64th Annual SSC meeting, in conjunction with the 2018 ISTH Congress in Dublin, Ireland Meeting Minutes

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Review of ISTH/SSC Secondary Coagulation Standard Lot #4 (A Hubbard)

Dispatch of Lot #4: Between the beginning of June 2017 and the end of May 2018 there were a total of 59 orders from 23 different manufacturers (16 Europe, 5 USA, 1 Canada, 1 Japan) and one external quality assurance scheme (College of American Pathologists). A total of 17,546 vials were issued. Lot #4 was also included in multi-centre studies for the value assignment of Lot #5 (1,246 vials). Remaining stock at the end of May 2017 was around 14,000 vials. It is predicted that stocks will be exhausted around mid-2019.

Calibration of Secondary Coagulation Standard Lot #5 (C Thelwell, A Hubbard)

Lot #5 comprises 100,000 screw-capped vials each containing 1ml of pooled normal plasma, freeze-dried. Stability testing based on an accelerated degradation protocol indicated mean loss per year at -20°C of less than 0.1% for four analytes (Factor V, Factor VII, Factor VIII, Antithrombin) - this indicates Lot #5 is very stable. More testing will be carried out prior to assigning the expiry date. Calibration of Lot #5 for over 20 analytes involved 3 large multi-centre exercises (77 laboratories) during 2017.

Proposed assigned values for 21 analytes have been accepted by the participants in the collaborative studies (Table 1). The proposed assigned value for factor V antigen is subject to WHO acceptance of this new analyte on the Proposed WHO 2nd IS Factor V, plasma. The number of datasets contributing to the individual value assignments ranged from 5 (factor V antigen) to 34 (factor V clotting). Assigned values for VWF activity by ristocetin cofactor (VWF:RCo) and the GPIb binding methods (VWF:GPlbR and VWF:GPlbM) will be confirmed when more laboratories have submitted results. Calibration for VWF:GPlbR and VWF:GPlbM methods will be carried out relative to the VWF:RCo value assigned to the WHO 6th IS FVIII/VWF plasma.

The calibration exercise also included tests on the current SSC Lot #4 for comparison with the original calibration values from 2010. Values for 18 out of 20 analytes differed between the two studies by less than 5%. The greatest difference was seen with VWF:collagen binding where a 6.7% difference was found. Overall these results agree closely with the original calibration and further support the excellent stability of SSC Lot #4.

Approval for the assigned values has been obtained from the study participants for the 21 analytes in Table 1. The calibration will be submitted for approval by the Executive Board of the Standing Committee and by SSC Subcommittee chairs and co-chairs before final endorsement by the ISTH Executive. The objective is to complete all approvals by the end of Q1 2019. There were requests to assign values for extra analytes to SSC Lot #5 including ADAMTS13, FXII, FXI antigen and FIX antigen. These calibrations can be carried out in a separate study.
Experience of EQA schemes with Lot #4

UK NEQAS (S Kitchen)

The use of Lot #4 for troubleshooting was reviewed. Between 2008 and 2017 there were 54 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 5 centres associated with UK NEQAS and for 2 centres for the WFH IEQAS scheme. There was an update on survey results for factor II, which previously indicated results 15% higher than expected when one commercial reference plasma was used. Tests indicated this was not related to the use of different thromboplastin reagents but to the reference plasma used. Surveys in September 2017 and April 2018 have shown that results relative to this reference plasma remain higher but there is a trend towards better agreement with the other reference plasmas.

SSC Lot#4 was dispatched as a test sample in a WFH IEQAS survey on factor IX tests in March 2018. Results were received from 97 centres. Two of the three main reference plasmas returned mean estimates close to the assigned value of 1.05 IU/ml whereas a third reference plasma returned a mean which was 12% higher; this is undergoing further investigation. The new SSC Lot #5 will be included in future UK NEQAS surveys on VWF activity methods and other analytes once it is released for use.

College of American Pathologists (R A Higgins)

SSC Lot #4 was issued in two surveys in 2017; one for thrombophilia testing and a special survey for VWF. Analytes are tested at the discretion of participating laboratories. Results are presented as mean and median 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 around 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 (111 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 by two methods were within 10% of the assigned value but a third method showed a larger discrepancy of around 15%. The mean value for VWF:RCo from the most popular method (n=14) differed from the assigned value by around 10%. The mean value for the VWF:GPIb method from one kit manufacturer was 12% higher than the assigned value for VWF:RCo. Mean estimates for VWF:antigen agreed very closely with the assigned value. Overall, the means of FVIII:C, 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.

Dr Craig Thelwell (NIBSC) was introduced as the new Chair of the Standing Committee.

There were 17 attendees.
Table 1
Proposed assigned values for SSC Standard Lot #5 (July 2018)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Value (IU/vial)</th>
<th>Inter-lab variability (GCV%)</th>
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<tr>
<td>Fibrinogen</td>
<td>3.19 mg/vial</td>
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TBC - to be confirmed
Animal, Cellular and Molecular Models

20 July 2018
8:00 – 12:30

Chairman: Jose A. Diaz

Co-Chairs: Brian Cooley, Margarethe Geiger, Laura Gutierrez, Nobuo Nagai, Laura Sanchez, Maxim Shaydakov.

RE: Session Report (Minutes)

- Room Capacity: 250 attendees (based on ISTH staff information).
- Attendance: The session achieved a constant presence of 3/4 of the room during the entire morning.
- Session coordinator: Jose A. Diaz.

Subject:

1. First Session: (08:00-10:00) **From preclinical models of venous thrombosis to in vitro to computational models:**
   The field of venous thrombosis is moving towards using more computational models. There is room for engineers working in coordinated efforts with vascular biologist and researchers. First, the session covered an update on where we are with animal models of VT, and the first consensus was presented for the first time. Then an update on where we are with in-vitro models of VT were presented in a very dynamic fashion. Finally, an update on where we are with computational models to simulate and study VT was presented, a presentation that truly captivated the audience.

2. Second Session: (10:30-12:30) **Finding new pathways to better understand venous thrombosis: Insights from Metabolomics, Proteomics and Genomics:**
   VT is a complex disease and despite our efforts to improve the knowledge we still have an epidemiology suggesting plenty of room for improvement. Thus, available technology can be applied to the VT field.
   The first presentation summarized the metabolomics methodology and current application in VT, from mice to humans. The last presentation introduced the genomics methodology and current applications in VT, providing a nice update on current data from mice to
humans. Unfortunately, the second presenter canceled for an important reason and I moved the last presentation to the second slot. That provided an hour for an in-depth discussion with the panel. All the speakers from both the early and late morning sessions got together on stage and a friendly and very professional discussion was established between the audience and the speakers. The level of the expertise from the speakers was outstanding, as was the level of the questions from the audience.

Below is a summary of the session including the content from each of the invited talks:

First Jose Diaz welcomed the audience and introduced Alison Michels from Canada, who presented the PB533 - Top Abstract Presentation entitled: von Willebrand Factor Interactions with Platelets and Leukocytes is Critical for Obesity-mediated Venous Thrombosis.

Introduction: Why We Developed This Program- The SSC Animal, Molecular and Cellular Models
Jose Diaz informed to the audience of the purpose of the session, announcing that at the end all the speakers will respond to questions from the audience in an open panel discussion format.

What Is New in VT Models? First Consensus in VT Models
Prakash Saha, United Kingdom

The first consensus in pre-clinical models of venous thrombosis (VT) was presented for the first time in our SSC on Friday morning. The presenter Dr. Saha, one of the authors of this consensus, introduced the audience to the details and purpose of this group to standardize the models of VT. There is a clear need for developing such an important document. Currently, there is no clear definition on how a model should be used, and a consensus was developed with a “decision-making format” based on the research question, which includes an algorithm to help researchers and reviewers choose the appropriate model to address a specific research question.

Can We Reproduce Thrombosis Conditions Using in-vitro Assays?
Jain Abhishek, United States

The primary source of modeling thrombosis has thus far been rodents. This is because other alternatives, such as in vitro methods or computational methods of thrombosis have not been predictive of the complex and multicellular signaling that occurs in humans in vivo. But the emergence of new microfluidic technology, known as “organ-on-a-chip” shows potential to serve as a more physiologically-relevant, quantitative and dissectible methodology to model thrombosis to the extent that it can contribute to gaining new knowledge of the disease and finding therapeutics. In this SSC session, Dr. Abhishek Jain presented two examples of application of thrombosis-on-a-chip, one of pulmonary microvascular thrombosis and another of Deep Venous Thrombosis. These examples demonstrate an interesting advance in modeling thrombosis in vitro.

Can We Reproduce Thrombosis Conditions Using Computational Models?
Speaker: Miguel Aguirre-Font, Spain

Computational mechanics is a branch of science that uses approximated numerical tools to solve equations of classical mechanics with two main purposes: a) virtual prototyping and b) virtual experimentation. Computational mechanics is now the predominant methodology (as compared, for example, to experimentation) in many fields of engineering such as the
aerospace industry. With the advent of computational power, these tools are now also becoming widely present in the medical field. Specifically, in vascular medicine, computational mechanics has been used with success in different patient-specific applications, mostly on the arterial side, such as preoperative planning, design of medical devices and vascular disease research. A computational model of DVT should be able to reproduce the thrombus formation process under patient-specific conditions, which involves biochemical and biomechanical phenomena at different temporal and spatial scales. This poses a series of challenges not only from the mathematical and numerical point of view, but also from the experimental and clinical side, which should be able to provide the necessary input and validation data. A non-exhaustive list of challenges have been identified: 1) to simulate the complex hemodynamics of veins, including the fluid-structure interaction phenomena with the vein wall, valves, and blood rheology 2) to simulate the multiscale process behind the coagulation cascade, 3) to better understand the tissue mechanics of the wall, valve and thrombus, 4) to define the right boundary conditions that connect the specific region of interest with the remaining vasculature and 5) to capture the influence of the surrounding tissue. Progress has been done in some of these areas, but to the best of the author’s knowledge, a patient-specific model of DVT has not yet been provided.

Can We Explore New Pathways in VT?

Insights from Metabolomics
Speaker: Sarah Onida, United Kingdom

Metabolomics is the study of the end products of cellular metabolism. This technology permits in-depth characterization of molecules in a given sample, and complements findings from other omic platforms, such as genomics, transcriptomics and proteomics. Much research has been performed in the mechanistic pathways involved in deep venous thrombosis (DVT) development and maturation. Metabolomics is providing further insight in this field both via animal and via human studies, with the aim of translating research findings into clinically relevant techniques for DVT diagnosis, prognostication and therapy, to improve patient care.

Insights from Genomics
Speaker: Chrissta Maracle, the Netherlands

The final presentation of the session entitled, “Insights from Genomics as a Tool for Studying Venous Thrombosis” was given by Chrissta Maracle. This talk succinctly summarized the history of genomic tools applied to venous thrombosis research, underscoring the rational transition from disease genetics to a genomics approach, as well as highlighting the successes and current limitations associated with each genomic study employed thus far. Specifically, she touched upon genome wide association studies (GWAS), next generation sequencing (NGS), including whole exome sequencing (WES) and moreover, shared her latest research findings on the role of SLC44A2 in venous thrombosis, exemplifying the utility of GWAS in identifying novel pathways linked to VT and embodying the theme of the session.

Panel Discussion: All Presenters - Models (First Session) and Explorative Tools (Second Session)
All the speakers from both the early and late morning sessions got together on stage and a friendly and very professional discussion was established between the audience and the speakers. The level of expertise from the speakers was outstanding, as was the level of the questions from the audience.
Keith Neeves has presented progress of the current Biorheology Subcommittee project on "Multi-center study on flow assay protocols and design." There has been recently a massive increase in the use of microfluidic devices in the field of Thrombosis and Haemostasis, which could generate issues in terms of variability and there is no information in the literature. The other objective of this study is to propose best practices and a benchmark of what should be expected on classical adhesion surfaces. Preliminary results using a custom four-channel microfluidic device were presented for whole blood perfused over fibrinogen and collagen surfaces in 5 different labs (SSC co-chairs). Overall, there is not much variation between donors and labs. Several issues were discussed: 1. Leakage from patterning and flow devices/2. Device-tubing connection/3. Significant variation in quality of microscopy image/4. Issues surrounding the exchange of fluids (blocking/blood/rinse)/5. Uploading and transferring of large file (>1 GB)/6. Protocol. Next goals: Additional studies on different anticoagulants, platelet count, and hematocrit have also been proposed. The study should be completed for the next ISTH meeting in Melbourne 2019.

Discussion: Q: include recommendation for fluorescence microscope use? A: Very good suggestion, this is indeed what we will propose.

The role of cytoskeleton in proplatelet formation in vitro has been well identified, but its role under in vivo conditions is not so clear and may differ from in vitro. This lab has used intravital bishoton microscopy to investigate in real-time how actomyosin and microtubules contribute to proplatelet formation under blood flow conditions. Catherine Léon showed that the profile of proplatelets were heterogeneous, with some thin and long ones, while other were thick and short. This was characterized and quantitated. The release of proplatelets was also studied and characterized. Big proplatelets were shown to be released. Proplatelets were identified in the lungs. Real-time
video-microscopy indicated that proplatelets were quite resistant to important changes in the flow that occurs in the sinusoids (inverted flow profiles were observed). They found that the cortical actomyosin regulates proplatelet length and thickness. Proplatelet elongation is balanced by myosin-mediated retraction forces and hemodynamic forces promoting elongation. The role of tubulin beta-1 was also evaluated. While it has been reported that tubulin beta-1 is critical for platelet formation in vitro, it is no so in vivo, suggesting that hemodynamic forces compensates for that.

Discussion:
Q: Do you think that all the proplatelets are able to release functional platelets? A: There is no scientific evidence for that, but the feeling is that the size of the proplatelets won’t modify the release of platelets
Q: Did you test the impact of platelet reactivity? A: This is difficult to evaluate.
Q: Contribution of the receptors to the release especially GPIb (remove this IC domain)? A: this has not been tested yet, but given that GPIb is a mechanosensor, it would be great to test it along with other sensors.
Q: Length and width could impact on proplatelet release? Did you try TPO ? A: this has not yet been done, but it is on the schedule.

Presentation title: Platelet Biogenesis: We Make Platelets
Presentation Start Time: 15:40  Presentation End Time: 16:05
Speaker: Jorge Valdez
Speaker Country: USA   Speaker Email: jvaldez@plateletbiogenesis.com

Platelet Biogenesis has developed a differentiation method to generate megakaryocytes (MK) using a clinical grade (cGMP) human induced pluripotent stem cell line (hIPSC). They seed the mature megakaryocytes generated through this processes into a millifluidic bioreactor, where the nucleated cells are retained and exposed to tightly regulated shear and pressure forces. MKs sit on a membrane and extend their proplatelets. These proplatelets are then exposed to shear forces to promote release of platelets. There has been an optimization of the bioreactor allowing to expose proplatelets to various flow forces. Once the ideal conditions will be identified, a scale up of the bioreactor will be performed: The chamber will have many different channels. The platelets generated through this process can be easily retrieved, and have been shown to exhibit many functional characteristics akin to those of human donor platelets. This group has looked various marker by epifluorescence and scanning electron microscopy. Presence of alpha granule and of the tubulin ring. The process of platelet release has also been studies by real-time video-microscopy. Single pre-platelets are released. The hypothesis is that these big platelets form smaller platelets later on.
Yield: today: 10^7-10^9/End of 2018: 10^9-10^10.
Current efforts are focused on the modular scale up of the millifluidic bioreactor to reach our goal of commercial scale production of platelets.

Discussion:
Q: Are images with platelets containing alpha-granule representative?/A: Yes images are representative.
Q: When measuring the yield/number of platelets produced, do you remove the platelets that were present at first (this modifies the yield)? A: Absolutely, the initial platelets were not counted to calculate the yield.
Q: Do these platelets produce thrombin in plasma? A: Don’t’ know. In the process of performing this experiment.
They used microfluidic chips to mimic and study the flow-driven elongation and rupture of megakaryocytes during the late stages of platelet production. MKs were injected into microchannels where adhesive pillars act as biochemical anchors while cells are exposed to flow. Of note, the pillars are covered with vWF to promote adhesion. 100 to 1000 pillars are found in one channel. The pillars help to anchor the MKs, which differs from the first version of the chamber in which MK were rolling over the surface. They provide evidence that VWF is required to release platelet-like particles. They visualize the entire platelet formation process, from the initial capture of a flowing MK upon its encounter with a pillar to the formation of elongations and finally to the release of (pro) platelets in the flowing media. This group has also provided evidence that the platelet-released particles have a tubulin ring. One remaining problem is the very poor yield. Anne Legoff proposes that the shear is not a critical parameter to release platelets, at least not in her device. A new goal is to elucidate the interplay between hydrodynamic forces and cytoskeleton remodeling during platelet formation. Therefore, a new chamber was designed. They believe that mechanical cues are required for elongation. Conclusion: immobilized VWF acts as an anchor in the pillar based bioreactor, this process is flow dependent; MK elongation is temporally and spatially inhomogeneous; Rupture and coiling occur at locations of maximal elongational rate.

**Discussion:**

Q: could you compute the actual forces on the proplatelet? A: This is ongoing work but an accurate description of the cell morphology is required.

Q: Could variations in shear rate explain the release? A: Most likely, there is some experimental evidence for that.

Q: Since you coat with VWF, do you see individual platelets adhering? A: difficult to answer, but no platelets appear to adhere.

Q: Size distribution of the produced platelets? Normal distribution? A: The size distribution is wide. Comment: “There is a huge controversy on the wide size distribution of the platelets and now, these observations of the different talks (today) appear to potentially explain why there is such a large heterogeneity”

The goal of mimicking bone marrow is to understand physiology and potentially to produce platelets. The bone marrow is like a sponge. The model Alessandra Balduini is working on is based on silk fibroin, derived from Bombyx mori silkworm cocoons. Silk is very similar to a sponge and could represent a very good biomimetic of the bone marrow. Importantly, silk can also be functionalized which is a great advantage. This is indeed a very promising biomaterial for bone marrow tissue engineering because of its tunable architecture and mechanical properties, the capacity of incorporating labile compounds without loss of bioactivity and demonstrated ability to support platelet production without premature activation. The group of Alessandra Balduini was also able to endothelialize the silk. Exploiting silk biomaterials, they have developed a series of 3D scalable systems that reproduce the structure of the human bone marrow,
supporting efficient platelet release from megakaryocytes. Their models provide useful technology for reproducing the bone marrow and studying thrombopoiesis ex vivo. Such a model has been used to better understand the mechanism of platelet production. They used AKT inhibitors as a proof of concept to show that their model can be used to understand platelet production at the molecular level.

**Discussion:**

Q: Have you modified the biophysical/biological properties of your silk models? A: Indeed, the last model they were careful to use sponge with a rigidity close to that of bone marrow.

Q: In vitro model produced platelets: what happens after recirculation? A: This has not been tested yet in our hands.

Q: You use MK produced in vitro, could you use a bone marrow derived MK to be used as a positive control in all the system described today? A: Indeed different types of MK can be used, and the aim of her lab is now to use IPS

Q: Contamination with platelets? A: No because they start with CD34+ cells and were careful to remove the contaminating platelets.

Q: What about platelet aggregation? A: No, this has not been performed yet.

Q: You used FN for the coating, have you used other adhesive proteins? A: yes various types of collagen

Q: Could silk be degraded and be present in the purified platelets. A: excellent question/we need to be careful at that!!

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**Biorheology Session on Friday, July 20th, 17:15-19:15**

**Top Abstract Oral Presentation**

**Moderator:** Judith Cosemans  
**Country:** NL  
**E-mail:** judith.cosemans@maastrichtuniversity.nl

**Presentation title:** Shear Enhances Fibrin-induced GPVI Shedding and Platelet ADAM10 Function  
**Start Time:** 17:15  
**End Time:** 17:30  
**Speaker:** S.J. Montague

S Montague discussed the interplay between fibrin and shear in the shedding of GPVI and ADAM10 function. She has discussed how fibrin exposure can increase ADAM10 activity and GPVI shedding and how this is enhanced with the exposure to shear stress. The role of these two physiological agonists may have in thrombus formation have also been introduced.

**Discussion:**

Q: Conditions in the viscometer: was there or not plasma? A: Coating with various adhesive proteins and then they used washed platelets or platelet-rich plasma.

Comment: you should use a system without VWF to strengthen the observations.

Q: Are you adding hirudin or PPACK to neutralize thrombin? Could explain the difference between fibrinogen and fibrin. A: Several controls have been performed (including using thrombin).

Q: Timeframe for GPVI shedding? A: We do not know because the method is not ideal yet. It is probably early but there is no proof yet.

Q: What is the best WSR for ideal ADAM10 activity? A: Ongoing experiments suggest that there is no real increase if you play around with shear.
Main session topic: Contribution of biorheology to platelet/cell aggregates  
Start Time: 17:30   End Time: 19:15
Moderators: Warwick Nesbitt, Mikhail Panteleev  
Moderator Country: AUS, RUS  
Moderators Email: warwick.nesbitt@monash.edu, mapanteleev@yandex.ru

Presentation title: Platelet ‘priming’ via VWF potentiates novel neutrophil interaction and mediates NETosis under flow  
Presentation Start Time: 17:30  Presentation End Time: 17:55  
Speaker: Kevin Woollard  
Speaker Country: UK  Speaker Email: k.woollard@imperial.ac.uk

This group explored platelet-leukocyte interactions under conditions of weak platelet activation, i.e. no P-selectin exposure. They demonstrate that binding of GPIb to VWF under flow ‘primes’ platelets resulting in $\alpha_{IIb}\beta_3$ activation. This priming does not lead to classical platelet activation or degranulation, but can bind neutrophils and T-cells (but not monocytes or B-cells) under flow, identifying a unique $\alpha_{IIb}\beta_3$ counter-receptor on leukocytes. Neutrophil binding leads to NETosis through a mechanosenstive mechanism as NETs are not formed under static conditions. These data demonstrate a novel mechanism for platelet-neutrophil cross-talk under flow and mechanosensitive NET production, which may be important in the pathogenesis of thrombotic disorders involving NETs.

Discussion:
Q: What is the significance of such a weak platelet/leukocyte interaction? A: most likely this interaction could be important in thromboinflammation/infection, but there is no proof of that for now.
Q: These observations are very interesting. It is surprising that you do not see Pselectin exposure under flow? A: From the assay we have done we did not see granule release, and what is difficult with this phenomenon is that it is only observed under flow.
Q: Netosis: are you looking at other markers? A: Yes, we are looking at MPO expression, but we think it is netosis, because you actually can observe it, with nets covering the channel
Q: Are beta2 integrins becoming activated? A: Ongoing work

Presentation title: The importance of using more physiological shear based methods for identifying hidden interactions following infection of the bloodstream  
Presentation Start Time: 18:20  Presentation End Time: 18:45  
Speaker: Steven Kerrigan  
Speaker Country: Ireland  Speaker Email: skerrigan@rcsi.com

This presentation was an overview of the significant contribution that biorheology has played in deciphering the intricate molecular pathways that exists between platelets /endothelial cells and bacteria. As a result of moving toward a more physiologically relevant model, new drug targets have been identified which may impact on the clinical approach to treating bloodstream infection and sepsis. The dynamic pathogen host response: the major problem is host response and the response is complex and very rapid. The drugs to treat sepsis have failed. This group has developed 2D and 3D models to look at bacteria adhesion under flow. The advantage of the 3D model is that you can observe microvessel leakage. Platelets adhered to S aureus at 800 s$^{-1}$ but not at 200 s$^{-1}$. Thrombi are forming in a FGN and IgG-dependent manner. It is actually the opposite with S sanguinis (adhesion but no thrombi at low but not high shear). They
provided evidence that this interaction was GPIb dependent. Thrombus formation is major issue of sepsis. The idea of this group was to prevent bacteria to bind to the surface.

Conclusion: No great progress in understanding platelet/endothelium/bacteria interplay. One reason is that biorheology is required.

Discussion:
Q: Any information of the molecular interaction, especially GPIb? A: Yes, this counterrector has been identified.

Presentation title: Platelet Count and Cancer Metastasis
Presentation Start Time: 18:45  Presentation End Time: 19:15
Speaker: Owen McCarty
Speaker Country: USA
Speaker Email: mccartyo@ohsu.edu

Platelets, components of hemostasis, when present in excess (>400 K/μL, thrombocytosis) have also been associated with worse outcomes in lung, ovarian, breast, renal, and colorectal cancer patients. In this talk, Owen McCarthy gave an overview of mechanistic studies on the link between platelet count, function and metastasis. Moreover, new data will be presented examining the prognostic power of platelet count-based predictions based on a cohort of 10,000+ cancer patients. Our study indicates the association of temporally derived platelet count features with a patients’ prognosis predictions, which may improve prognosis predictions in a stage and type of cancer-dependent manner. He used a spontaneous model of breast cancer, the MMTV-PyMT mouse (over 80 days). The platelet count was reduced after 40 days and this lead to a reduced size of primary tumors and less metastasis. Angiogenesis is reduced in primary tumors and proliferation is reduced in metastasis

Discussion:
Q: reduced primary tumor growth, is this why metastasis is reduced? A: Most likely since metastatic cells are released before day 40, but no proof yet.
Q: Have you tried different tumor cells? A: no.
Q: Could you block GPIb and other receptors? A: not for now, this approach is focused on lowering the platelet count.
Q: Have you looked at immune cells? A: infiltration of immune cells is the next step.

Presentation title: von Willebrand factor at platelet-endothelial cell junction under aberrant blood flow
Presentation Start Time: 17:55  Presentation End Time: 18:20
Speaker: JingFei Dong
Speaker Country: USA
Speaker Email: jfdong@BloodworksNW.org

Studying rheological impacts of blood flow on cells and proteins have been ongoing for decades, but in vivo models to mimic changes in blood flow pattern and force are limited and primitive. Ventricular assist devices provide an ideal way of studying how rheological changes. Most recognized laboratory change in patients with LVAD is the loss of large VWF multimers. Causes of this acquired von Willebrand syndrome in association with known phenotypic changes of endothelial cells/platelets and their clinical significance has been discussed. He showed that shear activates VWF, therefore loss of large VWF multimers can be caused by VWF activation without increase in ADAMTS3 cleavage.

Discussion: no time for discussion
Control of Anticoagulation

20 July 2018
8:00 – 12:30

Chairman: Mark Crowther

Co-Chairs: Julia Anderson, Chatree Chai-Adisaksopha, Benilde Cosmi, Adam Cuker, Jonathan Douxfils, Ismail Elalamy

-T van den Besselaar

Laboratory monitoring of VKA treatment: The purpose of our work is to define a Reference Measurement System for the Prothrombin Time including an International Standard (IS) for thromboplastin and a standardized Manual Tilt Tube Technique (MTT). Recently, a first external quality assessment of the MTT has been carried out, demonstrating the need for standardization.

8:45 to 9:15

"Is common calibration with Heparin/LMWH calibrator good enough for orientation about levels of Anti-Xa DOAC" (Henrik von Horn)

Dr von Horn presented results suggesting a common calibrator may be adequate for determining levels of anti-Xa specific direct oral anticoagulants.

9:15 to 9:45

"Is laboratory Monitoring of DOAC required in patients with APS; insight from the Canadian RAPS study" (Kim Legault)

Concern has been raised regarding the use of direct oral anticoagulants (DOACs) such as rivaroxaban in antiphospholipid syndrome (APS) due to case reports of recurrent thrombosis while on these agents. Our group at McMaster University in Hamilton, Canada undertook a multicenter feasibility study examining our ability to identify and consent patients for a study using rivaroxaban in APS, and achieve high levels of medication adherence. Rates of thrombosis and bleeding were collected as secondary outcomes. Overall we found relatively low rates of thrombosis and bleeding comparable to those seen in previous studies of warfarin in APS, however we sampled a relatively low-risk group of APS patients. Monitoring levels of DOACs may be unnecessary in this patient subset unless adherence is of concern, given the risk of under-anticoagulation compared to warfarin for patients who miss doses of rivaroxaban.

10:30 to 10:50

"Impact of DOACs on LAC testing: The Australian Perspective" (Jennifer Curnow)

Dr Curnow presented results suggesting that DOACs have important, and variable, impacts on thrombophilia testing, suggesting that such testing should rarely if ever be performed on samples that contain DOACs with the exception of genetic tests.
10:50 to 11:10
“Evaluation of the DOAC-Stop® procedure to overcome the effect of DOAC on several tests for thrombophilia screening” (François Mullier)

The impact of direct oral anticoagulants (DOACs) on laboratory assays used for thrombophilia testing is a well-known issue and may cause false-positive and -negative results. Therefore, the correct interpretation of tests that are performed in patients taking DOACs is mandatory to prevent misclassification and the subsequent clinical consequences. We aimed at evaluating the efficiency of a new and simple procedure (DOAC-Stop®; Haematex Research, Hornsby, Australia) to overcome the effect of all DOACs in real-life settings and to assess the percentage of erroneous results due to the presence of DOACs on thrombophilia screening tests. This study showed (on 135 patients and 20 controls) that the DOAC-Stop® adsorbent procedure appeared to be an effective and simple way to overcome the interference of DOAC on coagulation tests and should facilitate the interpretation of thrombophilia screening tests in patients taking DOACs (published in TH Open 2018; 02(02): e202-e209)

11:10
Unusual site thrombosis treated with DOACs Dr N Riva

Dr. Riva presented the protocol of a multicentre prospective international registry on the use of the DOACs in unusual site VTE (excluding DVT/PE). The aim is to evaluate the rationale for the use of the DOACs in this setting, and to assess the safety and effectiveness in real life clinical practice. This registry will be supported by ISTH and will use the online database technology REDCap. Any center willing to participate can contact Dr. Riva (nico.riva@hotmail.it) or Dr. Ageno (walter.ageno@uninsubria.it).

11:20
CVT Registry (Francesco Dentali)

Dr. Dentali presented additional information on the ongoing registry.

11:30
START Events registry (Sophie Testa)

The management of major bleeding in patients treated with direct oral anticoagulants (DOACs) is still not well established because of the limited clinical experience due to the relatively recent introduction of these drugs. START-Events, a branch of the START registry (Survey on anTicoagulatedpAtientsRegisTer) (NCT02219984), aims to describe the actual management of bleeding or recurrent thrombotic events in routine clinical practice. We presented results of 192 patients enrolled by 18 international centers: 143 patients with major bleedings and 49 patients with thromboembolic complications. Our data confirmed a high heterogeneity in the management of complications and the necessity to extend enrollment.

11:40
ORTHO-START registry (Elvira Grandone)

Dr Grandone presented an intermediate summary of the ongoing ORTHO-START registry
12:50
DOAC testing in Ontario: Results from IQMH (Karen Moffat)

International Council for Standardization in Haematology (ICSH) guidance document for DOAC measurement was presented at the Control of Anticoagulation SSC meeting in Dublin. The objective was to provide laboratory guidance addressing all three phases of DOAC testing: pre-analytical, analytical and post-analytical testing. The committee recommendations were consensus (100% agreement) and were vetted by appropriate pharmaceutical and in-vitro diagnostic manufacturers. The manuscript sections include background (drug characteristics), general patient and laboratory considerations, sample requirements (including stability), qualitative and quantitative measurements, method validation and external quality assurance. The manuscript is open access (Gosselin, et al Thromb Haemost 2018;118:437–450). The manuscript limitations were addressed, most notably the rapid changes in the field to include new drugs, reversal strategies, and emerging point-of-care methods. An updated guidance manuscript is proposed for early to mid-2019.

12:00
Venous stent registry (Sam Schulman)

Sam Schulman reported on behalf of James Douketis and Andrea Cervi from the Venous Stent Registry. There are now 27 registered cases and the target is 100. Additional centers are welcome. Interim analysis of the data shows a similar pattern to previous case series with almost as many regimen as cases.

15:15 to 15:45
DOACs measurements and clinical events: recent evidences from the literature and the presentation of the “MAS - Measure And See” Study (Sophie Testa)

Direct oral anticoagulants (DOACs) are administered at fixed dose without need for dose adjustment by lab testing. A high inter-individual variability in the drug blood levels was shown with all DOACs and a recent observation highlighted a relationship between DOAC levels and thrombotic complication. The MAS study is a cohort, observational, prospective, double blind, multicenter study in patients with NVAF treated with DOAC that aims to evaluate a possible relationship between drug anticoagulant levels and thromboembolic and bleeding complications.

15:45 to 16:50
The SAFIR registry: Rivaroxaban use in the in very elderly (Pr O. Hanon)

Dr Hanon presented the results of the SAFIR registry which examined the use of rivaroxaban in very elderly patients.

15:45 to 16:50
Management and Outcome of Life-threatening Emergency Situations in Patients treated with DOAC or VKA – Results of the RADOA-Registry (E Lindhoff-Last)
First national prospective multicenter non-interventional (NIS) registry (recruitment phase: March 2014 - May 2018 in 10 German sites) comparing consecutive patients (n=272) treated with DOAC or VKA providing 100% on-site source data verification (SDV) by external CRO and statistical analysis by statistician Prof. Dr. Eva Hermann, Goethe-University Frankfurt

Two subgroups of patients:

A) patients with severe bleedings while treated with DOAC (n=96) or VKA (n=97)
B) patients undergoing an emergency operation while treated with DOAC (n=44) or VKA (n=34) within 24 hrs after hospital admission (one subject received DOAC and VKA due to medication error and therefore has been excluded from most results)

Outcome: primary observation end point: In-hospital mortality within 30 days after admission

Analysis of pharmacokinetics of anticoagulants using residual blood samples (sample size n=1,473) – results to be determined

Results:

Severe bleedings on admission: Significantly increased unconscious VKA-patients while significantly more DOAC-patients presented with shock. Intracranial/intraspinal bleedings significantly increased in VKA-patients, GI-bleedings significantly more often observed in DOAC-patients. Application of PCC was significantly increased in VKA-patients > 40% of DOAC-patients received PCC. Idarucizumab has been applied in 5 of 8 dabigatran patients as well as in one rivaroxaban patient. Results to be determined. Severe bleeding: in-hospital mortality was significantly increased in VKA-patients compared to DOAC patients mainly caused by bleeding related deaths

17:15 to 17:45
Recommendations of the International Council for Standardization in Hematology (ICSH) for Laboratory Measurement of Direct Oral Anticoagulants (DOAC) (Bob Gosselin)

International Council for Standardization in Haematology (ICSH) guidance document for DOAC measurement was presented at the Control of Anticoagulation SSC meeting in Dublin. The objective was to provide laboratory guidance addressing all three phases of DOAC testing: pre-analytical, analytical and post-analytical testing. The committee recommendations were consensus (100% agreement) and were vetted by appropriate pharmaceutical and in-vitro diagnostic manufacturers. The manuscript sections include background (drug characteristics), general patient and laboratory considerations, sample requirements (including stability), qualitative and quantitative measurements, method validation and external quality assurance. The manuscript is open access (Gosselin, et al Thromb Haemost 2018;118:437–450). The manuscript limitations were addressed, most notably the rapid changes in the field to include new drugs, reversal strategies, and emerging point-of-care methods. An updated guidance manuscript is proposed for early to mid-2019.

17:45 to 18:15
The DaXa inhibition test: a concept for a universal and easily available aXa test that measures the direct inhibitory effect of all anti-Xa drugs. (Joost van Pelt)

The DaXa-inhibition assay: a concept for a readily available, universal aXa assay that measures the direct inhibitory effect of all anti-Xa drugs. In some clinical situations there is an urgent need to measure DOACs, but most laboratories don’t offer these tests yet. Available tests report the concentration of a specific DOAC, but technically they only measure the inhibition of exogenous FXa by any anti-Xa drug present in the patient’s plasma. Conversion of the result into a DOAC
concentration requires a specific calibration curve for each drug. As these assays don’t really 
measure DOAC concentrations, we propose to omit the calibration step and report FXa-
inhibition instead. Omission of the calibration curve based result conversion would greatly 
simplify the tests, lower their costs and presumably enhance wider availability.
DIC
18 July 2018
10:30 – 12:30; 15:00 – 17:00

Chairman: Toshiaki Iba

Co-Chairs: Bernd Jilma, Alessandro Squizzato, Jecko Thachil, Theodore Warkentin, Kazuma Yamakawa, Sacha Zeerleder

Pathophysiology of DIC (July 24, 2018 a.m.)

1. Miguel Cruz
   Baylor College of Medicine (USA)
   **Recombinant A2 Domain of von Willebrand Factor Attenuates Microthrombi Formation, Organ Injuries and D-dimer levels in a Porcine Model of Methicillin-Resistant Staphylococcus Aureus (MRSA) Sepsis-Induced Disseminated Intravascular Coagulation (DIC)**
   The A2 protein inhibited platelet interaction with fibrin(ogen). A2 prevented disseminated fibrin-rich microthrombi and decrease mortality in a lipopolysaccharide-induced DIC murine model. A2 could provide a novel therapeutic approach in critically ill patients with uninhibited activated coagulation and disseminated fibrin deposition such as DIC.

2. Cheng-Hock Toh
   University of Liverpool (UK)
   **Current pathological and laboratory considerations in diagnosing DIC**
   The pathophysiological mechanisms involve cross-activation among coagulation, innate immunity, and inflammatory responses. These events lead to consumption of both pro- and anticoagulant factors as well as endothelial dysfunction and disrupted homeostasis at the blood vessel wall interface. In addition to the release of tissue plasminogen activator (tPA) and soluble thrombomodulin (sTM) following cellular activation and damage, respectively, there is the release of damage-associated molecular patterns (DAMPs) such as extracellular histones and cell-free DNA.

3. Sacha Zeerleder
   University of Amsterdam (Netherlands)
   **Does cellular waste always harm? The role of cell-free DNA and histones in systemic inflammation**
   It is important to understand different ways how DAMPs induce inflammation (directly by cytotoxicity or via pattern recognition receptors) different mechanisms which protect us from harmful effects of DAMPS. In addition, the importance of heme as a DAMP will be discussed.

4. Hans Vink
   Maastricht University (Netherlands)
   **Glycocalyx and control of microvascular perfusion and capillary density**
   The endothelial glycocalyx plays significant roles for the maintenance of microcirculation. In the presentation, the method that was developed to monitor glycocalyx damage in patients introduced. Following these general introductions on glycocalyx, the glycocalyx changes in ICU patient with sepsis and the monitoring of glycocalyx was shown. Glycocheck will be a useful tool to identify patients at risk for organ failure / DIC / death. In addition, the actual data on the dynamic
changes of glycocalyx that can be measured in patients to monitor individual responses to general treatment strategies was presented. Finally, the possible implications of the observed glycocalyx changes in the ICU patients for (loss of) microvascular functions and will hypothesize on the potential therapeutic opportunity to target the endothelial glycocalyx was discussed.

5. Takashi Ito
Kagoshima University (Japan)
**Leukocyte-derived extracellular DNA as an underlying cause of extracorporeal circuit obstruction**
The cause of abnormal in-circuit pressure elevation in the ex vivo recirculation circuit system with a removable filter was investigated. It was found that extracellular DNA derived from activated leukocytes or senescent leukocytes caused in-circuit pressure elevation. Unexpectedly, heparin administration exacerbated in-circuit pressure elevation.

6. Florea Lupu
Oklahoma Medical Research Foundation (USA)
**Complement-coagulation interface in sepsis DIC**
Sepsis is characterized by robust activation of coagulation and complement enzymatic cascades, leading to DIC and contributing to organ failure and death. The presentation will summarize data from non-human primate models of sepsis, focusing on the pathophysiology of disease and testing novel therapeutic agents that inhibit complement at C5 level or the initiation of the intrinsic pathway of coagulation.

Clinical advances in DIC (July 24, 2018 p.m.)

1. Theodore E. Warkentin
McMaster University (Canada)
**Heparin-induced thrombocytopenia (HIT) versus non-HIT DIC**
“HIT vs non-HIT DIC.” Several lines of evidence indicate that immune heparin-induced thrombocytopenia (HIT) can be considered a DIC disorder: (a) patients with HIT have relative hypofibrinogenemia; (b) fibrinogen levels increase when HIT patients are treated with non-heparin anticoagulation; and (c) elevated PT [INR] values observed in some HIT patients are explained by reduced levels of coagulation factors (“consumption”). Further, DIC can be explained by HIT pathogenesis, which features HIT antibody-induced platelet activation (with formation of procoagulant platelet-derived microparticles) and monocyte activation (tissue factor expression). Clinical relevance includes diagnostic confusion between HIT and certain HIT-mimicking DIC disorders, particularly in critically ill patients with shock (septic or cardiogenic) complicated by “shock liver”, as this clinical presentation of non-HIT DIC, as well as HIT, can both result in ischemic limb injury requiring amputation(s).

2. Kazuma Yamakawa
Osaka General Medical Center (Japan)
**How to diagnose coagulopathy in sepsis patients**
Selecting an appropriate target population is essential to maximize survival benefits of anticoagulant therapy against sepsis. Our meta-analysis of three populations with sepsis and nationwide observational study in Japan showed that anticoagulants improved mortality only in sepsis-induced disseminated intravascular coagulation (DIC) but not in non-DIC.

3. Takashi Tagami
Duke-NUS Medical School (Singapore)
Antithrombin revisited
Positive results studies used the data only sepsis patients who were also diagnosed with disseminated intravascular coagulation (DIC), while the negative studies included all sepsis and/or critically ill patients in their analyses. When evaluating treatment efficacy for sepsis-associated DIC, the underlying diseases must be as homogeneous as possible.

4. Jerrold H. Levy
Duke University (USA)

Predicting mortality in patients with DIC after cardiac surgery
Cardiac surgical patients routinely demonstrate hemostatic changes consistent with disseminated intravascular coagulation (DIC) after cardiopulmonary bypass that may confound identification of severe coagulopathy. An evaluation of clinical and laboratory biomarkers consistent with postoperative DIC in the cardiothoracic surgical intensive care unit (CTICU) showed a 30-day mortality rate of 71%. Our evaluation also found the standardized JAAM DIC scoring system was useful to predict mortality.

5. Alessandro Squizzato
University of Insubria (Italy)
Nicole Jeffermans
University of Amsterdam (Netherlands)

International prospective registry for the diagnosis and management of sepsis-associated disseminated intravascular coagulation
There has been no large-scale international registry for diagnosis and therapy of DIC. It is important to understand the international difference and hopefully build a common strategy to fight against this critical condition. For this purpose, ISTH DIC SSC started the new registry program and the preliminary data showed the importance of keep continuing this project for the deep understanding.
Chairman: Guy Young

Co-Chairs: Manuel Carcao, Peter Collins, Alfonso Iorio, Gili Kenet, Johnny Mahlangu, Maria Mancuso

Top Abstract, Davide Matino

Davide Matino gave a talk on his research into the tissue distribution of FIX infusions in mice noting that FIX has a significant extravascular distribution and that plasma levels alone may not reflect the total efficacy of FIX infusions.

Practical issues with the use of emicizumab

Management of breakthrough bleeding, Peter Collins

Peter Collins discussed the approach to the management of breakthrough bleeding including reviewing data from the MASAC recommendations and the UKHCDO publication. He stressed the importance of better understanding the mechanism of action of the thrombotic microangiopathy complications in order to be able to treat all bleeds including serious ones safely.

Management of surgery in patients on emicizumab, Maria Elisa Mancuso

Elisa Mancuso reviewed published data on surgeries performed in patients receiving emicizumab demonstrating that many minor bleeds can be treated without prophylactic bypassing agents.

Laboratory issues with emicizumab, Stefan Tiefenbacher

Stefan Tiefenbacher gave a presentation discussing the available data on the performance of a variety of laboratory assays in plasmas spiked with emicizumab as well as a case study demonstrating that emicizumab significantly interferes with aPTT-based assays including the standard one-stage factor assays as well as Bethesda assays. He stressed the importance of additional research to address the issues of laboratory testing in patients on emicizumab.

Surveillance and monitoring for adverse events, Flora Peyvandi

Flora Peyvandi discussed the importance of post-marketing surveillance with all new hemophilia products and made specific points regarding monitoring for adverse events for emicizumab including thrombosis, TMA, and anti-drug antibodies.

Institute for Clinical and Economic Review, David Rind

David Rind reviewed a published report from ICER regarding the pharmacoeconomic impact of emicizumab prophylaxis in contrast to bypassing agents prophylaxis. The summary of the review demonstrated that emicizumab improved quality of life and reduced the cost in
comparison to bypassing agent prophylaxis. Importantly, this report is limited to the economic situation and pricing in the United States and may or may not be applicable in other countries.

SSC and other project updates

Calibration of SSC/ISTH plasma standard lot #5, Craig Thelwell

Craig Thelwell presented data on the calibration of the latest plasma standard (#5). This will then be sent to the Executive Board for Coagulation Standard, this SSC, and then the ISTH Executive Committee.

Calibration of WHO International Standard (IS) for factor V, Anthony Hubbard

Anthony Hubbard presented NIBSC data on the calibration of the new WHO standard for factor V. He demonstrated that this second IS performed well in comparison to the first WHO IS. This IS was endorsed at the SSC meeting and will move on to endorsement by the WHO.

Hemophilia Carrier Nomenclature, Robert Sidonio

Robert Sidonio presented the progress of his working group which will define a nomenclature for women/girls who are hemophilia carriers with respect to their factor levels and bleeding symptoms. The group plans to present their final proposal by the next ISTH meeting and then proceed with an SSC Communication paper.

Gene Therapy Working Group, Alok Srivastava

Alok Srivastava discussed the background and formation of this working group on gene therapy whose goal is to provide guidance on standardization and requirements/options for gene therapy trial outcomes. The goal is to post a draft document on the SSC website for comment sometime next year.

European Hemophilia Consortium EHC PARTNERS program, Brian O’Mahony

Brian O’Mahony presented information on the EHC PARTNERS program which is aimed at increasing the availability of factor for European countries with low per capita factor usage for both factor VIII and factor IX. He presented information on progress in several countries through this advocacy program.

Rare Bleeding Disorders

Plasminogen Deficiency, Charles Nakar

Charles Nakar presented data on this rare and serious multi-systemic disorder for which no specific therapy other than frequent FFP infusions was available. He presented clinical trial data on the use of topical plasminogen eye drops and data on an intravenous plasminogen concentrate. The topical plasminogen study was for patients with documented plasminogen deficiency and presence of the eye lesions known as ligneous conjunctivitis and demonstrated excellent results. The IV plasminogen concentrate was for patients with documented plasminogen deficiency and any clinical manifestations including but not limited to ligneous conjunctivitis. He also discussed a new research study moving forward to collect data on many more patients with this rare factor deficiency.
Diagnosing platelet disorders, Diane Nugent

Diane Nugent discussed novel methods to diagnose platelet defects including thrombocytopenia. She reviewed the many new genetic defects that have been found to cause inherited forms of thrombocytopenia, mostly with large platelets (macrothrombocytopenia). She then discussed an approach to diagnosing platelet function defects including platelet aggregation studies, flow cytometry, or assessing granules content via electron microscopy. She pointed out that this battery of tests is cumbersome and often confusing and difficult to assess. She then discussed the potential to make a more rapid and clear diagnosis using molecular testing.

New Frontiers in Immune Tolerance Induction

New approaches for Immune Tolerance Induction, David Lillicrap

David Lillicrap gave an overview of several new strategies that are being studied in vitro and in animals to either induce tolerance before the onset of an inhibitor (prevention) or to improve upon the outcomes of tolerance after the onset of the inhibitor (eradication). He discussed a variety of approaches from transplacental tolerance to oral tolerance approaches to gene therapy. Further research is needed to move some of these approaches into human clinical trials.

Extended half-life products and Immune Tolerance Induction, Manuel Carcao

Manuel Carcao briefly reviewed published data on in vitro and animal studies of rFVIII Fc and the induction of tolerance. He then reported on case reports and case series published over the last few years on the use of rFVIII Fc in both first-time and rescue ITI.

Prophylaxis with Immune Tolerance Induction, Carmen Escuriola-Ettingshausen

Carmen Escuriola-Ettingshausen reviewed past data and future possibilities of using bypassing agents and emicizumab. She reviewed the Bonn protocol of ITI with bypassing agents as the first attempt to control bleeding during ITI and speculated on the possibility of using emicizumab with ITI in the future.

Debate: ITI in the era of emicizumab—YES, Ri Liesner, and NO, Beatrice Nolan

Ri Liesner and Beatrice Nolan debated the pros and cons of performing ITI and provided their perspective on whether ITI should still be offered to patients who develop inhibitors. Both argued their side well and this was followed by a panel discussion.

Critical Appraisal of Novel Inhibitory Molecules for Hemophilia

Antithrombin, Craig Benson

Craig Benson presented data on the mechanism of action of fitusiran, a novel siRNA inhibitor of antithrombin synthesis. He then presented data from the phase 1 and 2 studies in patients with hemophilia with and without inhibitors.

Tissue factor pathway inhibitor, Patrick Fogarty
Patrick Fogarty presented data on the mechanism of action of TFPI inhibitors and reviewed some pre-clinical and early clinical data.

Protein C, James Huntington

James Huntington presented data on a molecule he and colleagues developed called SerpinPC which functions to interfere with the function of activated protein C. He demonstrated pre-clinical data demonstrating that this agent can control bleeding in hemophilia mouse. He also showed safety data in mice. He stated that they expect clinical trials to begin next year.

Expert Review, Peter Lenting and Steven Pipe

Peter Lenting provided an expert review of these novel inhibitory molecule approaches by evaluating the available scientific data and compared what is known about each of their biochemical effects on coagulation. Steve Pipe provided his perspective on these approaches from the clinical perspective regarding potential safety, efficacy, and conveniences issues.
Factor XI and the Contact System

20 July 2018
8:00 – 12:30

Chairman: Joost Meijers

Co-Chairs: Edward Feener, Heiko Herwald, Coen Maas, Owen McCarty, Stephanie Smith, Evi Stavrou

The Factor XI and the Contact System session on July 20 drew approximately 150-200 attendees.

The session started with the best-rated abstract presented by Michael Wallisch (USA). He discussed the use of a factor XII neutralizing antibody as adjunct therapy to heparin in a baboon model for ECMO. He concluded that supplementing heparin anticoagulation with inhibition of contact activation reduces thrombogenesis in membrane oxygenators during perfusion. This approach may allow for reduction of heparin dose and improve hemostatic safety of temporal extracorporeal activity.

Factor XIa is a promising target with the potential to obtain antithrombotic agents with very low bleeding liability. Anja Buchmüller (Germany) discussed the novel anti-FXIIa antibody BAY 1213790 that shows potent anticoagulant activity in vitro. Strong antithrombotic activity was observed in a ferrous chloride model in rabbits. BAY 1213790 did not increase bleeding in several models in rabbits, e.g. ear and gum bleeding and liver bleeding.

Zonne Hofman (the Netherlands) discussed her experiences using nanobodies to track down contact system activation. She presented data how nanobody-based FXIIa, C1INH-complex and cHK (or HKα) ELISAs can be used for both in vitro experiments (FXIIa) and as biomarker assays (C1INH-complex & cHK).

Simon Caulton (UK) investigated the molecular interaction between HK and gC1q-R. Interaction studies were performed to provide a binding model of HK domain 5 to the gC1q-R trimer. Truncation and mutation studies were used to further characterize this interaction to show which portions of the protein were important to facilitate the interaction between the two proteins.

Positive feedback activation of factor XI by thrombin contributes to normal hemostasis, however, direct activation of factor XI by thrombin is poor. Platelet receptors and surfaces such as polyphosphates (PolyP) and dextran sulfate (DXS) potently enhance factor XI activation, but are counteracted by sodium and calcium ions. Tilman Hackeng (The Netherlands) discussed the role of negatively charged phospholipids in thrombin-mediated factor XI activation, the effects of ionic strength, and comparison with polyP and DXS surfaces.

Reciprocal conversion of the plasma proteins factor XII (FXII) and prekallikrein to the fully active proteases FXIIa and kallikrein can occur in solution. The process is accelerated during contact activation by a variety of surfaces. The initiating events for reciprocal activation have been debated. David Gailani (USA) described his research that demonstrated that both FXII and PK have protease activity in their single-chain "precursor forms". In the case of FXII, the activity can convert PK to kallikrein in solution, and initiate PK, FXII and factor XI activation in the presence of a surface. For PK, the activity can initiate FXII activation in the presence of a surface, and cleaves the cofactor high molecular weight kininogen independently of a surface. These properties provide a mechanism for initiating activation of the kallikrein-kinin system.
Annually at our SSC subcommittee sessions since Montpellier (2016), Alvin Schmaier (USA) chaired a working group consisting of Drs. Joost Meijers, Coen Maas, David Gailani, James Morrissey, Thomas Renné, Allen Kaplan, José Govers-Riemslag, Jonas Emsley, Edward Feener and Johannes Sidelmann. This group at the Berlin SCC (2017) meeting and by email (January 2018) discussed the currently used wide variety of names and abbreviations of the contact system proteins. A consensus list of names and abbreviations based upon these discussions has been compiled by Alvin Schmaier. This list was sent out before the meeting to followers of our subcommittee and again presented and discussed by Alvin Schmaier during the business session. The suggestions of the committee were accepted by voting and will now be drafted into a report for publication as an SSC communication early 2019.

Nigel Mackman (USA) and his colleagues have determined the effect of generating mice with low levels of tissue factor combined with a complete absence of either F9 or F11. The key phenotypes of low TF mice is that they observed blood pools in late gestational placentas, ~80% of mice survive to wean and ~50% die of lung hemorrhages by 6 months of age. It was found that mice with low levels of TF and lacking F9 die at birth or shortly after birth and none of the mice are present at wean. However the absence of F9 did not affect the blood pools in the placentas. They also found the expected number of low TF mice lacking F11 at wean and the absence of F11 did not affect 6 month survival. Surprisingly, some mothers carrying low TF, F11 null embryos died due to placenta rupture indicating that an absence of F11 exacerbated the blood pools in the low TF placentas.

The presentation by Berhane Ghebrehiwet highlighted the interplay between the complement system and the Kinin Kallikrein system (KKS). In particular, he addressed the dual role of gC1qR, as both the receptor for the complement protein C1q and high molecular weight kininogen (HK) in the context of angioedema and other diseases such as cancer. Together with suPAR and Cyokeratin-1, gC1qR serves as a major platform for the assembly and activation of the KKS system thereby leading to the generation of bradykinin—the major cause of angioedema.

Although, inflammatory effects of factor XII (FXII) may be conventionally explained by its ability to activate the kallikrein-kinin system and the intrinsic blood coagulation pathway, direct effects of FXII on inflammation have recently emerged as an alternative hypothesis. Malgorzata Wygrecka (Germany) presented data that FXII directly regulates expression of inflammatory mediators and induces changes in the cell surface proteome in human lungs. Furthermore, she proposed that FXIIa may have distinct cellular activities more related to aberrant tissue remodeling.

Over the last several decades, the activation of the contact system has been observed in a wide variety of human diseases, however, in only a few instances is its role clearly defined. Moreover, the biological and physiological role of this system still remains elusive. Using genetically-modified mouse animals, Yi Wu (USA) determined the pathological role of each contact factor in arthritis, colitis, immunothrombosis and sepsis. His results demonstrated novel functions of the contact system in innate immunity. He further characterized the molecular and immunological mechanisms by which the contact factors regulate innate immune. Moreover, he investigated the participation of the contact system in host defense against gram-negative infection, revealing a physiological function of the contact system.

Sidney Strickland (USA) discussed his data on amyloid peptides and the contact system. Aβ, a driver of Alzheimer’s disease (AD), can activate FXII. AD patients have increased activated FXII and cleaved high molecular weight kininogen in their plasma. Depletion of FXII in AD-model mice
protects the animals from brain inflammation, neuronal loss, and cognitive impairment. These results implicate the contact system in AD pathogenesis.

The business meeting was concluded with a debate about the clinical role of factor XII, prekallikrein and high molecular weight kininogen. Three presentations were included and the first was by Nigel Key (USA) who introduced the clinical aspects of the contact system. While deficiency of any of the contact proteins can lead to prolongation of the aPTT that may be dramatic, their recently demonstrated role in animal models of thrombosis is challenged by an absence of evidence (but not definitive evidence of absence) in humans with deficiency states. While these individuals clearly do not have a bleeding propensity, there is currently no evidence that they are protected from thrombosis.

As several of the talks of our session showed novel agents for interference with thrombosis via targeting FXII, FXI, PK and HK, Thomas Renné (Germany) summarized recent developments and classical data that have established the role of the contact factors & FXI in thrombosis.

Factor XII is clearly dispensable for normal hemostasis in animals and man. Knocking out factor XII protects against thrombosis in animal experiments, although we lack convincing epidemiologic evidence for a role for factor XII in thrombosis in humans. James Morrissey (USA) discussed the evidence against the idea that the proteins that trigger the contact pathway of blood clotting are targets for antithrombotic drugs, except in cases in which blood is exposed to artificial surfaces.

We had a lively business meeting with a lot of discussion and comments from the audience.
Factor XIII and Fibrinogen

20 July 2018
15:00 – 17:00

Chairman: Verena Schroeder

Co-Chairs: Zsuzsa Bagoly, Marlien Pieters, Anetta Undas, Sanj Raut, Martin Guthold, Matthew J. Flick

Top Abstract Presentation

Nadezhda Podoplelova (Moscow, Russia): Binding of Coagulation Factor XIII Zymogen to Activated Platelet Subpopulations: Roles of Integrin αIIbβ3 and Fibrinogen

The aim of this study was to investigate interactions of zymogen fXIII with activated platelet subpopulations. In confocal microscopy and flow cytometry using fluorescently labeled factors, activated platelets bound 1000-1500 molecules/cell of fXIII at 100 nM, while both PS-negative activated platelets and resting platelets bound 200-400 molecules/cell. The binding was reversible, calcium-independent, and linear within the fXIII concentration range up to 1000 nM. Factor XIII predominantly binds to the caps of procoagulant platelets and colocalises with fibrinogen. Exogenous fibrinogen promoted fXIII binding by activated PS-negative platelets; this effect was abolished by the integrin αIIbβ3 antagonist monafram. The fXIII binding was 1.5- to 3-fold decreased for platelets from patients with gray platelet syndrome, and was variable for platelets from patients with Glanzmann’s thrombasthenia. In conclusion, strong platelet stimulation, fibrinogen, and αIIbβ3 play essential but not exclusive roles in fXIII binding. The preferential binding in the cap-like structures might be important for increasing local fXIII concentration in platelet thrombi.

Standardisation Topics

Craig Thelwell (NIBSC, UK): SSC/ISTH Secondary Coagulation Standard Lot #5: An Update on the Calibration of Factor XIII and Fibrinogen

The current Lot #4 of the SSC/ISTH secondary coagulation standard is depleted and will need to be replaced in 2019. The standard is calibrated for 21 analytes relative to WHO International Standards and is available in large numbers to manufacturers of diagnostic kits and reference reagents. An international collaborative study was organised to calibrate Lot #5 and the results for the calibration of fibrinogen and factor XIII (function and antigen) were presented. The proposed values were accepted by the study participants. Final approval from the SSC is anticipated by the end of Q1 2019 in good time for the replacement of Lot #4 when it expires.

Sanj Raut (NIBSC, UK): Update on Value Assignment of FXIII-B to the WHO 1st IS for FXIII Plasma
Data from a Feasibility/Pilot Study (involving 3 laboratories) carried out to additionally value assign the WHO 1st IS Factor XIII, Plasma (02/206) for FXIII-B subunit (Total) were presented. Monoclonal antibodies (MAbs) directed against Total FXIII-B subunit (kindly provided by Drs Katona & Muszbek, Hungary) were used to assess ELISA designs to calibrate the WHO 1st IS Factor XIII, Plasma, relative to locally collected Normal Plasma Pools. Study produced good data, with a combined geometric mean potency of 0.97 U/ml (GCV=11.9%), despite some inter-assay variability. The study also showed that there was no significant difference when assaying relative to fresh normal plasma pool compared to assaying relative to frozen normal plasma pool. Proposal to move onto larger main collaborative study (8-10 laboratories) to value assign the WHO 1st IS Factor XIII, Plasma (02/206) for FXIII-B subunit (Total) was made and endorsed by the ISTH/SSC FXIII & Fibrinogen Sub-committee. This study would be carried out in late 2018 and results reported at the next ISTH/SSC FXIII & Fibrinogen Sub-committee meeting in 2019.

Marlien Pieters (North-West University, South Africa): Update on the Standardisation of the Fibrin Clot Turbidity and Lysis Assays

The results of an international study investigating the feasibility of developing a standardised combined plasma clot turbidity and lysis assay were presented. This was a collaborative project between the FXIII and Fibrinogen, and the Fibrinolysis SSC subcommittees. The study concluded that it is feasible to combine the plasma clot turbidity and lysis assays and that it has potential for clinical use to determine fibrin clot properties in CVD. It furthermore identified analytical challenges that exist with this type of assay which should be carefully addressed when performing the assay. As a result of considerable variation between laboratories, it is suggested that patient data be reported relative to a reference plasma and that the use of standardised data analysis will help to improve this variability.

Alessandro Casini (University Hospitals of Geneva, Switzerland): Strategies for Diagnosis of Congenital Fibrinogen Disorders

Diagnosis of congenital fibrinogen disorders (CFD) is challenged by the low sensitivity and the high variability of standard haemostasis assays. The clinical phenotype of patients suffering from CFD is highly heterogeneous with an incomplete penetrance of the clinical phenotype and a poor segregation even among families with the same causative mutation. In this presentation the current diagnosis of CFD based on standard haemostasis assay and the genotype was reviewed and a step-wise procedure for an accurate diagnosis was suggested. Then, the clinical features of CFD patients were reviewed highlighting some specificity of each sub-type, and finally a new classification of CFD based on both the biological and clinical phenotype was proposed.

Verena Schroeder (University of Bern, Switzerland): Nomenclature of Genetic Variants of FXIII

Nomenclature of sequence variants should follow the internationally recognised HGVS recommendations. However, the traditional nomenclature of genetic variants of FXIII-A does not follow these recommendations. Following consultation with experts from the Sequence Variant Description Working Group and members of the Genomics in Thrombosis and Hemostasis SSC, it was proposed that recommendations on nomenclature of sequence variants of FXIII which
adopt the HGVS recommendations but at the same time take the traditional numbering of the mature protein into account should be developed and published as an SSC communication.

Scientific Topics

The presentation by Akitada Ichinose was cancelled.

Richard Fish (University of Geneva, Switzerland): Investigating Fibrinogen and Factor XIIIA Deficiencies in the Zebrafish

Zebrafish models of afibrinogenemia and dysfibrinogenemia were presented. Using laser-induced embryonic vessel injuries, afibrinogenenic embryos, homozygous for a frame-shift mutation in fga (fga-/-), did not support venous thrombosis and had a reduction in thrombocyte adhesion and aggregation compared to controls. When thrombocytes adhered to sites of injury in fga-/- animals, embolic cell aggregates were observed. The majority of dysfibrinogenemic fish, homozygous for a mutation in the fibrinogen Aα thrombin cleavage site (Aα R28C), also failed to show venous occlusion. When occlusion was measured it was short-lived and unstable. Thrombocyte binding and aggregation were also impeded by the dysfibrinogenemia mutation, even in heterozygosity. Both models recapitulate aspects of the phenotypes seen in patients with similar fibrinogen deficiencies.

Preparation of zebrafish with targeted factor XIIIA mutations was presented. There are three f13a genes in the zebrafish genome. The initial model targeted the f13a expressed in zebrafish embryos (f13a1b-87), because characterisation of in vivo hemostasis will take place in embryonic fish. The f13a1b-87 gene is expressed in what appears to be embryonic vasculature, macrophages and bone precursor cells. The functional characterisation of these animals is ongoing, with the aim of studying f13a1b deficiency in hemostasis, in particular for fibrin crosslinking and clot cell retention.

Laszlo Muszbek (University of Debrecen, Hungary): The Effect of Activated Factor XIII on Human Vascular Smooth Muscle Cells

FXIII-A could not be detected in human aortic smooth muscle cells (HAoSMCs) that have undergone osteoblastic differentiation. Activated recombinant FXIII-A (FXIIIa), but not the non-activated form, increased cell proliferation and collagen secretion in concentration dependent manner. In the in vitro wound-healing assay the time to reach 30% and 80% confluence was decreased to less than 1/7th and 1/3rd in the presence of 20 µg/mL FXIIIa. In parallel a highly significant (67%) decrease of thrombospondin-1 (TSP-1) concentration in the medium and a 2.5-fold increase in the cell associated TSP-1 were observed. TSP-1, c-Jun and Egr-1 mRNA did not change significantly, i.e. TSP-1 synthesis did not increase. In FXIIIa treated cell cultures granular, cell-associated precipitates appeared which contained polymerised TSP-1. During the course of plaque hemorrhage FXIII becomes activated and might exert its effect on vascular smooth muscle cells. This effect might be important in the pathogenesis of atherosclerotic plaques.

Ramzi Ajjan (University of Leeds, UK): The Fibrin Network in Diabetes: Mechanisms and Therapeutic Implications
Life expectancy is reduced in individuals with diabetes mainly due to cardiovascular complications. Increased thrombosis potential contributes to the high vascular risk in this population secondary to enhanced platelet activation, changes in fibrin clot structure and hypofibrinolysis. Interestingly, reduced fibrin clot lysis has recently been shown to be independent predictor of cardiovascular mortality following a coronary event and therefore improving hypofibrinolysis has the potential to alter clinical outcome.

The presentation covered some of the mechanisms responsible for hypofibrinolysis in diabetes including hyperglycaemia-induced reduction in plasmin generation and increased incorporation of complement C3 into diabetic clots. While hyperglycaemia is prothrombotic, over-treating high glucose levels and precipitation of hypoglycaemia is also associated with a hypofibrinolytic environment that can last for up to one week following an episode of low blood glucose. The last part of the presentation focussed on a novel targeted approach to improve the hypofibrinolytic milieu in diabetes with the use of non-antibody binding proteins that can modulate function of coagulation proteins.

Ameliorating the thrombotic environment in diabetes will help to reduce vascular events in this condition and improve mortality/morbidity in this high risk population.

Anetta Undas (Jagiellonian University Krakow, Poland): Fibrin Clot Properties as Predictors of Thromboembolic Events and Bleeding

The prothrombotic fibrin clot phenotype, including reduced clot permeability and lysability, is associated with arterial and venous thromboembolism. The unfavourable clot features can predict recurrent deep-vein thrombosis and pulmonary embolism after withdrawal of anticoagulation as well as the risk of cardiovascular death and recurrent myocardial infarction following acute coronary syndrome. Most cardiovascular risk factors are characterised by such unfavourable clot features measured in plasma. Plasma fibrin clot permeability may determine in part the amount of fibrin within arterial thrombi removed from patients with acute myocardial infarction. This clot permeability may predict the risk of stroke in anticoagulated patients with atrial fibrillation. Increased bleeding risk is observed in subjects with less compact fibrin clot structure, however major bleeds might be more common among subjects with dense clot networks, likely due to impaired wound healing. Plasma fibrin clot properties might be useful in identifying patients at risk of severe vascular outcome including life-threatening events.

Robert Ariens (University of Leeds, UK): The Role of Fibrin Crosslinking by FXIIIa in Clot Embolisation

The presentation showed that the cross-linking of fibrin by FXIII dramatically increased the clot’s mechanical properties. Cross-linking of both the alpha (60%) and gamma (40%) chains of fibrin contribute to an increase in clot elasticity. Cross-linking of the fibrin alpha chain by FXIIIa is responsible for the decrease in non-elastic deformation due to protofibril slippage. It was also shown that elimination of the fibrinogen gamma chain cross-linking sites in a murine model increases clot embolisation.
Update on WHO international standards projects
Colin Longstaff, NIBSC, UK

Colin Longstaff presented an update of WHO standardisation projects from the National Institute of Biological Standards and Control (NIBSC). The collaborative study to calibrate the 4th WHO International Standard (IS) for Streptokinase is complete and data have been analysed. The study, involved 15 laboratories from industry academia and regulators. Two candidate replacements for the 3rd IS (00/464) were included, plus a control sample (88/824) that has been measured in 3 studies over almost 30 years including 4 consecutive IS. The measured potency of 88/824 has varied over only 1.7% over this period, supporting the stability of the International Unit (IU) of Streptokinase. The next steps will be preparation of reports for the study participants, SSC experts and WHO which will propose a new 4th IS for approval in 2019. A new IS for TAFI/CPU in plasma is being prepared which will be labelled for antigen and activity. Sixteen laboratories are involved and two have agreed to establish a mass value for TAFI using Isotope Dilution Mass Spectrometry (IDMS) utilising recombinant TAFI containing heavy atom-labelled amino acids. The study is underway with planned submission to WHO in 2019. Work on establishing a gravimetric concentration of PAI-1 in plasma in ng/ml determined by IDMS, for a new antigen standard, has stalled following technical problems and lack of suitable equipment or collaborators. A project to investigate the stability of PAI-1 activity in the 1st WHO IS for PAI-1 activity (92/654) has been initiated. Preliminary results suggest there may be some problems with freeze-thawing of reconstituted samples and stability over several hours of solutions stored on ice, where 10-20% activity is lost. It is not yet clear that ampoules stored at -20 deg C since manufacture have also lost similar amounts of activity, which is a potential concern. A small collaborative study is planned to investigate these issues. Studies over several years have identified significant stability problems in potential standards for D-dimer after storage of freeze dried plasma at -20 deg C, but promising results have been observed using trehalose as a stabiliser. However, a study reported to the committee in 2017 suggested stabilised material may not work well as a representative standard to harmonise results from different D-dimer assay methods. Problems have been encountered in sourcing suitable D-dimer plasma for future work, but new studies are planned using commercially-sourced plasma to determine whether trehalose-stabilised plasma can act as a suitable reference material able to improve D-dimer assay variability and demonstrate adequate stability to become a WHO IS.

DIC in trauma and terminology standardization
Satoshi Gando, Sapporo Higashi Tokushukai Hospital, Japan

All insults, including trauma, sepsis, and cardiac arrest/return of spontaneous circulation, bring about the same non-specific body responses known as innate immunity, which is tightly coupled with coagulation systems. Pathologic over-expressions of these responses to trauma are considered to be the main pathophysiology of primary coagulopathy of trauma, namely
disseminated intravascular coagulation (DIC). After reviewing the pathophysiology of DIC, standardization of terminology on trauma-induced coagulopathy proposed by the ISTH SSC on fibrinolysis will be introduced.

Fibrinolysis shutdown – old dog: can we treat it with new trick?
Hunter Moore, University of Colorado, USA

Low fibrinolysis measured by viscoelastic hemostatic assays has recently been associated with increased mortality in severely injured patients. However, the original description of a trauma patients transitioning to fibrinolysis resistance dates back to the 1960s. Despite over half a century of appreciating this coagulation phenotype in trauma, strategies have not been clinically implemented to treat this pathology. New mechanistic evaluation supports historic observations that this process is driven early by plasminogen activator-1, supporting several potential therapeutic interventions.

Role of platelet function on TIC
Mark Walsh, Memorial Hospital of South Bend, USA

Mark Walsh gave a lecture entitled: Platelets, Fibrinolysis and Trauma Induced Coagulopathy (TIC). Like Real Estate: “Location. Location, Location.” A “Seamless Web of Intrigue.” In his talk he spoke of the Viscoelastic Tests (TEG/ROTEM) and “goal directed” blood component therapy for trauma resuscitation with an emphasis on platelet dysfunction and fibrinolysis. He discussed the importance of considering the gradient of activation of the platelet from the site of endothelial injury at the core of the clot to the lumen as well as the level of fibrinolysis locally at the endothelium and at the lumen as measured by TEG with Platelet Mapping (TEG/PM). He provided a history and comparisons of the ROTEM and TEG/PM uses in Massive Transfusion Therapy (MTP) in TIC with an emphasis on platelet dysfunction and fibrinolysis. Then he discussed the implications of local endothelial and systemic platelet function in TIC as well as the importance of fibrinolytic spectrum analyses in TIC as a function of “time and place” as well as the concept of “Phenotype Switching” along the fibrinolytic spectrum” that occurs within minutes in severe TIC. He presented the concept of the “Phenotype Switching” within the fibrinolytic spectrum which allows the traumatologist to negotiate the “Seamless Web of Intrigue” that fibrinolysis presents to the traumatologist and basic scientist alike as they attempt to apply the findings of bench science and clinical data to the bedside experience.

Use of TEG/ROTEM in liver transplantation
Fuat Saner, University Hospital Essen, Germany

Patients with end-stage-liver-disease (ESLD) are at increased risk of bleeding – an observation that dates back to the very first 100 liver transplantation performed in Denver (1963-1967). All patients died within 6 weeks. Although only approximately 30% of the cases died from uncontrolled bleeding, the majority of patients died from rejection. It was a period when appropriate immunosuppression was not available. Furthermore, bleeding was caused by lack of pathophysiology hemostasis disorder and sufficient treatment availability. In contrast to trauma patients or post partal hemorrhage, ESLD are chronic patients with complications beyond coagulopathy. However, a couple of studies showed that ESLD patients are more prone to thrombosis rather than bleeding. The reason for bleeding in these patients is not the pretended coagulopathy by standard laboratory tests (SLT), but more so the portal
hypertension. Therefore, ESLD-patients should follow a restrictive fluid regimen; particularly FFP transfusion should be avoided to treat unnecessary SLT values in a non-bleeding patient. Although YG Kang from Pittsburgh reported in 1985 that the use of TEG lowers the transfusion rate by 33% without increase of bleeding, many physicians are very reluctant to use visco-elastic tests (VET). ROTEM and TEG are able to reduce transfusion, which is associated with decreased morbidity and mortality. VET are also more reliable and have a shorter turn-around time to detect fibrinolysis. The incidence of fibrinolysis during transplantation is between 7-80%. It is supposed that during anhepatic phase, t-PA level is increased and PA1-I is decreased, which leads to fibrinolysis. Most fibrinolysis events resolve within 30 minutes. Treatment with tranexamic acid (TXA) should be considered only in bleeding patients. In conclusion, it should be stated that patients with ESLD are more prone to thrombosis than bleeding, and the treatment should be offered with the guidance of VET rather than blind FFP and platelet transfusions. Most importantly: DON’T TREAT A NON-BLEEDING PATIENT!

The fibrinolytic system and TIC
Dominik F. Draxler, Monash University, Australia

Hyperfibrinolysis has been described as an important contributor to trauma-induced coagulopathy (TIC), early after severe trauma. However, it is now widely accepted that within hours of injury the majority of patients develops fibrinolysis shutdown, a condition that can present itself with different mechanistic sub-phenotypes. The concept of fibrinolysis shutdown in trauma raised concerns about the use of the antifibrinolytic agent tranexamic acid (TXA), which has been demonstrated in the "Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage 2" (CRASH-2) trial to provide a survival benefit for bleeding trauma patients if administered early after trauma. However, while the correction of (hyper-) fibrinolysis might often not be possible or necessary, TXA might exert benefits by modulating plasmin generation unrelated to fibrinolysis.

Modeling of TEG, ROTEM and CCT algorithms for trauma resuscitation
Kjersti Baksaas-Aasen, Oslo University Hospital, Norway

Dr Baksaas-Aasen presented parts of the FP7 TACTIC project, namely the development of TEG, ROTEM and CCT algorithms for trauma resuscitation to be used in the multicentre randomized controlled trial iTACTIC. The algorithms are based on data from 2287 prospectively followed trauma patients, and combined with previously published evidence. The parameters applied in the algorithms are corresponding for TEG, ROTEM and CCTs, and at the same time the threshold values are available early and easy to remember. The algorithms were recently published in Annals of Surgery, the last iTACTIC patient was enrolled less than a month ago and we are looking forward to the publication of the results early 2019.

Disparities between INR/PTT and TEG
Ernest Moore, University of Colorado, USA

Trauma induced coagulopathy (TIC) was initially defined by the conventional coagulation assays (CCA), INR and PTT. The cell-based model of hemostasis, however, emphasizes that thrombin generation is only a component of the clotting process. Data from adult and pediatric trauma studies suggest that, while INR and PTT are strong biomarkers of injury severity, they are relatively poor indicators of the need for plasma resuscitation. Furthermore, randomized
trials indicate viscoelastic hemostatic assays (VHA) are superior to CCA for goal-directed resuscitation. Our recent studies suggest that variation in INR and PTT are only 50% explained by changes in their respective clotting factor activities, but the mechanisms remain unknown. Collectively, these studies question the role of monitoring INR or PTT in the injured patient at risk for uncontrolled bleeding.

Clinical applications of TEG and ROTEM and the need for improvement in the standardization and validation of these tests
Gabriel Dumitrescu, Karolinska University Hospital, Sweden

Viscoelastic tests (VETs) are clinically mainly used as point-of-care monitoring for haemostatic management in the bleeding patient especially in cardiovascular surgery, liver transplantation, severe trauma and obstetric haemorrhage. VETs have been investigated in other clinical areas but it is noted a slow adoption of this technology outside the management of massive haemorrhage. There has been some debate regarding the validation of VETs and highlights of the need for standardization and external quality control of these tests.

Need of a standardized test for the diagnosis and quantification of fibrinolytic resistance in sepsis patients
Ecaterina Scarlatescu, Institutul Clinic Fundeni, Romania

It is known that increased resistance to fibrinolysis is associated with higher mortality in critically-ill septic patients, however a specific intervention to correct hypofibrinolysis was not tested in large trials; the lack of routine simple tests for fibrinolysis probably contributed to this. Factorial biomarkers or plasma-based diagnostic assays for fibrinolytic activity quantification were studied in septic patients, however their use in clinical practice is limited, as they are cumbersome and time-consuming. Viscoelastic tests have proved useful for the real-time detection of 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. Modified viscoelastic tests with added plasminogen activators can detect hypofibrinolysis and are currently used for research purposes. Coagulation and fibrinolysis overlap in time, so measurements that include clot formation velocity may better capture the dynamic processes of clot formation and lysis in critically-ill patients and the use of velocity derived parameters could enable the identification of subgroups of septic patients with low fibrinolytic activity.
Selected abstract. Accumulation of Rare Variants in STAB2 in Type 1 von Willebrand Disease Patients. Eric Manderstedt - Kristianstad, Sweden

Eric Manderstedt worked on a Swedish VWF cohort (2016 families) and analysed SNVs previously associated with VWF levels, with the aim to estimate the frequency of these variants in the Swedish cohort. They did a GWAS analysis, considering extreme VWF levels. Results: some enrichment of these common polymorphisms in the VWD patients, stratification by O blood group showed a trend. They then looked for rare variants in the GWAS identified genes in the VWD patients (AmpliSeq + Ion torrent sequencing) and identified 147 variants. For those with MAF <0.5%: 8 rare variants in multiple genes associated with VWF levels. STAB2 was the most convincing locus identified – but rare variants did not localise in the EGF domain.

Conclusions:
- rare variant enrichment in STAB2 in VWD patients
- 9 other loci also show possible enrichment of low frequency variants in VWD patients.

SESSION 1 - NGS in Clinical Diagnosis of inherited bleeding, thrombotic and platelet disorders (part I) - Chair: Michele Lambert


The bone marrow is home to megakaryocytes, one of the largest cells in the body, each of which releases hundreds or thousands of platelets into the bloodstream. Platelets are essential for hemostasis, and they are key players in many other physiological functions. The speaker presented an overview of megakaryocyte development and platelet production, emphasizing what has been learned from studies of inherited platelet abnormalities. Discoveries in his laboratory regarding the biogenesis of platelet secretory alpha granules using “state-of-the-art” imaging technologies were featured (ARC syndrome and GPS).

The 100,000 Genomes Project. Karyn Mégy – Cambridge, UK

Karyn Mégy presented the UK 100,000 Genomes Project pilot data for Rare Diseases, taking as
example the Bleeding, Thrombotic and Platelet Disorder cohort. The whole genome of 13,037 rare disease patients and their family members were sequenced as part of the pilot. Patients were enrolled at 57 National Health Service (NHS) hospitals in the UK and 26 non-UK hospitals using standardized eligibility criteria. The primary aims of the project were: provide clinical feedback to the clinicians and their patient and identify new disorder-gene relationship. The talk focused on the former. Karyn lead the clinical interpretation of the whole genome sequencing results and explained the multi-disciplinary team based reporting process: (i) gene list selection; (ii) variant prioritisation; (iii) variant assessment and reporting to the referring clinician. She gave preliminary results: 13% diagnostic yield; few genes explain most cases; many of those genes were recently discovered. She insisted on the importance for data sharing.

Clinical correlation and result interpretation by a multidisciplinary team of experts provides meaningful and actionable reports to general providers when approaching a patient with a suspected inherited platelet disorder. Obtaining clinical and platelet phenotype information, incorporating the added time and cost of clinical correlation and maintaining clinical testing platforms is challenging. Further integration of clinical testing with research platforms via international collaborations that facilitate these connections, could assist in increasing access to different testing strategies and enhancing diagnostic capability.

SESSION 2 - Genome Wide Association Studies, Risk modifiers and eQTL studies. Chair: Pieter Reitsma

Hemostasis-related variants identified by GWAS in the Caerphilly Prospective Study. Benjamin Rodriguez – Framingham, US
Benjamin Rodriguez described their work on the first genome-wide association study (GWAS) of the Caerphilly Prospective Study (CaPs), a European ancestry population from South Wales, testing 7.8 million common and low frequency genetic variants for association with 48 hemostatic and platelet traits in 1,184 middle-aged male participants. CaPs is amongst one of the most deeply phenotyped cohorts in the world for broad hemostatic phenotypes including cell counts, platelet reactivity and function, coagulation, fibrinolysis, and plasma viscosity. This effort yielded hundreds of significant and thousands of suggestive variants with considerable impact on hemostatic phenotypes. Conditional analyses for 33 most significant associations are underway. Where applicable, traits will be replicated in the Framingham Heart Study and CHARGE consortia. As usual the vast majority of associated variants are localised in non-coding regions of the genome makes elucidating their mechanisms of action a challenging task. Integration of GWAS variants across hemostatic phenotypes and their high-confidence proxy SNPs with cell type matched epigenomic maps from EPIGENOMICS efforts, including histone mark H3K27ac and open chromatin DNAse accessibility, indicate significant enrichment of variants at megakaryocyte distal enhancers, but not at active promoter proximal elements. Mining of DNA-binding cistromes across ENCODE cell types predicts that variants impact on a surprising number of epigenetic modifiers and transcription factors implicated in controlling megakaryocyte-erythrocyte cell fate, suggesting specific and testable regulatory functions for many variants. These transcriptional regulators include EZH2, MYC, BRCA1, RCOR1, GFI1B, TCF3/E2A, HDAC8, and KDM5B. The role of selected variants in hemostasis mechanisms will be investigated by CRISPr-Cas9 deletion in iPSC-derived megakaryocyte cell models.
Exome Sequencing Studies Identify STAB2 Deficiency as a Genetic Risk for Venous Thromboembolic Disease. Karl Desch - USA.

Venous thromboembolic disease (VTE) is a complex genetic trait with several well described common and rare genetic risk factors. In order to discover new rare genetic variants that contribute to an individuals' risk for VTE, we performed whole exome sequencing in a clinical cohort of 400 individuals with unprovoked VTE and 6114 controls to conduct a collapsing analysis on 17,395 genes. Excess rare and damaging variants were identified in PROC, PROS1 and SERPINC1 as well as STAB2 which encodes a sinusoidal endothelial clearance receptor previously associated with plasma VWF level variation. Current efforts are focused on analysis in an independent replication cohort, development of a murine model of Stab2 deficiency and mutagenesis studies of STAB2 in mammalian cell culture.

Expression QTL studies in seven different blood cell types. Roman Kreuzhuber - Cambridge, UK.

Roman Kreuzhuber presented the concepts of GWAS (variants linked to specific traits) and eQTLs (variants linked to expression), and explained how he merged those 2 types of data to look at how expression level affected traits.

He used:

- GWAS in large populations (Astle et al, Cell 2016.)
  - 36 haematological traits
  - 33 millions variant tested
  - 2,700 associated variants
  - > 90% in non-coding space
  - -> link to eQTL to identify which gene it's linked to

- eQTL – seven purified human blood cell types from 4 different studies
  - 156 samples - BLUEPRINT epigenome project
  - Momozawa et al study (Nature, 2018)
  - Fairfax et al study (Science 2014)
  - Gain power by merging and reprocessing datasets
  - > 6,500 eQTLs identified

- Co-localisation GWAS and eQTL:
  - He presented data on the colocalization of GWAS variants for blood cell traits and blood cell eQTL variants. He illustrated the co-localisation for the ARHGEF3 locus with high expression of ARHGEF3 being causally associated with a low platelet count.

SESSION 3 - NGS in Clinical Diagnosis of INHERITED BLEEDING, THROMBOTIC AND PLATELET DISORDERS (BPD) (part II) – Chair: Willem H Ouwehand.

The implementation of a panel-based next generation sequencing approach in the diagnosis of inherited platelet disorders. Harald Schulze - Wurzburg, Germany
Harald Schulze reported on their experience with the implementation of a panel-based next generation sequencing (NGS) test to identify genetic variants in patients suspected to have inherited platelet disorders (IPD). An NGS panel designed using 6,800 probes that cover 59 selected genes that are known to be responsible for IPD was employed. DNA samples from 48 patients with suspected IPD were processed using the MiSeq Sequencing System. Common SNPs were excluded from further data analysis by a default filter. DNA from five patients with previously well-characterized genetic defects was used for quality control and process validation. The study was conducted in accordance with local Institutional Review Board guidelines. All five of the previously identified mutations in the quality control samples were identified and confirmed by the NGS approach. 15 samples revealed one or two unclassified genetic variants in the tested 59 genes indicating the diagnosis of 7 cases with defects in cytoskeletal proteins, 2 with membrane receptor mutations, 2 with transcription factors, 3 with genes involved in Hermansky-Pudlak Syndrome and one with a calcium signaling defect, respectively. All NGS-detected mutations have been confirmed by Sanger sequencing and subjected to familial segregation analysis. In one case of a novel likely pathogenic variant, segregation analysis ruled out that the variant was associated with the phenotype. Four samples failed the quality control. Our data suggest that NGS-panel is sensitive enough to identify genetic variants in platelet-specific genes of classified and unclassified patients. This approach will improve genetic testing of patients without substantiated diagnosis using conventional methods. For patients with unclear or no detected variants after this approach, whole exome sequencing (WES) or whole genome sequencing (WGS) should be considered with international consortia like the BRIDGE-BPD and ideally, with fully annotated clinical parameters by human phenotype ontology (HPO).

Whole-exome sequencing in evaluation of patients with venous thromboembolism.
Alfred Lee – New Haven, US

Genetics play a significant role in venous thromboembolism (VTE), yet current clinical laboratory-based testing identifies a known heritable thrombophilia (factor V Leiden, prothrombin gene mutation G20210A, or a deficiency of protein C, protein S, or antithrombin) in only a minority of VTE patients. We hypothesized that a substantial number of VTE patients could have lesser-known thrombophilia mutations. To test this hypothesis, we performed whole-exome sequencing (WES) in 64 patients with VTE, focusing our analysis on a novel 55-gene extended thrombophilia panel that we compiled. Our extended thrombophilia panel identified a probable disease-causing genetic variant or variant of unknown significance in 39 of 64 study patients (60.9%), compared with 6 of 237 control patients without VTE (2.5%). Clinical laboratory-based thrombophilia testing identified a heritable thrombophilia in only 14 of 54 study patients (25.9%). The majority of WES variants were either associated with thrombosis based on prior reports in the literature or predicted to affect protein structure based on protein modeling performed as part of this study. Variants were found in major thrombophilia genes, various SERPIN genes, and highly conserved areas of other genes with established or potential roles in coagulation or fibrinolysis. Ten patients (15.6%) had 1 variant. Sanger sequencing performed in family members of 4 study patients with and without VTE showed generally concordant results with thrombotic history. WES and extended thrombophilia testing are promising tools for improving our understanding of VTE pathogenesis and identifying inherited thrombophilia’s.
Carrier detection of inherited bleeding disorders in women; an Irish experience. Catriona Keenan – Dublin, Ireland

Catriona presented the array of 1,759 female sample requests for F8 (912) and F9 (277) gene analysis received in the Haemostasis Molecular Diagnostic (HMD) laboratory at the National Coagulation Centre at St. James’s Hospital Dublin, since the centre opened in 2001.

She showed that they have seen an increasing number of females presenting with personally reduced factor levels requiring full gene screening, but with no apparent family history. Likewise, many women from outside Ireland are being tested for the first time during pregnancy, some with reduced levels during pregnancy, and with little familial information available. She presented examples of solved cases: e.g. affected males whose variant is not detected in the mother (mosaicism? de novo?), a case of Turner Syndrome.

SESSION 4 – Variant interpretation & data sharing – Chair: Daniel Bellissimo

Variant interpretation: when is a negative result negative? Kate Downes – Cambridge, UK

Negative means no variant detected using the technique and criteria used to identify variants and ACMG guidelines for variant interpretation.

A report that only has a VUS finding should also be deemed as a “no variant detected report”. How do we ‘upgrade VUS’?

Co-segregation / parental samples will show:

- if variants co segregate with disease phenotype
- show for autosomal recessive disorders whether variants are in trans
- show whether causal variant is de novo

Kate highlighted the importance of data sharing.

Example 1 - Glanzmann Thrombasthenia case, homozygous for a VUS variant. Possible splicing variant ~+100bp intronic.

Parental samples and brother show co-segregation and that parents are consanguineous. RNA work shows variants alters splicing of gene. VUS upgrades to Likely Pathogenic based on ACMG guidelines.
Example 2- ITP patients refractory to 2nd/3rd line therapy screened on the ThromboGenomics NGS panel test for inherited thrombocytopenia. 7/24 cases have a likely pathogenic variant or VUS suggesting the possible usefulness such tests before treatment escalation to 2nd or 3rd line.

Example 3 – update of knowledge

Around 30 new genes and 6 new modes of inheritance identified since 2015. Most are new genes linked to thrombocytopenia. Example of new mode of inheritance is GP1BB (Sivapalaratnam et al, Blood 2018).

All together in excess of 100 patients who would have received a “no variant detected” diagnosis in 2015, now have a molecular diagnosis thanks to the new genes/mode of inheritance and improved panel tests.

In summary:
- 'No variant detected' report represents a finding at a particular time.
- We should be open to amending reports in the future.
- Variant sharing is key to provide evidence for pathogenicity of variants.

Data sharing: DECIPHER & MatchMaker. Kathleen Freson – Leuven, Belgium

Kathleen defined the notion of tier-1, tier-2 and tier-3 genes. She showed a comparison of the BPD gene lists for 4 gene panel platforms: the Spanish, Danish, UK-GAPP and ThromboGenomics. There is limited overlap between the gene content of the 4 platforms; mostly due to the different aims of the platform: some include thrombotic and coagulation genes, others Hereditary Haemorrhagic Telangiectasia or Ehler Danlos Syndrome genes, or again contain a large proportion of tier-2 genes. She then presented MatchMaker Exchange, a platform that allow to find patients with similar phenotype and variants in the same gene; this is highly useful in finding additional cases and move a gene from tier-2 to tier-1. She proposed 8 genes to be promoted to tier-1 (Annex 1), and 2 to be demoted from tier-1 to tier-2 (Annex 2).

Kathleen invited people to join a lunch discussion on gene/variant curation. This is linked to the Gold Variant grant obtained from ISTH by 6 SSCs, including the SSC for Genomics in Thrombosis in Haemostasis.


Willem H Ouwehand gave a summary of the work done by this SSC since the ISTH meeting in Kyoto in 2011, when the possible establishment of SSC for Genomics was first discussed. He thanked everyone for their support in establishing this new SSC for Genomics in Thrombosis and Haemostasis, and for the progress made so far. He presented data showing how the advent of NGS technologies has changed the way by which
a conclusive molecular diagnosis can be reached. Willem reviewed the cost development for WGS since 2008 (the first year that Illumina sequencing became feasible). He predicted that by 2022 a WGS analysis may become available at $200, making the wide use of WGS in the clinic for diagnosis feasible.

He encouraged the international sharing of data so that the level of pathogenicity assigned to variants would improve over time. He predicted that the genomes of millions of individuals will be sequenced over the next 5 years. This would include the genomes of healthy individuals and those with common or rare diseases. These new resources of genotype and phenotype will be at the basis of an improved annotation of the genome.

This was his last meeting as Chair and he passed on the Chair to Professor Kathleen Freson from the University of Leuven. Professor Freson had been selected as the preferred candidate for the Chair of the SSC after a formal selection process.

**Discussions**

Discussion and questions happened at the end of the sessions. Long discussion about feeding back of incidental findings, and how much information should be given to the patient: e.g. if a patient’s thrombocytopenia is explained by a RUNX1 variant, should he/she be told of the increased risk of leukaemia?

There were different views and it was agreed that each country had to develop its own policies which are aligned and influenced by the country’s legislation in relation to genetic testing. The SSC for Genomics in Thrombosis and Haemostasis could support the community in developing a consensus position.

**Annex 1 – List of new bleeding, platelet, coagulation and thrombotic disorder Tier 1 reportable genes.**

MOI: AR – Autosomal Recessive; AD – Autosomal Dominant; XR: X-linked recessive; GOF: Gain of Function
<table>
<thead>
<tr>
<th>Gene symbol (HGNC)</th>
<th>Gene name (HGNC)</th>
<th>Disorder</th>
<th>MOI</th>
<th>Category</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLKB1</td>
<td>Kallikrein B1</td>
<td>Fletcher syndrome</td>
<td>AR</td>
<td>Coagulation</td>
<td>PMID: 17598838, PMID: 14652634, PMID: 11344577</td>
</tr>
<tr>
<td>GBA</td>
<td>Glucosidase, beta</td>
<td>Gaucher disease</td>
<td>AR</td>
<td>Platelet</td>
<td>PMID: 15813845</td>
</tr>
<tr>
<td>PIGA</td>
<td>Phosphatidylinositol glycan anchor biosynthesis class A</td>
<td>Paroxysmal nocturnal haemoglobinuria</td>
<td>XR - Somatic</td>
<td>Thrombotic</td>
<td>PMID: 8557259, PMID: 8500164</td>
</tr>
<tr>
<td>CDC42</td>
<td>Cell division cycle 42</td>
<td>Takenouchi-Kosaki syndrome</td>
<td>AD</td>
<td>Platelet</td>
<td>PMID: 29394990</td>
</tr>
<tr>
<td>SRC</td>
<td>V-SRC avian sarcoma viral oncogene</td>
<td>Thrombocytopenia 6</td>
<td>AD - GOF</td>
<td>Platelet</td>
<td>PMID: 2693650 7, 2nd pedigree with de novo and functional data</td>
</tr>
<tr>
<td>ACVRL1</td>
<td>Activin A receptor, type II-like 1 A</td>
<td>Telangiectasia, hereditary hemorrhagic, type 2</td>
<td>AD</td>
<td>Bleeding</td>
<td>PMID: 8640225</td>
</tr>
<tr>
<td>ENG</td>
<td>Endoglin</td>
<td>Telangiectasia, hereditary hemorrhagic, type 1</td>
<td>AD</td>
<td>Bleeding</td>
<td>PMID: 9245986</td>
</tr>
<tr>
<td>SMAD4</td>
<td>Mothers against decapentaplegic, Drosophila, homolog of, 4</td>
<td>Juvenile polyposis/hereditary hemorrhagic telangiectasia</td>
<td>AD</td>
<td>Bleeding</td>
<td>PMID: 15031030</td>
</tr>
</tbody>
</table>
Annex 2 – List of demoted bleeding, platelet, coagulation and thrombotic disorder that are no longer Tier 1.

<table>
<thead>
<tr>
<th>Gene symbol (HGNC)</th>
<th>Gene name (HGNC)</th>
<th>MOI</th>
<th>Category</th>
<th>Rational for suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPM4</td>
<td>Tropomyosin 4</td>
<td>AD</td>
<td>Platelet</td>
<td>2 unrelated pedigrees but 1 pedigree also carried an ACTN1 variant, knock-out mouse model</td>
</tr>
<tr>
<td>ORAI1</td>
<td>ORAI calcium release-activated calcium modulator 1</td>
<td>AD</td>
<td>Platelet</td>
<td>TIER1 gene for PID/myopathy but not for BPD (the presence of ITP in a single patient led to the inappropriate labelling as a TIER1 gene)</td>
</tr>
</tbody>
</table>
Guidance Documents

1) Dr. Bethany Samuelson Bannow (United States) presented the final recommendation on the management of cancer-associated thrombosis in patients with thrombocytopenia. The new Guidance was published on June 16th 2018. Differences between the Guidance documents published 2013 and the new one (2018) were highlighted. A summary of the new suggestions are listed below.

   a. We recommend giving full therapeutic anticoagulation without platelet transfusion to patients with CAT and a platelet count of ≥ 50.

   b. For patients with acute CAT and severe thrombocytopenia (< 50) and a higher risk of thrombus progression, we suggest full-dose anticoagulation (LMWH/UFH) with platelet transfusion support to maintain a platelet count of ≥ 40–50.

   c. For patients with acute CAT and severe thrombocytopenia (< 50) and a lower risk of thrombus progression:
      i. We suggest reducing the dose of LMWH to 50% of the therapeutic dose or using a prophylactic dose of LMWH in patients with a platelet count of 25–50.
      ii. We suggest temporarily discontinuing anticoagulation in patients while the platelet count is < 25.
      iii. We recommend resuming full-dose LMWH when the platelet count is > 50 without transfusion support, in the absence of other contraindications.

   d. For sub-acute or chronic CAT (> 30 days since the index VTE) and thrombocytopenia (< 50):
      i. We suggest reducing the dose of LMWH to 50% of the therapeutic dose or using a prophylactic dose of LMWH in patients with a platelet count of 25–50.
      ii. We suggest temporarily discontinuing anticoagulation in patients while the platelet count is < 25.
iii. We suggest resuming full-dose LMWH, as indicated, when the platelet count is > 50 without transfusion support.

There was a good discussion with the SSC members. It was acknowledged that there is considerable variation in clinical practice and that the Guidance’s purpose is to provide a framework for clinicians (which characteristics to include in the risk benefit ratio) to work with when managing these patients.

2) Dr. Alok Khorana (United States) presented the final recommendation on the use of direct oral anticoagulants (DOACs) in the management of cancer-associated thrombosis. The new Guidance was accepted for publication and is expected to be published in September 2018. A summary of the new suggestions are listed below.

   a. The use of DOACs is suggested for cancer patients with an acute diagnosis of VTE, low risk of bleeding and no drug-drug interactions with current systemic therapy.
      i. Edoxaban and rivaroxaban are the only DOACs with current randomized trial evidence compared with LMWH in cancer patients.
      ii. LMWHs remain an acceptable alternative for these patients.

   b. LMWHs are suggested for cancer patients with an acute diagnosis of VTE and high risk of bleeding, including:
      i. Patients with luminal gastrointestinal cancers with an intact primary or patients with active gastrointestinal mucosal abnormalities such as duodenal ulcers, gastritis, esophagitis or colitis.
      ii. DOACs might be an acceptable alternative if no drug-drug interactions exist with current systemic therapy.

The Guidance document generated a good discussion with the SSC members. No concerns were highlighted.

3) Dr. Darko Antic (Serbia) presented a nice comprehensive review on: “Hematological Malignancies: Risk of Thrombosis and Use of Thromboprophylaxis. The risk of venous thromboembolism is high in this patient population. It remains unclear how these patients can be stratified and if an intervention (e.g. thromboprophylaxis) should be offered. This represents an important knowledge gap. The outline of a proposal on the prevention and management of venous thromboembolism complications in this patient population was also presented and generated discussion among members. The suggestions to use a risk stratification scores that has not been prospectively validated raised concerns about members. A meeting will be set in September to review and edit the outline further to account for the comments raised during the Discussion Period.
4) Dr. Jeff Zwicker (United States) presented a Guidance proposal on the management of thromboprophylaxis among acute leukemia patients receiving asparaginase chemotherapy. It was decided that the Guidance move forward. The writing committee will be composed of Drs. Jeffrey Zwicker (United States), Marc Carrier (Canada), Daniel DeAngelo (United States), Anna Falanga (Italy), Tzu-Fei Wang (United States), Mandy Lauw (Netherlands) and Gosia McMasters (Canada). The one-page summary is expected to be circulated among the co-chairs in August 2018.

Quality of Life Assessment Tool

Due to unforeseen circumstance, Prof Noble (United Kingdom) could not attend the SSC meeting in Dublin. Dr. Leslie Padrnos (United States) provided an elegant review of the literature on the assessment of quality of life in patients with venous thromboembolism. She identified an important knowledge gap. Cancer patients are underrepresented in the studies assessing quality of life among patients with venous thromboembolism. Furthermore, these scores were not validated in this patient population. A meeting with be set in September 2018 (Prof Noble and Drs. Carrier and Padrnos) to discuss the next steps for the Quality of Life Assessment Tool Project (Team and process)

ISTH SSC Malignancy & Haemostasis Registry

Dr. Wang (United States) presented a proposal to develop an international registry to evaluate the outcomes (safety and efficacy) of patients on concurrent targeted cancer therapies and DOACs. Primary outcomes (recurrent VTE events and major bleeding) would be collected over a 6-month follow-up period. The proposed sample size was a convenient sample size of 100 patients. The audience was enthusiastic with the proposal and brought a few comments to consider. It was felt that Dr. Wang should consider including cancer patients with either atrial fibrillation or VTE (and not restrict it only to VTE patients). It was also felt that the sample size might be too small and to consider increasing it if enrolment is successful. It was decided that a final draft of the proposal will be distributed to the co-Chairs for review in early September 2018.

Basic Sciences

Professor Godfroid (Belgium) provided an excellent educational session and update on IrCPI, a new potent inhibitor of thrombosis and cancer-associated thrombosis.

Professor Falanga provided the audience with the rational and methods of the HyperCan study. The study includes two main aims (Project A and B). The first project involves a large (>10,000) cohort of healthy volunteers prospectively followed for cancer occurrence. Project B involves patients with NSCLC, gastric, colorectal and breast cancer (> 4000) followed for up to 5 years. Blood samples are collected at baseline and at different time intervals. Samples are currently (and will be analysed) for a panel of hemostatic proteins, clotting activation biomarkers, thrombin generation, pro-coagulant microparticles and thrombophilic polymorphisms. Prof Falanga presented early updates and results and formal analyses will be done in the future.
Professor Gerotziafas provided a comprehensive review on the use of biomarkers for VTE risk stratification in patients with lung adenocarcinoma. Important knowledge gaps in risk stratifications and the different biomarkers assays was identified and highlighted.

Finally, Profs. Mackman and Dubois led a new initiative to generate a consensus of the biomarkers that should be measured in different cancer types as predictors of cancer-associated thrombosis. A one-hour meeting was held (Liffey Room 3A Friday July 20th 14:00-15:00) to generate discussion about exchanging samples so that a full dataset for all the different biomarkers can be generated and different levels compared in different tumour types. A list a potential biomarkers and participating group was generated. Follow-up on this meeting will be done through the last quarter of 2018.
Pregnancy Outcomes in Women With Antiphospholipid Antibodies: Interim Results From a Single Centre Cohort Study

Speaker: Margreta Frishman (United Kingdom)

Persisting antiphospholipid antibodies (aPL) are associated with adverse obstetric events including recurrent miscarriage, late fetal loss or early delivery due to pre-eclampsia or placental insufficiency (obstetric APS) and also thrombosis. We are a tertiary referral centre for those with aPL, and have a management protocol for women with aPL during pregnancy and the puerperium with the aim of preventing obstetric complications and maternal thrombosis.

Aim of the study is to report the fetal and maternal outcomes from a single centre cohort of 511 pregnancies in 372 women over a period of eight years.

This is an ongoing retrospective observational study registered as an audit. Data was collected from clinic lists of patients attending the pregnancy clinic at the Thrombosis centre of St. Thomas' Hospital in London, UK between Jan 2010 to December 2017. Women persistently positive for aPL were included if pregnancy outcome data was available.

511 pregnancies in 372 women were included in the study. The overall live birth rate was 79%. Fetal outcomes were as follows: rate of preterm delivery < 34 weeks gestation and < 37 weeks was 2.5% and 10%, respectively. Birthweight < 1500g and < 2500g was 1% and 11%, respectively. The rate of pregnancy loss at < 10 weeks was 14%. Intrauterine death after 24 weeks occurred in 1%. Maternal outcomes were as follows: 5.5% developed pre-eclampsia and 33% had a caesarean section.

Projects

Antiphospholipid antibodies in lymphoma

Speaker: Cristina Belizna (France)

This project is a proposal of creation of an international registry of lymphoma associated with antiphospholipid antibodies (aPL). Recent findings reported elevated levels of aPL in various malignancies. In addition, several studies suggested an increased prevalence of certain
malignancies in aPL-positive patients. Nevertheless, it is suggested that aPL positivity and titers do not reflect their pathogenicity; therefore, their pathological significance in patients with solid cancer or hematological malignancy is still unclear and controversial. In patients with lymphoma, although for some authors the presence of aPL has been reported as associated with an increased risk of thrombosis, several data are contradictory.

Data are also controversial as regards the eventual role of aPL as markers of disease activity and progression in hematological malignancies. Some authors suggested that aPL positivity could not predict disease prognosis and treatment response in lymphoma patients; however these data were contradicted by other reports. Therefore, some authors found a high prevalence of aPL in lymphoma patients (around 40%), and suggested that aPL are correlated with shortened event-free survival.

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 as regards the potential role of aPL as markers of worse prognosis in lymphoma and with respect of the increased risk of thrombosis in this population. Therefore, we propose a prospective three years study register (one year of inclusion and 2 years of follow up) in lymphoma patients with positive aPL. We have chosen to concentrate this study only in non-hodgkinian lymphoma patients in order to insure as much as possible 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.

**Thrombin generation**

*Thrombin generation and APS: overview, new results and efforts of standardization*

**Speaker: Romy Kremers (The Netherlands)**

Thrombin generation has been recognized as an global hemostasis assay that correlates with bleeding and thrombotic events. Currently, many methods are used to measure thrombin generation, which range from experimental to fully automated. Last year, a working party was started within the Lupus SSC subcommittee to standardize the measurement of thrombin generation. We have send out a questionnaire to inventory the methods and protocol that laboratories use to measure TG and the preliminary results were presented in this presentation at the SSC meeting.

**Towards a thrombin generation based assay to identify high risk APS patients**

**Speaker: Hilde Kelchtermans (The Netherlands)**

Looking at the laboratory criteria of the antiphospholipid syndrome (APS), lupus anticoagulant (LAC; the only functional assay included) correlates best with the clinical symptoms of APS. An adapted version, the β2-glycoprotein I (GPI) dependent LAC, correlates even better with thrombosis. We aimed to go two steps further: (1) by measuring thrombin generation instead of a clotting time; (2) by the addition of thrombomodulin to include the activated protein C (APC) system. We developed an assay that enables the discrimination between anti-prothrombin and anti-β2GPI induced APC resistance, possibly leading to a better risk stratification in APS patients. This assay was validated using monoclonal antibodies, patient-derived antibodies and
a small cohort of APS patients and healthy controls. Interestingly, discrimination with other types of APC resistance such as oral contraceptive usage was possible. Our assay requires further confirmation in a large APS patient cohort.

**Lupus anticoagulant**

**Taipan Snake Venom/Ecarin Time for LAC in Anticoagulated Patients: Preliminary Results from a Multicenter Study**

**Speaker: Gary Moore (United Kingdom)**

Snake venom FII-activators were first proposed for use in LAC testing as they are insensitive to the effects of VKA anticoagulation, although the assays did not achieve widespread use due to lack of availability. The appearance of direct FXa inhibitors in the therapeutic anticoagulant armoury has prompted a resurgence of interest since the venoms bypass FXa. 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. Preliminary data suggest similar reference ranges and low imprecision from a variety of platforms. There are few data yet from clinical samples, results from one centre suggest moderate sensitivity and high specificity, and another centre has demonstrated high detection rates of LACs in established triple-positive APS patients.

**LAC Testing during DOAC: Neutralizing the Culprit?**

**Speaker: Julien Favresse (Belgium)**

The impact of DOACs on laboratory assays used for lupus anticoagulant is a well-known issue and may cause false-positive results. Therefore, the correct interpretation of results that are performed in patient taking DOACs is mandatory to prevent misclassification and the subsequent clinical consequences. Several strategies were proposed to minimize the impact of residual DOACs on coagulation assays: (1) the use of DOAC insensitive assays, (2) the addition of idarucizumab to the plasma sample (Praxbind, Boehringer Ingelheim) to specifically neutralize the in vitro activity of dabigatran, or (3) missing one (for once-daily fixed-dose regimens) or two (for twice-daily fixed-dose regimens) DOAC intake in patients with low thromboembolic risk. More recently, a new solution (namely the DOAC-Stop® treatment, Haematex Research, Hornsby, Australia) appeared to be a valuable alternative to overcome the effect of DOACs. The aim of this study was to evaluate the efficiency of this new and simple procedure in real-life settings.

**Need for Update of the 2009 Recommendations for LAC Detection? Results of a Questionnaire and Panel Discussion**

**Speaker: Hannah Cohen (United Kingdom)**
The update of the ISTH SSC guidelines for lupus anticoagulant (LAC) testing of 2009 have proven to be very useful to reach more uniformity in the performance and interpretation of LAC testing. However, some points, including LAC testing in samples from anticoagulated patients, cut-off values, interpretation of results, merit reconsideration. A questionnaire on LAC testing was circulated to the ISTH SSC on LAC/antiphospholipid antibodies (aPL) and also ECAT (External quality Control for Assays and Tests) Foundation members. The results of the questionnaire were presented at the ISTH 2018 SSC meeting, followed by a Panel Discussion on the need for an update of the 2009 recommendations for LAC testing.

There were 185 responses to the questionnaire. The results are encouraging in that there was agreement for some key aspects of current guidelines for LAC testing e.g. sample processing, principles of testing, choice of LAC tests, interpretative reporting. There was less agreement for some other aspects of LAC testing, including timing of testing in relation to thrombosis or pregnancy, cut-off values, interpretation of results, LAC testing in patients on anticoagulation - VKAs, heparin (LMWH/UFH) and DOACs. The variable opinions in part reflect limited evidence base, and more studies needed to inform evidence-based recommendations. The questionnaire responses have identified areas to focus on to encourage further uniformity in performance and interpretation of LAC testing.

Panel discussion

Panel: Cristina Belizna (France), Hannah Cohen (United Kingdom), Philip de Groot (the Netherlands), Bas de Laat (the Netherlands), Katrien Devreese (Belgium), Doruk Erkan (United States), Masahiro Ieko (Japan), Ian Mackie (United Kingdom), Thomas Ortel (United States), Jacob Rand (United States), Denis Wahl (France), Vittorio Pengo (Italy)

A selection of points was discussed:

• Timing of LAC testing in relation to a thrombotic event or during pregnancy
• LAC in patients on VKA
• LAC in patients on DOAC
• LAC in patients on heparin
• Methodology: calculation of cut-off value
• Confirmation of a first LAC result

The SSC subcommittee will prepare a guidance/guideline on interpretation of LAC and will take into account the different opinions of the panel members and remarks of the audience. On the methodology on LAC measurement only minor changes should be made based on the 2009 recommendations.

Antiphospholipid antibodies-solid phase assays

Antiphosphatidyl Serine/Prothrombine Antibodies: Pro's and Con's to Include in the Diagnostic/Classification Criteria: Pro
**Speaker: Maria Laura Bertolaccini (United Kingdom)**

Measurement of aPS/PT antibodies can detect additional patients, currently classified as seronegative patients based on the criteria lab tests. Also, according to own studies and literature the speaker illustrates that aPS/PT antibodies have an additional value being an independent risk factor. Arguments pro aPS/PT antibodies to include in the diagnostic criteria are: these are pathogenic antibodies, an independent risk factor, independent of LAC, with high diagnostic accuracy. Commercial tests are available and compare well.

**Antiphosphatidyl Serine/Prothrombine Antibodies: Pro’s and Con’s to Include in the Diagnostic/Classification Criteria: Con**

**Speaker: Vittorio Pengo (Italy)**

To diagnose thrombotic APS we have now sufficient evidence that triple positivity (LA+, aCL+, abeta2+, same isotype) is the aPL profile associated with thromboembolic events in patients with APS, in carriers and in obstetric APS. Moreover, most if not all catastrophic APS occur in triple positive patients. Thus, the addition of aPS/PT to the panel of aPL test seems premature and needs further studies.

**Antiphosphatidyl Serine/Prothrombine Antibodies: Pro’s and Con’s to Include in the Diagnostic/Classification Criteria: Discussion**

**Speaker: Maria Laura Bertolaccini and Vittorio Pengo**

Further pros and cons are discussed. Including aPS/PT antibodies may identify patients at higher risk and be more complete identifying APS.

**Role of IgM in APS: Results of the Solid Phase Assays Multicenter Study**

**Speaker: Walid Chayoua (the Netherlands)**

The antiphospholipid syndrome (APS) is characterized by thrombosis and/or pregnancy morbidity with the persistent presence of lupus anticoagulant, anti-cardiolipin (aCL) and anti-b2glycoprotein I (ab2GPI) IgG or IgM antibodies. In literature, the association of IgM antibodies with thrombosis and obstetric complications are contrary. Therefore, the relevance of aCL and ab2GPI IgM antibodies in APS is debated. In a multicenter study we investigated the diagnostic and clinical role of IgM antibodies in APS. We found that IgM antibodies are not of added diagnostic value in APS but rather be useful for risk stratification in APS.

**Anti-Domain I Chemiluminescence Detection in a Prospective Cohort**

**Speaker: Denis Wahl (France)**

The speaker gives an overview of the use of anti-domain I antibodies (aDI). The definition of cut-off values is crucial in the interpretation. This determines the distribution of aDI in patients and controls. Equally, the definition of medium and high titer could be useful. A meta-analysis of all published data could be useful to compare study results, including reanalysis with different cut-off values.
Diagnosis of APS

Update on the New APS Classification Criteria Development Efforts

Speaker: Doruk Erkan (United States)

An international multidisciplinary effort to develop new rigorous, consensus-based criteria to identify patients with high likelihood of having Antiphospholipid Syndrome (APS) has been initiated and in progress. There is a distinction between diagnostic and classification criteria. The latter goal is to define study samples representative of the majority of patients and to identify a homogenous group, not intended to capture the entire universe of possible patients but rather to capture the majority of patients who share key features. There have been important advances in the methodology of classification criteria development and ACR/EULAR published new recommendations for development and validation of criteria sets, based on contemporary standards of measurement. The speaker illustrates all the phases that have already been performed and the steps to be done in the future.

New Insights on APS Diagnostics Derived from Imaging APS Immune Complexes

Speaker: Jacob Rand (United States)

The antiphospholipid syndrome (APS) is an autoimmune thrombotic condition that is marked by autoantibodies against phospholipid-binding proteins. A defining aspect of the disorder is positivity for clinical laboratory tests that confirm antibody binding to anionic phospholipids. The speakers illustrates a totally other approach to identify APS patients. The binding of proteins from plasmas of APS patients to phospholipid can be imaged with high resolution microscopy-based imaging techniques, namely atomic force microscopy and scanning electron microscopy. These findings demonstrate how high resolution microscopic techniques can contribute to advancing the understanding of this enigmatic disorder.
The focus of the Subcommittee is to address issues of thrombosis and hemostasis in children and neonates by fostering international collaboration in research and clinical trials, registries and to develop clinical standards and recommendations relating to patient care for the pediatric population. Work within the SSC is organized through project-related working groups lead by one of the co-chairs.

1. Administrative issues:

Dr. Heleen van Ommen stepped down as co-chair in 2017. Dr. Tina Biss (UK) and Dr. Leslie Raffini (US) have been appointed as new co-chairs. The new co-chair team has met by TC in January 2018 and, apart from the public SSC meeting, had an internal face-to-face meeting in Dublin to discuss ongoing and future projects.

2. SSC communications/reports:

Two communications and one guidance papers have been published by the SSC since the last meeting.


**Recommendations for future research and standardization of definitions in relation to pediatric pulmonary embolism: Communication from the SSC of the ISTH**


3. SSC business meeting in Dublin:

First part 10.30-12.30:

3.1. Top abstract presentation: **Platelet hyporeactivity combined with enhanced coagulation in healthy term and preterm newborns during the first days of life.**

Speaker: Ekaterina Koltsova (Russian Federation)

Abstract: Background: Preterm newborns are a group of high risk of bleeding and thrombosis in the early neonatal period. The laboratory diagnostic tools at the disposal are not sufficiently sensitive to the manifestation of impairments in neonates. Aims: To assess hemostasis in preterm and term newborns using global coagulation testing and flow cytometry characterization of all platelet functional responses. Methods: Ten preterm neonates (33-34 gestation weeks) and ten term neonates (>37 gestation weeks) were enrolled. Platelet functional activity was estimated by flow cytometry, coagulation was assessed using integral assay of thrombodynamics and standard coagulation assays (activated partial thromboplastin time (APTT), prothrombin, fibrinogen, D-dimer, antithrombin III and protein C concentrations). All parents gave their written informed consent; the protocol was approved by ethics committee. Results: All platelet functions (specifically, lack of all granules, of their release, phosphatidylserine exposure and integrin activation) were profoundly (by 50-70%) impaired in newborns. Preterm infants did not differ from term in glycoprotein Ib, P-selectin release or mepacrine uptake/release, but had decreased glycoprotein IIb-IIIa, lower glycoprotein IIb-IIIa activation, and phosphatidylserine externalization upon stimulation. The shape change of platelets after activation was smaller in newborns compared to adults and in preterm compared to term. Clot growth rate in thrombodynamics was increased compared to adult values. Conclusions: Hemostasis of newborns is characterized by pronounced plasma hypercoagulation and reduced functional activity of platelets in comparison with the adults. There is no significant difference in the parameters of coagulation, but some of the platelet functional responses are depressed in preterm newborns compared with terms.

3.2. **DIC Survey: Project Update and Future Steps.**

Speaker: A. Chan (Canada)

Working group: Responsible past co-chair: A. Chan

Revathi Rajagopal (lead), Paul Monagle, Ziad Solh

Collaboration between Pediatric SSC and DIC SSC (chair: Jecko Thachil)

Survey results were presented, publication is pending:

- Variable practices among pediatricians
Many labs are still not compliant with ISTH recommendations to report age-appropriate values

Adult guidelines are not widely accepted in pediatrics

A guidance paper in Pediatric DIC is planned.

3.3. Survey on the management of isolated distal deep vein thrombosis

Speaker: Mihir Bhatt (Canada)

A questionnaire was distributed among attendants prior to the Pediatric SSC meeting and responses collected at the end of the meeting. The results will be analyzed and reported.


Speaker: M. Rajpurkar (US)

Working group: responsible past co-chair: Neil Goldenberg; Madhvi Rajpurkar, Tina Biss, Heleen van Ommen, Susan Williams, Anthony Chan.

Report:

- Systematic review and SSC position paper have been published (see above).
- Presentation of an ongoing ‘Survey on current practice(s) for diagnosis and management of PE amongst pediatric hemostasis/thrombosis physicians’; interim data on 99 respondents so far were presented

Further planned projects:

- Utility of diagnostic prediction rules for PE
- Recommendations about development of a PERT (pulmonary embolism response team)/clinical care pathway system
- Increase awareness of PE amongst other specialties; review article(s) on PE in emergency medicine or other general pediatric journals planned

3.5. Database and DNA Biorepository for Congenital Antithrombin Deficiency: Status Update.

Speaker: R. Kumar (US)

Responsible past co-chair: A. Chan

Working group: Riten Kumar (lead), Suzan Williams, Mike Tarantino, Vicky Price

Update presented at meeting:
Phase I (Redcap database set-p) and phase II (feasibility study in few centres) completed. Phase III will invite multiple international centres to participate. Participation will involve data entry; collection of samples will be contingent on further funding.

Special Topic: Use of DOACs in Children and Adolescents: Is It Time?

3.6. An Update of DOAC Clinical Trial Data
Speaker: Sarah O'Brien (United States)
Brief overview of ongoing paediatric development plans for DOACs and available data so far

3.7. Debate: Are They Ready for Routine Use? No
Speaker: Anthony Chan (Canada)

3.8. Debate: Are They Ready for Routine Use? Yes
Speaker: Leslie Raffini (United States)
Pointed debate on the pros and cons of using the DOACs in children and adolescents prior to paediatric efficacy & safety data being available and before they are licenced for children. The debate is still open but the field is changing rapidly.

3.9. Proposal for Pediatric DOAC Registry: North America
Speaker: Leslie Raffini (United States)
Report on an evolving paediatric subgroup of the International Network of Venous Thromboembolism Clinical Research Networks, planning to collect real-world data on DOAC use in children. Core group of the Venous thromboEmbolism Network U.S. has developed a protocol and received funding via the American Thrombosis/Hemostasis Network (ATHN).

3.10. Proposal for Pediatric DOAC Registry: Europe
Speaker: Susanne Holzhauer (Germany)
Proposal for a paediatric real-world DOAC registry on ‘Focus-cohorts’, focusing on special disease populations, i.e. cancer, chronic kidney disease, neonates, long-term anticoagulation in cardiac disease, antiphospholipid antibodies, inherited thrombophilia. The registry will be set-up within the International Pediatric Thrombosis Network.

3.11. Task Force for Pediatric Anticoagulant Drug Development – Update
Speaker: Christoph Male (Austria)
Responsible chair: Christoph Male

Working group: P. Monagle, A. Chan, H. van Ommen, T. Biss; S. O’Brian, L. Raffini

Goal: To ensure that pediatric developments of new anticoagulant drugs target paediatric needs and are feasible.

A progress report was presented: The European Medicines Agency (EMA) is planning a ‘Paediatric addendum on the existing guideline on clinical investigation of medicinal products for treatment or prevention of thromboembolic disease’. The working group responded to a call for comments, supporting the paediatric addendum, highlighting some of the specific needs and challenges for paediatric development plans, and referring to the recommendations already published by the SSC. Next steps are to draft a position statement, and to get in contact with the US Food and Drug Association.

Second part 15.00-17.00:

3.12. The Identification of At-Risk Patients and Prevention of Venous Thromboembolism in Pediatric Cancer

Speaker: Jay Michael Balagtas (United States)

Responsible past co-chair: A. Chan

Cooperation between the Pediatric SSC and the SSC on Hemostasis in Malignancies.

Brief report on the already published guidance paper ‘The identification of at-risk patients and prevention of venous thromboembolism in pediatric cancer: guidance from the SSC of the ISTH’ (see above).


Speaker: Sanjay Ahuja (United States)

Responsible co-chair: Shoshana Revel-Vilk

Working group: Anjali Sharathkumar (lead), S. Ahuja, Tina Biss, Ketan Kulkarni, Matt Regan

Report on a systematic review on incidental VTE in children. Preliminary results were presented. The group refined the definitions of incidental vs asymptomatic VTE. There was little information on incidental VTE in the literature, therefore the group focused on asymptomatic VTE regarding clinical symptoms, acute complications, chronic sequelae, and the effect of primary or secondary thromboprophylaxis on the clinical course. The results or the systematic review will be published.

Speaker: Marisol Betensky (United States)

Working group on paediatric PTS; responsible past co-chair: Neil Goldenberg

Members: Leonardo Brandao, Manuela Albisetti

Report on a systematic review and meta-analysis; preliminary results were presented, identifying occlusive VTE and CVC-related VTE to be associated with PTS. The analysis will be further refined and sensitivity analyses performed.

3.15. Survey on Anticoagulation Management during ECMO

Speaker: Meera Chitlur (United States)

Working group: responsible co-chair: H. van Ommen; members: M. Chitlur, P. Monagle

An International ECMO interest group was formed in the Perioperative Hemostasis SSC, with involvement of the working group of the Pediatric SSC. A survey was performed on routine practice of anticoagulation and monitoring in ECMO among members of this international group (n=15), demonstrating substantial heterogeneity. Survey data set and methodology (RAND survey) will be refined and extended to larger number of centres.

3.16. Development of Appropriate and Necessary Care Consensus Guideline for Adolescents with Heavy Menstrual Bleeding and Bleeding Disorders - an Update

Speaker: Ayesha Zia (United States)

Responsible co-chair: S. Revel-Vilk

Working group and collaborators: Ayesha Zia, Meera Chitlur, Peter Koides, Susan Halimeh, Maha Othman, Dvora Bauman

Presentation on the development steps of the Consensus Guideline.

Phase I: systematic review of existing literature and guidelines and development if background reports (completed);

Phase II: in-person meeting of HBM working group to finalize gaps, definitions, and care statements (took place at ISTH meeting in Dublin);

Phase III: RANC/ExpertLens panel voting on appropriateness and necessity of HBM care by international panel of experts (to start October 2018).

Special Topic: Quality of Life and Adherence to Anticoagulant Therapy in Children and Adolescents: Proposal for Working Group
3.17. Introduction to the Session and Speakers
Speaker: Tina Biss (United Kingdom)
− Few data on the impact of childhood thrombosis and anticoagulant therapy on QoL
− Lacking data on adherence to anticoagulant therapy in children:- Quantitative data; Knowledge of barriers and facilitators; Usefulness of educational tools
− Suboptimal anticoagulant control is likely to have an impact on the safety and efficacy of treatment
− Tools to assess QoL and adherence while on anticoagulant therapy would be useful in addressing the impact of DOACs in both clinical trials and routine practice
− Opportunity to improve education and influence adherence of young people

3.18. Adherence to Anticoagulant Therapy in Pediatrics: Quantitative Study
Speaker: Fiona Newall (Australia)
− 42% of families missed at least one warfarin dose in preceding month
− Parents rated their efficacy in warfarin management as predominantly ‘very confident’
− No clear patterns connecting efficacy and management practices or demographics was evident
− Frequent contact with the clinical team was reported to be important to supporting adherence to therapy

3.19. QoL and Adherence to Anticoagulant Therapy in Adolescents: Qualitative Study
Speaker: Kay Rooney (United Kingdom)
Report of a qualitative study exploring barriers and facilitators to treatment adherence in adolescents engaged in anticoagulant therapy.

3.20. International Pediatric Thrombosis Network
Speaker: Heleen Van Ommen (the Netherlands)
Responsible co-chair: H. van Ommen
Working group: Manuela Albisetti, Mihir Batt, Suzanne Holzhauer, Christoph Male, Paul Monagle, Shoshana Revel-Vilk, Elizabeth Chalmers
Mission:
To develop an international trial network to conduct epidemiological research, to link academic and industry partners to initiate and conduct clinical trials, and to incorporate translational research into clinical trials

To harmonize clinical care via educational tools, lectures and publications.

Progress report:

A Pediatric thrombosis registry (Thromb-PED) has been created in RedCap and a webpage created at the ISTH SSC homepage. Interested colleagues can register to become members via the webpage and, after IRB approval, enter data on children with thrombosis. In addition, specific projects will be run via the network, currently a project on neonatal renal vein thrombosis and a registry of DOAC use in children.

4. Further ongoing projects:

4.1. Catheter-related arterial thrombosis

Responsible co-chair: Neil Goldenberg, Manuela Albisetti

Working group: Mariana Bonduel, Shoshana Revel-Vilk, Mattia Rizzi

- A systematic review has been published:

**Catheter-Related Arterial Thrombosis in Neonates and Children: A Systematic Review.**

- A SSC position paper has been prepared, reviewed and supported by the SSC co-chair team, but was rejected by the editor of JTH; an appeal by the Pediatric SSC chair was unsuccessful; the manuscript will be submitted to another journal.

**Recommendations for Future Research in Catheter-Related Arterial Thrombosis in Children.** Albisetti M, Rizzi M, Bonduel M, Revel-Vilk S, Goldenberg NA.

4.2. Diagnostic criteria for venous thrombosis in children

Responsible past co-chair: L. Brandao

Working group: J. Journeycake, C. Male, A. Chan, R. Krishnamurthy, C. Loewe

The project targets four areas:

a. Central vein thrombosis

b. Pulmonary embolism
c. Cerebral venous thrombosis
d. Imaging of VTE in Vascular Abnormalities

which are planned to be covered in a series of respective clinical guidance papers, currently being drafted under the lead of L. Brandao.

The project is ongoing.

4.3. **Registry of Congenital Severe Purpura Fulminans**

Responsible past co-chair: L. Brandao

Working group: Vicky Price (CA), Adrian Minford (UK), Paul Monagle (AU),

Progress Presented at SSC meeting in 2016

Received an ISTH grant of ~ 6,000 CAD. The RedCap database has been established. Several cases of neonatal PF have already been identified in in various countries, but the process of setting up centres to enter data is ongoing.

4.4. **Guidance paper on catheter-related thrombosis prevention and treatment in the pediatric population**

Responsible past co-chair: A. Chan

Working group: Ketan Kulkarni (lead), L. Brandao, P. Monagle, V. Faustino, C. Male

- Systematic review in progress
**Perioperative and Critical Care Thrombosis and Hemostasis**

18 July 2018  
10:30 – 12:30; 15:00 – 17:00

**Chairman:** Marc Samama  
**Co-Chairs:** Pierre Albaladejo, James Douketis, Andreas Greinacher, Beverley Hunt, Jerold Levy, Alex Spyropoulos

**Review of the Business and Educational Sessions:**

The sessions took place on Wednesday, July 18th, 2017, in room Liffey A, Dublin convention center for the business session, and on Thursday in the same room for the educational session. On Wednesday, the room was packed with around 500 attendees during the morning sessions and, at least, 300 people during the second part of the program. On Thursday, during the joint educational session with two concurrent SSC sessions, 500 attendees were in the room.

Marc Samama (Paris, France) chaired the first part of the business session (2 hours), with the following topics discussed:

**Top abstract presentation:** *Development of a New Point-of-care Test for the Detection of Direct Oral Anticoagulants (DOACs)*, by G. Frydman et al, Massachusetts Institute of Technology and Massachusetts General Hospital, Boston

A point-of-care test has been developed for the detection of DOACs in whole blood and plasma as well as identify the specific type of DOAC. This test overcomes the current limitations of coagulation testing, and could be useful as a tool for the detection and monitoring of DOACs in emergency and critical care settings.

**Morning business session:**

*Tranexamic acid (TXA) in the prevention of surgical bleeding.* Jerrold Levy (Duke University School of Medicine, Durham, USA). Prof. Levy performed a detailed overview of hemostatic agents, mainly TXA, but also aprotinin, and recombinant factor VIIa, focusing on the rational for their use and clinical applications for the prevention of surgical bleeding.

*TXA in the management of bleeding Beverley Hunt.* (St. Thomas' Hospital, London, UK). Prof Hunt covered the utility of tranexamic acid for established bleeding in the areas of trauma, surgery and post-partum haemorrhage, summarizing the benefits and lack of risk of the compound.
New evidence for perioperative management of OAC and anticoagulants. Jim Douketis (McMaster University, Hamilton, Canada), Pierre Albaladejo (Grenoble University Hospital, France), Thomas Thiele (Universitätsmedizin Greifswald, Germany). These three lecturers addressed this topic following different approaches:

Prof. Jim Douketis discussed Ongoing and Planned Studies on the Perioperative Management of DOACs for Elective Surgery, both from the perspective of clinical patient management and as regards the role of perioperative laboratory for coagulation function testing.

Regrettably, Prof. Spyropoulos was unable to attend the session due to travel delays and his lecture was given by Prof. Douketis.

Prof. Albaladejo then discussed the Perioperative Management of DOACs for Urgent Surgery, discussing results from an ongoing French registry assessing clinical management and outcome.

Prof. Thiele addressed the Perioperative Management of antiplatelet therapy. His talk briefly summarized current concepts of the perioperative management of patients receiving dual antiplatelet therapy with an indication for elective or urgent surgery, specifically in patients with coronary stents.

Afternoon business session:

This session was chaired by Jun Teruya (Baylor College of Medicine, Texas Children's Hospital, Houston)

Prof Teruya first gave an Overview of extracorporeal devices and hemostasis:

Recently indication for ECMO has expanded substantially that includes ECPR, extracorporeal cardiopulmonary resuscitation, a new modality of resuscitation of cardiac arrest refractory to conventional CPR. However, bleeding and thrombosis are ongoing problems during ECMO. Dr. Teruya discussed possible causes of bleeding and thrombosis and their management. Among them, intravascular hemolysis, i.e., plasma free hemoglobin, is thrombogenic and a predictor of poor outcome. Bivalirudin may be used in the setting of heparin induced thrombocytopenia or when heparin anticoagulant effect is not adequate despite increasing dose. Plasma exchange may be considered in order to reset hemostasis.

Impact of VADs and ECMO on primary hemostasis and consequences of therapeutic approaches. Prof. Barbara Ziegler (Children's Hospital, University of Freiburg, Switzerland). Acquired von Willebrand syndrome (AVWS) develops in VAD- and ECMO-patients and can increase the bleeding risk. The HM III device causes less serious AVWS. Platelet secretion defects should be investigated in VAD- and ECMO-patients. The awareness of these acquired disorders may lead to an optimized therapeutic approach and the development of improved devices.
Anticoagulation and monitoring for ECMO and VADs: targets and drugs. Paul Monagle (Royal Children's Hospital, Melbourne) emphasized the need for a common language/set of descriptors to compare inter-institutional ECMO and VAD. A uniform agreement of clinical outcome measures would also be welcome and better tests are needed in order to implement comparisons of drug and monitoring strategies using the agreed clinical outcomes.

Anticoagulation for ECMO and VADs other than heparin and warfarin: direct thrombin inhibitors and novel agents Thomas Renne (University Medical Center Hamburg) showed brand new data establishing that targeting FXIIa is a safe thromboprotection in bypass systems and provide the first anticoagulant strategy in a clinical setting that is not associated with excess hemorrhage.

At the end of the session, the panel opened the discussion with the audience (about 60 people). The published guidance documents and projects of the SSC were described. A discussion was launched, with several questions. Some suggestions came from the floor including:

- management of DOACs for multiple procedures
- bleeding complications, implementation of a large registry
- surveys with more granularity

The shared education session on the Thursday was dedicated to: How to Manage Antithrombotic Drugs in Patients Undergoing Invasive Procedures and chaired by Jim Douketis and Fionnuala Ni Ainle. On behalf of the SSC, Marc Samama gave a case-based presentation on: Determinants of Bleeding in a Perioperative Setting. He developed on non-specific reversal agents and also focussed on antidotes and especially on the issues raised by andexanet-α. Five other speakers addressed the topic.

Perioperative Mechanisms of Thrombosis
John-Bjarne Hansen (Norway)

The Role for Laboratory Testing Before Urgent Surgery (Case Based Presentation)
Adam Cuker (United States)

Determinants of Acute Coronary Syndromes in a Perioperative Setting
Ursula Rauch-Krôhnert (Germany)

Perioperative Prevention of Thromboembolism: An Asian Perspective
Heng Joo Ng (Singapore)
Why It Is Safe to Continue Antithrombotics for Some Procedures and Not Others?

Peter Verhamme (Belgium)

Overall, this was a very well attended session (standing room only) with >500 attendees and good audience engagement.
Plasma Coagulation Inhibitors
19 July 2018
8:00 – 12:30

Chairman: Jun Teruya
Co-Charis: Ian Jennings, Hiroko Tsuda, Eriko Mosishita, Cecilia Guillermo, Herm-Jan Brinkman, Richard Mahler

Top abstract: Effect of andexanet-tissue factor pathway inhibitor (TFPI) interaction on in vitro clot formation and lysis via different coagulation pathways

Dr. Genmin Lu (USA)
Andexanet (AnXa) is a modified, recombinant human factor Xa (FXa) being developed as a specific reversal agent for all of the FXa inhibitors. AnXa retained high binding affinity to FXa inhibitors, including TFPI. They characterized the differential effect of AnXa on clot formation and lysis via different coagulation pathways in the context of recombinant tissue plasminogen activator (rtPA). They found the differential effect of AnXa on CT and LT via three coagulation pathways showed that the AnXa-TFPI interaction in the Tfpathway could be compensated in the presence of rtPA.

Clinical significance of TFPI

Dr. Alan Mast (USA)
TFPI is expressed in two major isoforms in humans, TFPIα and TFPIβ, which have different cellular locations, inhibitory activities and effects on human disease. The biochemical activities of TFPIα and TFPIβ were presented. The clinical significance of the TFPI isoforms was covered by discussion of the role of TFPI in hemophilia and in the procoagulant state induced by factor V Leiden.

TFPI: How Amsterdam found its own factor V

Dr. Saskia Middeldorp (The Netherlands)
They investigated a family with bleeding tendency with prolonged PT and PTT. They were found to have an elevated TFPI level. They performed whole exome sequence analysis and found a novel gain-of-function mutation in the f5 gene (c.C2588G). Factor V is short, missing 623 amino acids from the B domain, which they called factor V Amsterdam.

Post veni-puncture cleavage of protein S and the influence thereof on protein S testing

Dr. Herm-Jan Brinkman (The Netherlands)
The most troubling aspect of the protein S activity assay is the sporadic falsely low protein S activity levels, reverting to normal when repeated. Reason for these erroneously decreased
activity levels may be proteolytic degradation of protein S. Several proteases have been identified, including factor Xa, thrombin, elastase and protac, that are able to target the so called thrombin sensitive region in protein S, thereby modulating its anticoagulant activity. Extent of cleavage, conditions for cleavage, proteases involved and influence on protein S diagnostics are issues currently under investigation.

**Investigation into racial differences in genetic risk factors for venous thromboembolism**

**Dr. Hiroko Tsuda (Japan)**

They established a global network of experts from Japan, South Korea, Singapore, Hungary, and Brazil to investigate worldwide distribution of three gene mutations representing type II deficiency, protein S (PS) Tokushima (PS p.Lys196Glu), protein C (PC) p.Arg189Trp, and PC p.Lys193del. PS Tokushima was found only in Japanese. PC p.Arg189Trp was found in Japanese and Singaporean patients suffered from venous thromboembolism (VTE), being most prevalent in patients of Chinese origin, and PC p.Lys192del was in Japanese, South Korean, and Singaporean VTE patients. In contrast, none of these mutations was found in either VTE patients or healthy individuals of Hungary and Brazil, indicating the racial differences in genetic risk factors for VTE.

**Prevalence of protein S deficiency in Latin America**

**Dr. Cecilia Guillermo (Uruguay)**

Protein S deficiency is one of the inherited thrombophilia associated with risk for first thromboembolic event, especially in young people. The knowledge about the prevalence of this deficiency in Latin-American and how it is diagnosed, is limited. To increase awareness of this topic we conducted a survey through Grupo CLAHT and members of other Scientific Societies, addressed to Clinicians and Laboratory specialists. They asked Clinicians about how many patients with thrombotic disease (venous and/or arterial) treated in last year, and how many of them have diagnosis of protein S deficiency, while they asked to Laboratory doctors, how many requests of protein S they received in last year, how many deficiencies they diagnosed and what methodologies they used.

They received 88 answers, from 15 countries. From the clinical point of view, they validated 42 answers of physicians, who reported a median of 2050 (range 1381 - 2790) patients treated in last year with thrombotic event (venous and/or arterial), between this selected population they reported 159 Prot S deficiency, thereby a prevalence of 7.7% (5.6-11.5), higher in comparison to prevalence reported in international literature in thrombotic patients.

From the Laboratory point of view, 17 laboratories reported 6063 requests of protein S determination (median 356 requests/laboratory/year), with a median of 168 deficiencies (range 152-185). Consequently, the prevalence from the Laboratory was 2.77% (2.5-3.05).

They concluded the prevalence of protein S deficiency it seems to be higher to the reported internationally in thrombotic population, probably due to a wrong timing of the request, in relationship with acute event or other issues. It could be interesting to investigate prevalence of protein S deficiency prospectively, with special attention in the ethnic diversity of Latin America.
There are an excessive number of requests of protein S: it is necessary to work in guidelines to correct this problem.

**Molecular basis and thrombotic manifestations of antithrombin deficiency in Japanese patients**

**Dr. Eriko Morishita (Japan)**

Genetic analysis and clinical background of antithrombin deficiency analyzed in their laboratory. Congenital antithrombin deficiency is a typical thrombophilia predisposition in Japan. They summarize causative genetic mutations for antithrombin deficiency analyzed in their laboratory and their clinical background (clinical symptoms of thrombosis, age at onset, family history, and contributing factors for thrombosis).

**Debate: Antithrombin replacement during heparin therapy: Pro**

**Dr. Meera Chitlur (USA)**

Anticoagulation management in ECMO is challenging. Bleeding and thrombosis are major complications due to contact of the cellular and plasma coagulation proteins in blood with artificial surfaces resulting in activation and consumption. This combined with the underlying disease state of the patient makes managing anticoagulation extremely difficult with significant variability in ACT’s and PTT’s used to monitor heparin anticoagulation. Since heparin accelerates antithrombin (AT) activity, one question has been whether maintaining adequate levels of AT can assist in decreasing the variability in heparin dosing and potentially decrease the thrombotic risk. She reviewed information which supports the use of AT to assist with managing anticoagulation and discuss the potential benefits.

**Debate: Antithrombin replacement during heparin therapy: Con**

**Dr. Paul Monagle (Australia)**

Antithrombin replacement therapy has become commonplace over recent years for children on ECMO. The principle is to increase heparin effectiveness. However, there is little data to support its benefit and in fact much data suggests antithrombin replacement may cause harm. Antithrombin is expensive and without clear evidence of benefit then it is hard to justify the frequency with which it is currently used.

**Heparin-like substance – is it myth or reality?**

**Dr. Rocky Hui (USA)**

He focused on a recent case of Heparin-like substance (HLS) encountered at Texas Children’s Hospital. He discussed the clinical symptoms, laboratory findings, treatments and outcomes of the patient. In addition, he presented the characterization of the HLS in found in the patient and how it compares to other reported cases. It was mostly heparan sulfate. There was also a brief review of what’s known and published about HLS under various clinical settings.
SSC/ISTH Plasma Standard Lot #5: An Update on the Calibration of Protein S, Protein C and Antithrombin

Dr. Craig Thelwel (UK)

The current Lot #4 of the SSC/ISTH secondary coagulation standard is depleted and will need to be replaced in 2019. The standard is calibrated for 21 analytes relative to WHO International Standards and is available in large numbers to manufacturers of diagnostic kits and reference reagents. An international collaborative study was organised to calibrate Lot #5 and the results for the calibration of Protein S (function, free antigen and total antigen), Protein C (function and antigen) and Antithrombin (function and antigen) were presented.

Ongoing SSC activities/future projects

- Drs. Mahler, Jenning, and Tsuda retired from co-chairs after this meeting.
- Dr. Morishita is a new Co-Chair approved in 2017 and Ignjatovic is a candidate for a new Co-Chair pending approval in 2018.
- Four papers will be submitted to JCH soon – antithrombin, APC resistance, protein C, and protein S.
- Protein S typing using cofactor function for TFPI, not as a cofactor activity of activated protein C.
- Function and assay of thrombomodulin
- Anti-antithrombin and anti-TFPI therapy for hemophilia
- Revisiting alfa 2 macroglobulin
- Congenital antithrombin registry
Chairman: Donald M. Arnold
Co-Chairs: Tamam Bakchoul, Philip Choi, Brian Curtis, Shigeki Miyata, Francois Mullier, Rachel Petermann

15:00 Top Abstract Presentation: Type I Interferons Upregulate IFITM3 in Human Platelets and Megakaryocytes to Control Viral Infectivity
Robert Campbell, United States

15:15 Overview of Platelet Immunology SSC Activities
Donald Arnold, Canada
DA highlighted ongoing work of the platelet immunology SSC over the last year:
• Submitted an SSC communication on the investigations of fetal/neonatal alloimmune thrombocytopenia (under review)
• Co-chairs have received 2 ISTH grants: 1) Towards precise and rapid diagnosis of HIT: a prospective, multicentre cohort study (Nagler, Bakchoul et al); 2) International survey of H. Pylori testing and management in ITP (Vishnu, Arnold et al).
• Educational Module on Thrombocytopenia, which is nearing completion.

15:25 Thrombocytopenia Learning E-Module
Tamam Bakchoul, Germany
- 5 modules are already submitted
- Prefinal version will be available within the next 8 weeks
- Milestones will be achieved in planned time
- Reviewing process is almost completed

Prakash Vishnu, United States
Title: International Survey of H. pylori testing and treatment in patients with Immune Thrombocytopenia (Principal Investigator: Donald M. Arnold, MD, MSc, FRCPC; co-investigator: Prakash Vishnu, MD, FACP)

The objective is to understand patterns of practice regarding testing and treatment of H. pylori in patients with ITP across various geographic regions. This study is funded by ISTH and currently underway.

Design and methodology
1. Web-based survey consisting of scenario-type and short answer questions
   Survey validation through vetting; and pilot testing with a small group of select experts from the Platelet Immunology/Physiology SSC
2. Link to the survey delivered by email and responses tracked anonymously
3. Data collected in ISTH-REDCap database
4. Project timeline:
   a. Project set up: 5 months (January 2018 – June 2018)
   b. Launch: July 2018
   c. Survey duration: 4 months (July 2018 – October 2018)
   d. Finalization/analysis: 2 months (November 2018 – December 2018)
   e. Reporting: January 2019
5. Expected outcomes:
   a. Data presentation - ISTH SSC meeting/congress
   b. Publication of survey results - SSC Communication

If you would like to be included in the H. Pylori ITP survey please contact Joanne Duncan (duncanj@mcmaster.ca). We anticipate that the survey will be rolled out in September 2018.

15:55 ITP Virtual Biobank - Proposal
Donald Arnold, Canada

DA discussed important limitations with current basic experiments in ITP:
1. Rarity of patients and samples for experiments
2. Potential for inaccurate clinical diagnosis (e.g. exclusion of other causes)
3. Case mix among patients with ITP (disease severity, platelet count, recent/previous treatments)

A proposal for the SSC is a multi-faceted project to improve the standardization of samples and methods for basic investigations. Dr. Arnold discussed 2 components to such a proposal:

1. Virtual biobank:

This would be a collection of well-characterized patient samples that include key clinical information on platelet count levels, treatments, diagnosis, etc. Samples would be stored locally (e.g. virtual) at key participating centres and catalogued. The proper consent will be required and privacy issues will need to be addressed. Ultimately this would be a resource for sharing among academic investigators and collaborators.

2. ITP Summit

The objective of such a summit would be to establish 2 – 4 research priorities on the advancement of disease mechanisms. Goal is to develop new ideas, lines of inquiry and methods of investigation. It would consist of a panel of experts in ITP and related fields e.g. platelet immunology, megakaryocyte biology, T-cell biology, autoimmunity, molecular biology (n= 10 – 12 individuals) at a 1 – 2 day meeting.

16:15 Platelet Microparticles in HIT and ITP

Francois Mullier, Belgium

Prof F. Mullier, Mr Nicolas Bailly, Ph. Hughes Jacqmin, Prof B. Chatelain

Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC-NARILIS), Laboratory of clinical biology

In immune thrombocytopenia, platelet-derived microparticles (PMPs) may provide additional information to platelet counting. However, current methods available to measure PMPs are expensive and only available in specialized laboratories. Therefore, we have developed a PMPs gate in XN-20 PLT-F channel for the measurement of PMPs. Concentration of PMPs was determined on XN-20 and by flow cytometry, using international recommendations. Preliminary results on healthy subjects and few patients with immune thrombocytopenia will be presented during this talk.

16:35 Standardization of NAIT Testing - SSC Communication

Rachel Petermann, France

The position paper of the members of the Platelet Immunology Scientific and Standardization Committee of ISTH on investigations to be done for Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT) proposes five recommendations to manage FNAIT diagnosis and testing which were felt to be priority areas: clinical definition of FNAIT, blood samples requirements for testing, methods for serological investigations and human platelet antigens
Immunotherapy and immunomodulation are revolutionising the landscape of therapeutics for both chronic autoimmune diseases as well as cancer. Alemtuzumab has new life as a powerful tool to halt the progression of relapsing remitting multiple sclerosis, but it has been associated with a rare immune mediated thrombocytopenia which can be severe, refractory to first-line treatments, and life threatening. Quite distinctly, a more immediate immune thrombocytopenia is seen with checkpoint inhibitors used increasingly in malignancy. These therapies are increasingly seen as indispensable, and managing thrombocytopenia will benefit from organised research beyond scattered retrospective case series. These scenarios represent a new class of iatrogenic immunotherapy induced autoimmunity. Due to the low incidence of these complications, we propose an international multi-site collaboration to better catalogue and study these effects. Studying these complications may offer additional perspectives to our current knowledge on the pathogenesis of primary ITP more broadly, as they represent human models of autoimmunity. [For further enquiries on collaboration opportunities, please contact me at info.nprc@anu.edu.au]

Laboratory assays have limited sensitivity
- Future research should focus on:
  1) Testing drug metabolites as target antigens
  2) Identifying fingerprints of Drug-Antibodies (activation, Apoptosis, desialylation, MKs, etc)
  3) Investigations of the impact of used material for antibody testing

Diagnosis of Heparin-induced thrombocytopenia (HIT) is challenging and currently available diagnostic tests are associated with important drawbacks. Functional assays such as the serotonin-release assay (SRA) and heparin-induced platelet activation test (HiPA) are rarely available in a timely manner. Clinical scoring tools such as the 4Ts score are observer-dependent and the accuracy in clinical practice is limited. The accuracy of different immunoassays is varying among tests and manufacturers and strongly depends on the
threshold. The risk of misclassification of non-diseased patients is high. In addition, it is not precisely defined how to process the information obtained at bedside and rule-in or rule-out HIT. Combining all the information mentioned above in a combined algorithm might improve diagnostic procedures, but algorithms using Bayes theorem have limitations as well. A promising strategy is development of a clinical prediction rule for the diagnosis of HIT, but high-quality data are necessary to do this. We developed a study protocol for a registry-based prospective, multicenter cohort study for the diagnosis of HIT. With the present study, we aim to (A) to assess the association between clinical characteristics as well as routine laboratory test results and the presence of HIT, (B) to develop a clinical prediction rule for the diagnosis of HIT that can be applied at bedside, (C) to additionally study the diagnostic accuracy of other diagnostic instruments under development, and (D) to observe outcomes in patients treated with different anticoagulants and treatment strategies. During the presentation we will present all the details of the study, which are available at the webpage www.isth.org/page/toradihit.

18:15  HIT Functional Testing Workshop- Proposal
Ishac Nazy, Canada

Heparin-induced thrombocytopenia or HIT is a serious and potentially life-threatening immune mediated platelet disorder that follows exposure to heparin. Diagnosing HIT relies on confirmatory laboratory testing for the presence of platelet activating antibodies that bind complexes of platelet factor 4 (PF4) and heparin (PF4/heparin). The serotonin release assays (SRA) is the most studied functional assay for the diagnosis of HIT and it has the highest sensitivity and specificity. However, recent studies have reported discrepant sensitivity results by the SRA. Padmanabhan et al, 2016 and Vayne et al, 2017 showed lower SRA sensitivity in HIT-positive samples. However, Nazy et al., 2015 showed 36% of HIT-negative samples have low-levels of platelet activating antibodies using a PF4-dependent functional assay but the samples were negative in the traditional heparin-dependent SRA.

The objective of this abstract was to propose a multi-laboratory workshop to assess the sensitivity of the SRA when testing samples that are positive in the newer PF4-dependent functional assays but negative in the SRA.

18:35  Heparin-Independent Platelet Activation in HIT Functional Assays
Tamam Bakchoul, Germany

- HIT is not a rare complication undergoing extracorporal circulation (ECC) treatment
- Sera from patients with ECC could cause unspecific platelet activation -> impairment of functional assay by immune complexes which mimic HIT-antibodies
- Implementation of inhibition steps (ELISA with high concentration of heparin and HIPA with IV.3) could be useful to detect HIT in this clinical setting.
18:55 Future Directions, Collaborations, Education

Donald Arnold, Canada

The discussion captured ideas from the chair, co-chairs and SSC attendees on future directions regarding both content and format of the SSC sessions. With the time slot being shortened to approximately 2 hours for future SSC meetings starting in Melbourne 2019, we will have to focus these sessions on the most active projects. This might mean that not all topics (ITP, DITP, HIT, FNAIT) are covered at each annual SSC session.
Chairman: Paolo Gresele

Co-Chairs: Marie-Christine Alessi, Marina Camera, Andrew Frelinger III, Marie Lordkipanidze, Jose Rivera, Matthew Rondina

SSC Platelet Physiology Session Minutes

The Platelet Physiology SSC organized a 2+2 hour session on Thursday July 19th 2018, room Liffey A.

The 8.00 to 10.00 am session was opened by a 15 minutes selected abstract presentation by Madhumita Chatterjee (Germany) on the topic: “Platelet chemokine receptor CXCR7 mediates an anti-thrombotic and anti-thromboinflammatory effect”


Ongoing projects and projects about to be started were listed:

- Evaluation of the Bleeding Assessment Tool (BAT) for the bleeding history of inherited platelet disorders (P. Gresele, P. Harrison): cross-sectional phase: completed; second year follow-up: almost completed, report in preparation
- Assessment of the correlation between the ISTH/SSC bleeding assessment tool score and result of laboratory tests employed for the diagnosis of inherited platelet function disorders (BAT-LAB substudy) (P. Gresele) completed: analysis ongoing and report by end 2018.
- Generation of guidance on the measurement of platelet dimensions (P. Noris) concluded: report in preparation.
- Prospective evaluation of the bleeding phenotype in PT-VWD to support evidence-based diagnosis and management (M. Othman) concluded: report in preparation.
New projects about to be started were also listed:
- Measurement of soluble markers of platelet activation: methods and use (M. Camera).
- Diagnosis of inherited platelet disorders on a blood smear (A. Greinacher).
- Standardization and inter-laboratory comparison of platelet aggregation reagents: a multicentre comparison (M.C. Alessi)

Moreover, a joint project with the EHA for the generation of a Guidance on the management of Glanzmann Thrombasthenia, coordinated by G. Di Minno, P. Gresele for the Platelet Physiology SSC and C. Balduini for the EHA SWG on Thrombocytopenia and Platelet function disorders is ongoing. It was also mentioned that two of the proposals made by the Platelet Physiology SSC to the call of the ISTH for topics for the production of clinical practice guidelines were shortlisted for possible further consideration, and these are: 1) Management of bleeding at delivery (M.C. Alessi, P. Gresele) 2) Guidelines for the measurement of platelet procoagulant activity (M. Camera, P. Gresele)

The audience was invited to interact with the SSC Platelet Physiology about the ongoing and new projects and to send expressions of interest to participate in the new projects to be started.

Maha Othman (Canada) then presented the final results of the study “Guidance on the diagnosis and management of PT-VWD based on expert opinion: a project from the platelet physiology subcommittee of the ISTH”. Only 53 patients have been so far reported with the rare but potentially life threatening bleeding disorder PT-VWD and literature is scarce in providing information required to establish standards for diagnosis and management of the disease. A RAND based approach was thus designed to obtain a formal consensus among experts. A panel formed of 11 experts (10 countries, 4 continents) was established. A series of 46 survey statements on history and clinical presentation (14), diagnosis (21) and management (11) of PT-VWD were prepared, rated by the experts and analyzed. Experts have agreed largely on most statements related to history, clinical features, methodology for diagnosis and treatment approaches. Few statements were judged uncertain/ inappropriate, in which a small percentage of experts were represented. Analysis shows that some areas remain uncertain and more information/ research is needed. An agreement was made towards the suggested approach to reduce mis/under diagnosis and a simple algorithm for treatment was suggested. An SSC communication manuscript to convey this expert consensus for a better diagnosis and treatment of PT-VWD world wide is underway.

Subsequently, a part of the session was devoted to the presentation of some unconventional platelet functions, with special attention to methodological aspects and to pathophysiological implications, involving 4 invited speakers:

- Dr Florian Gartner (Germany), who highlighted that adherent platelets actively migrate at sites of inflammation and showed that migration allows platelets to scavenge and bundle fibrin-bound material, including intravascular bacteria.
- Dr Eleonora Petito (Italy), who showed that platelets, apart from their ability to move randomly, undergo in vitro polarization and migration in the direction of a chemotactic stimulus. This ability explains platelet recruitment in tissue in disease conditions. In particular, platelets undergo polarization and chemotaxis in the direction of active MMP-2. Active MMP-2 present in carotid plaque extracts acts as a platelet chemotactic stimulus and induces platelet recruitment in plaque tissue.
- Dr Randall G. Worth (USA), who showed that platelet-mediated bacterial killing is a well characterized function of agonist-stimulated platelets releasing microbicidal peptides. When studying these types of functions, several things should be considered: 1) the nature of bacteria-platelet interaction, 2) the relative strength of agonist, 3) contaminating leukocytes, 4) presence
of plasma proteins or proteins released from platelets. Controlling for these conditions is essential for interpreting the nature of platelet-mediated bacterial killing.

- Dr Simon C. Pitchford (UK), who showed that platelets are a requisite part of the innate immune response during infection. This role requires platelets to function in atypical ways, for example: to possess the ability to migrate, and recognize pathogens for efficient killing, which may not require classical aggregation responses. He described methods he developed for advanced quantitative immunohistochemistry, radioactive tracers, and fluorescent platelet multiphoton microscopy to uncover these behaviours of platelets in response to bacterial infection in vivo.

The second part of the session, from 10:30 to 12:30, moderated by Marina Camera (Italy) and Matthew Rondina (USA), was opened by Andrew L. Frelinger III (United States) who, also on behalf of Jose Rivera Pozo, presented the results of the project “Standardization of flow cytometry for the assessment of platelet disorders”. Flow cytometry is increasingly used particularly in the study of platelet function in inherited platelet disorders (IPDs). However, wide variation exists in specific reagents, methods, and equipment used for flow cytometric analysis of platelet function making interpretation and comparison of results difficult. Therefore, the goal of the project was to provide guidelines for the use of the flow cytometric technique for the evaluation of platelet function in patients with possible inherited or acquired disorders of platelet number and function. Results of a previous RAND survey of experts were reported at the 2017 SSC meeting. A follow-up survey was conducted and final results were presented. A few areas of disagreement remained regarding the use of heparin and EDTA as anticoagulant for testing platelet surface levels of GPIb-IX, GPIb/IX/V, GPIaIIa, and GPVI. A lack of expertise on flow cytometric tests for ristocetin-dependent VWF binding suggests additional studies are needed.

This speech was followed by two other talks on some innovative methodological developments for the use of flow cytometry in the study of platelets.

Thomas A. Blair (USA) showed that mass cytometry uses mass spectrometry to simultaneously detect multiple metal-conjugated antibodies on single-cells, thereby enabling a significantly greater number of cellular parameters to be simultaneously analyzed compared to fluorescence flow cytometry. Mass cytometry can be used to identify unique platelet subpopulations and novel alterations in surface glycoproteins on platelets from inherited platelet disorder patients.

Khaleed M. Naseem (UK) presented how fluorescent barcoding of whole blood allows multiplexed phosphor-flow cytometry to be performed on blood platelets. After the appropriate reagent selection and optimisation, this method enables rapid, large scale signalling profiling and drug screening in whole blood.

Paul Harrison (UK) presented the results of a new ICSH standardization study on mean platelet volume (MPV) measurement. The study investigated the potential of some commercial platelet standards (a fixed porcine platelet preparation with or without added fixed human RBC) prepared by Biotechne. These were distributed to a mixture of commercial and academic/pathology laboratories around the world (18 laboratories in total – 7 different manufacturers and 18 different instruments). Samples were measured 5 times upon receipt, 14 days and 28 days after storage at 4 degrees C after warming and mixing to a standard protocol. The results demonstrated that the MPV values were reasonably stable within each instrument over 28 days and that the presence or absence of RBC affected the MPV and platelet count measurements. There were differences between MPV values between instruments based upon differences in the measurement principle (impedance, optical or imaging), gating of the platelet distributions and the calculation of MPV values. The study shows the potential of a standard for calibrating MPV although there is no reference method for MPV measurement available at present to assign
values. Future studies are planned and may involve using standards with different MPV values (from low to high) to see if the differences observed between methods are consistent. Harmonization of results between current commercial analysers may not be possible as it is unlikely that a manufacturer will change their methods of gating and deriving the MPV. However, it may be possible to access raw data files and determine if harmonization has potential in the future. The design of the next phase of the study will be discussed at the next ICSH meeting in October and will hopefully be undertaken in the next year.

Marie Lordkipanidzé (Canada) presented an exhaustive review of the literature on the conservation and shipment of platelets for platelet function studies. She reported the plan to launch an online survey on international practices in terms of conservation and shipment in winter 2019, through the ISTH website, and to follow an expert grading of recommendations in spring and summer 2019.

Marie Christine Alessi (France) presented a project aiming to evaluate, in a multicentric way, the performances of commercially available agonists of platelet aggregation. She gave details on the type of reagents, case series to be studied (healthy controls, inherited platelet function disorders and cardiologic patients under antiplatelet treatments) and characteristics required for the participating laboratories.

Andreas Greinacher (Germany) showed that the platelet phenotype can be characterized by immunofluorescence using standard blood smears and that this can be used for enabling the diagnosis of several hereditary platelet disorders on shipped blood smears: Bernard Soulier syndrome, Glanzmann thrombasthenia, Wiskott-Aldrich syndrome and X-linked thrombocytopenia, GFI-1B thrombocytopenia, Gray platelet syndrome, disorders with lacking dense granules, ETV6 associated thrombocytopenia, the MYH9 disorders, beta-1 tubulin defects, alpha-tubulin defects, Filamin thrombocytopenia, HDAC6 deficiency. Two major challenges have to be solved: 1) validate whether the immunofluorescence method allows the diagnosis robustly in patients with these disorders, 2) standardization of the antibodies used and the staining method used. This requires collaboration between centres who care for patients in whom one of these disorders has been confirmed genetically and centres working on fluorescence microscopy.

Attendance to the session was excellent, ranging from approximately 200 to possibly more than 400 attendees in the different phases of the SSC session. Discussion from the audience was active and lively. The room session was good, the organization and facilities were excellent, apart from the position of the screen for the moderators which was in part not visible. Technical assistance in the room was reasonably good. The timing of the session was respected, except for a small delay of the first part, with the session ending around 10 minutes beyond schedule, but not interfering with other activities because in the coffee break interval.
Predictive and Diagnostic Variables in Thrombotic

19 July 2018
8:00 – 12:30

Chairman: John-Bjarne Hansen
Co-Chairs: Cecilia Becattini, Suzanne Cannegieter, Geert-Jan Geersing, Paul Kyrle, Gregoire Le Gal, Marc Righini

The program was divided into four sessions. The first session was devoted to updates of SSC projects. After a short introduction by the chair, Professor Hansen, the best abstract related to our Subcommittee was presented by Dr. Parpia from McMaster in Canada. He presented an individualized prediction model for risk of first DVT using clinical assessment and D-dimer.

Thereafter followed updates on ongoing SSC projects.

First, Dr. Kraaijpoel presented on standardized definition for the diagnosis of Death from VTE in clinical studies. The definitions of pulmonary embolism (PE)-related death used in clinical studies vary widely and the comparability between studies is therefore suboptimal. During her presentation, the audience was asked to participate in a survey to define pulmonary-related death where the final results will be proposed as guidance statement in the following year.

Second, Dr. Klok talked about the subsegmental PE project. The project aims to derive a widely accepted radiological definition of subsegmental PE which can be used in current and future studies as well as in clinical practice to determine optimal treatment for this patient category.

The second session was on ongoing projects related to our SSC. The third session was on diagnosis of VTE. In the first talk, Dr. Geersing talked about why we miss PE so often. He focused on the pre-diagnosis phase where he addressed how we get a suspicion in the appropriate patient and why we miss it so often. Finally, he focused on the influence of the prevalence on diagnosing PE.

Thereafter Dr. Righini presented modern recommendations for diagnosis of PE. His talk was followed by Dr. de Wit who focused on why emergency physicians often are not compliant with algorithms for diagnosis of PE. Her explanation was that they were too time consuming, complex and modified to many times for widespread adoption by emergency physicians. She argued that emergency physicians should be involved in future diagnostic studies.

The fourth session was devoted to biomarkers and risk prediction of VTE. First, Dr. Selby presented on the significant number of clinical and laboratory challenges in implementing D-dimer in the real world of outpatient VTE diagnosis, including inherent variability of D-dimer assays, understanding and implementing conventional and adjusted cut-off values, and the significant knowledge to action gap that still exists in using D-dimer appropriately with validated clinical probability assessment tools.

Second, Dr. Cannegieter presented data on internal and external validation of a prediction model, including both clinical risk factors and biomarkers, for recurrence in all patients with a first VTE. In the third and last presentation, Dr. Eichinger presented data on internal and external validation of a risk prediction model for patients with a first unprovoked VTE. She
argued that it is most relevant to predict the recurrence risk in this subgroup of patients with first VTE. She is also running a clinical study to test the prediction model.

The meeting room was packed throughout the entire 4-hour program and each presentation was accompanied by constructive discussion.
Chair: Chris Gardiner

Co-Chairs: Alexander Brill, Emma Josefsson, Juan Melero-Martin, Rienk Nieuwland, David Smadja and Johannes Thaler.

Extracellular vesicle session. Moderators Chris Gardiner & Rienk Nieuwland

1. Rienk Nieuwland, the Netherlands - Update on ISTH/ISEV/ISAC Working Party on Flow-Cytometry of Extracellular Vesicles

Detection of single extracellular vesicles (EV) by flow cytometry has been a longstanding interest of the SSC on Vascular Biology. Although we have been aware already a long time that we were detecting only “the tip of the iceberg”, that is the largest EV (“microparticles”) due to relative insensitive instruments, it was not until 2014 that the size distribution of EV in normal human body fluids was published. This size distribution is a “power-law function”, meaning that there are many small (<200 nm) and relatively few large EV. Since then, progress has been made in single EV detection by flow cytometry, and for example in our laboratory we measure over 1,000-fold higher concentrations of platelet EV in normal human plasma compared to about 15 years ago. In 2015, three international societies (ISTH, ISEV and ISAC) realized that they were working independently from each other but with a same goal: trying to improve detection of EV by flow cytometry, and trying to standardize identification and concentration measurements of EV. In 2017, several comparison studies have been performed, including a fluorescence calibration study organized by John Nolan, and a standardization of EV measurements through vesicle diameter approximation. This last study showed that many flow cytometers are still sufficiently sensitivity to detect EV. At present, the working group is working on “minimum information for EV flow cytometry”, which can be seen in the current trend to publish minimal requirements trying to improve the reliability and reproducibility of results from EV measurements.

2. Johannes Thaler, Austria - Mechanisms of VTE in Cancer

VTE is a multifactorial disease and patient, tumor and treatment related factors determine the overall risk of developing VTE in cancer patients. Moreover, during the course of disease the risk of VTE varies in cancer patients and is particularly high during hospitalization when cancer treatment is initiated, decreases when remission is achieved and rises again if recurrence of malignancy occurs. It was demonstrated that laboratory parameters that globally reflect coagulation activation and fibrinolysis (e.g. peak thrombin generation and D-dimer) are strong predictors of occurrence of VTE in cancer patients. However, there also cancer type specific parameters for VTE prediction, that point to distinct pathways of VTE in specific cancer types. Evidence indicates for example that tissue factor (TF)-exposing extracellular vesicles (EV) play a role primarily in the prothrombotic state of pancreatic cancer patients. Podoplanin expression, which is a potent platelet activator, predicts VTE in malignant primary brain tumors, which are the most prothrombotic of malignancies. For neutrophil extracellular traps (NETs) an important
role was found in lung cancer in experimental and clinical studies. Polyphosphates, which initiate contact activation, seem to play an important role in prostate cancer. Johannes concluded that certain parameters that generally reflect coagulation activation or fibrinolysis, like D-dimer, are likely to be useful tools for risk prediction of VTE in patients with different malignancies and are therefore of interest for the clinical use e.g. as part of risk prediction models. More experimental parameters like TF-exposing EV, NETs, podoplanin and polyphosphates are of interest because they point to cancer-type specific pathways of VTE.

3. Rienk Nieuwland. Prediction of Venous Thromboembolism in Cancer Patients Using a Extracellular Vesicle-Based Clotting Assay

Universal thromboprophylaxis of cancer patients is not recommended because the number of patients to treat does not outweigh the risk of bleeding. Thus, there is a need to identify cancer patients at risk of VTE in whom thromboprophylaxis is justified. To identify such patients, clinical risk prediction scores are being used. Recently, we compared four scores in a prospective multicentre cohort study (Haematologica 2017; 102: 1494-01). In total, 876 cancer patients were included, of which 53 patients developed VTE (6.1%). The main conclusion is that “the present findings do not support the use of any of the examined scores to select patients for thromboprophylaxis.”

There is increasing awareness that the mechanisms underlying the development of VTE differ between cancer types. For example, in pancreatic cancer there is increasing evidence that tumour-derived extracellular EV exposing coagulant tissue factor (TF) are involved, whereas in lung cancer neutrophilia and neutrophil-extracellular traps (NETs) formation are involved (Blood 2017; 130: 1499-1506). Therefore, in the before mentioned multicentre study, we measured the TF-EV coagulant activity in fresh patient plasma to identify patients at risk of developing VTE. The assay was performed in 648 patients, of which 40 (6.2%) developed VTE. Overall, the performance of the assay was comparable to risk prediction scores. In pancreatic cancer patients (n=100), however, the clotting assay had a specific hazard ratio (SHR) of 4.18 (P=0.05), which out-performed Khorana (SHR 0.42, P=0.42) and Vienna scores (SHR 1.71; P=0.44). The assay showed a certain specificity, because the assay was unable to predict VTE in lung cancer patients (n=91; SHR 0.92, P=0.92). Taken together, these results strongly suggest that measuring the TF-EV coagulant activity may be useful biomarker to predict VTE in pancreatic cancer patients (Thromb Res 2018; 166: 54-59).

4. Nigel Mackman, United States Overview of Procoagulant Extracellular Vesicle Assays

Dr Mackman gave an overview of procoagulant EV assays, outlining the factors which influence these assays and common problems. When measuring EV-associated tissue factor (TF) it is imperative that the appropriate control are used; specifically TF inhibition. There are many commercially available anti-TF antibodies available but most do not adequately block TF activity. Some laboratories use corn trypsin inhibitor to block contact activation but this is very expensive and is not necessary if the appropriate controls are used. Preanalytical variables are of key importance, particularly the time taken between sampling and separation of plasma. He reiterated that detection of TF antigen by flow cytometry does not reflect TF activity and is of little or no use in clinical studies. Current methodologies for measuring TF activity are hampered by the lack of an appropriate EV-TF standard.
5. Rienk Nieuwland Nigel Mackman, Ali Amirkhosravi, United States, Françoise Dignat-George, France, Johannes Thaler, Barry Woodhams, United Kingdom, Francois Mullier, Belgium. Round Table Discussion on Standardisation of Procoagulant Extracellular Vesicle Assays

As a first step towards standardization of assays measuring the procoagulant activity of EV, a questionnaire was sent to an expert panel. The panel discussed the responses and an SSC workshop initiative proposed by François Dignat-George.

The questionnaire covered the following topics: The relevance of pre-analytical variables; different assays used to measure the procoagulant activity of EV; specificity of assays for tissue factor and phosphatidylserine; the problem of contact activation; the need for a tissue factor standard; whether to combine assays to measure tissue factor activity and antigen; The need for standard operating procedures and multi-centre studies. Françoise Dignat-George proposed a two-year workshop to compare the sensitivity and specificity of assays to measure the presence of coagulant TF-exposing EV in human plasma and this was agreed by the panel.

The outcome of the panel discussion and consensus reached will be published in the near future.

Neutrophil Extracellular Traps session. Moderators: Alex Brill United Kingdom, Emma Josefsson Australia

1. Alexander Brill. Proposed Guidelines for Studying NETs

Dr Brill briefly introduced NETs and presented topics covered in draft document which was circulated to the panel members prior to the SSC and formed the basis for discussions.

2. Alexander Brill, Simon De Meyer, Belgium; Paul Harrison, United Kingdom; Emma Lefrançais, France; Cheng-Hock Toh, United Kingdom; Grace Thomas, France. Round Table Discussion on Standardisation of Study of NETs

The panel discussed the quantification of circulating biomarkers of NET formation. It was unanimously agree that cell-free DNA (cfDNA) alone is not a specific biomarker for NETs (since also other sources than NETs: bacteria, host dying cells). It may be useful if combined with visualization of the structure of DNA or with myeloid peroxidase (MPO). It was however concluded to be useful on its own in order to assess burden of disease.

Citrullunated histone H3 is regarded a specific marker of NET formation. AA Arg is replaced with citrulline. The panel concluded that this is a good marker in tissues but very difficult to use in fluids. The panel considered demonstration of nucleosomes, myeloperoxidase (MPO) or neutrophil elastase (NE) to be non-specific as standalone tests specific when complexed with DNA or histones.
The preparation of blood for measurement of NETs was discussed at length. There are differences when using plasma or serum and the choice of anticoagulant and centrifugation speed could also influence result.

Microscopy and imaging of NETs remain the gold standard but this is not always possible but a combination of biomarkers can be used in combination.

Circulating Endothelial Progenitor Cells session. Moderators: David Smadja France, Juan Melero-Martin United States


Dr. Medina described preclinical data about potential use of endothelial colony-forming cells (ECFCs) as a cell therapy targeting the ischemic retina, including dose response, delivery route, and toxicity. The findings presented provide supportive preclinical evidence for the development of ECFCs as an efficacious cell product for ischemic retinopathies. Discussions were mainly about ECFC mechanism of action in this indication – i.e., paracrine effect with microvesicles secretion or growth factor synthesis from ECFC and the potential for future injections of ECFCs in retina with perivascular cells. Involvement of ECFCs in cancer was also discussed. Finally, issues regarding ECFC immunogenicity and limitations of the model used in Dr. Medina’s results were discussed

2. Gian Paolo Fadini, Italy. Endothelial Progenitor Cells as Biomarkers

Circulating progenitor cells (CPCs), including endothelial progenitor cells (EPCs) are biologically related to many aspects of cardiovascular disease, as they promote angiogenesis and vascular repair. Dr. Fadini presented results from his group on cardiovascular disease and recent data on diabetes. Over years, the most predictive phenotype was CD34 (+) CD133 (+) cells and not the putative KDR (+) EPCs. Dr. Fadini emphasized the existing heterogeneity among studies according to the CPC/EPC phenotype. His main conclusion was that hematopoietic cells could be more predictive than putative EPC as a significant biomarker of cardiovascular events and death in patients, and also correlated with microalbuminuria and retinopathy in diabetic patients. Discussion was about EPC and drug induced diseases, in particular in vascular diseases. Moreover, ontogeny of EPCs and their involvement as liquid biopsy was also discussed, with the conclusion that a link between EPC origin and function was currently not possible.


Dr Guerin's talk was about EPCs and vasculogenic stem cells in bone marrow, cord blood, and in mobilized and non-mobilized peripheral blood. The aim of the study presented was to evaluate the distribution and to thoroughly phenotype immune cells and circulating vasculogenic stem cells by mass cytometry. Dr. Guerin introduced this technology and reported a robust protocol that could enable a new definition of vasculogenic stem and progenitor cells for human clinical studies and models of vascular disease and tumor angiogenesis. Discussions also included the issue of KDR expression in vasculogenic stem cells and its potential intracellular expression.
4. Juan Melero-Martin, David Smadja. Update on Standardization of CEPCs

Dr. Smadja discussed the current effort by this SCC subcommittee with regards to a position paper on ECFCs. This effort originated from the SSC results and discussion gathered during a round table of experts that took place in our previous 2017 ISTH SSC meeting in Berlin. This position paper is in its final stage and will be shortly submitted to the *Journal of Thrombosis and Haemostasis*. In addition, Dr. Smadja discussed plans for a similar round table/workshop covering flow cytometry, EPCs, and ECFCs that could take place in our next ISTH SSC meeting in Melbourne.
Von Willebrand Factor

18 July 2018
10:30 – 12:30; 15:00 – 17:00

19 July 2018
8:00 – 12:30

Chairman: Sandra Haberichter


Top Abstract presentation: Patients with Aortic Stenosis Have von Willebrand Factor Abnormalities and Aberrant Angiogenesis in BOEC
Soundarya Nivedita Selvam (Canada)

Dr. Selvam presented data on patients with aortic stenosis (AS) who may bleed due to acquired von Willebrand syndrome and experience gastrointestinal bleeding from angiodysplasia. Twenty-eight patients with severe AS undergoing valve replacement and 10 healthy controls were enrolled. Blood was obtained from patients prior to, 3-5 days, and 6 months after surgery. VWF and FVIII levels were elevated in patients with AS compared to controls and significantly increased immediately after surgery. This was associated with significantly higher VWF:GPIbM and collagen binding after surgery. VWF multimer analysis indicated a loss of high molecular weight multimers prior to surgery. Normal multimer profiles were restored shortly after surgery. BOECs isolated from five patients prior to surgery had no difference in VWF and Ang-2 storage or release compared to controls. Forty-eight hours after seeding, AS BOECs were almost twice as proliferative as controls, however, tubule formation in Matrigel did not vary after eight hours. Quantitative and qualitative VWF abnormalities associated with increased BOEC proliferation are observed in patients with AS.

VWF Clearance and Clinical Implications
David Lillicrap (Canada)

Dr. Lillicrap reported on the basic understanding of how and where VWF is cleared, which has advanced significantly in the past few years with many remaining questions. A range of methodologic strategies have been used to demonstrate that at least eight different lectin and scavenger endocytic receptors expressed on macrophages, hepatocytes and sinusoidal endothelial cells contribute to VWF clearance. The role of these different receptors in physiologic and pathophysiologic VWF clearance requires further study. The enhanced knowledge relating to VWF clearance has also impacted the diagnosis and clinical care of VWD. 15-20% of type 1 VWD cases show accelerated VWF clearance, and enhanced clearance is also seen in types 2A and 2B VWD. This information highlights that the use of desmopressin to treat these VWD subtypes must always be preceded by a therapeutic trial that includes sampling out to 4 hours post-desmopressin administration.

ADAMTS13 Levels in WiN Cohort
Johan Boender (Netherlands)

Dr. Boender presented his data on ADAMTS13 levels in patients enrolled in the Willebrand in the Netherlands (WiN) study. ADAMTS13 levels were shown to have no correlation with VWF:Ag or VWF activity. However, the data showed a negative association with VWF
propeptide (VWFpp) levels. In addition, ADAMTS13 levels were shown to decrease with age at a rate of approximately 2% per decade.

**Standardization and challenges of ADAMTS13 activity and antibody testing in the Asia Pacific Region**

**Ross Baker (Australia)**

Dr. Baker reported on ADAMTS13 testing as the cornerstone in the diagnosis and treatment of patients with life threatening thrombotic microangiopathy and thrombocytopenia. A large number of ADAMTS13 activities, antigen and antibody tests are available using different methodologies and platforms. The result of an ADAMTS13 activity and antibody test will define a diagnosis of TTP (<10% activity) or aHUS (>10% activity), whether the TTP is immune mediated (iTTP) or congenital (cTTP), guide therapeutic decisions. He described the first Asia Pacific Microangiopathic Thrombocytopenia Network (APMAT) standardisation of ADAMTS13 activity and antibody testing with 24 centres from 10 AP countries to address the unmet need of accurate diagnosis of microangiopathic thrombocytopenia in a large AP population cohort of 3.2 billion people. Lyophilised samples were sent in 2 surveys using the same platform (chromogenic activity and ELISA antibody assays) and compared with the same samples used in the ECAT QA program. Overall the performance and CV’s were similar to the ECAT QA program and the implementation between laboratories were acceptable with appropriate z-scores for inter- and intra-laboratory measurement. However precision and clinical interpretation of the ADAMTS13 activity assay around the 10% cut off level is still problematic and will lead to variation in clinical decisions and management. Interpreting anti-ADAMTS13 antibody assay is even more difficult because the definition between normal and abnormal titres is unclear leading to misdiagnoses of a pathological antibody affecting ADAMTS13 function. Further work is required to standardise ADAMTS13 testing for the different clinical situations of the new TMA diagnosis.

**The Interactive Registry of Acquired von Willebrand Syndrome (INTREAVWS)**

**Augusto Federici (Italy)**

Dr. Federici presented an updated version of the Interactive Registry on Acquired von Willebrand Syndrome (INTREAVWS) that will be proposed on behalf of the WG on AVWS. INTREAVWS will be planned in three different steps similarly to what done for the 3WINTERS-IPS. Retrospective information (First step=2 years) 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 (Third step) for the response to different therapeutic approaches and progression of the disease.

**Update on 3Winters-Ips Project**

**Augusto Federici (Italy)**

Dr. Federici presented data on a total of 202 VWD3 patients that were observed in the prospective phase of the study. Results of the centralized genotypic and phenotypic analyses of this cohort were presented. The number of bleeding episodes treated with VWF Concentrates was described together with the number of VWD3 receiving short-term prophylaxis for surgery and secondary long term prophylaxis for recurrent bleeds. Since the frequency of bleeds was lower than expected, an extended prospective observation (from 2 to 5 years) will be required.

**Removing the Diagnosis of VWD from patients**

**Giancarlo Castaman (Italy)**

Dr. Castaman reported that the diagnosis of VWD should always rely on the demonstration of the presence of a bleeding history, a deficiency of VWF and inheritance. While this requirement
is easily fulfilled in families with clear autosomal dominant inheritance and severely reduced FVIII/VWF measurements, the picture could be rather complicated in presence of borderline levels and dubious bleeding symptoms. The new proposed category “Low VWF” should be carefully used when evaluating a subject without the possibility of family investigations and the collection of a standardized bleeding history. When this occurs, VWD could be again considered in the presence of a demonstrated family trait. For several other subjects, antifibrinolytics and judicious use of desmopressin can safely prevent significant bleeding symptoms when a positive bleeding history is documented.

Clinical Challenges in Low VWF diagnosis
Michelle Lavin (Ireland)
Dr. Lavin reported on the diagnosis of low VWF where assessment of both plasma VWF levels and bleeding phenotype are critical. The threshold for low VWF remains unclear but no significant difference in bleeding scores has been observed between those patients with baseline plasma VWF levels in the 30-39 IU/dL or 40-50 IU/dL range. Physiological increases in plasma VWF levels in pregnancy and with aging may complicate evaluation of laboratory parameters in these clinical situations. In assessment of bleeding phenotype Bleeding Assessment Tools (BATs) provide a standardized method to quantify patient reported symptoms. BATs differ in their sensitivity, with the Condensed MCMDM-1 VWD score reliant on physician consultation rather than symptoms to accrue scores in the menorrhagia domain. Mild reductions in plasma VWF levels are common, affecting up to 1% of the population. However, bleeding associated with these low VWF levels is found in only 0.1% - 0.01% of the population. Ultimately, the discrepancy between prevalence of reduced plasma VWF levels and number of patients registered suggests that additional phenotypic modifiers contribute, with low VWF levels acting as a risk for bleeding.

Low VWF - Meta Analysis
Alberto Tosetto (Italy)
Dr. Alberto Tosetto presented data about an individual-patient meta-analysis aimed at evaluating the bleeding phenotype in patients having type 1 VWD (defined as VWF:Ag below 30 IU/dL) or "Low VWF" (defined as VWF:Ag 30-50 IU/dL). 1411 patients enrolled in the EU MCMDM-1 VWD, the Canadian Type 1 VWD, and the Zimmerman Program for the Molecular and Clinical Biology of VWD studies were analysed, 449 and 962 having a VWD and "Low VWF" phenotypes, respectively. The main findings were: “Low VWF” patients are more frequently blood group O, older females; have lower average bleeding scores and number of symptoms; are possibly selected because of menorrhagia. A group of “severe bleeders” was also identified (n=270, 19%), having a similar distribution of “low-vWF” and “VWD” phenotypes.

Alloantibody Formation in type 3 subjects in Zimmerman Program
Pamela Christopherson (USA)
Dr. Christopherson presented data from the Zimmerman Program Type 3 VWD Study that sought to determine the presence and impact of alloantibodies by developing a sensitive and specific ELISA to detect IgG and IgM anti-VWF antibodies in the type 3 VWD cohort using directly plated rVWF. To detect and characterize these alloantibodies, they recommend performing a screening assay and then determine the titer, evaluate the inhibition of function by competition assays (VWF:GPIbM, VWF:CB3, VWF:CB4, VWF:F8B) and to follow and retest all patients with a positive alloantibody. In this type 3 cohort they found 7 subjects who were positive for IgG and 1 with IgM alloantibody. 2 subjects (4.4%) showed signs of presence of an inhibitory antibody to collagen 3 and FVIII. 1 subject had a high titer non-inhibitory antibody that rendered treatment ineffective. None of the subjects with positive alloantibody had large deletions, therefore type 3 VWD, regardless of genetic cause, appears to carry a risk of alloantibody formation.
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Diagnostic Algorithm: a look to Latin America
Analia Sanchez Luceros (Argentina)
Dr. Sanchez-Luceros presented a look at the countries in Latin America with the largest population and the current situation of VWD in Latin America. Many of the efforts in LA are related to the most severe forms of VWD, with limited access to complex laboratory diagnosis and few facilities available for diagnosis in cities in every country. The diagnosis of VWD in these areas is substantially less frequent than expected compared to current literature on VWD diagnosis. This data indicates that many subjects with potentially Low VWF or type 1 von Willebrand disease remain undiagnosed. Dr. Sanchez-Luceros also presented on the Latin America von Willebrand disease registry that has been initiated.

Diagnostic Algorithm – BloodCenter of Wisconsin, Sandra Haberichter (USA)
Dr. Haberichter reported on the diagnostic algorithm used at BloodCenter of Wisconsin to diagnose and subtype von Willebrand disease. Initial testing includes measurement of VWF:Ag, VWF:GPIbM, VWF:CB3 and FVIII activity. Further testing is dependent upon the results of these four initial assays and is driven by the Lab Information System. The physician receives a customized interpretation of the data. The benefits to the automated algorithm are reduced time from sample delivery to result receipt, increased laboratory efficiency, elimination of unnecessary testing, and reduced cost to the patient.

Calibration of ISTH Lot#5 and Standardization of VWF:GPIbM, Anthony Hubbard (UK)
Dr. Hubbard reported on the increased adoption of VWF:GPIbR and VWF:GPIbM methods as alternatives to the ristocetin cofactor method which presents an urgent need for standardisation in order to avoid locally-derived units and the risk of divergent test results between laboratories. Assigning unitage to the WHO 6th IS FVIII/VWF Plasma (07/316) for the VWF:GPIbR and VWF:GPIbM methods will provide traceability for working standards and promote harmonisation of test results. The proposal to adopt the VWF:RCo value on the WHO 6th IS for both methods was supported in a survey of the VWF sub-committee carried out in 2017 and also by a panel of 13 SSC experts and chairs. It is proposed that values of 0.87 units per ampoule for VWF:GPIbR and VWF:GPIbM are added to the assigned values on the WHO 6th IS. This proposal will be submitted to WHO for acceptance in October 2018. Calibration of the new version of the SSC/ISTH Secondary Coagulation Standard Plasma (Lot#5) has been completed for VWF:antigen, VWF:collagen binding and VWF propeptide. The calibration study for VWF:RCo indicated that many laboratories have already adopted the VWF:GPIbR and VWF:GPIbM methods and assigned values for these new methods will be included when more laboratories have submitted results.

Variability in VWF:RCo results: data from the UK NEQAS (Blood Coagulation) proficiency testing programme, Ian Jennings (UK)
Dr. Jennings presented data on several different methodologies employed by centres performing VWF activity assays in the UK NEQAS (Blood Coagulation) proficiency testing programme. In 2012, the SSC plasma standard (lot #4) was distributed to >200 centres for VWF assay, and differences were observed between aggregometry-based and immunoturbidimetric assay-based methods. A further distribution of the same material in 2017 revealed very similar findings. Discrepant results are also present between the different immunoturbidimetric assays when testing samples from type 1 and type 2 VWD donors, although the pattern of results is inconsistent. Standardisation though value assignment for immunoturbidimetric assays of the WHO International standard for VWF, and of the SSC plasma standard lot #5 is recommended, along with genetic data to elucidate the discrepant results observed in UK NEQAS (Blood Coagulation) exercises.
VWF:GPIbM assay assessment of DDAVP Response, Kenneth Friedman (USA)

Dr. Friedman presented data on the VWF ristocetin cofactor assay, which is the traditional assay used to measure the platelet-binding activity of VWF. This assay is used in the diagnosis of von Willebrand disease, to label the potency of VWF concentrates and to assess clinical responses to therapy. In the past few years, several additional clinical assays have become available to assess the platelet-binding activity of VWF for the diagnosis of VWD, but these assays have not been validated for assessment of response to therapy. Dr. Friedman presented pilot data showing that the VWF:GPIbM assay performed similarly to the VWF:RCo assay in assessing the therapeutic response of VWD patients to DDAVP.

Von Willebrand Disease and Pregnancy: A Review of Evidence
Paula James (Canada)

Dr. James presented data on von Willebrand disease, von Willebrand factor, and pregnancy. Pregnancy is a time of profound physiologic changes in hemostatic factors including VWF and FVIII. VWF and FVIII levels increase over the course of pregnancy, although not to the same degree in women with VWD compared with controls. Antepartum and postpartum bleeding remain important risks. Strategies for managing pregnancy, labour and delivery and the postpartum period were reviewed.

Antifibrinolytic therapy for preventing VWD-related postpartum hemorrhage: indications and limitations
Peter Kouides (USA)

Dr Kouides reported on fibrinolysis as a key component of the pathogenesis of postpartum hemorrhage. Yet, it’s only been in the last year that the use of antifibrinolytic therapy, tranexamic acid, clearly reduces the mortality for postpartum hemorrhage in general. This can probably be extrapolated to VWD patients with PPH but what’s not clear is if in the general population and in patients with VWD there’s clearly a beneficial role for prophylactic treatment. Intuitively this would appear to be the case in severe VWD but more substantiating data is needed as well as data for its use prophylactically postpartum in cases of type 1 VWD with a historically increased bleeding score but correction of the VWF levels in the third trimester. Dr. Kouides reviewed the above issues and proposed a survey with a registry of tranexamic acid use in Type 1 VWD with the intent to ultimately carry out a prospective controlled trial.
Chairman: Maha Othman

Co-Chairs: Rezan Abdul-Kadir, Patricia Casais, Emmanuel Favaloro, Ian Greer, Susan Halimeh, Predrag Miljic

Dr. Maha Othman; the SSC chair (began chairmanship July 2017) gave an introduction on the subcommittee’s activities and highlighted the ongoing projects. She first introduced the committee co-chairs: Rezan Kadir (UK), Susan Halimeh (Germany), Predrag Miljic (Serbia), Ian Greer (UK) with the two new members: Patricia Casais (Argentina) and Emmanuel Favaloro (Australia). She then reminded of the ISTH SSC mandate and focus of the women’s SSC. She also invited for active participation in the committee and registering as members. Dr. Othman listed all current ongoing and completed SSC projects. There are currently 10 projects coordinated under the auspice of the women’s SSC; 3 of which are joint projects with other SSCs (the Paediatric, FVIII, DIC SSCs). Six new projects have been introduced in the past year; three of which are registry-based. Three publications (SSC communications) are currently under review or in revisions for JTH. Dr. Othman also announced the success of the Women’s SSC in obtaining ISTH $50k Grant titled: “Gold Variants: Defining a high-quality set of clinically relevant DNA variants with, and for the Thrombosis and Haemostasis Community”. This is in collaboration with 5 other SSC with the Genomics in Thrombosis and Hemostasis being the head SSC on the grant. Dr. Othman finally reviewed the SSC agenda and invited for active participation in the discussion.

Top Abstract: Deeksha Khialani: The Joint Effect between Different Types of Combined Oral Contraceptives and Genetic Risk Factors on Venous hrombosis Risk: Results from the MEGA Case-control Study

Deeksha Khialani presented data from female participants of the MEGA study, a large population based case-control study. She aimed to investigate the joint effect of combined oral contraceptive pills (COCs) and FV Leiden (FVL), Prothrombin 20210 mutation (PT20210) and fibrinogen gamma (FGG) mutation, on venous thrombosis risk; both in general and for the different progestogen types. Results showed the joint effect of FVL and PT20210 mutation with progestogen type OCP demonstrate the highest risk of VTE when combined with cyproterone, Gestodene and desogestrel; and the risk is lowest with levonorgestrel. The presentation generated interesting discussion around the method of analysis and the types of OCPs used.

Peter Collins-UK: Point of Care Monitoring of Hemostasis During Postpartum Hemorrhage

Professor Peter Collins gave an excellent overview of the concept and the role of point of care monitoring of hemostasis during postpartum hemorrhage (PPH). The presentation included previous studies that showed the association between hypofibrinogenenaemia and severe hemostatic impairment, and that fibrinogen less that 2g/L is a good predictive biomarker for progression of PPH. To overcome the limitations of standard tests of coagulation, point of care viscoelastometric testing has gained popularity in the management of acute bleeding. In the setting of PPH, studies have shown that Fibtem A5 <10 mm performed on the Rotem machine can be available in 10 minutes and roughly equal to fibrinogen <2 g/L. Fibtem>12 mm or
Fibrinogen >2 g/L is adequate for hemostasis during PPH. It was highlighted that hemostatic impairment in PPH is uncommon and accounts for less than 10% of causes. Thus, Formulatic “shock pack” replacement results in unnecessary use of FFP in many women. Prof Collins also reviewed randomized controlled studies that investigated the role fibrinogen replacement during PPH. These studies have shown that pre-emptive treatment with fibrinogen without hemostatic assessment does not improve outcomes. Similarly, improved outcomes have not been shown with infusion of fibrinogen concentrate triggered by Fibtem A5 ≤15 mm. Data suggest that fibrinogen replacement is not required if Fibtem is >12 mm or fibrinogen is >2g/L, but it is unknown whether point of care testing and fibrinogen given at 2 g/L affects outcomes and is cost effective. Further studies in this subgroup of women are required to answer this question.

Rachel Collis-UK: Point of Care Testing and Fibrinogen Therapy during Postpartum Hemorrhage: Clinical Applications

Dr. Rachel Collis then discussed the clinical applications of point of care testing and fibrinogen administration during PPH and how their 10-year programme of research in the area has been translated into a nation quality improvement programme in Wales. The quality improvement programme included structured protocol for management of PPH with risk assessment of all women, gravimetric measurement of blood loss at delivery and a specific escalation plan at 500,1000,1500 ml PPH in addition to Rotem-guided blood product replacement using a simple algorithm. Comparison of their data for the first and the second half 2017 showed improved outcome. The programme proves the feasibility of introducing point of care-guided algorithm for hemostatic management of PPH. However, Prof Collins highlighted that amalgamation of all interventions in the protocol led to the improved care and outcome.

Ekaterina Koltsova-Russia: Classic and global hemostasis testing in pregnancy and during pregnancy complications

Dr Ekaterina Koltsova discussed changes in hemostasis during pregnancy that leads to a procoagulant shift in the hemostatic system. Dr Koltsova presented the challenges of interpreting laboratory assays of hemostasis in pregnancy, indicating that classic routine coagulation/platelet assays are unable to detect early haemostatic changes in obstetric complications such as PET. The use of global assays of hemostasis appears to be promising in several aspects of pregnancy management. There is good data to show that global hemostasis assays have a good sensitivity to hypercoagulation during normal pregnancy, reflect procoagulant shift in preeclampsia, during ovulation induction in IVF and after caesarean section. Global assays can also serve as an alternative for anti-Xa assay, conventionally used for low molecular heparin prophylaxis or therapy. However, additional development and research are needed to implement these results into clinical practice.


Dr Predrag Miljic gave an excellent talk on the challenges of managing women with type 2B VWD in pregnancy with excellent case presentations. Increased binding of VWF with platelets leads to platelet clumping in circulation with thrombocytopenia, which is observed in about 30% of individuals. In pregnancy, thrombocytopenia may emerge, or worsen significantly. Qualitative abnormality of VWF Further impairs platelet function. The risk of bleeding may be significant, not only postpartum but also prenatal period. However, data on the exact risk of bleeding and the management of pregnancy and postpartum in women with 2B VWD is scarce and limited to case reports and small case series. Presenting two cases managed in his center highlighted
these challenges. This followed by a review of cases reported in the literature as well as current management guidelines. Current recommendations suggest a multidisciplinary team approach for management, level of vWF:RCoF maintained at 100 IU/dL at the time of delivery and > 50 IU/dL several days afterwards. Platelet transfusion is recommended if platelet count is less than 50x10^9/L in case of C-section or less than 30x10^9/L in case of vaginal delivery. These recommendations are based on expert opinion and lack strong evidence. Thus, he highlighted the need for multicenter collaborative projects aimed to obtain meaningful data on phenotypic expression and management of 2B during pregnancy.

**Maha Othman: A woman’s life-long bleeding condition and expert opinion**

Dr. Othman moderated a panel discussion on a challenging case of life long unresolved case of heavy menstrual bleeding in a 47 years old who has a strong family history of similar bleeding and a 19 year old daughter who started to show similar condition. No gynecological pathology found and almost all haemostasis tests were negative. The patient underwent endometrial ablation to stop bleeding. All other measures only partially helped. The panel aimed to seek consultation from experts from different areas of the world to capture various practices with respect to diagnosis and management. The patient (from Montreal, Canada) attended the session and gave a few min presentation on her life long bleeding, experience and quality of life. The session began with introduction and objectives followed by presentation of the case, after which each panelist (Prof Paolo Gresele (Italy), Prof Anne Goodeve (UK) and Dr. Analia Sanchez-Luceros (Argentina) provided their views on what to do to support the diagnosis/management. Dr. Gresele’s: This is a primary hemostatic disorder, diagnosis of the cause is important and is possible. There are three major considerations: 1) Some lab tests are missing and need to be completed: eg platelet alpha granule/secretion tests. For some of these disorders there are no known genes. 2) DNA tests on Next-Gen sequencing platforms can/should be done. 3) Therapy is lagging behind; patient should be treated like having a hemostatic disorder. Dr. Sanchez-Luceros: Overall, labs are not quiet complete. Platelet function tests can be furthered so as fibrinolytic testing. Platelet aggregation traces need to be carefully examined. These can provide extra information on platelet function. Euglobulin lysis time and intraplatelet VWF, fibrinogen level to be checked and genetic consultation can be considered. Vitamin C therapy may be considered for treatment. Endometrial ablation is not a common gynecological practice for this purpose in Argentina. In recent years embolization by hemodynamics of local lesions became more acceptable to women, reduce uterus size and is less invasive than endometrial ablation. A prior MRI angiogram to delineate lesions is to be done. Prof. Goodeve: Patient should go further with genetic testing with Next-Gen or the whole genome sequence (NGS or WGS). Attention needs to be paid to the descriptive terminology to define the patient’s and her daughter's bleeding symptoms. It is important to note while NGS, WGS or exome sequencing may be useful, will almost certainly pick up several possible disorders. The patient has a dominant disorder. It could be tricky to pick the correct disorder if there are a number of disorders that result in bleeding. Prof. Goodeve also agreed patient would benefit from a genetic consultation. Several attendees also shared their experiences from their practices in their own country. An action plan is currently being discussed with the patient and her treating physician in Montreal to implement suggestions made at the session in order to reach the diagnosis.

**Erik Klok: Artemis study – diagnostic management study in pregnant women with clinically suspected PE**
Dr. Klok discussed his progress report of an ongoing trial to study safety and efficiency of the YEARS diagnostic algorithm for management of suspected PE in pregnant patients. The YEARS algorithm combines a simplified version of the Wells rule (only 3 variables) and a D-dimer test in all patients, with a pre-test probability dependent D-dimer threshold. The last patients have been now included and final results are expected by the end of this year.

Maria T DeSancho: Hematologic alterations in patients undergoing controlled ovarian stimulation for in vitro fertilization: A prospective observational pilot study

Dr. DeSancho explained that In vitro fertilization (IVF) offers a unique model to study hematologic alterations due to the rapid changes in endogenous estradiol (E₂) levels during controlled ovarian stimulation (COS). The purpose of the study is to prospectively evaluate the hematologic alterations in patients undergoing COS for IVF at a university medical center. Women ages 18 to 40 in their 1st IVF cycle are recruited to participate in the study. Data including demographics and COS and hematologic parameters including complete blood count, global and special coagulation testing, and platelet function assay (PFA) testing are being collected. The hematologic data are collected on day 1 of COS and after completion of COS. Enrollment started in January 2015 and to date, 39 patients were screened and hematologic data is available for 21 patients. We found a statistically significant shortening of the closure time measured by the PFA with both collagen epinephrine and collagen ADP. Additional patients need to be recruited to confirm this finding.

Saskia Middeldorp: Registry of pregnancy in patients exposed to direct oral anticoagulants (DOACs).

Dr. Saskia Middeldorp presented the registry of pregnancy in patients exposed to direct oral anticoagulants (DOACs). This project aimed to register all women who have been pregnant while receiving anticoagulant treatment with a DOAC, and to assess the effects of exposure to DOACs in utero on the fetus and the child on the long-term. This is a multicenter, international, observational cohort study. The registry is designed to collect both retrospective and prospective data. All women with confirmed use of DOACs, and confirmed pregnancy test during DOAC use are to be included. Dr. Middeldorp stated that the registry started in 2015 and currently there are 28 cases reported (only 14 with complete CRFs). Cases are from Austria, Canada, Chili, France, Germany, Israel, Italy, the Netherlands, UK, Poland, Portugal, New Zealand, Spain, and Switzerland. Recruitment has been very slow and difficult and it is hard to predict yet how many cases will be reported to us in the next coming years. There were no questions from the audience.

Amparo Santamaría Ortiz: WiTEAM: International registry on women with placenta-mediated complications and thrombophilia.

Dr. Santamaría explained her new study; The WiTEAM, an observational, prospective, international and non interventional phase IV registry based study. A working group in the Spanish Society of Thrombosis and Haemostasis http://www.witeamproject.org developed the project initially. The project was then submitted and approved by the ISTH’s Subcommittee of Women’s Health Issues in Thrombosis and Haemostasis. The questionnaire was developed and data were entered in the ISTH REDCap database. The registry is now available on ISTH website and at https://redcap.isth.org/surveys/?s=AY8H7WXXJR. The goal of the project and registry is to capture current practices with the management of women with thrombophilia and placenta mediated complications. The talk generated interesting discussion around the hereditary thrombophilia in relation to PMC and previous evidence about the treatment.

Offer Eerez: DIC in pregnancy: Nomenclature, Scoring and a global registry.
Dr. Erez explained the concept of Disseminated intravascular coagulation (DIC) as a complex disease with thrombosis resulting from systemic activation of the coagulation and bleeding due to consumption of coagulation factors and platelets. He discussed the challenge with defining DIC in obstetrics and that it is currently based on the clinical impression of the attending physician, and loosely on some laboratory tests. Moreover, considerable controversy exists between obstetric and hematology specialties regarding these definitions and whether a "true" DIC exists in obstetrics. The debate -not evident in literature- but rather comes across in oral communications, remains to be resolved. Dr. Erez’s previous published work with respect to DIC scoring system in sepsis has provided a foundation to developed similar work in Obstetrics. An international registry for cases of DIC in pregnancy has just been launched on the ISTH RedCap database and is available at https://redcap.isth.org/surveys/?s=KFC8RN8XWC. This registry will collect the demographic, clinical, and laboratory data of all cases and it is aimed to characterize the etiologies for DIC in pregnancy, the main clinical presentations of this syndrome, the pharmacological and surgical treatment that were applied, and their outcome, and finally maternal and neonatal outcome. This information will allow us to better define what is DIC in pregnancy, what are the risk factors for this complication, can diagnostic score help the clinician make a more accurate and timely diagnosis, what are the recommended treatments, and to propose and algorithm that will assess the physician to tailor the proper intervention for DIC in pregnancy based on the above mentioned parameters.

Marc Blondon: Registry For Invasive Treatments For Massive Pulmonary Embolism In Pregnancy And Postpartum Period.

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. Recruitment of 10-15 cases per year, with an achievement of 80 included women within 5-8 years is anticipated. It is believed that the international effort and the use of the ISTH platform will help answer important clinical questions for the care of these patients. There was interest from the audience in participation in the registry. The questionnaire is currently being finalized and will soon be posted on the ISTH website with an invitation to participate.

Deborah Obeng-Tuudah: Obstetrics and gynaecological outcomes of women with platelet function disorders.

Dr. Obeng-Tuudah explained that the presentations and management of women with known platelet function disorders can be varied at times in the obstetrics and gynaecology settings. This is due to the rarity of these conditions and the different types of platelet function disorders culminating into limited clinical experience. Therefore it was suggested to take advantage to study the presentations, complications and management regimes established in the many women with known platelet function disorders, referred to the tertiary haemophilia and thrombosis centre at Royal Free Hospital, London, United Kingdom. The project is set as an ISTH SSC endorsed international data registry so that interested clinicians across the world can
contribute. The international platform will strengthen the project by increasing patient recruitment. Dr. Tuudah presented the goals of the study and methodology and reviewed the questionnaire, which will be used for data collection. The registry will soon be established using the ISTH REDCap database and will be available on the ISTH website with an invitation to participate.

**Robert Sidonio: Title: Hemophilia Carrier Nomenclature: Proposed terminology to improve communication.**

Dr. Sidonio explained that currently, there is no formal terminology to apply to hemophilia A and B carriers regarding whether they have normal (>50% FVIII/FIX) or deficiency in the mild, moderate and severe range creating confusion in academic discussion and conveying data to the consumer population. Furthermore, there are limited guidelines on how to approach and manage those patients. On July 2017 Dr. Sidonio proposed this project to the SSC on FVIII, FIX and rare factor. The goal was 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, payers and community members. In his presentation, Dr. Sidonio discussed the formal literature review he completed in phase one of this project, then he proposed the nomenclature as follows: Asymptomatic hemophilia carrier (Hemophilia A/B carrier with >50% without bleeding), Symptomatic hemophilia carrier (Hemophilia A/B carrier with >50% with bleeding), Mild hemophilia carrier (Hemophilia A/B carrier with >5-50%), Moderate hemophilia carrier (Hemophilia A/B carrier with 1-5%), severe hemophilia carrier (Hemophilia A/B carrier with <1%). This nomenclature generated discussion with some debate. The proposed terminology will be presented/discussed at the upcoming NHF meeting and WFH meeting in 2019 with the goal to come to a consensus in the summer of 2019 in preparation for publication of an official stance. Given its intimate relation to the women’s SSC, this is currently a joint project between both SSCs.

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

Dr. Zia explained the rationale of the project. 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 diagnosis and management, reflecting the lack of high-quality evidence demonstrating the superiority of any one approach. The project aims to assess clinicians’ perceptions of the appropriateness of various diagnostic strategies, approach to decision-making, and perceived clinical equipoise in HMB evaluation. The project will use a web-based survey using the RAND/Expert Lens model to capture expert judgment over three to four rounds of survey questions, to 50 HMB and bleeding disorders national and international experts from three different specialties (hematology, OB-GYN and adolescent medicine). The primary outcome will be perceived appropriateness and necessity of HMB evaluation and management strategies in four clinical vignettes. The final deliverable will be a consensus guidance document emanating from this survey, clearly delineating the subset of adolescents for whom it is appropriate and/or necessary to work-up for a bleeding disorder, the type and timing of work-up, and the optimal management approach. Dr. Zia explained the methods she will use for developing appropriateness and necessity criteria set for HMB care in adolescence, milestones of the project and the progress thus far. A working group meeting took place during ISTH to finalize definitions and survey vignettes. Survey start date is September/October, 2018.
130 physicians agreed to be part of the survey. Fifty final respondents will randomly be selected for the final survey. This was initially a project submitted to the ISTH Paediatric SSC. Given the overlapping interest and scope with the Women’s SSC, it is now endorsed as a joint project between both SSCs.

**Erik Klok: The TEAM-VTE study: Risk, predictors, impact and outcome of anticoagulation-associated abnormal menstrual bleeding in patients with VTE**

Dr. Klok explained the rationale of the TEAM-VTE study. There is little evidence on the incidence and impact of abnormal menstrual bleeding in anticoagulated female VTE patients in their fertile age. This subject has been discussed intensively with the introduction of the DOACs, which may increase the risk of abnormal menstrual bleeding compared to vitamin-K antagonists. Published studies to date involve very small case series of limited scientific value or unplanned post-hoc analyses of large phase 3 trials. The TEAM-VTE is a prospective observational international study dedicated to this subject, which should be the starting point of future outcome trials to decrease the current burden of abnormal menstrual bleeding after initiation of anticoagulant therapy.