in adolescents across specialties; Adapting the algorithms to the electronic medical record to facilitate screening and management

2. **Hospital Guidelines on Pediatric Venous Thromboembolism Prevention – a survey**

Speakers: Brian Branchford (US), Julie Jaffray (US)

Review of risk factors associated with hospital-associated venous thromboembolism (HA-VTE) to identify children at the highest risk of VTE; validated VTE risk assessment model. Quality Improvement Initiative: network of U.S. children's hospitals, working together to decrease hospital acquired conditions in children; VTE is the #2 hospital acquired condition. Review of risk assessment tools and VTE prevention programmes from various pediatric hospitals in the US.
Presentation of the Children's Hospital-Acquired Thrombosis (CHAT) Project:
Phase I: risk assessment model and stratified scoring system established from a large scale, multi-institutional registry including pertinent medical data from children
• Phase II: to validate risk model at multiple institutions (started 2019)
• Phase III: RCT of risk-adjusted thromboprophylaxis measures.

3. **Pediatric Pulmonary Embolism Working Group – project report.**

Speaker: M. Rajpurkar (US)
Responsible past co-chair: Neil Goldenberg; Working group: Madhvi Rajpurkar, Tina Biss, Heleen van Ommen, Susan Williams, Anthony Chan.

- Presentation of completed '**Results of a Multinational Survey of Diagnostic and Management Practices of Thromboembolic Pulmonary Embolism in Children**':
  - PPE is rare at individual centers -5-10/year->highly variable management
  - 8% reported the existence of a specific pulmonary embolism response team (PERT) or a clinical care pathway
  - 40% had a specific management protocol for thrombolysis/thrombectomy
  - 4% indicated that they use a prognostic risk score for classification of PE.

  The study has recently been published: **Results of a Multinational Survey of Diagnostic and Management Practices of Thromboembolic Pulmonary Embolism in Children**. Rajpurkar M, Williams S, Goldenberg NA, Van Ommen CH, Chan AK, Thomas R, Biss T. *Thrombosis Research 2019 (in press)*

- Ongoing project: '**Development of guidance for development institutional clinical care pathways/Pulmonary Embolism Response Team (PERT)**'
  Goals: to help individual institutions develop practice pathways, to i) achieve uniformity in management of PPE, ii) improve patient outcomes at individual centers. Opportunity for prospective collaboration and data collection.
  - Clinical pathway: identify patients at risk, diagnosis, risk assignment, management, follow-up with measurement of outcomes
  - Pulmonary Embolism Response Team (PERT): central coordination where all involved specialists can be alerted simultaneously; builds upon the rapid response team concept by incorporating a process, whereby immediate multidisciplinary consultation is utilized to achieve consensus regarding the optimal, individualized care for each complex clinical scenarios

4. Clinically unsuspected VTE - working group update

Speaker: Meera Chitlur, on behalf of Anjali Sharatkumar (United States)
Working group: responsible co-chair: Tina Biss; members: Anjali Sharathkumar (lead), Sanjay Ahuja, Ketan Kulkarni, Matt Regan

Goal: to provide evidence-based recommendations for evaluation and management of clinically unsuspected VTEs (uVTE) in pediatric population
Specific Aims: i) to understand epidemiology of uVTEs, ii) write a consensus guidance statement about management of uVTEs, iii) identify knowledge gaps and provide recommendations for future research.
Methodology: Systematic review of literature
Outcome of interest: Prevalence of Clinically unsuspected VTE
Results: i) Asymptomatic VTE detected by surveillance for VTE, ii) Incidental VTEs detected during evaluation of medical condition unrelated to clinical suspicion for VTE.
- CVL remains the primary risk factor for uVTEs
- Asymptomatic VTEs detected during surveillance are not associated with adverse outcomes: unnecessary to perform surveillance imaging, thrombophilia testing thromboprophylaxis or treatment
- Insufficient evidence to support treatment for iVTE

5. Prognostic Factors of Post-thrombotic Syndrome in Pediatric Patients: Systematic Review and Recommendations for Future Research

Speaker: Marisol Betensky (US)

Working group on paediatric PTS: Responsible past co-chair: Neil Goldenberg
Members: Leonardo Brandao, Manuela Albisetti

Results:
– Incidence of any PTS 46.3%; moderate to severe PTS 5.4%
– Most common prognostic factors assessed by studies: i) DVT-related, ii) Patient-related
– Prognostic factors associated with PTS: i) CVC, ii) occlusive DVT at diagnosis, iii) Incomplete DVT resolution

Recommendations:
- Continue to develop / refine validated instruments that evaluate functional limitations and QoL-impact of PTS
- Future studies should include PTS as an important endpoint of interest with particular focus on clinically significant PTS
- Future studies should investigate the role of DVT treatment-related factors as prognostic indicators for the development of PTS

6. International Pediatric Thrombosis Network

Speaker: Heleen Van Ommen (the Netherlands)
Working group: Responsible co-chair: H. van Ommen; Members: Manuela Albisetti, Mihir Batt, Suzanne Holzhauer, Christoph Male, Paul Monagle, Shoshana Revel-Vilk, Elizabeth Chalmers

Aims:
1. Throm-PED registry: prospective, observational disease registry: long-term outcome studies, safety/efficacy of drugs in real life, evaluate new management strategies etc.
2. Task force for anticoagulant drug development; Throm-PED clinical trial network

Progress report:
A Pediatric thrombosis registry (Thromb-PED) has been created in RedCap and a webpage created at the ISTH SSC homepage: www.isth.org/iptn. 53 participating centers: currently seeking IRB permission/waiver. Each center has one representative. General Assembly annually during ISTH congress. Consortium agreement.
Specific projects: i) neonatal renal vein thrombosis, ii) registry of DOAC use in children.

7. Special Topic: Are controlled clinical trials of anticoagulation necessary and feasible in children?

7.1. Debate: No

Speaker: Paul Monagle (Australia)

Few clinical trials in paediatric anticoagulation have been successful. RCT are difficult because paediatric VTE is rare, heterogenous regarding age, pathophysiology, comorbidity. Lack of knowledge of natural history, of validated diagnostic tools, of clarity about outcome measures. RCT do not provide all the information we need to improve outcomes for children requiring anticoagulation. We need i) rare disease model of thinking; ii) registries, iii) cohort/cross sectional studies, iv) longitudinal follow up studies, v) mechanistic studies

7.2. Debate: Yes

Speaker: Christoph Male (Austria)

RCT provide the least biased and confounded estimate of the benefit/risk of interventions. Not all study questions require RCT in children given existing (adult) evidence. Other questions will never be definitely solved for children without RCT; e.g. the recent Thrombotect study for the first time demonstrated definite evidence of a positive benefit/risk of anticoagulant prophylaxis to prevent CVC-related VTE. Given the challenges and limitations, RCT in children should be performed in the context of observational studies, e.g. prior registries/cohorts to establish natural course, response to standard care; parallel-cohort RCT; long-term observational treatment and follow-up studies, particularly to identify any off-target drug effects on the developing organism.

Perioperative and Critical Care Thrombosis and Hemostasis

6 July 2019
14:30 – 16:30

Chairman: Jerrold Levy

Co-Chairs: Pierre Albaladejo, James Douketis, Marc Samama Beverley Hunt, Jerold Levy, Alex Spyropoulos, and Thomas Theile

The first part of the session was moderated by Drs Alex Spyropoulos and Jerrold Levy, and began with an introduction of our current projects, publications, and other work in progress in the past year that included the following:

Projects underway and pending:
SSC Statement: Thromboembolic Patient Risk and Procedural Bleed Risk Stratification in patients on Chronic OAC Needing Elective Procedures (Alex Spyropoulos, lead)

Communications pending:
1. Management of emergency procedures for patients on antiplatelet therapy (Thomas Thiele lead), In Preparation.
2. Tranexamic acid communication: (B Hunt and J Levy), In Preparation.
3. Ischemic limb necrosis in septic shock. Joint submission with DIC committee (Toshiaki Iba and Ted Warkentin, In Press.
4. ECMO/VADs: We are creating a joint communication with Biorheology and vWF and our SSC. In Preparation.

5. Publications:

1) Godier A, Greinacher A, Faraoni D, Levy JH, Samama CM. Use of factor concentrates for the management of perioperative bleeding: guidance from the SSC of the ISTH. J Thromb Haemost. 2018 Jan;16(1):170-174.

2) Thomas W, Samama CM, Greinacher A, Hunt BJ; Subcommittee on Perioperative and Critical Care. The utility of viscoelastic methods in the prevention and treatment of bleeding and hospital-associated venous thromboembolism in perioperative care: guidance from the SSC of the ISTH. J Thromb Haemost. 2018 Nov;16(11):2336-2340.

Additional presentations included:

A summary of our two publications in the last year. Dr Jerrold Levy discussed our guidance document regarding factor concentrates for perioperative bleeding. The presentation included a review about the importance of factor concentrates in the management of critically ill bleeding, the lack of alloimmunization, lack of transfusion risk, and potential for storage and availability without cross-matching and rapid deployment in emergent bleeding during major surgery or traumatic injury. Despite surgical measures to minimize bleeding, patients develop acquired hemostatic defects and although allogeneic blood products are often administered, increasing studies have reported the use of factor concentrates as a

therapeutic approach for restoring hemostasis. He also described the different agents reviewed in the document including fibrinogen concentrates, prothrombin complex concentrates, factor XIII, and recombinant factor VIIa, the supporting data for their use in a perioperative and critically ill patient population, and conclusions regarding their administration.

Next, Dr Andreas Greinacher from Germany reviewed the recommendations on the use of viscoelastic monitoring in perioperative and critical care hemostasis. Perioperative and critical care bleeding is a common clinical scenario that requires monitoring as part of therapeutic approaches. Although the utility of viscoelastic methods in managing bleeding continues to be debated, studies report that viscoelastic monitoring in bleeding patients reduces transfusion and potentially improves outcomes. Dr. Greinacher summarized the published evidence, and reviewed our pragmatic recommendations reflecting current evidence for the use of viscoelastic methods, and need for additional evidence demonstrating improvement in clinical outcomes beyond cardiac surgical patients.

Subsequently, Dr. Deborah Siegal, the lead on the "Timing and Management of Patients Needing Urgent and Emergent Procedures: PAUSE ER" study reviewed the rationale, perspectives, and other important aspects of the study. Of note is there are limited data regarding the perioperative management and outcomes of patients treated with oral anticoagulants (OACs) requiring urgent surgery or procedures, common clinical scenarios for which best practices are uncertain. The Perioperative Anticoagulant Use for Surgery Evaluation (PAUSE) study is designed to address this unmet clinical need. PAUSE-ER is a prospective cohort study evaluating treatments and outcomes of OAC-treated patients (any oral anticoagulant for any indication) requiring urgent surgery or procedures within 24 hours of presentation. The study objectives are (i) to determine the frequency of adverse events (thrombosis, major bleeding, mortality), (ii) to identify and compare determinants of adverse events, and (iii) to describe and compare the use of OAC reversal strategies, hemostatic products, and resource utilization. Patients will be followed for 30 days. This is an exploratory, hypothesis-generating study with a convenience sample size of 200 patients treated with direct oral anticoagulants (DOACs) and 200 treated with vitamin K antagonists.

Following this presentation, Drs. Jean Marie Connors and Lisa Baumann Kreuziger moderated the subsequent sessions.

First, Dr. Beverley J. Hunt provided an overview and commentary regarding managing bleeding in the critical care patient. As the management of bleeding in critically ill patients remains a major clinical challenge, the underlying causes of coagulopathy in this setting are often complex with limited diagnostic testing and potential management strategies. Critically ill patients in the intensive care unit often have a multiplicity of problems ranging from septic shock, thrombotic microangiopathies, disseminated intravascular coagulation, shock liver, and other pathologic responses that contribute to major bleeding. ICU patients commonly have thrombocytopenia that may be due to multiple causes, including thrombotic microangiopathic states to DIC. The issue of renal failure further contributes to the complex coagulopathic state as well as the use of multiple anticoagulants in elderly patients. As Dr. Hunt summarized, managing bleeding in critically ill patients remains a major clinical challenge.

Dr. Alex Spyropoulos discussed his report currently *in review* in JTH of standardized reporting of patient-related thromboembolism risk and procedural-related bleed risk in periprocedural antithrombotic and bridging therapy. As noted in his presentation, periprocedural management of patients receiving chronic oral anticoagulants (OAC) are at risk for bleeding, and subsequent

interruption over time also poses a thromboembolic risk. Further, additional important patient- and surgery-specific risk factors for thromboembolism and bleeding are important to consider and should be assessed and risk-stratified in any periprocedural anticoagulant management strategy and study. Dr. Spyropoulos also noted how patient-specific thromboembolic risk influences whether an aggressive periprocedural antithrombotic approach such as bridging therapy with unfractionated heparin (UFH) or low-molecular-weight-heparin (LMWH) should be used during warfarin /VKA interruption to prevent perioperative thromboembolism in high-risk patients. As a result, the risk for periprocedural bleeding highly influences whether anticoagulants need to be interrupted and if anticoagulant interruption is deemed necessary, the timing of perioperative interruption and resumption. He also noted how prior publications on the topic report a lack of uniform definitions of patient-specific thromboembolic risk and procedural/surgical bleeding risk, thus identifying a need to apply a standardized risk stratification approach.

The objective of this official SSC communication of the ISTH using a multidisciplinary panel of internists, hematologists, anesthesiologists, vascular medicine specialists, and surgeons is to recommend a standardized risk stratification scheme for reporting of both patient-specific thromboembolic risk and procedural/surgical bleed risk for patients on chronic OAC – either VKA or the DOACs – who need an elective procedure or surgery. This document further builds on prior work of standardized reporting for periprocedural antithrombotic and bridging therapy as part of the Scientific and Standardization Committee of the ISTH.

Plasma Coagulation Inhibitors

9 July 2019
16:30 – 18:30

Chairman: Jun Teruya

Co-Chairs: Eriko Morishita, Herm-Jan Brinkman, Cecilia Guillermo, Vera Ignjatovic, and Zsuzsanna Bereczky

Moderator: Zsuzsanna Bereczky and Vera Ignjatovic

New classification of protein S deficiency, role as a co-factor for tissue factor pathway inhibitor

Hem Jan M Brinkman, The Netherlands

Problem with the current protein S deficiency classification, introduced in 1990 by Philip Comp and adjusted by Rogier Bertina during the ISTH-SSC meeting in 1991, is the type II classification for an isolated functional defect. The current type II classification only takes into account the activated protein C (APC) cofactor activity of protein S. During the last decades it became clear, however, that protein S is also an important cofactor for Tissue Factor Pathway Inhibitor alpha (TFPIa). Although commercial tests for the TFPIa cofactor activity are not available yet, it seems adequate to condole with current knowledge and future commercial available assays and to propose an adjustment of the Bertina classification as follows:

- to classify a deficiency in APC cofactor activity as type II-APC
- to classify a deficiency in TFPI cofactor activity as type II-TFPI.

During the SSC-PCI meeting, the audience was asked to contribute to the discussion by email.

Natural changes in natural anticoagulants

Vera Ignjatovic, Australia

The aim of this presentation was to outline to the audience the state of knowledge when it comes to age-specific changes in natural anticoagulants in terms of their concentration, activity and post-translational modifications.

The presentation started with key facts around the lack of evidence for the majority of drugs that are used in children and outlined the major age-specific differences in the hemostatic system that are encompassed under the term Developmental Haemostasis. The fact that children are epidemiologically and physiologically diverse but fundamentally different from adults was highlighted.

A/Prof Ignjatovic then discussed reference ranges and the importance of having appropriate age-specific reference ranges, particularly for children.

The presentation then moved onto knowledge of age-specific changes in natural anticoagulants and the fact that only 9 studies to date investigated this concept and those studies included natural anticoagulants as part of the studies, not as the main focus.

A/Prof Ignjatovic outlined the evidence for frequent lack of correlation between the quantity and function of natural anticoagulants across the age spectrum, supporting the idea of age specific post-translational modifications for these proteins. Specific evidence of age-specific structural differences in natural anticoagulants was provided for Antithrombin, Alpha-2macroglobulin and Heparin Cofactor ll.

The main conclusion was that, when it comes to children, we are working in a different physiological system; At present we know little about that system and that we are solving pieces of the puzzle every day.

In terms of future studies A/Prof Ignjatovic proposed investigation of age-specific changes in endogenous heparinoids (e.g. Heparan sulphate) as proteins with important physiological functions that could have impact on the endogenous bleeding and clotting potential of individuals across the age spectrum and which could potentially interact with drugs administered to children.

Laboratory diagnosis and phenotypic characterization of antithrombin deficiency;

Antithrombin deficiency registry

Zsuzsanna Bereczky

In the presentation, the major diagnostic issues were summarized including molecular genetic diagnosis and laboratory diagnosis of antithrombin (AT) deficiency. At present more than 300 different mut mutations are described in the databases, among which missense variants are the most frequent. Based on the type and typical localization of the mutations, type I (quantitative) and type II (qualitative) deficiencies are distinguished, the latter can be further divided into type II RS (reactive site), type II I-IBS (heparin binding site) and type II PE (pleiotropic effect) deficiencies. Interpretation of mutation should follow the recommendation of the Human Genome Variation Society. In case of novel variants, indirect and direct pieces of evidence should be collected to confirm the pathogenic nature. In "mutation negative" cases by Sanger sequencing MLPA analysis and investigation of other genes with potential effects on AT levels (eg. hypoglycosylation of AT due to mutations in genes involved in N-glycosylation) is recommended. There are some founder mutations in AT gene (SERPINCI); the most frequent is AT Budapest 3, which is the most common variant in the Hungarian population.

Concerning functional laboratory assays in AT deficiency heparin cofactor assay or progressive assay can be performed. The latter is useful in distinguishing type IIHBS deficiency from other defects. In case of type II I-IBS deficiency, there are big differences in the sensitivity of the commercially available reagents, as it was confirmed by different studies. Moreover, different subtypes of type II HBS deficiency (AT Budapest 3, AT Basel, AT Padua I) may give different results in the different functional assays. It is worthy of investigation, which assay conditions may have major impact on assay sensitivity issues.

The clinical nature of AT deficiency is heterogeneous, within type ll HBS deficiency the most severe form is homozygous AT Budapest 3, even more severe than type I heterozygous

deficiency. AT Budapest 3 heterozygous form was more severe than heterozygous AT Basel and AT Padua. There are arterial thrombotic complications described, mostly in AT Basel and AT Padua.

As several clinical and laboratory questions emerge in AT deficiency, an international registry could help to solve the problems. International AT registry is aimed to collect clinical, laboratory and genetic data on patients with AT deficiency. By analyzing data from a big international cohort of patients, so far unresolved problems and unanswered questions will be clarified. The registry is focusing on the types of thrombotic events, the laboratory diagnostic issues, the treatment strategies and on patient management in special situations. With the help of ISTH we can open this next year.

Conclusions:

1. Molecular genetic studies are useful in AT deficiency, since they help in:

   - establishment of AT deficiency diagnosis in uncertain cases (AT levels in the grey zone, transient AT deficiency)
   - patient management by determining the prognosis of the disease, since it may have a significant prognostic value (clinical picture correlates better with genotype than with the results of laboratory assays)
   - establishment of diagnostic algorithms and in patient management in case of founder mutations
   - discovering new genetic factors in AT deficiency, which may modify disease severity and clinical appearance
   - obtaining important novel pieces of information on structure/function of AT and identifying new functions of AT

2. Factors that may have major impact on assay sensitivity in type II HBS deficiency are:
   source of substrate (anti-FXa is superior to anti-FIIa) heparin concentration (lower concentration is superior to high concentration) pH (higher pH is superior to lower pH) incubation time (shorter time is superior to longer time) sample dilution (seems not to be the most important factor)

3. International AT registry is under construction and ISTH (SSC) members are invited to collaborate.

Moderator: Cecilia Guillermo and Jun Teruya

Plasma protein inhibitors in patients on DOACs: to test (and how) or not to test? Interference with classical methods

Cecilia Guillermo

At present, we have two types of Direct Oral Anticoagulants (DOACs): anti lla like dabigatran and the "Xabans" (Rivaroxaban, Apixaban, Edoxaban and Betrixaban recently approved in USA), which target directly factor Xa.

When we review the pharmacology of DOACs, is important to highlight two characteristics: the short half-life, between 5 to 9 hours for rivaroxaban in the young patient, to 17 hours for dabigatran, and the other important point is their clearance, 80% by kidney for dabigatran and 27% for Apixaban. Therefore, we can easily conclude that with a day or a couple of day of discontinuation, in a patient with normal renal function, we can skip the interference of DOACs in coagulation test, but that depends on the clinical situation

So, why would it be necessary to test Plasma Coagulation Inhibitor (PCI) during DOAC treatment? In the vast majority of cases it is not necessary: the primary management of thrombosis will not change: it would be interesting to know if the patient has a deficiency, to decide the duration of anticoagulation in some clinical scenarios, but in this cases we can wait to test without DOACs.

However, the laboratory receives samples from patients on DOACs to test, or worse, samples from patients we do not know what medication they take.

This is probably because some clinicians do not know the interference of DOACs in laboratory tests as Drs. Favaloro and Lippi mentioned.

What is the effect on PCI tests?

About antithrombin (AT) assays, Dr Van Blerk, demonstrated in a National Belgian survey, tests based on thrombin activity will overestimate the concentration of AT, so deficiencies could be missed, while tests based on activity, are not affected. Here we can see the same interference by dabigatran showed by Dr Adcock and colleagues. In her review, Dr Devreese showed the high variability of AT determination. We can see the opposite with anti Xa anticoagulants. AT level is not affected if the assay is thrombin based, but it will be overestimated if the assay is FXa based. The same results were observed with edoxaban and apixaban on AT assay.

In conclusion, FXa based AT assay is unaffected by dabigatran, but overestimated by anti Xa, and the opposite when we use FIla based AT assay
Regarding this subject, PCI subcommittee is working on this topic since the beginning of DOACs era: in 2012, with Dr Steve Kitchen as a Chairman, subcommittee minutes were published and can be accessed at ISTH web site.

We can observe that clot-based protein C (PC) and protein S (PS) are overestimated in the presence of anti Xa, but Chromogenic PC and free PS antigens are not affected.

About the effect of dabigatran, Dr Gosselin and Dr Douxfils showed that even small amount of dabigatran can overestimate PC or PS activity, while chromogenic assay and free antigen are not affected.

In summary, the result depends on the type of assay and the type of anticoagulant.

Recently, in vitro removal agents have been developed (DOAC STOP, DOAC Remove); both are able to neutralize all DOACs with minimal effect on hemostasis tests. However, there is little evidence to recommend their use to test PCI.

From the literature, the majority of authors suggest performing an algorithm to rule out the presence of DOACs, for example, using an anti Xa and/ or a thrombin time, and prefer testing through even if interference is possible.

Dr Favaloro advised against testing patients for thrombophilia on anticoagulant therapy, because the risk of a wrong diagnosis exceeds the benefice.

Conclusions:

1) If it is possible, do not test patients on DOACs therapy. The use of in vitro removal agents could be an option.

2) If the laboratory could be at risk to receive samples without clinical information, we suggest to perform a pre testing algorithm to rule out the presence of DOACs, and if it is necessary we can select an appropriate test, according the type of DOACs: for dabigatran, based assay for AT, chromogenic and free antigen for PC and S, respectively. For anti Xa, FIIa based assay for AT and chromogenic and free antigen assays for PC and PS, respectively.

New method to test protein S

Eriko Morishita

Background

- The APC-cofactor activity of Protein S (PS) measurements by the clotting method are more affected by DOACs than the antigenic measurements, which are performed by immunoassay. For this reason, free PS antigen is often recommended as the first-line test for PS deficiency, although free PS antigenic assay would miss the type II deficiency.
- Recently, Tsuda et al has developed a novel assay system for accurate simultaneous determinations of APC-cofactor activity of PS and total PS antigen levels to screen for both type I and II PS deficiencies.

Aim

- The aim of this presentation was to examine the influence of anti-Factor Xa DOACs on the novel chromogenic assay to measure the level of APC-cofactor activity of PS by comparing the results with the clotting assays.

Results

- APC-cofactor activity of PS was measured using four reagents by the clotting methods. PS activity was influenced by rivaroxaban and edoxaban and increased in a concentrationdependent manner. On the other hand, there was no significant association between the plasma concentration of apixaban and the PS activity, suggesting that apixaban does not influence the measurement.
- None of the three anti-FXa DOACs had any effect on PS total activity when it was measured using the novel chromogenic assay.

Conclusion

- The type of anti-FXa DOACs and the measurement principles influence the levels of PS activity.
- Given that DOACs has little influence on the chromogenic assay, this assay would be suitable to screen for hereditary thrombophilia in patients taking DOACs in Asia as it would not miss type II PS deficiency

Use of Protein C (PC) Concentrates

Leonardo Brandao

PC concentrates have been in clinical use since the late 80's and there are mainly two plasmaderived compounds, Ceprotin@ and Protexel@. A third compound named Anact-C@ is available in Japan/Asia. They have been licensed for the treatment of severe inherited PC deficiency in 2001 (Dreyfus M et al. Vox Sanguinis 2007) in Europe, and in 2007 in North America (Manco-Johnson M et al. Thromb. Haemost 2016).

Practical clinical tips regarding the use of PC concentrates in severe congenital PC deficiency:

1) Response to PC replacement with PC concentrate was documented even with severe cutaneous lesions provided that no significant delay to start PC replacement was in place.
2) PC replacement >60 IU/kg/dose during maintenance phase was associated with less breakthrough cutaneous purpura fulminans (PF) lesions.
3) With re-initiation of vitamin K antagonist, PC replacement for 3-5 days is indicated.
4) PC replacement confers better response in comparison to historical controls treated with plasma.
5) The administration of PC concentrates subcutaneously seems to facilitate both acute and long-term management. A wide range of dosing has been documented.

Guideline-based recommendation for their use dates back to 1989 (Marlar R et al. J Pediatr

1989). However, the level of evidence and number of recommendations has continued to be low, including the recommendations made in the recent ASH 2018 guideline, reflecting the rarity of the condition and the knowledge gaps pertaining to this clinical scenario. Despite available literature, there are clinical scenarios for which clinical recommendations could be considered including the use of PC concentrates in pregnancy, periprocedural management, venous thromboembolism without PF lesions, and breakthrough PF lesions. In the instance of newborns suspected to have severe congenital PC deficiency, diagnostic and treatment algorithms should be put in place as soon as the diagnosis is confirmed.

Discussion of the Current and Future Projects of SSC PCI

Jun Teruya

Cecilia Guillermo will rotate off after this meeting. Two new co-chairs will be recruited. Current chair and co-chairs are from USA, The Netherlands, Australia, Japan, and Hungary. Female to male ratio is 3:2.

There were 3 applicants for 2 co-chair positions. We recommended Dr. Elizabeth Van Cott and Dr. Gary Moore for new co-chairs.

Publication

- APC resistance paper is accepted by JTH for publication
- Protein C paper was submitted to JTH and is currently under review.
- Antithrombin paper was submitted to JTH.
- Investigation into racial differences in genetic risk factor for venous thromboembolism is in preparation by Dr. Tsuda.

Projects

- New classification of protein S deficiency (Herm-Jan Brinkman)
- International registry of antithrombin deficiency (Zsuzsanna Bereczky)

Platelet Immunology

8 July 2019
16:30 – 18:30

Chairman: Donald M. Arnold

Co-Chairs: Tamam Bakchoul, Philip Choi, Brian Curtis, Shigeki Miyata, Maria Ahlen, and Rachael Grace

Welcome and Introductions (Donald Arnold, Canada)

The Chair, Donald M. Arnold, welcomed all attendees and summarized the mandate of the Platelet Immunology SSC.

New Antigens and Mechanisms in ITP (Ulrich Sachs, Germany)

Ulrich J. Sachs (Giessen, Germany) gave an update on antigens targeted by ITP autoantibodies with a focus on glycoprotein V. Such antibodies are detected in the majority of ITP patients and have been demonstrated to induce platelet clearance. The relative share of different platelet clearance mechanisms remains to be elucidated. He also outlined some aspects of autoantibodies against alphaVbeta3 which appear to interfere with platelet production. He concluded that a simple dichotomy between anti-GP Ib/IX positive and anti-GP Ib/IX negative ITP patients is unlikely to exist; and that ITP therapy can currently not be tailored according to serological test results.

There was discussion around the need to improve diagnostic tests for ITP given that a significant proportion of patients test negative in autoantibody assays.

Immunotherapy-induced ITP: Cohort study (Phil Choi, Australia)

Phil Choi presented his proposal on The Immunotherapy-Induced thrombocytopenia Register (TIIR). He described the clinical features of alemtuzumab-induced ITP in patients with MS, as well as describing reported outcomes from patients receiving checkpoint inhibitors who subsequently develop ITP. Phil explained the case that these phenomena do not represent classical drug-induced thrombocytopenia as the thrombocytopenia may occur even years after the use of alemtuzumab. Although a prospectively recruiting biobank would potentially provide insights into a human model for ITP, this is not currently feasible and a REDCap register to better explore the treatment options and outcomes for patients with these phenomena is being launched with the assistance of the ISTH.

The current registry is open through the ISTH portal. When the audience was polled, at least 7 people had experience with immunotherapy-induced ITP. Each of them (and others) was encouraged to submit their data to the registry.

Standardization of clinical outcomes in adult and pediatric ITP: Proposal (Donald Arnold, Canada)

Donald Arnold gave a presentation about a new project led by himself and Rachael Grace (Co-Chair). The problem to be addressed is the lack of standardized outcomes for ITP clinical studies and trials, specifically related to: 1) Platelet count response, 2) Bleeding; and 3) Health

related quality of life. Standardization of these outcomes in ITP trials is needed to compare data and evaluate effects of treatments.

The objectives of this project are: 1) To describe the definitions of platelet count response, bleeding, HRQoL used in adult and pediatric ITP studies to date; 2) To establish standard definitions.

Aim 1: We will conduct a systematic review of the literature to describe how platelet count response, bleeding and HRQoL have been reported across pediatric and adult ITP treatment studies. We will begin the search from 2009, when the IWG published the first standardization of terminology paper (Rodeghiero et al. Blood 2009). Our proposed search strategy will capture full text manuscripts published from 2010, using Medline search (terms to be agreed upon), to capture studies that reported on any of the outcomes of treatment response. We will include all ages, and all study designs.

Once a description of these outcomes is done, we will establish standardized definitions. This will be a joint initiative with Francesco Rodeghiero and the planning committee of the second IWG for ITP terminology. Either a Delphi method or GRADE methodology will be used to establish consensus from a panel of clinical experts and patient representatives.

Towards Standardization of Functional HIT Assays Using 5B9, a humanized monoclonal antibody: Proposal (Yves Gruel, France)

5B9 is a chimeric IgG antibody specific to PF4/heparin complexes with a human Fc fragment which has been developed by the lab of Y Gruel in Tours (France), and it fully mimics the cellular effects of human IgG antibodies developed in HIT patients.
In this context, a multicentre project aiming to investigate whether 5B9 can be used as an internal quality control in functional assays used for the diagnosis of HIT is proposed by Y Gruel. For each assay evaluated (platelet aggregation test, HIPA, SRA, flow cytometry, HIMEA...), each participating center will have to test several concentrations of 5B9, with and without heparin, and with samples (PRP, washed platelets or whole blood) from 10 different donors. 10 participating laboratories have been identified so far and the study is planned to be performed in 2020.

TORADIHIT: Report (Tamam Bakchoul, Germany)

Dr Tamam Bakchoul (Tübingen, Germany) Reported on the TORADI Project on behalf of himself and Dr. Michael Nagler. In this ongoing study, members of the SSC Platelet Immunology submit data of patients from their hospitals who were suspected to have Heparin induced Thrombocytopenia (HIT). Dara from almost 500 patients from three Countries (Switzerland, Germany, USA) have been submitted online by June 2019. The incidence of HIT according to the functional assay and 4Ts score was 5%. Members of the SSC discussed in this session different possibilities to improve the recruitment into the study, since the study is only at about 50% of the enrolment target after 3 years.

A call to participate has been initiated by the chairman of the SSC who encouraged members to contribute. Spontaneous agreement at the session was made by three centers (Yves Gruel, Ulrich Sachs, D Arnold). TB and Michael Nagler will coordinate coaching to new the centers to accelerate the recruitment process.

Another suggestion that was raised during the discussion was to use existing HIT registries, such as the one from Japan (see next) to complete patient recruitment retrospectively.

Treatment strategy for patients suspected of having HIT in Japan (Shigeki Miyata, Japan)

Dr. Miyata reported on the HIT registry in Japan. To confirm the efficacy and safety of argatroban therapy with ethnicity-specific initial doses, which are less than half of those recommenced in Western countries, we have conducted by a nationwide post-marketing survey of 605 patients who were clinically suspected of having HIT and were treated with argatroban. The incidences of major clinical outcomes including thromboembolic events and serious bleeding are consistent with those reported in the previous similar clinical studies performed in North America. This result suggests that ethnicity-specific doses may be considered for argatroban therapy in Asian patients with HIT.

In addition, we have conducted another nationwide registry of patients with clinically suspected HIT (n = 837). Approximately one-fourth of these patients were ultimately diagnosed as having HIT, since they tested positive in the washed platelet activation assay. This registry shows the following results:

1. Acute HIT patients who possessed HIT antibodies with higher platelet activation properties developed thromboembolic events with a significantly higher incidence rate than those with lower platelet activation properties, suggesting that we might need different treatment strategies for patients with strong HIT antibodies and those with weak HIT antibodies.
2. DOACs may be effective even in Japanese patients with HIT, although we cannot get any conclusion so far due to a small sample size.
3. High-dose IVIg and therapeutic plasma exchange look promising for the treatment of severe HIT patients, especially who are at high risk of bleeding or urgently require the surgery using cardiopulmonary bypass.

Dr. Miyata proposed an international multicenter clinical trial for the approval of high-dose IVIg and plasma exchange for the treatment of severe HIT patients. There was discussion from the group around this idea, which was felt to be exciting and timely, but also posed significant feasibility challenges especially around recruitment.

Discussion and closing remarks (Donald Arnold, Canada)

Dr. Arnold recapped the new and existing projects discussed at this session, and called on members of the SSC to participate.

Platelet Physiology

7 July 2019
16:30 – 18:30

Chairman: Marie Lordkipanidzé

Co-Chairs: Paolo Gresele (past Chair), Marie-Christine Alessi, Marina Camera, Sofia Ramström, Jose Rivera, Matthew Rondina

The session was moderated by Matthew Rondina (Italy) and Jose Rivera (Spain).

- Overview of the Platelet Physiology SSC and Projects

Marie Lordkipanidzé

The business session started with an introduction by Marie Lordkipanidzé (Canada) who gave an overview of the mandate of the Platelet Physiology SSC, illustrated the Platelet Physiology subcommittee webpage within the ISTH site, including the options for interaction by registered members, and encouraged the audience to register. The new procedure for more transparence in selecting future Co-Chairs was discussed and a call for application for the next cycle (2020-2021) was made.

A list of the concluded projects and associated publications was given:

- Standardization of flow cytometry for the assessment of inherited and acquired disorders of platelet number and function, led by Larry Frelinger and Jose Rivera Pozo: first draft circulated to co-authors and publication likely by end of 2019
- Consensus/guidance on the methods for the study of platelet secretion, led by Diego Mezzano: in final stages of analysis with publication expected in early 2020
- Guidance on the measurement of platelet dimensions: methods and clinical use, led by Patrizia Norris: in final stages of analysis with publication expected in early 2020

Ongoing projects were presented in this session (as described below) and in the joint SSC session with Genomics in Thrombosis & Haemostasis (Standardizing Platelet Transcriptomics for Discovery, Diagnostics, and Therapeutics in the Thrombosis and Haemostasis Community (StriDe Study)), held on 6 July 2019.

- Evaluation of the ISTH Bleeding Assessment Tool (BAT) for the assessment of inherited platelet disorders: laboratory findings and 2 year follow-up

Paolo Gresele

The cross-sectional analysis of the ISTH-BAT study is completed, with a draft manuscript being circulated to co-authors. Results were briefly presented and publication is expected in the Fall of 2019.

The longitudinal arm of the study (2 year follow-up) was completed in December 2018. Loss to follow-up was minimal (85% completion) with 308 bleeding events reported. The draft manuscript to be prepared in Fall 2019.

The BAT-LAB sub-study, which looked at laboratory findings in patients in whom bleeding history and outcomes were assessed, is also completed. Preliminary results were shown and discussed with the audience, and a formal analysis with a draft manuscript is to be completed by the end of this year.

- Diagnosis of inherited platelet disorders on a blood smear: an update Andreas Greinacher

Andreas Greinacher presented an update on the ongoing project to diagnose inherited platelet disorders (IPDs) on a blood smear using immunofluorescence (IF). The systematic validation study on anticoagulants and storage times gave the following results:

- EDTA anticoagulated blood provides the most reproducible results for (IF) of blood smears
- Blood smears that were not fixed were stable for about 3 days. Thereafter the IF signal decreased.
- Fixed blood smears can be stored at -20°C for weeks (probably months?)

Blind comparison was performed on diagnoses made from blood smears and Next-Generation Sequencing (NGS) results of patients in the Mini Bridge study in Denmark headed by Eva Leinoe and the Spanish collaboration project on IPDs, headed by José Rivery Pozo, Murcia, Spain. In total, the results from 48 individuals with diverse genetic diagnoses of IPD were compared with outcomes from blood smears assessed with IF. The most clinically-relevant IPDs could readily be diagnosed by IF. In 6 patients where NGS found no definite mutation, IF provided a detailed phenotype of the platelets suggestive of a platelet defect. During the discussion, the audience was impressed with the preliminary findings and a call for an international study was made.

- International survey on practices surrounding the conservation and shipment of platelets for platelet studies

Marie Lordkipanidzé

Marie Lordkipanidzé presented an update on the systematic scoping review on the practices surrounding the conservation and shipment of platelets for platelet studies. The systematic search of the literature came up with 9494 records to be assessed. Title and abstract screening (Imane Boukhatem and Marie Lordkipanidzé) resulted in 149 papers to be assessed in full text; of these,

107 were retained for qualitative synthesis. The main findings were presented. The scoping review is being finalized for publication by the end of this year. A discussion ensued regarding an international survey of practices. The scoping review revealed a high heterogeneity of practices that will not be easily transposed into consensus-forming statements. Pierre Fontana in the audience pointed out that an international survey is not likely to give a clearer portrait of the best practices and an international study may be required. The Platelet Physiology SSC will need to decide whether an international survey is to be carried out or not.

- A multicentric comparison of platelet aggregation agonists against NIBSC standards

Marie-Christine Alessi

The “Platelet Aggregation Standardization Project – PAPS Study” is jointly led by the Scientific and Standardization Committee on Platelet Physiology and the National Institute for Biological Standards and Control (NIBSC). The primary objective of this project is to compare commercial suppliers of platelet agonists and determine the extent of variability between reagents. This study includes comparators from the NIBSC and a commercial supplier. The prepared comparators will help to assess the behavior of commercial reagents across laboratories. The project will evaluate the main agonists used for diagnosis of platelet dysfunction. A questionnaire has been sent to potential investigators allowing to identify the reagents and the equipment used by investigators wishing to participate in the study. Each investigator site will have to perform a 5-point dilution series of the in-house and the comparator agonists in different platelet-rich plasma (PRP) samples. The number of PRP samples will be adapted to each investigator site/supplier to meet statistical requirements. EC50 will be determined and compared between suppliers, aggregometers etc. By the time of the project presentation, 28 laboratories agreed to participate in the project. Ethics approval is mandatory and must be obtained according to each participating centre’s local regulations. The study is planned to start during the fourth quarter of this year. Lively discussion with the audience showed support for this ongoing project, with more centers willing to participate.

- Biomarkers of in vivo platelet activation: a systematic review and meta-analysis

Marina Camera & Sofia Ramström

Marina Camera & Sofia Ramström presented a proposed revision of the previous project “Measurement of platelet activation markers: usefulness, current practices, future”, that will also include collaboration with the Vascular Biology SSC. Instead of the previous questionnaire approach on a very broad subject, the current proposal is to perform a systematic review and meta-analysis of available data considering platelet activation markers. Due to the amount of data available, the suggested focus this project will be exclusively on the soluble platelet activation markers. The performance in predicting ischemic events in patients with coronary artery disease will also be assessed. Marina Camera & Sofia Ramström showed the preliminary analysis on some of the markers of interest to demonstrate the feasibility.

The revised project seemed to be well received; only a few comments were received during and after the presentation. The audience advised the co-PIs to pay extra attention to preanalytical issues of potential interest to the results. As the prospective studies might be few, the co-PIs don't expect to be able to give specific advice regarding this in the proposed communication, but to be able to give society members interested to start a study a thorough overview of the available options and the performance of the markers reported so far. A draft of the communication is planned to be sent to the committee members by March-April 2020.

- Procoagulant platelets: a collaborative project with the Vascular Biology SSC Marina Camera & Sofia Ramström

Marina Camera & Sofia Ramström presented a new project aiming to establish guidance for markers and methods to define a procoagulant platelet as compared to an apoptotic platelet. As the field today contains many varying definitions and as the nomenclature used is far from standardized, the co-PIs propose this to be a suitable project for the SSC. Several researchers with active research in the area did approach the co-PIs during the discussion period and were positive to participate in this work. In addition, this project will involve Emma Josefsson from the Vascular Biology SSC, who has expertise in platelet apoptosis and animal models in this area. The co-PIs believe that a RAND expert survey might be a way forward will this project and will construct a draft for such a survey. Proposed revised name for the project is **"Procoagulant/apoptotic platelets – call for standardization of methods and nomenclature".**

Another issue presented was the role and measurement of tissue factor in platelets, a controversial area in the field needing more attention. Commentators from the audience questioned how to merge these two subjects in one project, and after the meeting with the chair and co-chairs of the committee, we decided on proposing this as a separate project. The proposed plan is to produce a protocol and perform a multi-center study in selected international laboratories where these collect and analyze donors to establish a normal range. Proposed revised name for the project is **"Recommendations for studies of tissue factor-positive platelets".**

- Call for collaboration with the Platelet Immunology SSC

Paolo Gresele, Donald Arnold & Tamam Bakchoul

Tamam Bakchoul (Platelet Immunology SSC) was a guest speaker in the Platelet Physiology SSC business session to outline areas of need for collaborative projects. He first presented the mandate of the Platelet Immunology SSC with their current areas of research / interest. Proposals for collaboration included areas of antibody-mediated effector functions, which included 4 major aims:

- to combine resources from both SSC to establish an external quality assessment for platelet function assays

- to develop predictive tools for the clinical outcomes of thrombocytopenic patients
- to implement novel laboratory assays in the diagnostic algorithms
- to evaluate suitable platelet function assays in thrombocytopenic patients

These projects were well received and the SSCs will work together to implement them in the coming years.

Attendance was excellent at the beginning of the session, but only the core attendees of the Platelet Physiology SSC remained at the end. This may have been a result of the new congress format (with many ISTH delegates who may not be aware of the business session format) and timing of the session in the late afternoon (as fatigue was palpable). Discussion from the audience was active and lively when interactions were solicited. Many participants came up to the speakers after the session to carry on with more in-depth discussion on their specific projects. The room was good-sized, the organization and facilities were excellent, apart from the some technical difficulties in projecting some of the PowerPoints. Technical assistance in the room was reasonably good. Each speaker kept to time, with the session running the expected 2 hours. Some ongoing projects could not be presented in this session due to time constraints. The joint session with the SSC on Genomics in Thrombosis and Haemostasis alleviated some of this difficulty, as a key new collaborative project led by Matthew Rondina (co-chair) was presented on 6 July 2019.

Platelet Physiology Subcommittee at the 2019 ISTH Congress in Melbourne, Australia

Predictive and Diagnostic Variables in Thrombotic

9 July 2019
8:00 – 12:30

Chairman: John-Bjarne Hansen

Co-Chairs: Cecilia Becattini, Kerstin de Wit, Geert-Jan Geersing, Gregoire Le Gal, Marc Righini, and Emmanuel Morange

The program was divided into two sessions. The first session was devoted to updates on on-going projects. After a short introduction by the chair, Professor Hansen, Dr. Tritschler presented on a standardized definition for PE-related death and classification of the cause of death in VTE studies, a SSC project that was initiated 2 years ago. The development process of the proposal included a systematic review of current definitions used for PE-related death, two subsequent surveys with VTE experts, and consensus meetings held within the SSC working group. During his talk, Dr. Tritschler presented the final proposal which will be submitted as a SSC communication within the next weeks. Second, Dr. Klok talked about the subsegmental PE project. The management of subsegmental pulmonary embolism (ssPE) is challenging, not at least because the diagnosis is often unsure: the interobserver agreement of ssPE between expert chest radiologists has been reported to be poor. In an attempt to develop a diagnostic definition of ssPE, which should allow for a more reproducible diagnosis, two Delphi analyses were performed. The first was among expert chest radiologists, who were asked to reach consensus on a radiologic definition of ssPE. The second one involved clinical PE experts, who were asked whether they agreed on the provided diagnostic definition, and provide their best practices with regard to the treatment of ssPE in specific clinical settings. The results of both Delphi analyses were presented at the 2019 ISTH meeting.

The second session was on new project initiatives. First, Dr. de Wit presented a project on standardization of diagnostic assessment for venous thromboembolism in cancer. In this project, the best evidence on PE and DVT diagnosis in cancer patients will be systematically reviewed and information from physicians and patients will be gathered, in order to update the standards for PE and DVT testing in cancer patients. Thereafter, Dr. Geersing talked about a new project on standardized assessment of bleeding risk in patients treated with anticoagulants for venous thromboembolism. The number of patients on extended anticoagulation after a first VTE event is clearly increasing. Albeit likely clinically effective in terms of reducing the occurrence of recurrent VTE, inevitably, this increases the risk of bleeding. Physicians caring for these patients are thus increasingly confronted with patients experiencing a bleeding event under anticoagulant treatment, or they need to manage patients at (dynamically increasing) risk of experiencing such a bleeding event. Bleeding events are in a way thus an inevitable consequence of prolonging anticoagulant treatment, yet also have an important impact on treatment adherence, treatment persistence and overall quality of life for patients on these drugs. Assessing bleeding risk will become an integral part of thrombosis medicine and we need therefore novel biomarkers and improved prediction models to identify risk of major bleeding during anticoagulant treatment. Then Dr. Morelli presented a new project initiative on biomarkers for and prediction of venous thromboembolism after stroke. Identification of patients at high risk of venous thromboembolism (VTE) after acute ischemic stroke would form the basis

for targeted thromboprophylaxis. However, current data on VTE prediction in stroke patients are scarce, and prediction models based on clinical factors alone discriminate poorly between stroke patients at high and low risk of VTE. Therefore, the overall aim of this project is to identify and combine the best predictors for VTE after acute ischemic stroke in order to improve patient stratification and aid clinical decisions on therapeutic intervention. As a first step, a systematic review on clinical factors and biomarkers for VTE after ischemic stroke will be performed to inform the main gaps in knowledge related to the topic. The systematic review will help to direct further steps based on cohort studies aimed to identify clinical factors and biomarkers that can used to develop a prediction model for VTE occurrence after ischemic stroke. Lastly, Dr. Barco presented a project under development on the prediction of death in acute pulmonary embolism.

The meeting room was well attended throughout the entire 2 hrs program and each presentation was accompanied by constructive discussions.

Vascular Biology

7 July 2019
16:30 – 18:30

Chair: Johannes Thaler

Co-Chairs: Emma Josefsson, Juan Melero-Martin, Rienk Nieuwland, David Smadja, Romaric Lacroix and Kimberely Martinod

Extracellular Vesicles

Workshop Update on the Comparison of the Assays to Measure Procoagulant Extracellular Vesicles in Plasma Samples

Speaker: Romaric Lacroix, Aix-Marseille University, Marseille, France

Dr. Romaric. Lacroix presented an update about the SSC project to compare assays which measure tissue factor (TF)-exposing extracellular vesicles (EVs). Despite the fact that EVs may have a potential to predict cancer associated thrombosis, the broad methodological heterogeneity and the lack of standardization among assays impede further clinical development. About antigenic assays, the capacity of flow cytometry (FCM) to detect TF is questionable and the specificity of current protocols has not been tested so far. Regarding functional assays, new assays with improved sensitivity and reproducibility are being proposed, including the use of immunomagnetic separation to isolate EVs from plasma. However, none of these assays have been validated at a multicenter level. Thus, a collaborative project with the objective to compare the analytical performance (sensitivity, specificity and repeatability) of the assays to measure TF-EVs in plasma samples is timely and relevant. 14 labs already registered accounting for 31 assays in 7 groups of methods. The first 4 being FMC, FXa assays, Clotting assays and Zymuphen. Results are expected to be presented at Milano ISTH 2020 (More details on the SSC VB web page)

Extracellular Vesicles

Standardization of Vesicle Concentration Measurements by Calibration of Flow Cytometers: METVES II

Speaker: Johannes Thaler, Medical University of Vienna, Vienna, Austria

All body fluids contain extracellular vesicles (EVs), an umbrella term for all vesicles including “microparticles” (“microvesicles”) and exosomes. Because EVs are an intrinsic component of body fluids, and because measuring the concentration, cellular origin, composition and function of EVs may provide clinical relevant information, the interest in EVs is growing exponentially.

Flow cytometry is the most widely used instrument to measure EVs, and the SSC on Vascular Biology of the ISTH has organized and supported standardization studies comparing EV concentrations in provided samples between flow cytometers. There are still several problems, however, that hamper standardization of EV concentration measurements: flow cytometers differ in sensitity, light scatter signals are complex and depend on (light) collection angels and particle

refractive index, and data are in “arbitrary units which hamper data interpretation, standardization and data comparison.

METVES II will overcome the before mentioned problems by (1) developing a reference materials and procedures to calibrate all flow cytometer aspects (scatter, fluorescence, flow rate; “*one reference material to calibrate them all*”), and (2) developing an easy-to-use (stable) EV-containing biological test sample. By calibrating flow cytometers, the currently generated signals in arbitrary units can be translated into SI units, thus making then a direct comparison of measurement results possible between flow cytometers.

The METVES II consortium is a collaboration between European metrology institutes, academic partners and industry, and our main goal will be “to get EVs into the clinics” by making reference materials, procedures and easy-to-use software available to perform calibrations of flow cytometers for EV research.

Neutrophil extracellular traps

Can we standardize measurement of neutrophil extracellular traps in patient samples?

Speaker: Kimberly Martinod, KU Leuven, Leuven, Belgium

Neutrophil extracellular traps (NETs), first named in 2004, represent a “hot topic” of research. As of July 2019, there were >2500 publications on NETs, and specifically 384 on NETs in thrombosis. NETs consist of either nuclear chromatin or mitochondrial DNA along with neutrophil proteins such as myeloperoxidase and neutrophil elastase. NET formation is now established as more than one active biological processes which can either result in cell death or generation of functional cytoplasts. The released NETs can have antimicrobial function or contribute to disease pathologies (i.e. by binding platelets/red blood cells/coagulation factors in thrombosis; by histone disruption of endothelial/epithelial cells). Methodologies to study NETs are evolving: including techniques of microscopy involving identification of DNA/histones/neutrophil proteins, citrullinated histones, decondensed chromatin; flow cytometry including imaging cytometry, identification of extracellular DNA/(citrullinated) histones/neutrophil proteins; plate-based assays including SytoxGreen, measurements of DNA by intercalating dyes, sandwich ELISAs; and others. These various techniques can be applied to isolated cells, tissue samples, or biological fluids and there is inconsistency in the literature, including in thrombosis/hemostasis, about identification of NETs. This is likely due to the different pathways/natures of the NETs being released.

The SSC of the ISTH thus proposes a standardization study with the aim of generating a recommendation paper in the JTH, specifically focusing on measurement of NETs in blood/biological fluids in human samples. To begin, a public survey will be released in August via the ISTH RedCAP project to assess how labs in thrombosis/hemostasis are currently measuring NETs, and to gauge interest in active participation in a full standardization study. Depending on funding decisions due Dec 2019, the first arm of a standardization study will begin with the preparation of standardized NET samples (containing NETs or non-NET nucleosomes, prepared from a mixture of NETs induced by different stimuli (infectious vs sterile, lytic vs. vital, etc) which will be sent to participating groups to measure using their current techniques. A comparative evaluation will be performed on this to determine if a uniform NET profile can be established either from a single or a combination of different assays. If the first arm is successful, a second arm would involve sending standardized samples to the same groups, along with standardized reagents and protocols to repeat NET measurements. A second comparative study will be

performed on these results, from which the goal is to submit an SSC recommendation to the ISTH. The timeframe for this proposed study is 3 years.

Please look out for the ISTH RedCAP survey in August, and share to all researchers who you may know who are currently studying NETs in their labs. Anyone interested in participating in a full standardization study is welcome to contact Kim Martinod (email: kim.martinod@kuleuven.be, Twitter @kmartinod).

Neutrophil extracellular traps

Quantification of H3Cit in human blood samples.

Speaker: Charlotte Thålin, Karolinska Institutet, Stockholm, Sweden.

Citrullinated histone H3 (H3Cit) is the product of PAD4-mediated citrullination of arginine to citrulline at the histone H3 N-terminal. Histone tail citrullination promotes chromatin decondensation, involved in the release of neutrophil extracellular traps (NETs).

H3Cit has recently been detected and quantified by means of ELISA in several patient populations, but there are important challenges with currently used H3Cit ELISA assays:

- The widely used polyclonal H3Cit antibody (ab5103) varies between lots and is unspecific for citrulline.
- The current use of PAD4-citrullinated histone peptides in standard curve generation is problematic. The enzymatic generation may introduce off-target modifications and lot-to-lot variability, and histone peptides are notoriously unstable in plasma.

Monoclonal anti-citrulline antibodies bind specifically to the reported target with < 10% cross-reactivity. Nucleosomes are close to 100% recovered when spiked into plasma, and the 3D structure of nucleosomes mimics the physiologic setting in which H3Cit circulates in vivo. Employing monoclonal and specific antibodies, and synthetically generated citrulline-containing nucleosomes (H3R2/8/17cit dNucs) for standard curve generation allows for accurate quantification of H3Cit in plasma. This standardized ELISA assay may be a valuable tool for comparison of the levels of H3Cit in different patient populations and between labs.

Circulating Endothelial Progenitor Cells

The Combinatorial Impact of Endothelial Colony-Forming Cells and Mesenchymal Stem Cells on Treating Vascular Disease

Speaker: Jatin Patel, The University of Queensland, Brisbane, Australia

Dr. Patel presented recent preclinical data from his group describing the potential of endothelial colony-forming cells (ECFCs) and mesenchymal stem cells (MSC) for cell therapy of ischemic vascular diseases. Considerations about how to improve allogeneic stem cell engraftment with and without immunosuppression were also discussed. Dr. Patel described how in his xenograft mouse model (hind limb revascularisation via implantation of ECFC and MSC) the use of cyclosporine had a significant decrease in reperfusion based on laser Doppler imaging compared to vehicle controls and had poorer limb survival. He also discussed data suggesting that in the presence of cyclosporine ECFCs show a loss of function and how this deleterious impact of cyclosporine could significantly impact ECFC-based

allogeneic cellular therapies. Finally, issues regarding ECFC immunogenicity and limitations of the model used in Dr. Patel's results were discussed.

Circulating Endothelial Progenitor Cells

Distinct Expression of Neuregulin-1 in Human Endothelial Colony-Forming Cells

Speaker: Juan Melero-Martin, Boston Children's Hospital, Boston, United States

Dr. Melero-Martin presented recent results from his group on human Endothelial colony-forming cells (ECFCs). Specifically, he discussed work performed in his lab to elucidate distinct phenotypic characteristics of ECFCs and their comparison to tissue-resident endothelial cells (ECs) and iPSC-derived ECs. Dr. Melero-Martin described how transcriptional RNA-seq analysis revealed particularly high expression of the cardioprotective growth factor neuregulin-1 (NRG1) in ECFCs and how this high level of expression was confirmed by Real-time qPCR, ELISA, Western blot, and immunofluorescent staining. Furthermore, Dr. Melero-Martin presented data on distinct cardioprotective properties of ECFC-derived NRG1. In his concluding remarks, Dr. Melero-Martin proposed that ECFCs are unique in their high expression of NRG1, which confers to these cells a distinct cardioprotective ability. He also described how he envisions the use of NRG1 expression as a marker for ECFC identity in comparison to other ECs. Finally, issues regarding ECFC identity, specific markers, and related limitations in the field were discussed.

Circulating Endothelial Progenitor Cells

Closing remarks

Speaker: David Smadja, Faculté de Pharmacie de Paris, INSERM UMR-S 1140, Paris, France

Dr. Smadja discussed the current effort by this SCC subcommittee with regards to standardization and the publication in JTH recently available (DOI: 10.1111/jth.14462). This publication is the result of round table discussion in Berlin. In addition, Dr. Smadja discussed plans for a survey in the next month to go deeper in the standardization process:

https://drive.google.com/open?id=1x2QwdPQc7Zloioo5T1Y_Q7Bx9lYIVTATc1j6cid4mLw

Results of this survey will be presented our next ISTH SSC meeting in Milan.

Platelets

Introduction to platelets - and a new collaboration project

Speaker: Emma Josefsson, The Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia

Dr. Emma Josefsson reported on the traditional roles of platelets. Then two collaborative projects with the Platelet Physiology SSC were presented. The title of the first project was "Identification of platelet activation markers predictive of cardiovascular events (a systematic review and meta-

analysis)". It is the aim of this project to improve thrombotic risk stratification of coronary artery patients. The aim of the second project is the investigation of procoagulant platelets. Specific features of procoagulant in contrast to apoptotic platelets were discussed. The need for guidelines was pointed out because there are many different markers/methods used to assess procoagulant platelets.

Finally, a new project to establish guidelines on procoagulant platelet markers/methods was proposed. Aims of this project are:

- Find combinations of markers/methods that can identify and separate procoagulant platelets from apoptotic platelets.
- Identify tools that can be used to address differences in signaling pathways
- Identify the experimental setting for TF-associated platelet measurement (sample preparation, antibodies, experimental conditions)

Von Willebrand Factor

7 July 2019
16:30 – 18:30

Chairman: Sandra Haberichter

Co-Chairs: Ross Baker, Simon De Meyer, Veronica Flood, James O'Donnell, Analia Sanchez-Luceros, and Michelle Lavin

Guidelines for the Diagnosis and Management of von Willebrand Disease

Nathan T. Connell (United States)

Dr. Connell reported on the new VWD guidelines currently being developed. ISTH is collaborating with the American Society of Hematology, National Hemophilia Foundation, and the World Federation of Haemophilia to develop updated guidelines for the diagnosis and management of von Willebrand disease (VWD). Two panels have been convened to prioritize questions for a systematic review of the literature and build evidence-to-decision frameworks for key recommendations to improve the diagnosis and care of those living with VWD. To inform the work, the partner organizations collaborated on an international survey of physicians, patients, and stakeholders as to what the key questions were facing the VWD community and these data will be presented separately during the meeting. Guideline publication is anticipated in 2020.

International Microangiopathic Thrombocytopenia ADAMTS13 Standardisation (IMATAS) Collaborative Study

Ross Ian Baker (Australia)

Dr. Baker reported on a new, collaborative study, IMATAS. Deficiency of ADAMTS13 is increasingly being recognized as a common cause of microangiopathic thrombocytopenia (MAT) and in particular at a level of <10% is diagnostic of thrombotic thrombocytopenic purpura (TTP). ADAMTS13 testing is now the cornerstone in the diagnosis, prognosis and treatment of patients with life threatening thrombotic microangiopathy and thrombocytopenia. However, precision and clinical interpretation of the ADAMTS13 testing is still problematic and will lead to variation in important clinical decisions and management. Further work is required to standardise ADAMTS13 testing for the different clinical situations of the new MAT diagnosis, whether it is immune mediated TTP or congenital TTP, guide TTP treatment, monitoring and prediction of relapse. In order to address this unmet need, the ISTH VWF subcommittee will coordinate the International Microangiopathic Thrombocytopenia ADAMTS13 Standardization Collaborative study (IMATAS) to examine the standardisation, analytical performance and clinical interpretation of ADAMTS13 testing

Examining Current Practices in the Care of Pregnant Women With Low von Willebrand Factor / von Willebrand Disease – Survey

Michelle Lavin (Ireland)

Dr Lavin provided an update on recent survey results. While the increased risk of postpartum hemorrhage (PPH) in pregnant women with Von Willebrand Disease (VWD) is well established, the optimal antepartum and postpartum management strategies are a source of current debate. Working in collaboration, the ISTH VWF and Women's Health SSCs conducted an international survey to assess current practices in the management of pregnant women with VWD. Data from 132 respondents globally were analyzed with marked variations observed in the antenatal and postpartum monitoring of plasma VWF levels, the advice given regarding neuraxial anesthesia, use of hemostatic therapy at delivery and the definition of postpartum hemorrhage. These data highlight the differences in approach to the management of pregnancy in women with VWD and underscore the need for consensus guidelines to harmonize care.

Von Willebrand Factor Calibration Studies: A Report on SSC/ISTH Lot #5 (GpIb-Binding Methods); A Proposal to Replace the WHO 2nd International Standard for von Willebrand Factor

Craig Thelwell (United Kingdom)

Calibration of the SSC/ISTH Secondary Coagulation Plasma Standard Lot #5 for VWF activity was presented by Dr. Thelwell. The result from VWF:GPIbM methods (0.80) was not significantly different to VWF:RCo (0.82; p=0.532) however VWF:GPIbR was significantly higher (0.95; p=0.001). Values were assigned relative to the WHO 6th IS FVIII/VWF Plasma, which was established as a WHO Reference Reagent for VWF:GPIbR and VWF:GPIbM in 2018. Lot #5 has now been approved and will replace Lot #4 which is almost depleted. Stocks of the WHO 2nd IS for VWF Concentrate are low and a proposal to calibrate a replacement was approved by the WHO Expert Committee on Biological Standardisation. A collaborative study is being organised for 2019/20 for potency estimation of three candidate replacement materials for the existing analytes (VWF:RCo; VWF:CB and VWF:Ag) and assigning values for VWF:GPIbR and VWF:GPIbM will be investigated.

Enhanced Local Disorder in a Clinically Elusive von Willebrand Factor Provokes High-Affinity Platelet Clumping

Matthew Auton (United States)

Dr. Auton presented data from a published international collaboration (PMID 28533135) initiated by Drs. Juan Frontroth and Matthew Auton that provides a biophysical basis for high-affinity platelet agglutination during a desmopressin DDAVP (D-amino D-arginine vasopressin) trial on two patients with Von Willebrand Disease (VWD). The case study involving two young girls, initially thought to have type 2A VWD, revealed a type 2B phenotype as a result of two mutations located in the disulfide bond of the Von Willebrand Factor (VWF) A1 domain. Patient data from Dr. Frontroth showed a transient thrombocytopenia and platelet clumping in blood smears during DDAVP administration indicating high-affinity VWF mediated platelet adhesion. Biophysical structure/function studies confirmed the high-affinity, established a mechanism for high affinity, and demonstrated a loss of native structure in the A1 domain, a conformation lacking a structured alpha 2 helix. This is the first study to experimentally quantify residue-specific conformational dynamics of a pathological disease state of VWF as it relates to patient manifestations of VWD.

ADAMTS13 Conformation in Thrombotic Thrombocytopenic Purpura
Elien Roose (Belgium)

Dr. Roose reported on immune-mediated TTP (iTTP) that is diagnosed based on severe thrombocytopenia, haemolytic anaemia, severely decreased ADAMTS13 activity and presence of anti-ADAMTS13 autoantibodies. However, those signs and symptoms can overlap with other diseases, like HUS and sepsis. Additionally, anti-ADAMTS13 autoantibodies are not always detected in iTTP patients. Dr. Roose's group has shown that determination of the ADAMTS13 conformation could aid in the correct diagnosis of patients with a (severely) decreased ADAMTS13 activity. While ADAMTS13 adopts a folded conformation in sepsis and HUS patients (similar to healthy donors), iTTP patients have an open ADAMTS13 conformation. Interestingly, the open conformation in iTTP patients appears before a severe drop in the ADAMTS13 activity and is induced by anti-ADAMTS13 autoantibodies. Therefore, the ADAMTS13 conformation can be used as a biomarker in the diagnosis of iTTP.

Project Update: 3Winters-Ips and Interactive Registry on Acquired von Willebrand Syndrome Projects
Augusto B Federici (Italy)

Dr. Federici presented an updated version of the Interactive Registry on Acquired von Willebrand Syndrome (INTREAVWS) proposed on behalf of the WG on AVWS. Retrospective information will be collected using Electronic Case Reports (ECR) by ISTH Members and Investigators who would like pseudonymously to submit their patient data. The Second step of the study will include the central confirmation of AVWS. Only after this confirmation, patients will be observed prospectively for the response to different therapeutic approaches and progression of the disease. Dr. Federici also reported an update on the 3Winters-Ips project. Since the frequency of bleeds was lower than expected, an extended prospective observation (from 2 to 5 years) will be required.

Women's Health Issues in T&H

7 July 2019
16:30 – 18:30

Chairman: Maha Othman

Co-Chairs: Patricia Casais, Emmanuel Favaloro, Ian Greer, Susan Halimeh, Predrag Miljic, and Robert Sidonio Jr.

Welcome, outline of the women's SSC and overview of activities- Chairman of SSC: Maha Othman (Canada)

Dr. Othman gave an introduction on the subcommittee's activities and highlighted the ongoing projects. She first introduced the current committee co-chairs: Robert Sidonio (USA), Susan Halimeh (Germany), Predrag Miljic (Serbia), Patricia Casais (Argentina) and Emmanuel Favaloro (Australia). She then reviewed the ISTH SSC mandate and explained the focus of the women's SSC. She also invited for active participation in the committee and registering as members. Dr. Othman listed all ongoing and completed SSC projects. There are currently 11 projects coordinated under the auspice of the women's SSC; 4 of which are joint projects with other SSCs (the Pediatric, FVIII, DIC and VWF SSCs). Two new projects have been introduced in the past year. Three SSC publications were completed in the past year; 2 for JTH and one for RPTH. Dr. Othman highlighted the value and the success of the joint Session with LP/aPL SSC and DIC SSC on: "Antiphospholipid Antibodies and DIC in Pregnancy" in the current ISTH meeting. She explained the ISTH's new process for selection of co-chairs and encouraged members of the hemostasis community to submit expression of interest and apply for co-chair positions as they become available. Dr. Othman finally reviewed the SSC agenda and invited for active discussion and feedback to all topics.

<u>Part I: Women and bleeding: This section was moderated by Emmanuel Favaloro and Maha Othman</u>

Challenges in the management of VWD in pregnancy: results from an international survey-Michelle Lavin (Ireland)

Dr. Lavin first provided the background and explained the rationale and objective of this project. While the increased risk of postpartum haemorrhage (PPH) in pregnant women with Von Willebrand Disease (VWD) is well established, the optimal antepartum and postpartum management strategies are a source of current debate. Working in collaboration, the ISTH VWF and Women's Health Issues in Thrombosis and Haemostasis SSC conducted an international survey to assess current practises in the management of pregnant women with VWD. Data from 132 respondents globally were analysed with marked variations observed in the antenatal and postpartum monitoring of plasma VWF levels, the advice given regarding neuraxial anaesthesia, use of haemostatic therapy at delivery and the definition of postpartum haemorrhage. These data highlight the differences in approach to the management of pregnancy in women with VWD and underscore the need for consensus guidelines to harmonise care. Common bleeding disorder affecting women. Dr. Lavin concluded: there are different approaches in care to pregnant women with VWD and only few questions with consensus. Current challenges are the standardisation of management approaches and data collection and a consensus guidance.

Hemophilia carrier nomenclature: proposed terminology to improve communication- Robert Francis Sidonio, Jr. (USA)

Dr. Sidonio and his team sought to propose terminology and a conceptual framework for categorization of hemophilia A and B carriers accounting for personal bleeding history, genetic determinants and baseline factor level, which will ultimately improve communication between providers, researchers, payors and community members.

Hemophilia Carriers with normal levels (as defined by international standards; >40%) may have an increased bleeding phenotype despite having a factor level in the normal hemostatic range. They suggest using the term “symptomatic hemophilia carrier” and to avoid utilizing this terminology to characterize all hemophilia carriers. Furthermore, during a woman’s lifetime she may have prolonged periods of no significant bleeding and we suggest to the use the term “asymptomatic hemophilia carrier.” This is of course acknowledging that this bleeding tendency may change during her lifetime thus the bidirectional arrow in the figure. They suggest that the term “hemophilia carrier” be reserved for use in discussions regarding genetic counseling and prefer to use the terms “women” and girls with hemophilia (WGH)”. Therefore for a hemophilia carrier with factor levels >5-40%, 1-5% and <1% should be referred to as a mild hemophiliac, moderate hemophiliac and severe hemophiliac respectively.

Setting standards for appropriate and necessary care for young women with heavy menstrual bleeding and bleeding disorders: an international panel survey- Ayesha Zia (USA)

Dr. Zia introduced the proposal of this project at the SSC 2018 meeting in Dublin. She first provided the background and objective of the project. She then reviewed the methodology and discussed the results and data collected so far.

Unpredictable, prolonged or heavy menstrual bleeding (HMB) may be expected for many adolescents soon after menarche. A decade of clinical experience and research has now established firmly that bleeding disorders (BD) are common in adolescents with HMB. Guidelines for HMB and BD evaluation in adolescents suggest a variety of strategies for screening, diagnosis and management, reflecting lack of high-quality evidence demonstrating the superiority of any one approach. This results in under-recognition of BD in adolescents, and years of suffering and mismanagement.

The objective of this project is to assess clinicians' perceptions of the appropriateness of screening, diagnostic and management strategies for optimal detection of BD in adolescents with HMB.

An online survey using the RAND/ExpertLens platform was used to capture expert judgment encompassing three specialties (hematology, OB-GYN and adolescent medicine), over three rounds of survey questions.

The results of the survey clearly delineate the subset of adolescents with HMB for whom it is appropriate and/or necessary to work-up for BD, the type and timing of work-up, and the optimal management approach. Additionally, the results of the survey highlight areas where future research is needed.

Part II: Women and thrombosis & complex pathologies. This section was moderated by Predrag Miljic and Robert Francis Sidonio, Jr.

The MAPP registry: thrombolysis and invasive treatments for MAssive Pregnacy-related Pulmonary embolism- Marc Blondon (Switzerland)

At the SSC 2018 in Dublin, Dr. Blondon discussed the lack of specific data and weak evidence around treatment of massive pulmonary embolism (PE) in pregnancy and the postpartum period. While about 50-100 massive PE is estimated to occur every year in Europe and the same number in North America, only 127 cases were found in a recent systematic review of the literature. While maternal, obstetrical and neonatal outcomes appeared good in that study, but findings are prone to a high risk of publication bias. Dr. Blondon has proposed a new SSC-supported international registry to prospectively collect data on women suffering from pregnancy-related massive PE. The registry will explore maternal effectiveness and maternal/obstetrical safety of intravenous / intraarterial thrombolysis, mechanical thrombectomy. Such data would help inform the care of these very ill patients and future guidelines.

Dr. Blondon has now launched the MAPP registry and invited for participation. This is an international registry that seeks to collect data on women with pregnancy related massive PE focusing on the risk factors and efficacy of three treatment modalities: IV/IA thrombolysis, thrombectomy and ECMO. The plan is to recruit 10-15 cases per year, with an achievement of 80 included women over 5-8 years period that could lead to a future clinical trial. Suggestions from the group included clarifying the time to thrombolysis, including cases of surgical thromboectomy and systemic anticoagulation. The registry is available on ISTH REDCap website.

The role of tissue plasminogen activator in reproductive and depressive disorders- the hibernation model- Sivia Hoirisch Clapauch (Brazil)

Dr. Clapauch intended to bring a new perspective to the women SSC and that is the complex relationship between coagulopathies in women and psychiatric disorders. She believes there is potential for creating useful projects with international aspect.

In her talk, she discussed literature on the complex pathologies of metabolic impairments, anxiety and depression and tPA related coagulopathy in women in relation to PCOS, placental impairment, other women related disorders. Questions raised were: why do women with PCOS, first-trimester losses or preeclampsia are at high risk for cardiovascular disorders and why depressive disorders increase the risk of both reproductive and cardiovascular disorders. She started by highlighting the role of tPA and general coagulation in the role of metabolic abnormalities, anxiety, depression, autism and general mental illness. Dr. Clapauch presented 2 posters at this ISTH meeting; one discussing the role of anxiety and depression in the pathogenesis of "unprovoked" thromboses and the other explaining why schizophrenia patients have a huge prevalence of thrombophilia. She advocates for creating space at the SSCs for addressing issues at the interface Hematology-Psychiatry, such as it is the case in Psychiatry meetings.

Dr. Clapauch was encouraged to submit ideas/ a formal proposal to investigate the role of coagulation making this connection, which can be examined as potential SSC projects aiming to identify priorities re psychiatric issue and coagulopathies in women.

A new algorithm based on thrombin generation to assess hormone-related prothrombotic changes- Jonathan Douxfils (Belgium)

Dr. Douxfils provided a thorough background on how hemostasis in women is influenced by physiological changes in hormonal status associated with the menstrual cycle and pregnancy and the explained the rationale and objective for this new SSC project proposal.

Hormone-based contraceptives (including combined hormonal contraceptives (COC)) and hormone replacement therapy (HRT) preparations also affect hemostasis. These hormonal variations may increase in the risk of venous thromboembolism (VTE) due to various impact including changes in the levels of hemostasis factors but also an acquired resistance of activated protein C (aPC). The risk of VTE is also higher in the presence of intrinsic risk factors (such as older age, obesity, etc.). Risk factors for VTE change over time suggesting that an individual's risk should be re-evaluated periodically. Accumulating epidemiological studies highlighted the association of COCs with thrombotic events. Differences exist between COCs in their risk of VTE depending, among others, on the type of progestogen they contain. This led to an update of the summary of product characteristics and information of these products by regulatory bodies worldwide to help women make informed decisions about their choice of contraception together with their healthcare professional. Biological variables that may reflect different pharmacological effects, possibly related to VTE risk, should be investigated in the development of a new combined (oestrogen-progestogen) contraceptive product, including aPC resistance, protein S, protein C and SHBG. However, no such biomarker alone has been shown as a good predictive marker of the VTE. Contra-indications of the use of COCs are however proposed in cases of hereditary or acquired predisposition for VTE, such as activated protein C (APC) resistance, antithrombin deficiency, protein C deficiency, and protein S deficiency although they are not routinely measured before treatment initiation or during treatment.

The first goal of this project is to investigate if women with prothrombotic events have higher hemostasis changes than matched controls without event. The three regulative pathways of the coagulation (protein C/protein S, antithrombin and TFPI pathways) will be investigated and integrated together in an algorithm that will take all these variables into account. This global exploration of the coagulation, using the thrombin generation as final endpoint, could facilitate the processing of data's and raise the interest of using this technology in clinical routine. Finally, the aim will be to propose to physicians, regulatory bodies and stakeholders an all-in-one solution for the investigation of prothrombotic states. This will simplify the characterization of the patients and potentially help the physician to guide the patient's care. As in the case of a new contraceptive therapy, it would allow to go directly for the safer treatment if the patient has risk factors such as a genetic mutation or a deficiency of a coagulation factor.

Dr. Douxfils is proposing a registry to investigate if women with thrombotic events have higher hemostasis changes than matched normal controls utilizing thrombin generation to predict

thrombotic events in a future developed algorithm. In addition, he seeks to standardize an endogenous thromboin potential based APC resistance assay.

International registry on DIC in pregnancy- Offer Erez (USA)

DIC is one of the leading causes of maternal mortality. This complication is always secondary to pregnancy complication that will lead to intravascular coagulation, depletion of clotting factor and hemorrhage. The classical obstetrical disorders associated with DIC are placental abruption and amniotic fluid embolism. DIC can also develop in women with preeclampsia, HELLP syndrome, sepsis, retained stillbirth and acute fatty liver of pregnancy. Due to maternal thrombocytopenia antiphospholipid syndrome is part of the differential diagnosis of patients with DIC in pregnancy. In order to improve our knowledge on DIC during pregnancy the ISTH SSCs of DIC and Women's Health in Thrombosis and Hemostasis joined hands to establish an international registry of these patients that will assist in further understanding the clinical phenotype, associated obstetrical disorders, accuracy of diagnostic score and outcome of patients who developed DIC all over the world.

Dr. Erez has initiated and launched an international registry to gather data on the various approaches to the diagnosis and treatment of DIC during pregnancy. He currently has >50 records and he presented the preliminary data. He also highlighted the recently published SSC communication in JTH that is a call to action. This registry will gather information mainly on the epidemiologic characteristics of the patient, clinical conditions leading to DIC, how DIC was diagnosed and treated, and the subsequent maternal and fetal outcomes. No suggestions for any changes given.

SSC Joint Session: Perioperative and Critical Care Thrombosis and Hemostasis,

Biorheology, and Von Willebrand Factor

8 July 2019
16:30 – 18:30

Chairs : Jerrold Levy (Perioperative and Critical Care Thrombosis and Hemostasis)

Pierre Mangin (Biorheology)

Sandra Haberichter (Von Willebrand Factor)

The combined the session was moderated by the Chairs of the respective SSCs that included Professors Pierre Mangin, Sandra Haberichter, and Jerrold Levy

The first presentation was “Mechanical Support Essentials: what the coagulation expert needs to know” by Jean Connor, MD, Harvard, Brigham and Women's Hospital, Boston. Doctor Connor reviewed the continued development of ventricular assist device designs to improve hemocompatibility, an important finding that should further decrease thrombotic complications. She described a pilot trial of using lower targets and discussed new strategies to inhibit thrombosis without affecting hemostasis are in various stages of development and include preventing activation of the contact pathway factors XII and IX, and have yielded promising preclinical results in animal models of ECMO. As she noted, the coagulation consultant can play a role in the management of these patients by understanding the devices used, engaging with MCS care teams before thrombotic or bleeding crises develop, and guiding the coagulation management of these patients.

The second presentation was “Arterial Pulsatility and Circulating von Willebrand Factor in Patients on Mechanical Circulatory Support by Sophie Susen, MD, Centre Hospitalier Régional Universitaire de Lille, Lille, France. Doctor Susen describe how pulsatility defines a flow with a periodic variation. When defining that blood flow in the cardiovascular system she described how pulsatility quantifies the amplitude of the pulse and the degree of expansion of the vascular system and it is usually characterized by arterial pulse pressure. She noted aortic pulse pressure is defined as the difference between the systolic and diastolic blood pressure, therefore the maximum pressure and the minimum pressure measured in the aorta during a cardiac cycle. Pulsatility index is also used to define arterial pulsatility and is defined as the difference between maximum and minimum blood flow velocity. The pressure gradient quantifies the amplitude of the pulse and the degree of expansion of the vascular system.

The third presentation was “Hemostatic management in VAD patients: too thick or too thin, how do we get it right? Lisa M Baumann Kreuziger, MD, Medical College of Wisconsin. Doctor Baumann described how mechanical circulatory support (MCS) provides a bridge to heart transplant in patients with life-threatening heart failure and sustains patients either ineligible for transplant or as a bridge to heart transplantation. She also discussed how extracorporeal membrane oxygenation (ECMO) provides temporary support for patients in cardiac or pulmonary failure through external gas exchange and continuous flow of blood. Because the

median time to heart transplant exceeds event-free time on ECMO, pulsatile left ventricular assist devices (LVADs) are used to as longer-term support. Despite the improvements in survival, major bleeding occurs in one-third of patients with a LVAD and ischemic stroke and LVAD thrombosis can affect 12% of adults and 29% of children. An antithrombotic strategy to mitigate LVAD bleeding and thrombotic complications have been tested in randomized trials in children, but intensity of antithrombotic therapy in adults varies widely. Consensus guidelines for antithrombotic therapy during ECMO were created due to significant differences in management across centers. Because of the high risk for both bleeding and thrombotic complications, she reviewed how experts in hemostasis can impact the care of patients requiring mechanical circulatory support and are a necessary part of the management team.

David Ku, MD, PhD, from Georgia Tech discussed “Biorheological aspects of thrombosis in ECMO and mechanical support.” Because thrombosis can form on the surface of artificial materials used in these devices, the type of thrombus differs for materials exposed to high shear stress compared to stagnant blood. Three common components of artificial circulation components are described. First, ECMO circuits from children typical exhibit blood clots. These clots are hard to see as it depends on the strength of a flashlight shining through blood. He noted how they harvested the circuits to determine where the nidus of the clots formed. The long lengths of tubing are generally free of clot. The clots were almost always present at the ends of the connectors joining the tubing. The clots formed in a circumferential manner at the edge of the connectors. Using computational fluid dynamics (CFD), we show that these edges have stagnant zones of blood which does not wash clean. The clots are predominantly fibrin red clots by histology. These clots, when started, can grow into the lumen and become macroscopic. This pattern where over 80% of the clots in a circuit originated from connectors is borne out by circuits from 3 independent centers with different configurations and anticoagulation protocols. The clots at the connector edges were reproduced in an in vitro system using whole blood from an animal model perfused through a typical circuit with multiple connectors. He described how they were able to reproduce the clots in the same time scale as human circuits with the same histological appearance, and the stagnant blood zone as the culprit was confirmed by changing the connectors with a streamlined design. These new connectors exhibited no clotting in the in vitro system compared to the old connectors in the same circuit

Jun Teruya, MD, DSc, Texas Childrens Hospital/Baylor discussed “Biorheological aspects of thrombosis in ECMO and mechanical support.” Dr Teruya focused on unique aspects of pediatric coagulation issues, anticoagulation for extracorporeal membrane oxygenation, and other novel aspects of the pediatric patient population.

The final Q&A was lively and many interesting and important questions were asked by the audience. The dynamic interaction of the different speakers and subject matter created a thoughtful and interesting discussion after these excellent presentations.

SSC Joint Session: Lupus Anticoagulant/Phospholipid Dependent Antibodies, Women's Health Issues in Thrombosis and Hemostasis, and Disseminated Intravascular Coagulation

8 July 2019
16:30 – 18:30

Chairs: Katrien Devreese (Lupus Anticoagulant/Phospholipid Dependent Antibodies)
Maha Othman (Women's Health Issues in Thrombosis and Hemostasis)
Toshiaki Iba (Disseminated Intravascular Coagulation)

Classical International Consensus Clinical Criteria with Low Positive Anticardiolipin Antibodies or Anti-β2-glycoprotein I Antibodies Present Between the 95th and 99th Centiles or Intermittent Antiphospholipid Antibodies

Hannah Cohen, University College London Hospitals NHS Foundation Trust, UK

In clinical practice, we often see patients who do not fulfil the International Consensus criteria for diagnosis of obstetric antiphospholipid syndrome (APS). The focus in this talk was on non-criteria laboratory manifestations of obstetric APS: low positive aCL and/or aβ2 GPI antibodies, between the 95th and 99th centiles or intermittent antiphospholipid antibodies (aPL). Placental thrombosis is not universal in obstetric APS and there is evidence that at least some of the pathogenic mechanisms for obstetric manifestations of APS may differ from thrombotic APS. It is therefore plausible that there may be differences in aPL phenotypes in these conditions. Several studies suggest that low positive aCL and aβ2GPI are clinically relevant in women with classical International Consensus clinical criteria of obstetric APS. However, the majority of studies are retrospective with small numbers and a wide variation in the determination of reference ranges. aPL may fluctuate during pregnancy and this phenomenon may explain at least some intermittent aPL. A prospective multicentre observational study was proposed, to clarify the role of low positive aCL/aβ2GPI in obstetric APS. This study should include a validation exercise to standardise cut-off values in participating centres and could consider inclusion of other non-criteria antiphospholipid antibodies.

What is the Association Between Antiphosholipid Antibodies and DIC During Pregnancy or Postpartum Period?

Stéphane Zuily, University Hospital of Nancy, France

Disseminated intravascular coagulation (DIC) is an acquired syndrome characterized by systemic intravascular activation of coagulation. There are several obstetric causes of DIC

during pregnancy and postpartum. For instance, placental abruption, post-partum hemorrhage, severe pre-eclampsia, acute fatty liver, and sepsis are main risk factors for DIC during pregnancy. Antiphospholipid antibodies (aPL) are recognized risk factors for pregnancy morbidity (e.g. pre-eclampsia or HELLP syndrome) defining the antiphospholipid syndrome (APS). Although DIC and APS share a similar pathophysiology in part, coexistence of DIC and APS is uncommon, except in cases of the catastrophic variant. The purpose of this presentation will be to perform an overview of all available evidence regarding the association between aPL and DIC during pregnancy.

Do the role of isotypes and antibody profile of antiphospholipid antibodies differ for thrombosis and pregnancy morbidity?

Walid Chayoua, Synapse Research Institute, Maastricht, Cardiovascular Research Institute Maastricht, Maastricht, the Netherlands

Clinical criteria for the antiphospholipid syndrome (APS) include either thrombosis or pregnancy morbidity. However, the same antiphospholipid panel is tested for the classification of APS, independent of the underlying clinical cause. In literature more data becomes available that points towards distinct aPL-profiles in thrombotic and obstetric APS. We aimed to investigate the role of isotypes and antibody profile of aPL in thrombosis and pregnancy morbidity.

From eight European medical centers 1068 patients were enrolled: 259 thrombotic APS patients, 122 obstetric APS patients, 204 non-APS thrombosis patients, 33 non-APS obstetric patients, 60 APS patients of which the local center could not specify their clinical manifestation, 196 patients with autoimmune diseases and 194 normal controls. To minimize inter-assay and inter-laboratory variation, aCL and aβ2GPI aPL were detected with four commercially available assays: Bioplex 2200®, ImmunoCap®EliA, ACL AcuStar® and QUANTA Lite ELISA® by a single technician. Manufacturer's cut-off values were used. LAC was determined by the local center.

Positivity for aCL and/or aβ2GPI antibodies are significantly correlated with both thrombosis and pregnancy morbidity, independent of the isotype and solid phase assay. Higher odds ratios were obtained for IgG compared to IgM positivity for thrombosis or pregnancy morbidity. Isolated IgM was rare in thrombotic APS, but more frequent in obstetric APS, ranging from 3.5%-5.4% and 5.7%-12.3%, respectively, dependent on the solid phase assay. In a multivariate logistic regression analysis of aPL, IgM positivity was found to be associated with pregnancy morbidity. However, detection of IgM was not independently associated with thrombosis. Combined positivity for LAC, IgG and IgM increased OR for thrombosis compared to the current classification criteria, from 2.8 (95% CI, 2.1-3.7) up to 9.6 (95% CI, 3.4-27.1). While in pregnancy morbidity, the odds ratios for combined LAC, IgG and IgM positivity were mostly similar to the current criteria.

Thrombotic and obstetric APS patients have distinct aPL profiles. Our data supports testing for aCL and aβ2GPI IgM in women suspected of obstetric APS. However, no added value was found for testing IgM in patients suspected of thrombotic APS. Still, IgM aPL might be useful as

a second line test to improve thrombotic risk stratification. Combined aPL positivity does not increase the association with pregnancy morbidity.

Treatment of obstetric APS

Beverly Hunt, King's College London, UK

The speaker gives an update on the treatment of obstetric APS.

The standard of care for individuals with obstetrical APS is low dose aspirin (LDA), intermediate-dose LMWH or unfractionated heparin to prevent antiphospholipid antibody-related obstetrical complications. Mothers with a previous history of thrombosis require intermediate or full-dose anticoagulation (usually LMWH) throughout pregnancy to prevent further thrombotic events. As the presence of antiphospholipid antibodies increases the risk of hypertensive disorders in pregnancy, LDA is given to all individuals who have antiphospholipid antibodies. Treatment options to improve pregnancy outcomes refractory to LDA and heparin include low-dose prednisolone in recurrent first-trimester pregnancy loss. Some studies suggest that hydroxychloroquine reduces the rate of antiphospholipid antibody-related adverse pregnancy outcomes. The HYPATIA study, a multicentre RCT of hydroxychloroquine versus placebo in addition to standard of care in women with persistent antiphospholipid antibodies planning for pregnancy has started.

Disseminated Intravascular Coagulation in Sepsis

Satoshi Gando, Sapporo Higashi Tokushukai Hospital, Japan

Disseminated intravascular coagulation (DIC) is recognized as dysregulated coagulofibrinolytic responses against the insults including sepsis. Neutrophil activation-released neutrophil extracellular traps and histones release due to cell damage cause inflammation, platelet and coagulation activation, suppression of anticoagulation pathways, and inhibition of fibrinolysis, which has been considered main pathophysiology of DIC. Importantly, DIC plays pivotal roles in the development of multiple organ dysfunction, leading to poor prognosis in patient with sepsis.

Disseminated Intravascular Coagulation in Obstetrics: the Japanese score

Kobayashi Takao, Hamamatsu Medical Center, Shizuoka, Japan

The obstetrical DIC score developed by Maki is common and prevalent among obstetricians throughout Japan for the last several decades. In order to allow a prompt diagnosis and to start early treatment before obtaining the results of coagulation tests, the obstetrical DIC score is very useful. This score consists of underlying diseases, clinical symptoms, and laboratory findings. The obstetrical DIC score was validated through clinical trial. The obstetrical DIC score

was ≥8 points in 70 of 77 obstetrical DIC cases (90.9%) accumulated through nationwide collaboration by 110 facilities in Japan between 1984 and 1985. The most common were 38 cases of placental abruption, followed by 23 cases of postpartum hemorrhage (PPH). The obstetrical DIC score and the diagnostic criteria for DIC by the Japanese Ministry of Health and Welfare (JMHW) showed a positive correlation, validating this score as useful in initiating therapy for DIC.

Among the underlying diseases, placental abruption, amniotic fluid embolism and atonic bleeding (PPH) are characterized by typical acute obstetrical DIC with a severe decrease in plasma fibrinogen. The obstetrical DIC score and plasma fibrinogen level showed a negative correlation, and DIC scores of ≥8 had 97% sensitivity and 36% specificity for FBG < 150 mg/dL (1.5 g/L) in 43 DIC cases reported by Seto et al.

Transfusion guidelines for patients with massive bleeding (Ver.2) backed by the Japan Agency for Medical Research and Development were published in 2019, and the recommendations in obstetric cases were as follows: Cryoprecipitate and fibrinogen concentrate are effective for patients with peripartum massive bleeding, and plasma fibrinogen levels of 150-200 mg/dL (1.5-2g/L) are a suggested indicator for the timing of administration. Therefore, 1.5-2 g/L of plasma fibrinogen are thought to be critical levels of massive bleeding.

According to a national survey in 2019, the obstetrical DIC score is still useful; however, it became clear that the appropriate revisions in accordance with the present situation are necessary. The purpose of obstetrical DIC score is to prevent maternal death. Now we are going to develop the obstetrical DIC score that can predict plasma fibrinogen 1.5-2.0 g/L with high probability based on items that can be urgently examined, such as an underlying disease, clinical symptoms, and blood count, even in primary facilities.

DIC in Obstetrics and an International Registry

Offer Erez, Soroka University Medical Center, School of Medicine Faculty of Health Sciences; Ben Gurion University of the Negev, Maternity Department Division of obstetrics and Gynecology, Beer Sheva, Israel

DIC is one of the leading causes of maternal mortality. This obstetric complication is secondary to pregnancy complication that will lead to intravascular coagulation, depletion of clotting factor and hemorrhage. The classical obstetrical disorders associated with DIC are placental abruption and amniotic fluid embolism. DIC can also develop in women with preeclampsia, HELLP syndrome, sepsis, retained stillbirth and acute fatty liver of pregnancy. Due to maternal thrombocytopenia antiphospholipid syndrome is part of the differential diagnosis of patients with DIC in pregnancy. In order to improve our knowledge on DIC during pregnancy the ISTH SSCs of DIC and Women's Health in Thrombosis and Hemostasis joined efforts to establish an international registry of these patients that will assist in further understanding the clinical phenotype, associated obstetrical disorders, accuracy of diagnostic score and outcome of patients who developed DIC all over the world.

SSC Joint Session: Genomics in Thrombosis and Hemostasis and Platelet Physiology

6 July 2019
16:30 – 18:30

Chairs: Kathleen Freson (Genomics in Thrombosis and Hemostasis)

Marie Lordkipanidzé (Platelet Physiology)

Standardizing Platelet Transcriptomics for Discovery, Diagnostics, and Therapeutics in the Thrombosis and Hemostasis Community

RNAseq to aid clinical diagnosis of platelet disorders

Michele Lambert

Next generation DNA sequencing technologies have greatly contributed to the diagnostic abilities of clinicians today for many rare inherited bleeding disorders especially in the inherited platelet disorders. However, there is still a gap between the diseases that exist and the molecular mechanisms that have been defined. RNAseq was important in helping to define novel mechanisms of inheritance (ie in the Thrombocytopenia Absent Radii syndrome and Gray Platelet Syndrome) and provides a way to interrogate additional molecular mechanisms within particular cellular populations. RNAseq technology may provide a way to help validate novel VUS identified in DNA sequencing and understand ways in which these variants may contribute to disease. However, these technologies are probably not yet ready for “prime time” in routine diagnostic laboratory use.

Statistical integration of RNA-seq data in genetic studies of rare platelet disorders Ernest Turro

Whole-genome and targeted DNA sequencing with platforms such as the ThromboGenomics platform now provides genetic diagnoses to patients with a wide range of platelet disorders. Nevertheless, only 40-50% of cases with a platelet count abnormality and about 20% of cases with a platelet function defect can expect to receive a genetic diagnosis. The aetiologies of unexplained cases may be non-genetic, polygenic (involving many genes) or oligogenic (involving a few genes), yet there are grounds for thinking that many unexplained cases have a monogenic basis. In my talk I described two ways in which RNA-seq of platelets and other blood cells can aid monogenic discovery. The first approach is a statistical framework for genetic association in which expression levels estimated by RNA-seq are treated as mediators of disease risk. This type of

model can boost power if altered expression is the mechanism by which disease is caused. The second approach uses RNA-seq to study the mechanisms underlying a novel putative genetic association. I described this approach using a concrete example from a recently published article on the gene IKZF5. Certain types of missense variant in IKZF5 cause widespread and pathway-specific misregulation of expression in platelets but not in other blood cell types (CD4+ T cells, monocytes, neutrophils), giving an indication of the mechanisms underlying disease risk.

Using Platelet Transcriptomics for Novel Gene Element Discovery

Jesse Rowley

The within and between-individual variation and repeatability of platelet RNA-expression and alternative splicing was examined in platelets isolated repeatedly from healthy individuals over 4 years. Platelet gene expression and splicing were generally stable between individuals, and repeatable within individuals over time. Known and novel eQTLs were enriched among the most repeatable genes. A novel platelet splice QTL for SELP was identified that was shown to directly affect exon 14 skipping and the ratio of transmembrane versus soluble P-selectin protein production.

Platelet transcriptome and cancer

Thomas Würdinger

Dr Wurdinger presented the latest results of his team on tumour-educated platelets and cancer diagnostics. The results of the standardized thromboSeq protocol as recently published in Nature Protocols (Best MG et al, Nature Protocols, 2019) revealed that various tumour types can be detected via distinct profiles of spliced platelet RNA. Initial results of the pan-cancer study were presented showing early stage cancers could be detected at high specificity.

De novo protein synthesis induced by platelet activation and its impact on the platelet transcriptome

Loredana Bury

Platelets synthesize proteins, both constitutively or after stimuli and this mechanism is regulated at a post-transcriptional level by several mechanisms, such as pre-mRNA splicing and the action of miRNAs. Dicer is a protein with a crucial role in miRNA maturation and during the talk it was shown that Dicer neo-synthesis was triggered in human platelets by stimulation with thrombin. Dicer neo-synthesis leads to the increased maturation of microRNA 223 and to the downregulation of the expression of its main target, the P2Y12 receptor, thus suggesting that complex feedback interactions between transcriptome and proteome occur upon platelet activation and may regulate platelet function.

Impact of miRNA on Antiplatelet Responsiveness

Pierre Fontana

Platelet microRNAs (miRNAs) expression profiles correlate with platelet reactivity and cardiovascular risk prediction. PF presented the impact of one of those candidate miRNAs, miR-126, on thrombus formation using a zebrafish model and on platelet reactivity using human progenitor cells. The results point out to a regulation of platelet-mediated thrombin generation mediated by miR-126, rendering thrombus formation sensitive to anticoagulant but not to antiplatelet drugs. This hypothesis was strengthened by the investigation of aspirin-treated cardiovascular patients, suggesting that miRNA profile could help tailoring antithrombotics in those patients.

Standardizing Platelet Transcriptomics for Discovery, Diagnostics, and Therapeutics in the Thrombosis and Haemostasis Community

Matthew Rondina

Matthew Rondina presented the joint proposal from the ISTH SSC on Genomics in Thrombosis and Haemostasis and SSC on Platelet Physiology on standardization of approaches for platelet transcriptomics. The open discussion period has allowed the PI to receive constructive feedback from the community in order to enhance the proposal, as it was planned for submission at the 2019 ISTH SSC 50K competition. The outline of the project is as follows:

“With the advance of techniques such as next-generation RNA-sequencing (RNA-seq), platelet transcriptomics are increasingly utilized for discoveries on novel aspects of platelet biology, as diagnostic and prognostic markers, and for therapeutic development efforts. Despite the interest in using platelet transcriptomics for discovery and diagnostic efforts, methods for platelet isolation and transcriptomic sequencing and bioinformatics are not uniform. The main objective of the “*Standardizing Platelet Transcriptomics for Discovery, Diagnostics, and Therapeutics in the Thrombosis and Haemostasis Community: The STRIDE Study*” is to establish standardized and validated techniques for platelet isolation for transcriptomic studies in the field. The secondary objectives of this project are to disseminate bioinformatic code investigators may use in analyzing platelet transcriptomic datasets and to educate and train the ISTH community and beyond in best practices in platelet transcriptomic research. If funded, this study will enable data-driven recommendations by the ISTH-SSC on best practices in platelet transcriptomic research.”