# Table of Contents

Biorheology ................................................................................................................. Page 2
Control of Anticoagulation .......................................................................................... Page 4
Factor VIII, Factor IX and Rare Coagulation Disorders ........................................... Page 10
Factor XIII and Fibrinogen ......................................................................................... Page 13
Fibrinolysis .................................................................................................................. Page 19
Genomics in Thrombosis and Hemostasis ................................................................. Page 24
Hemostasis and Malignancy ....................................................................................... Page 28
Lupus Anticoagulant/Antiphospholipid Antibodies .................................................... Page 29
Models of Thrombosis and Hemostasis ................................................................... Page 32
Plasma Coagulation Inhibitors .................................................................................. Page 39
Platelet Physiology ..................................................................................................... Page 45
Predictive and Diagnostic Variables in Thrombotic Disease ................................... Page 51
Women’s Health Issues in Thrombosis and Hemostasis ........................................... Page 53
SSC Biorheology session 2021 Virtual meeting:

Talk 1: Valve Thrombosis in Trans-Catheter Aortic Valves: Influence of Patient Anatomy (Lakshmi Prasad Dasi, GeorgiaTech, USA).

Valve thrombosis remains a major concern after transcathether aortic valve replacement (TAVR). While the material exposed to blood flow has some prothrombogenic properties which are difficult to avoid, Lakshmi Prasad Dasi, highlighted how disturbed flows occur around prosthetic aortic valves and their prothrombogenic nature by using notably numerical approaches such as computational flow dynamics (CFD).

Talk 2: Thrombosis on Artificial Materials: Extracorporeal Circuits and Beyond (Anna Waterhouse, University of Sydney, Australia).

Blood-contacting medical device materials are largely chosen based on device function, e.g. flexibility, and location. Currently, biocompatibility of these materials is largely handled through drug treatments. Dr. Waterhouse is focused on investigating surfaces in the context of thrombosis and how to modulate hemocompatibility of these surfaces, while focusing on protein adhesion in extracorporeal circuits consisting of hydrophobic polymers. She is interested in clot structure, looking at hydrophobic, mildly hydrophilic, and hydrophilic (through plasma treatment) surfaces, as quantified through contact angles. Activation of FXII was found on hydrophilic surfaces, but was reduced for hydrophobic surfaces. Clotting time was also reflected by these results based on turbidity measurements with faster clotting on hydrophilic surfaces. Fibrin formation was less dense on hydrophobic surfaces when compared to hydrophilic, except directly at the surface. Correspondingly, lysis time was also faster on hydrophobic surfaces. Results are attributed to less FXIIa activation on hydrophobic surfaces when compared to hydrophilic.

Talk 3: Thrombosis in Mechanical Circulatory Support (Sophie Susen, Lille medical school Lille, France)

Hemostasis is impacted by flow disturbances, shear, and pulsatility. Dr. Susen is focused on thrombosis found in short term mechanical circulatory support devices, specifically ECMO. High shear is found in the pump, surface stasis on connectors in the circuit, and low flow with high blood-material surface area in the oxygenator of these circuits. Thrombosis and bleeding remain as common complications in ECMO. Thrombus accumulation occurs in the device and can lead to systemic complications, such as stroke. Clotting complications are found in 20-30% of patients, despite antithrombotic prophylaxis through unfractionated heparin. This leads to various problems. aPTT, ACT, and Anti Xa are used to monitor heparin usage, with some heterogeneity in usage among centers and among populations – based on age. Direct thrombin inhibitors like bivalirudin may serve as an alternative treatment, which was better than heparin. Targeting FXIIa reduces fibrin and platelet deposition the oxygenator in animal models without increasing bleeding time. However, due to different flow associated with ECMO tubing, pump, and oxygenator, there can be
different process of thrombosis and therefore different/multiple targets may be appropriate. In Dr. Susen’s work, she found a distribution of thrombus with 13 pump thrombi, 15 oxygenator thrombi, and 23 tubing thrombi based on 15 patients. Fibrin and VWF are major components in these thrombi, which were overall highly heterogenous. NETs does not appear important in thrombus formation in ECMO. There are some differences in COVID-19 vs. non-COVID-19 patients. Future studies should consider the thrombotic distribution in ECMO circuits.

Talk 4: Minimizing Thrombus Formation in a Novel Polymeric TAVR Valve and Modeling Thrombus Initiation Across the Scales (Dany Bluestein, Stony Brook University, NY, USA).

Dany Bluestein first reviewed the mechanism by which platelets become activated and initiate coagulation and result in thrombosis. He next described post-procedural complications of tissue or bio-prosthetic valves with notably thrombotic events. He described a novel polymeric valve as an alternative to current devices used in clinic. The valves were designed to achieve better hemodynamics to generate less thrombogenicity. He provided evidence that these valves presented less calcification, so are potentially less thrombogenic with better mechanical stability and a lower potential to activate platelets when compared to TAVR device. Dr. Bluestein also described a “platelet multiscale model” they developed to model thrombosis initiation across the scales. He also reported a flow model used to study platelet adhesion under flow used to calculate platelet kinematics.

Talk 5: Thrombosis Related to Artificial Valves (Cécile Oury, Cardiovascular Sciences Unit at the GIGA Institute, University of Liège, Belgium)

Cécile Oury started by highlighting the medical concern related to valvular heart diseases and the increased need to prosthetic heart valves to treat these patients. Cécile Oury presented the advantages and limitations of mechanical heart valves and bioprostheses with a focus on thrombosis and the risk of bleeding. The group of C Oury aims to develop hemocompatible prosthetic valves using bioactive surface polymer coating technology while maintaining the structural integrity and performance of the valve with the objective to: i) reduce the need of anti-thrombotic therapy and the linked risk of bleeding; ii) provide new anti-vegetation preventive and therapeutic option; ii) increase prosthetic valve durability. The strategy of the lab of Cécile Oury is to develop Mosaic bioprosthesis with encapsulation of antibiotic and antiplatelet drugs and they provided first evidence for the feasibility of the coating and used a device to show that after 200 million cycles the coating and its chemistry was still preserved with still antibacterial and anti-platelet effects. Finally the hemodynamic performance were not impacted by the coating

Q&A: There was a Q&A session for speakers to receive comments and questions.
<table>
<thead>
<tr>
<th>Lecturer</th>
<th>Title</th>
<th>Summary</th>
</tr>
</thead>
</table>
| Omri Cohen    | International Registry on the Diagnosis and Treatment of Inferior Vena Cava Thrombosis | Thrombosis of the inferior vena cava (IVC) is a rare form of deep venous thrombosis (DVT). Optimal treatment strategies and clinical outcomes are unknown.  
Data from the prospective GARFIELD-VTE indicates that patients with IVC thrombosis are younger, and have higher all-cause mortality rates than LEDVT patients, only partly attributable to malignancy.  
The “SIVECT” registry on IVC thrombosis will be a multicenter, international, observational study aimed to assess risk factors, and the effectiveness and safety of current treatment options in patients with IVC thrombosis, and describe the long-term outcomes of patients with IVC thrombosis.  
Patients with an objective diagnosis of IVC thrombosis, either with or without proximal lower extremity DVT will be included. Information should be collected on baseline characteristics, risk factors for thrombosis, symptoms, mode of diagnosis, presence of concomitant lower limb DVT, PE, IVC filter or unusual site thrombosis (splanchnic, gonadal and renal veins), treatment modalities (anticoagulation and/or thrombolysis), choice of anticoagulant, dose and duration of treatment, recanalization assessment (if available), recurrence of VTE during follow up, bleeding according to ISTH criteria, PTS according to Villalta score and mortality during follow up. Patients should be followed up for 24 months from diagnosis. The number of visits is left to the discretion of the treating physician, but information on clinical outcomes at two intermediate time points is requested.  
Delays in recruitment have been encountered due to the COVID-19 pandemic, however an ethics committee submission package is available, and will be sent on request.  
For additional information and involvement please feel free contact Dr. Omri Cohen at omricmd@gmail.com. |
<p>| Nicoletta Riva | International Registry on the Use of the Direct Oral Anticoagulants for the Treatment of Unusual Site Venous Thromboembolism | Dr. Riva presented an update on the International registry on the use of the DOACs for the treatment of unusual site venous thromboembolism (DUST study). It is an international, prospective, observational study enrolling adult patients with objectively diagnosed unusual site venous thromboembolism (VTE, e.g. splanchnic, renal, ovarian, cerebral, retinal vein thrombosis) and treated with any of the DOACs. Aims are to evaluate the rationale for the use of the DOACs for the |</p>
<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Abstract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ravi Sarode</td>
<td>Toward a Universal Anti-Xa Assay</td>
<td>Approximately, 2-3.5% of patients on DOAC (Factor Xa Inhibitors) present with major bleeds. Andexanet and prothrombin complex concentrate (PCC) are often used to manage these bleeds. DOACs have a much shorter half-lives (~12 hrs) than VKA, therefore, not all patients (~30%) present with a very high DOAC levels. Andexanet inhibits TFPI, thus, there is a potential prothrombotic effect when given in the absence/low levels of DOAC. ANNEXA-4 study included patients with levels &gt;75 ng/ml (excluded 28% patients with levels &lt;75 ng/ml) for hemostasis efficacy evaluation. Two ongoing clinical trials (one each with andexanet and PCC) require &gt;100 ng/ml DOAC level as an inclusion criterion. Both studies also allow “equivalent” anti-Xa activity expressed in IU/mL. We prospectively studied 85 apixaban and 41 rivaroxaban patients for specific DOAC levels as well as LMWH anti-Xa activity by Siemens and Instrument Laboratory’s heparin reagents that use a universal single curve for UFH/LMWH. There was an excellent correlation (R² 0.94-0.98 and Pearson correlation coefficient 0.97-0.99) between DOAC specific levels and both LMWH anti-Xa assay. These finding suggest that locally calibrated LMWH anti-Xa against DOAC specific assay can be used in clinical practice to guide strategy to manage major bleeds. Also, ISTH should propose a universal anti-Xa assay for measuring UFH, LMWH and FXaI levels using IU/mL unit, which is familiar to all physicians.</td>
</tr>
<tr>
<td>Jovan Antovic</td>
<td>First 5 Years of New Oral Anticoagulant Analyses at Karolinska University Laboratory – a Study of Patients With High and Low Drug Concentrations at a Tertiary Care Center</td>
<td>The use of DOAC drugs has increased in recent years, even in patients with relative high risk of bleeding such as the elderly (40% of patients with AF treated with DOAC in Stockholm are &gt; 80) and patients with cancer. Monitoring DOAC can be of importance in situations such as bleeding, thrombosis, trauma, pre-surgery and interventions and in case of a suspected drug interaction. The methods to monitor DOAC concentrations used at the Karolinska University laboratory includes measurement of apixaban, rivaroxaban and dabigatran 24/7 by indirect measuring of the concentration by anti-FXa and anti-FIIa and measurement of apixaban, rivaroxaban, dabigatran and edoxaban by LC-MS/MS.</td>
</tr>
</tbody>
</table>
The two methods differ regarding the need for immediate centrifugation and frozen samples during transport; the time of analyzing the test and providing test result to clinician; test result assessed and commented by lab physician; precision of concentrations < 30 ng/mL. The aim of the project is to identify

1) Indication for NOAC analysis?
2) Clinical situation in relation to high and low drug concentration?
3) Was the appropriate laboratory method for NOAC analysis used?

All DOAC-tests analyzed at the Karolinska University laboratory between January 1st 2014-December 31st 2018 were included. We identified all DOAC analyses with high concentrations (>250 ng/mL) and low concentrations (<30 ng/mL). There were in total 747 DOAC analyses with low concentrations (n= 521) and high concentrations (n=226). The majority of all DOAC tests were analyzed by the anti-factor IIa/Xa method (82%).

The patient files are examined, and the following variables are identified for each patient: Age, sex, weight, indication for DOAC, type of DOAC and dose, presence of bleeding or thrombosis at the time of test, comorbidities, eGFR, concurrent drugs with a potential to interact with DOAC, laboratory data such as platelet count, hemoglobin, PT, APTT.

Interim analysis of 250 patients was performed and it was observed that referrals for DOAC testing come predominantly from the emergency department, internal medicine, neurology and surgery. 31-33 % of NOAC tests had high or low concentrations.

We expect to finalize interim analysis and analyze additional 250 patients’ files to the next SSC meeting.
Hierarchical Bayes analysis was used to estimate preference coefficients (utilities) for each attribute. Preference groups were identified using latent class analysis.

Results: We conducted four FGDs involving 29 participants. The five most important factors identified in the FGDs were included in the survey. There were 250 survey respondents (mean age 45 years, 53% male). The most important factor was re-bleeding risk followed by thrombosis risk, index bleed severity, indication for OAC, and patient characteristics. Two preference groups were identified, a majority group (87% of respondents) placed the highest utility on re-bleeding risk followed by thrombosis risk, while a minority group (13% of respondents) placed the highest utility on OAC indication.

Conclusions: Overall, the most important factor influencing provider decision making was re-bleeding risk followed closely by thrombosis risk, although the indication for OAC was most important for a minority of respondents. This highlights variability among providers in an area lacking high-quality data to guide practice. Further research is needed to determine absolute rates of outcomes and patient values and preferences (J Thromb Haemost 2021;19:153-160).

Background: Gastrointestinal bleeding frequently complicates anticoagulant therapy causing treatment discontinuation. Data to guide the decision regarding whether and when to resume anticoagulation based on the risks of thromboembolism and recurrent bleeding are scarce. Therefore, we aimed to retrospectively evaluate the incidence of these events after anticoagulant-related gastrointestinal bleeding and assess their relationship with timing of anticoagulation resumption.

Methods: Patients hospitalized because of gastrointestinal bleeding during oral anticoagulation for any indication were eligible. All patients were followed up to 2 years after the index bleeding for recurrent major or clinically relevant non-major bleeding, venous or arterial thromboembolism, and mortality.

Results: We included 948 patients hospitalized for gastrointestinal bleeding occurring during treatment with vitamin K antagonists (n=531) or direct oral
anticoagulants (n=417). In time-dependent analysis, anticoagulant treatment was associated with a higher risk of recurrent clinically relevant bleeding (hazard ratio [HR] 1.55; 95% confidence interval [CI] 1.08 to 2.22), but lower risk of thromboembolism (HR 0.34; 95% CI 0.21 to 0.55), and death (HR 0.50; 95% CI 0.36 to 0.68). Previous bleeding, index major bleeding, and lower glomerular filtration rate were associated with a higher risk of recurrent bleeding. The incidence of recurrent bleeding increased after anticoagulation restart independently of timing of resumption.

**Conclusions:** Anticoagulant treatment after gastrointestinal bleeding is associated with a lower risk of thromboembolism and death, but higher risk of recurrent bleeding. The latter seemed to be influenced by patient characteristics and less impacted by time of anticoagulation resumption (J Thromb Haemost 2021; DOI: 10.1111/jth.15476).

<table>
<thead>
<tr>
<th>Nakisa Khorsand</th>
<th>Standardization of the Definition of Clinical Outcome for Patients Treated for a Major Oral Anticoagulant Related Bleed</th>
</tr>
</thead>
<tbody>
<tr>
<td>In 2014 the SSC of the ISTH composed a working group to formulate a standardized definition for hemostatic effectiveness after treatment of major, anticoagulant-associated bleeding. This resulted in definitions for effective haemostasis being proposed in 2016 on behalf of the subcommittee. The next step was to validate the proposed definitions. The results of this validation were published in 2019. For the virtual ISTH SSC meeting, for this subject, the recommendations have been updated based on the results of the validation study and experience gained with using the proposed definition of clinical effectiveness. The update includes clarification of classifying a bleeding type as a visible or non-visible bleed, a clear time frame to assess haemoglobin level and additional explanation on how to use the criteria when other measures than antidotes (eg. Invasive interventions) are used to manage the bleed. Adopting this revised ISTH-SSC standardized definition for assessing good effectiveness of haemostatic management in future studies on any agent used to treat anticoagulant associated major bleed, is highly recommended (J Thromb Haemost 2021;19:1112-1115).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Karlyn Martin</th>
<th>Use of Direct Oral Anticoagulants in Obese Patients With Venous Thromboembolism Updated Guidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Although direct-acting oral anticoagulants (DOACs) have widespread first-line use for treatment and prevention of venous thromboembolism (VTE), uncertainty remains regarding their efficacy and safety in patients with obesity. We reviewed</td>
<td></td>
</tr>
<tr>
<td>From the SSC on Control of Anticoagulation</td>
<td>available data for use of DOACs for VTE treatment and prevention in patients with obesity, including phase 3, phase 4, meta-analyses, and pharmacokinetic and pharmacodynamics studies. In addition, we reviewed available data regarding DOACs in bariatric surgery. We provide updated guidance recommendations on using DOACs in patients with obesity for treatment and prevention of VTE, as well as following bariatric surgery (J Thromb Haemost 2021;19:1874-1882).</td>
</tr>
</tbody>
</table>
REPORT OF THE FVIII, FIX AND RARE BLEEDING DISORDERS SCIENTIFIC AND
STANDARDIZATION COMMITTEE MEETING AT THE ISTH 2021 VIRTUAL CONGRESS

The FVIII, FIX and Rare bleeding disorders Scientific and Standardization Committee program at ISTH 2021 comprised three themes: nonfactor therapies, gene therapy, and SSC new projects. There were ten presentations given by speakers from Canada, India, the United Kingdom, the Netherland and the United States of America. The SSC co-chairs moderated the sessions.

NONFACTOR THERAPIES THEME

Dr Carcao and Dr Alimelu addressed the contentious question of whether, in patients receiving nonfactor therapy, exposure to replacement therapy should be early or not. Both speakers argued convincingly through different historical data sets that exposure should be early. However, the form the early exposure will take remains unclear as there is currently no data to support the argument that low dose exposure is tolerogenic. An argument for early exposure is the challenge of immunogenicity of replacement therapy, which negatively impacts treatment outcomes in people with haemophilia. Both speakers added that there are other benefits of early exposure, including the ability to treat breakthrough bleeds when these occur when using nonfactor therapy prophylaxis and other perceived benefits of factor replacement, such as a physiological role in bone mass density.

In the debate on “Nonfactor Replacement Therapy Versus Factor Replacement Therapy Risks -There Is No Free Lunch”, Dr Doshi and Dr Kenet gave opposing views on the pros and cons of nonfactor therapies. The limitation of this debate is that many of the NFT are still investigational products; therefore, our experience with their use is limited. While NFTs effectively address the challenge of immunogenicity associated with replacement therapies, they have also sharpened the concern of thrombosis, which was never an issue with replacement therapies. Dr Doshi argued that there are many shortcomings of NFTs, including poor understanding of their interaction with other hemostatic agents, lack of monitoring modalities, potential for thrombosis, and lack of data on long-term safety and efficacy. Dr Kenet argued using her real-world data set that there are many benefits of NFTs that cannot be ignored. These include their high efficacy in bleed preventions, high target joint resolution rates and the fact that mitigating measures are in place to prevent the occurrence of thrombosis.

Dr Alok Srivastava addressed the question of “What Should Be the Alternative Endpoints for Non-Replacement Therapies beyond ABRs”. He proposed that in addition to measures to evaluate the extent of haemostasis achieved with NFT, we should also be considering the ICF-WHO framework outcomes measures. These include a combination
of assessment of joint function, activities, participation and quality of life. In addition, these measures should include the evaluation of patient-reported outcomes.

**GENE THERAPY THEME**

The first talk on the Gene Therapy theme was given by Dr Lillicrap, who addressed standardization in anti-AAV therapies. He argued that there is a critical need for AAV assay standardization for various reasons, including the fact that this standardization will influence decisions on patient eligibility for gene therapy. Standardization will also help better define gene therapy success and explain variability seen in a number of programs and eliminate the uncertainty regarding effective vector delivery.

Dr Cooper shared UniQure data informing the rationale for dosing patients with pre-existing anti-AAV antibodies with gene therapy. Part of the reason for this approach's success was that AAV5 and AAV8 were the least prevalent in the healthy donor population. But, more importantly, the avidity of AAV5 IgG created the weakest antibody-antigen complexes compared to other AAVs. Overall, in the limited experience so far, pre-existing anti-AAV5 had no impact on the short term gene therapy outcomes.

Dr Konkle presented the WFH Gene Therapy Registry governance structure, which included the WFH, ISTH, EHC, NHF and industry partners constituting the Steering Committee, Scientific Advisory Committee and the Patient Panel. The core data collected in the WFH GTR included demographics data, medical history, safety and efficacy data. In addition, the registry will also collect longitudinal patient-reported outcomes and mortality data.

**NEW SSC projects**

Dr Young presented a proposal for a new nomenclature for previously untreated patients. The challenges with the current PUPs definition are that it does not include the patient age and makes no distinction between no exposure and minimal exposure to replacement therapy and does not cater to nonfactor therapies. The proposed working group members on this project include pediatric treaters from the US, Canada, Germany and Australia. Anyone interested in this project should contact Dr Young at GYoung@chla.usc.edu.

Dr Raut presented the proposal for value assignment to the WHO 9th International standard for FVIII. The project aims to calibrate and assign a single value for the FVIII suitable for both clotting and chromogenic assays. This will be a multi-assay, multicentre international collaborative study. Recruitment for participants will start in 2021, and the project should be completed in November of 2022. Anyone interested should contact Dr Sanji Raut at sanj.raut@nibsc.org.
Overall, the 2021 SSC business meeting was a successful education and provocative platform we have all come to expect despite the limitations imposed by using the virtual platform and the limited time available for the program.

Report compiled by Johnny Mahlangu- SSC Chair

Sanj Raut, on behalf of Matthew Locke (NIBSC, Potters Bar, UK)

Stocks of the 2nd IS for Thrombin (01/580) were running low, so an international collaborative study was organised to calibrate a replacement. Twenty laboratories from 13 countries took part in the study and measured the potency of two candidate replacement standards (coded 01/578 and 19/188) relative to the 2nd IS. In total, 111 valid assays were returned, which were a combination of plasma/fibrinogen clotting assays and chromogenic assays. For 01/578, potency estimates by clotting assays (101.1 IU/ampoule) were significantly lower than estimates by chromogenic assays (111.5 IU/ampoule). Mean potency estimates for 19/188 were 90.4 IU/ampoule by clotting assay and 88.1 IU/ampoule by chromogenic assay, which was not a statistically significant difference. The close ratio between clotting and chromogenic assay potency estimates for 19/188 suggests it has a higher alpha-thrombin content than 01/578 and is equivalent to the current IS (01/580). Based on the results of this study, the WHO Expert Committee on Biological Standardization established 19/188 as the 3rd IS for Thrombin with a potency of 90 IU/ampoule in August 2020.


Andrew Riches, Sanj Raut (NIBSC, Potters Bar, UK):

**Background:** The current WHO 1st International Standard (IS) for FXIII plasma (02/206) was established in October 2004 with an assigned potency for activity of 0.91 International Units per ampoule (IU/ampoule) and for antigen (A2B2 complex) of 0.93 IU/ampoule. The WHO 1st IS was subsequently additionally assigned with a Total FXIII-B subunit antigen value of 0.98 IU/ampoule, in October 2019.

**Rationale:** The current WHO 1st IS Plasma (02/206) is used for FXIII potency measurement, both activity and antigen (FXIII-A2, FXIII-A2B2 complex & Total FXIII-B Subunit), in patient’s plasma for diagnosis of FXIII deficiencies and in FXIII therapeutic concentrates (P.D. & Rec) & Fibrin Sealants (Potency Labelling). Stocks of the current standard are running low & will be exhausted by 2022/23. This standard is primarily used by hospitals, clinical laboratories, manufacturers of plasma products and assay kits and by regulatory authorities. Approximately 200 ampoules are dispatched each year.
**Aims & Objectives**: To calibrate & assign values for FXIII activity, FXIII (A2B2) antigen and Total FXIII-B subunit to the proposed WHO 2nd IS for FXIII plasmas, suitable for all methods, by assays relative to the WHO 1st IS, in a multi assay, multi center, international collaborative study.

**Study design**: Participants to carry out 4 independent estimates of the candidate material(s) following strict assay designs. Assays of FXIII activity, FXIII (A2B2) antigen or Total FXIII-B subunit (preferably all 3) are proposed to be carried out. Raw data will be analysed at NIBSC, where primary comparison will be of the candidate vs WHO 1st IS FXIII Plasma (02/206).

The study will be launched in Q3/Q4 2021 and the objective will be to submit to WHO/ECBS for establishment in November 2022. Participants are invited to express their interest by contacting Andrew Riches-Duit (NIBSC, UK): e-mail: andrew.riches-duit@nibsc.org

**Standardisation of SEM image analysis of fibrin clots - SSC project**

Marlien Pietes,1,2 Martin Guthold3 (1Center of Excellence for Nutrition (CEN), North-West University, Potchefstroom, South Africa; 2Medical Research Council Unit for Hypertension and Cardiovascular Disease, North-West University, Potchefstroom, South Africa; 3Department of Physics, Wake Forest University, Winston-Salem, USA)

A large discrepancy in fibrin fibre diameter has been reported from scanning electron microscopy (SEM) analysis, in healthy individuals. The absence of an internationally accepted standardised method has resulted in numerous different approaches being described in the literature which varied considerably in terms of activator, the concentration of reagents (particularly thrombin) used, SEM preparation and setup. This exceptionally large variation precludes inter-laboratory comparison and prevents the establishment of normal and disease ranges which are available for other CVD risk factors. This necessitates the need to establish an internationally accepted standardised method that would 1) facilitate interpretation of results, 2) allow direct comparison of data from different laboratories and 3) permit the determination of fibrin fibre diameter for healthy (normal) individuals and individuals with altered clotting. We aim to develop a standardised protocol to form fibrin fibres for SEM diameter measurements.

**Proposed study design**:

- Obtain protocols from laboratories currently performing SEM analysis on fibrin clots.
- Develop a proposed standardised method based on the above protocols and in consultation with researchers working in the field. The following details will be specified: fibrinogen/thrombin ratio and concentrations, buffer conditions, sample preparation, coating of fibres, SEM conditions (resolution, pixel size, magnification)
- Obtain pool plasma that will be sent to participating labs (10-15) to analyse using their in-house methods and the proposed standardised method in order to compare the variability in the data.
- Data will be analysed manually by each participating laboratory and images will also be analysed centrally using an automated measurement tool.
**Scientific & Clinical Topics**

**Bleeding of unknown cause - evidence for impairment of the hemostatic potential.**

Dino Mehic, Johanna Gebhart, Ingrid Pabinger. (Clinical Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria)

Despite extensive laboratory investigations of coagulation and platelet function, in a majority of patients with mild-to-moderate bleeding tendency no diagnosis can be determined. Patients with bleeding of unknown cause (BUC) are indistinguishable from patients with definite diagnoses (e.g. von-Willebrand-disease, platelet function defects) in regards of their bleeding phenotype. The lack of targeted treatment options places a high psychological strain on patients and treating physicians. We recently identified an impaired thrombin generation in BUC patients, with prolonged lag time and time to peak, as well as decreased maximum thrombin generation. In line, analyses of the plasma clot formation revealed a lower clot formation rate, which resulted in a longer time to peak, increased absorbance and a shorter clot lysis time, when comparing to healthy controls. In a previous study, we also found evidence for dysregulation of fibrinolytic factors in patients with BUC, which was partially in line with previous studies on bleeding cohorts. Furthermore, we also found that tissue factor pathway inhibitor (TFPI) is elevated in BUC patients, which was associated with a mild delay in thrombin generation (prolonged lag time and time to peak). One of our very recent studies identified blood group O to be an independent risk factor for more severe bleeding in BUC patients. This was underlined by impairment of parameters in ROTEM and plasma clot formation analysis. BUC patients with blood group O interestingly showed evidence for denser, firmer clots with reduced lysis, when comparing to non-O patients. Our comprehensive investigations on BUC patients provide evidence for an impaired hemostatic potential, where hyperfibrinolysis, natural anticoagulants as well as the ABO blood group might play a role within a multifactorial holistic approach of several risk factors.

**Automated measurement of fibrin fiber diameters in images obtained using Fluorescence microscopy.**

J.J. de Vries¹, J.A. Slotman², C. Martinez-Torres³, G.H. Koenderink³, M.P.M. de Maat⁴. (¹Department of Hematology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands; ²Erasmus Optical Imaging Centre, Department of Pathology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands; ³Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Delft, the Netherlands)

Accurate quantification of fibrin fiber diameters is important when studying clot properties. There is a range of imaging modalities to determine fibrin diameter. Often used methods include scanning electron microscopy and fluorescent (confocal) microscopy. The first method gives a high-resolution image, but sample preparation might affect clot properties, while the later has a limited resolution and cannot reliably measure thin fibers. Furthermore, quantification is often done manually. Therefore, we aimed to develop a method to reliably determine fiber diameter using the super-resolution method: stimulated emission depletion (STED) microscopy. STED microscopy selectively switches off fluorophores around the focal point, thereby increasing resolution and improving the differentiation of separate fibrin fibers. Clots were prepared by adding thrombin (1U/ml) and CaCl₂ (17mM) to platelet-poor plasma supplemented with fluorescently labelled fibrinogen. Using the Leica Navigator software, multiple images per clot were recorded automatically, both at STED and confocal settings at a Leica TCS SP8 microscope. After applying a bandpass filter (from 3-40 pixels) to the
images, fiber diameters were measured using DiameterJ or Local Thickness plugin in ImageJ. STED images showed more pronounced details in the fibrin network compared to confocal images. In addition, fiber diameters measured were consistently smaller in STED compared to confocal images, and the absolute values are comparable to values known from SEM images. Finally, the correlation between fiber diameters measured using the two plugins was strong (r=0.923). In conclusion, we show an automated method to reliably measure fiber diameter in images obtained by STED microscopy.


A Daraei1, M. Pieters2, 3, S. R. Baker1, 4, Z. de Lange2, 3, R. I. Litvinov5, C. S. B. Veen6, M. P. M. de Maat6, J. W. Weisel5, R. A. S. Ariens4, M. Guthold1. (1Department of Physics, Wake Forest University, Winston-Salem, USA; 2Center of Excellence for Nutrition (CEN), North West University, Potchefstroom, South Africa; 3Medical Research Council Unit for Hypertension and Cardiovascular Disease, North-West University, Potchefstroom, South Africa; 4Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK; 5Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA; 6Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands).

Background. Scanning Electron Microscopy (SEM) is a powerful, widely used, high-resolution imaging technique to analyze the structure of fibrin networks. Currently, structural features, such as fiber diameter, length, density and porosity, are mostly analyzed manually, which is tedious and may introduce user bias. A reliable, automated structural image analysis method would mitigate these drawbacks.

Methods. We evaluated the performance of DiameterJ (an ImageJ plug-in) for analyzing fibrin fiber diameter by comparing automated DiameterJ outputs with manual diameter measurements in four SEM data sets with different imaging parameters. We also investigated correlations between biophysical fibrin clot properties and diameter, and between clot permeability and DiameterJ-determined clot porosity.

Results. Several of the 24 DiameterJ algorithms returned diameter values that highly correlate with and closely match the values of the manual measurements. However, optimal performance was dependent on the pixel size of the images – best results were obtained for images with a pixel size of 8-10 nm (13-16 pixels/fiber). Larger or smaller pixels resulted in an exaggeration or suppression of diameter values, respectively. The correlation between clot permeability and DiameterJ-determined clot porosity was only modest, likely because it is difficult to establish the correct depth of focus in the clot images.

Conclusions. Several DiameterJ algorithms perform well for diameter determination in SEM images, given the appropriate imaging conditions (13-16 pixels/fiber). Determining fibrin clot porosity via DiameterJ is challenging.

M1 protein from Group A Streptococcus impacts fibrin clot formation, structure and fibrinolytic potential.

Sophie Cherrington1, Lewis Hardy3, Robert J. Francis2, Cedric Duval2, Azhar Maqbool3, Helen Philippou3, Craig Thelwell1. (1NIBSC, Haemostasis Group, Division of Biotherapeutics, Blanche Lane, South Mimms, EN6 3QG UK; 2NIBSC, Biological Imaging Group, Blanche Lane, South Mimms, EN6 3QG UK; 3Leeds Institute of Cardiovascular & Metabolic Medicine (LICAMM), University of Leeds, Leeds, West Yorkshire, United Kingdom).
Background: Fibrin formation is an essential part of innate immunity. The fibrin clot network traps bacteria to limit dissemination and reduce the severity of invasive infections and a protective fibrin biofilm covers the external surface of clots acting as the first line of defense. The surface anchored M1 protein is a major virulence determinant of a highly prevalent and virulent Group A streptococcus (GAS) strain, M1T1. During early infection M1 is cleaved from the cell surface resulting in soluble M1 at the site of infection. M1 is known to form a supramolecular complex with fibrinogen however the impact of this on fibrin formation and structure is unknown.

Aims: Investigate the impact of GAS M1 protein on fibrin clot formation, structure and fibrinolytic potential.

Methods: Fibrin clots incorporating recombinant M1 (rM1) were made by the activation of human blood, plasma, and purified fibrinogen. Fibrin structure was assessed using imaging techniques (confocal microscopy and scanning electron microscopy), densitometry analysis of reduced cross-linked fibrin clots and permeation assays (gel porosity). Fibrin clot formation, mechanical strength and lysis rates were measured kinetically using fibrinolysis profiles (turbidity), thromboelastography (ROTEM) and KC4 analysis.

Results: M1-bound-fibrinogen produced clots with remarkably different structures and properties. rM1 protein (0.47-60 µg/ml) produced heterogeneous clots, with irregular fibre bundles and compacted fibrin that lacked mechanical strength. Clot porosity increased (above 15 µg/ml rM1) up to 12-fold at 60 µg/ml with increased fluid permeability and susceptibility to lysis by plasmin with a 2.2- 2.7-fold reduction in lysis times. rM1 disrupted the formation of the protective fibrin biofilm, increasing porosity and biofilm entirely absent at higher rM1 concentrations.

Conclusions: GAS strains of M1-type are commonly associated with invasive infections. M1-bound fibrinogen at infection sites may contribute to the severity of infection by forming fibrin clots with a compromised protective film at the surface, that are mechanically weaker, more porous, and less resistant to lysis by plasmin.

Clinical features in afibrinogenemia: insights from the QualyAfib study.

Alessandro Casini (Geneva University Hospitals, Switzerland):

Congenital afibrinogenemia is a rare and severe coagulation disorder characterized by the complete absence of fibrinogen. Data from the QualyAfib study confirm that the bleeding phenotype is severe in afibrinogenemia. Among the 204 patients (119 adults and 85 children) included, the median ISTH BAT was 14 (8-21). Overall, 168 (82%) patients reported bleeding requiring a medical intervention and 68 (33%) had at least one bleeding episode per month. Peri-operative bleeding (n=82, 40%), muscle hematomas and hemarthrosis (each n=78, 38%), cerebral bleeding (n=48, 23%) were particularly common. We also observed a high risk of thrombosis as total of 37 (18.1%) patients experienced a thrombotic event. Venous thromboses occurred in young patients with a mean age at first event of 27 years, including 6 children (7%). Arterial thromboses were also observed in young adults, with a mean age at first event of 36 years. Miscellaneous symptoms included spleen rupture (5.4%) and painful bone cysts (17.6%) especially in younger patients. Women were particularly affected by heavy menstrual bleeding and obstetrical complications.
Comprehensive evaluation of coagulation and fibrinolysis parameters in patients with congenital factor XIII deficiency in Pakistan - an introduction and outline of the SSC Project.

Munira Borhany¹, Verena Schroeder². (¹National Institute of Blood Disease & BMT, Karachi, Pakistan; ²Department for BioMedical Research, University of Bern, Switzerland):

Congenital factor XIII (FXIII) deficiency is a rare bleeding disorder associated with significant bleeding manifestations. However, this rare bleeding disorder makes the meaningful studies with large patient numbers very difficult. In addition, most patients in the developed world receive prophylactic FXIII replacement therapy, which can bias the investigations. Moreover, in Pakistan majority of our patients are on demand treatment with plasma. The aim of the study is to investigate whether there are any changes in the coagulation and fibrinolytic system as a compensation mechanism of congenital FXIII deficiency, and to study if any changes in these are related to the severity of bleeding complications or other medical conditions. We are collecting samples from FXIII deficient patients and control group (blood donors). Data including clinical and laboratory parameters will be recorded and will be evaluated by using SPSS version 23. So far we have run basic tests (CBC, PT, APTT, Fibrinogen, and D.dimer) of 27 FXIII deficiency patients and 23 controls. For further analysis samples are saved to be performed later at Dr. Schroeder’s lab in Switzerland. After completing this study we expect that our results will give a comprehensive picture of the state of the coagulation and fibrinolytic system in a large collective of patients with congenital FXIII deficiency. It will help us to update and improve our recommendations/guidelines for the diagnosis and management of these patients.
tPA for COVID-19 ARDS
Ernest E. Moore, University of Colorado, USA

Early in the course of COVID-19 respiratory failure patients have relatively normal lung compliance with elevated dead-space ventilation, a hallmark of diffuse pulmonary microvascular thrombosis that was confirmed in autopsy findings of deceased COVID-19 patients. Clinical studies suggest the underlying pathogenesis is a prothrombotic state compounded by fibrinolysis shutdown. Consequently we conducted a phase 2 trial (NCT04357730) with the hypothesis that tPA with therapeutic heparin would improve oxygenation and reduce ventilator free days.

Eligible patients were: 1) age 18-75 years; 2) confirmed diagnosis of COVID-19 and 3) severe respiratory failure requiring mechanical ventilation (MV) with PaO2/FiO2 ratio <150 for >4 hours. Stage1 patients (1 to 36) were randomized to an IV 50mg bolus of tPA (Alteplase) followed by IV unfractionated heparin to maintain aPTT at 60-80 for the next 7 days. Stage 2 patients (37-50) were randomized to a 50 mg bolus followed by a 50 mg tPA-drip plus heparin 500 U/hour over 24 hours, then heparin to maintain aPTT 60-80s/7 days. The results of this trial show that tPA as a bolus followed by therapeutic anticoagulation for severe COVID-19 respiratory failure is safe; there were no major bleeding events. Further, this regimen may be efficacious, with substantial observed differences in PaO2/FiO2 ratios at 48 hours, ventilator-free days, and mortality compared to controls. While these differences did not reach statistical significance, they were consistent trends present at every interim analysis, and importantly in the comparisons within group (compared to baseline) the tPA-Bolus group sustained significantly higher PaO2/FiO2 ratios (p<0.017) at 6, 12, 24, 48, 72, 96, 144 and 168 hours post-randomization. We believe these findings warrant a phase 3 trial.

SSC project update: validation of the t-AUCi parameter using ROTEM to assess fibrinolytic resistance in septic patients
Ecaterina Scarlatescu, Institutul Clinic Fundeni, Romania

Detection of subtle changes in fibrinolysis is important to the clinical management of critically-ill septic patients as increased resistance to fibrinolysis is associated with increased mortality. In clinical practice, fibrinolysis can be detected on viscoelastic tests (VET) as a decrease of clot firmness related to the maximum clot amplitude. Resistance to fibrinolysis is more difficult to diagnose using VET, as in most clinical situations characterized by low-grade fibrinolysis the decrease in clot firmness after reaching the maximum amplitude is not visible during the limited measurement time. However, since fibrinolysis begins before the clot reaches its maximal firmness, it should also be reflected by the kinetics of clot formation before reaching the point of maximal amplitude.

Based on this assumption, in our study we calculated a new early kinetic parameter (t-AUCi) representing the time required to reach maximal clot amplitude after maximal clot formation velocity has been reached. The main objective of the study is to evaluate the correlation between the degree of fibrinolytic activation and the newly calculated parameter (t-AUCi), by using standard and tPA spiked ROTEM analysis in patients with sepsis and in healthy controls. We finished patient and control inclusion and the first analysis of the ROTEM data. The results from...
the biomarkers reflecting fibrinolytic activity are not yet analyzed, and the data analysis from ROTEM is only preliminary. The parameters included in the new parameter calculation are very sensitive to all kinds of small artifacts and all the traces have to be manually checked and the values verified together with the raw data. Our preliminary results showed that t-AUCi values decrease when the amplitude of the clot decreases after reaching MCF. tAUCi values decreased in EXTEM tests performed with increasing tPA concentrations, while such a decrease was not noted in APTEM. These results need to be confirmed by final ROTEM data analysis and by correlation with the plasmatic markers of fibrinolytic activity measured in our study group.

The Basis and Application of Assays Measuring Fibrinolytic Parameters
Ze Zheng, Medical College of Wisconsin, Versiti Blood Research Institute, USA

The purpose of this talk is to summarize the basis and application of assays measuring fibrinolysis, and how to choose proper assays based on the scientific questions. We also hope to create a “cheat sheet” for fibrinolysis assays as a SSC group effort.

Here are a list of scientific questions that we might ask while doing research related to fibrinolysis:

1. **How much tPA is available?**
   a. tPA total concentration
   b. PAI-1-free tPA: by ELISA, free tPA forms a covalent complex with the biotinylated PAI-1, which is bound to avidin on the plate
   c. tPA/PAI-1 ratio
   d. tPA release = [tPA antigen after thrombotic occlusion] – [resting tPA antigen]

2. **Is the tPA functional?**
   a. tPA enzymatic activity assay: Chromophore p-nitroaniline (pNA) is covalently bound to the C terminus of Val-Leu-Lys and is released upon plasmin digestion with a yellow color. The intensity of color is proportional to tPA activity. This assay is sensitive to endogenous tPA changes (basal and injury-induced), and is sensitive to high PAI-1
   b. Plasmin-generation assay

3. **Are the inhibitors of fibrinolysis functioning normally?**
   a. Total PAI-1 antigen, tPA-free PAI-1, uPA-free PAI-1
   b. α2-antiplasmin concentration, plasmin-α2-antiplasmin (PAP) complex
   c. α2-macroglobulin
   d. Thrombin-activatable fibrinolysis inhibitor (TAFI)

4. **What is the overall fibrinolytic activity?**
   a. Plasma-based clot lysis time (CLT)
   b. Euglobulin clot lysis time (ECLT)
   c. D-dimer generation assay
   d. tPA-induced plasma clot lysis time

5. **Factors that affect the properties of fibrin clot?**
   a. Clauss assay (clottable fibrinogen)
b. Thrombin time

c. Thrombin-antithrombin (TAT complex)

Application of Fibrinolytic Assays: Strengths and Limitations
Tetsumei Urano, Hamamatsu University School of Medicine, Japan

The fibrinolytic activity is fully expressed only when fibrin is formed. Thus in order to adequately assess fibrinolytic potential in plasma, fibrin clot formation is necessary and several different clot lysis assays have been established. In this talk, I explain both strengths and limitations of three different clot lysis assays: euglobulin clot lysis assay (ECLT), tPA-induced plasma clot lysis time (tPA-PCLT) and plasmin generation assay. As we reported before, ECLT well represents tPA activity in plasma which is simply determined by the balance between tPA and PAI-1 in plasma. When calcium ion is supplemented in the assay (Ca\textsuperscript{++}-ECLT), ECLT was dramatically shortened as a result of PAI-1 inactivation by endogenously generated thrombin. Thus the difference between regular ECLT and Ca\textsuperscript{++}-ECLT indicates PAI-1 activity in plasma which appeared negative values in two distinct PAI-1 deficient patients. tPA-PCLT was prolonged by soluble thrombomodulin (sTM). Inhibitor of activated thrombin-activatable fibrinolysis inhibitor (activated TAFI; TAFIa) significantly shortened both regular- and sTM supplemented-PCLT to similar time, and the differences represent the endogenous TAFIa activity obtained by a tiny amounts of soluble TM contained in plasma and the total TAFIa activity obtained by supplemented sTM, respectively. Plasmin generation assay is similar to tPA-PCLT, and plasmin activity at a certain time point can be obtained as a sum of its generation and its inactivation. These two assays of tPA-PCLT and plasmin generation assay are global fibrinolytic assays and suitable to analyze coagulation/fibrinolysis relationship in which TM/TAFI plays essential role.

In summary, it appears that clot formation is essential to correctly evaluate fibrinolytic potential. In these clot lysis assays, ECLT well represents tPA/PAI-1 activity. Both tPA-PCLT and plasmin generation assay are global fibrinolytic assay and are influenced by many factors including PAI-1, TM/TAFI system, and alpha2-antiplasmin. Employing TAFIa inhibitor, however, we may assess TM/TAFI specific activity in these assays.

Plasmin Generation Assays: Tailoring Experimental Design
Alisa Wolberg, University of North Carolina Chapel Hill, USA

Global assays are a workhorse for understanding coagulation and fibrinolytic potential; however, additional information on thrombin generation and plasmin generation parameters is essential for defining mechanisms that lead to coagulopathy and fibrinopathy. The Wolberg lab has developed a plasmin generation assay based on principles from calibrated automated thrombography. The assay is triggered by tissue factor and tissue plasminogen activator (tPA) and is specific for plasmin and sensitive to fibrin cofactor activity. The assay can be used to detect antifibrinolytic activity of pharmacologic therapies to prevent bleeding, including tranexamic acid. The assay is not sensitive to endogenous initiators of fibrinolysis (tPA or urokinase plasminogen activator) or their canonical inhibitor plasminogen activator inhibitor-1. However, the assay detects thrombomodulin-mediated activation of thrombin-activatable fibrinolysis inhibitor and may be
useful for studies of the crosstalk between endothelial (dys)function and fibrinolysis. In concert with other global and kinetic assays, the plasmin generation assay may be useful for defining mechanisms contributing to pathology in obesity, COVID-19, and trauma.

**Euglobulin Lysis Assay Standardization: Project Proposal**  
Anton Ilich, University of North Carolina Chapel Hill, USA

Euglobulin fraction was first used in global fibrinolytic assays in 1941 and is still often referred as the gold standard for hyperfibrinolysis measurement. Principle of euglobulin clot lysis time (ECLT) is based on the rebalancing of all key fibrinolysis factors in favor of activators, by osmotic pressure reduction and acidification of plasma. This allows to detect spontaneous clot lysis without addition of exogenous fibrinolysis activators.  
Originally, the clot from euglobulin fraction was formed in the glass tube and manually tilted to detect the time of clot liquefaction. This approach had several limitations: high variability of results, low throughput, time consuming. Thus, several groups came to the decision to automate the assay by forming a clot in the well of the 96 well plate and recording the lysis time by measuring the optical density changes over time.  
We have performed an automated ECLT in various factor deficiency conditions, namely hemophilia, congenital PAI-1, plasminogen, and fibrinogen, as well as on a large number of healthy controls and trauma patients (Ilich *RPTH* 2020, Ilich *Tromb Res* 2021). The conditions to precipitate euglobulin fraction vary significantly between published automated ECLT protocols. To compare analytical variables, we performed ECLT on the same set of samples using 2 protocols: from Hamamatsu University and from UNC Chapel Hill. Shiny App for clot lysis time, developed by Dr. Colin Longstaff was used to generate clot lysis time values based on curve analysis. Both protocols yielded equivalent results, with a strong correlation between those two methods ($R^2 = 0.9803$). However, additional work on standardization is required to test for inter-laboratory variabilities.

**Standardization of a Functional Soluble Thrombomodulin Assay: Project Proposal**  
Michael Boffa, University of Western Ontario, Canada

Thrombomodulin (TM) is a transmembrane protein primarily expressed on endothelial cells with broad anticoagulant, anti-inflammatory, and antifibrinolytic activities. These are largely mediated by its cofactor activity for protein C and thrombin-activatable fibrinolysis inhibitor (TAFI) activation. Soluble TM (sTM) in plasma results from proteolysis of TM and shedding of the extracellular domain, and sTM is elevated in disease states such as sepsis and severe COVID-19. The function of sTM remains unclear but may be modified by oxidation of Met388 which eliminates protein C cofactor activity while retaining TAFI cofactor activity. To complement existing assays of sTM antigen and PC cofactor activity in plasma samples, I propose the development and standardization of assays that probe the TAFI cofactor activity and/or the extent of Met388 oxidation. (1) Based on the protein C cofactor activity assay, sTM can be captured from plasma in antibody-coated wells, followed by the addition of thrombin and TAFI and subsequent measurement of TAFIa activity. (2) An ELISA to specifically detect sTM oxidized at Met388 could be devised, after the raising of a specific antibody against the oxidized
Met388-containing epitope. (3) Detection and quantification of modified and unmodified Met388-containing peptides could be performed using LC-MS/MS of trypsinized plasma samples; quantitation is achieved by the inclusion in the reaction of stable isotope-labeled peptides corresponding to the native or oxidized peptides.

**NIBSC Standards Updates: D-dimer, PAI-1 and TAFI**
Colin Longstaff, NIBSC, UK

A project to make a new WHO International Standard (IS) for thrombin-activatable fibrinolysis inhibitor (TAFI; proCPU or CPB2 gene product) has been coordinated by C Thelwell at NIBSC. The aim of this project is to make a standard suitable for assays that generate active TAFI and provide an antigen assignment in µg/ml. The gravimetric antigen value will be determined using Isotope Dilution Mass Spectrometry (IDMS) which uses TAFI enriched in $^{13}$C and $^{15}$N arginine and lysine. There is also a project to replace the current WHO IS for PAI-1 activity (92/654) and at the same time assign an antigen value using the IDMS approach to label this standard in ng/ml. Trials have begun of *E.coli*-expressed PAI-1, (provided by Paul Declerck, University of Leuven, Belgium) added to plasma and trehalose has been included in the formulation to improve long term stability. Attempts to make a standard for D-dimer have been proceeding for many years and are made difficult by the complex nature of the D-dimer antigen (a mixture of different sized fibrin breakdown products) in clinical samples; the diversity of monoclonal antibodies used to measure this analyte; and different calibrators used by method developers. We also reported previously on instability of freeze-dried D-dimer, which we presume is prone to aggregation. We have evidence that freeze-dried D-dimer in plasma can be stabilized by added trehalose and work continues to show that such a preparation may be a useful as a standard to harmonize results from different methods.
Minutes from SSC meeting, 17th July 2021.

Introduction and Gene Curation Update
Kathleen Freson (Leuven, Belgium)

Gene Curation - upgrade of gene to Tier1 gene for rare gene disease NGS panel list:
TMP4 - AD macrothrombocytopenia
PTGS1 (COX1) - Bleeding disorder platelet type 12

Tier 2 genes on webpage
MAST2 added to Tier 2 gene list.
Update will become available on ISTH webpage: https://www.isth.org/page/GinTh_GeneLists

GoldVariant Database
Karyn Megy (Cambridge, United Kingdom)

There are several resources for sharing disease causing variants such as ClinVar, Locus Specific Databases (EAHAD, LOVD, GT), still many labs that use NGS for diagnostics
The SSC-GinTH GoldVariants interface was released and variants can be submitted by the community. Submitted variants will become available from the ISTH website (https://www.isth.org/page/GinTh_GeneLists) and they will be transferred to the ClinVar database. Data sharing is very important to improve variant classification.
The resulting manuscript from this project was published in JTH in July 2021.

Status of the GoldVariant database in July 2021:
814 submissions from 30 centres across the world. One third have been submitted as Variant of Uncertain Significance (VUS).

Variant Curation for Clinical Practice
Shruthi Mohan (North Carolina, United States)
Presentation of the ClinGen coagulation factor deficiency variant curation expert panel who are currently generating and piloting ACMG/AMP variant interpretation guidelines for F8 and F9. These criteria can be gene- or disease-specific. Defining phenotype criteria is very important in this curation process. For F8: plasma factor levels can be in the severe or mild/moderate range and with X-linked inheritance after exclusion of VWD-2N. For F9: abnormal low F9 levels are detected. Segregation data will also be used. Examples of variant curation for F9 and F8 were provided and it was shown how difficult it is to use these curation rules. The F8 Tyr365Cys results in lower FVIII plasma values but most patients with this variant have no bleeding. If the phenotype is considered low FVIII, there is strong evidence (PS4_very strong) while if bleeding is taken into account, this would only be a VUS (PP3). Many variants are also remaining VUS because co-segregation data are often lacking. Curation rules for F8 and F9 will be published in the fall.

**Variant Curation for Clinical Practice**

**Justyne Ross (North Carolina, United States)**

Presentation of the ClinGen platelet disorder variant curation expert panel who developed the ACMG/AMP variant interpretation guidelines for Glanzmann thrombasthenia. The curation rules (again gene- and disease-specific) and the first 70 variants in ITGA2B and ITGB3 were recently published: see Specifications of the variant curation guidelines for ITGA2B/ITGB3: ClinGen Platelet Disorder Variant Curation Panel. Ross JE, Zhang BM, Lee K, Mohan S, Branchford BR, Bray P, Dugan SN, Freson K, Heller PG, Kahr WHA, Lambert MP, Luchtman-Jones L, Luo M, Perez Botero J, Rondina MT, Ryan G, Westbury S, Bergmeier W, Di Paola J. Blood Adv. 2021 Jan 26;5(2):414-431. At this moment 250 variants (24% are VUS, and 12% are (likely) benign) were curated and examples of variant curation were discussed. This expert panel has now started with setting up the curation rules for the genes (GP9, GP1BB, GP1BA) for Bernard Soulier Syndrome.

**Diagnostic Strategy to Search for Rare Inherited Thrombophilia in Clinical Setting**

**Bengt Zöller (Sweden)**

Genetics of rare thrombophilia involves variants in PROS1, PROC, SERPINC1 and very specific variants in F5 (APC resistance), F2 (Antithrombin resistance), F9 Padua and more recently, STAB2 variants. Diagnostic also involves measurements of protein S, protein C and antithrombin plasma levels using antigen but also functional assays can be used. It is however known that not all pathogenic variants in these genes are associated with abnormal plasma levels. NGS screening can detect variants, also common, that can be used for polygenic risk scoring. The detection of novel variants requires additional functional studies. The pros and cons were discussed for targeted sequencing, WES and WGS. Thrombogenomics was able to detect variants in nearly 50% of patients with thrombophilia. Precision medicine for thrombosis will consist of NGS screening and can in the future be expanded with DNA methylation, transcriptomics, metabolomics, proteomics, AI and deep learning.
Human Mutational Constraint as a Tool to Understand Variants in Platelet-Associated Genes
Joe Oved (United States)

Aim of this project was to develop a new algorithm to determine pathogenicity of (new) genes for platelet disorders with a focus on loss of function (LoF) pathology. The analysis pipeline was developed for bone marrow failure as described in Oved et al, Blood Advances, 2020, Vol4. The constraint for LoF variants was used via the pLI score (genes with pLI score >0.9 are likely to cause pathology when LoF variants occur). The frequency of LoF variants (dominant versus recessive) was determined for the different genes for platelet disorders according to the role of their protein function. The data are published: Oved JH, Lambert MP, Kowalska MA, Poncz M, Karczewski KJ. Population based frequency of naturally occurring loss-of-function variants in genes associated with platelet disorders. J Thromb Haemost. 2021 Jan;19(1):248-254. Constraint of certain genes can be high such as in genes coding for transcription factor and cytoskeletal proteins, for others the constraint was low. A gene that is highly constraint is not because of the platelet dysfunction but because of other phenotypes such as leukemia (RUNX1 and ETV6) or neuropathology (NBEA). Example of how the tool was used for newly discovered genes: WIPF1 (pLI = 0.9 for bi-allelic disease) suggesting other (subclinical) phenotypes associated with LOF in this gene than only platelet dysfunction.

VarioPath project: Leveraging UK Biobank Data to Advance Variant Pathogenicity Interpretation
Janine Collins (Cambridge, United Kingdom)

The frequency of (Likely) pathogenic variants reported in the in house pathogenic database, HGMD and ClinVar in coagulation and platelet disorders genes were investigated in the UKBB dataset (131K participants) with WES and EHR data available. This database will therefore allow to study the phenotypic effects of (L)PV on phenotypes in the general population. About 1/26 participants carry a (L)PV in 51 known platelet genes. Some variants were illustrated that are causing thrombocytopenia and in this cohort significantly reduced the platelet count in some but not all carriers of these variants. The hypothesis is that the common variants play a role in this phenotypic difference. Examples were provided that show how polygenic risk scores interfere with the effect of a rare variant (Collins, Br J Haematology, 2021).

Thrombosis Genetics at Scale: Lessons from Electronic Health Record Biobanks
Scott M. Damrauer (Pennsylvania, United States)

Million Vetran program (MVP) for common traits - eg. VTE are powerful when linking to electronic health records to capture biomedical data. This not only provides the opportunity to perform GWAS on multiple traits (different phenotypes) but also the longitudinal data collection allows for cases to be identified over time.
The MVP currently includes data from more than 850K participants of which 650 K have been typed with custom array and EHR data collected over 15-20 years (median of 186 visits). This cohort is investigated for VTE using ICD9/10 codes. Controls are subjects that are not cases. Within time controls can become cases if VTE occurs.

A Genome-Wide Association Study Meta-Analysis for Venous Thromboembolism
Florian Thibord (United States)

The third and largest meta Analysis of Venous Thromboembolism of the INVENT consortium: 55,000 cases and 1 million controls: 86 loci were identified of which 51 are novel. These include 13 (anti)coagulation loci, 10 immune/inflammatory loci, 37 platelet trait loci and 26 remaining loci with wider functions. This discovery meta-analysis will be repeated in a validation cohort (28K cases). Next GWAS studies will be performed in African population. Fine-mapping of the loci is required. A transcriptome wide analysis and heritability studies will be performed in the future.
Dr. Zwicker provided introduction to the session
Dr. Ni Ainle provided an overview of the use of DOAC in patients with cancer. Highlighted some of the data limitations to date including use in patients taking oral chemotherapeutic agents.
Dr. Wang described recently published study funded through ISTH. The registry included over 200 patients from 6 institutions who received targeted oral agents along with DOACs. The overall rate of hemorrhage was 4% but there was variability between diagnosis and oral agents. Highlighted the need for additional data.
Dr. Khorana reviewed the evidence to data supporting the prognostic role of the Khorana Score to predict cancer associated thrombosis. It remains the most validated predictive score developed. Predictive accuracy lower within specific cancer subgroups. He identified newer adjuncts to the score and future directions
Dr. Sanfilippo reviewed results of an ISTH SSC project on the development and validation of risk assessment models in CAT. There have been a large number of studies published evaluating different risk models but very few published have followed the TRIPOD methodological standards.
Dr. Zwicker presented the data on safety of anticoagulation in patients with primary and secondary brain tumors.
Dr. Leader and Dr. Hamulyak described an ongoing project looking at outcomes with DOACs in patients with brain tumors across a number of institutions. If sites are interested in participating, should contact Dr. Leader at avileader@yahoo.com
Dr. Lee described the management options available when recurrent VTE occurs in cancer patients on therapeutic anticoagulation. She provided a treatment algorithm for management of recurrent VTE.
Dr. Bosch provided rationale and updates on a prospective study that is supported by ISTH evaluating anticoagulation management of recurrent VTE in cancer
Dr. Le Gal provided SSC instructions on how to implement common data elements developed with ISTH and available online for use
Dr. Soff described the use of trombopoietin mimetic agents in different cancer populations and the rationale for a guidance document on their use for this indication
Dr. Mahe provided a touching remembrance of our colleague Dr. Guy Meyer

Last 25 minutes were dedicated to Q&A
MINUTES SSC Session July 17th 2021 - SSC lupus anticoagulant/antiphospholipid antibodies by Katrien Devreese (chair)

Introduction- Katrien Devreese
An overview of the current projects of the SSC lupus anticoagulant/antiphospholipid antibodies is presented, as well as a call for new projects. The current projects can be found on the SSC webpage.

Registry on augmented antithrombotic treatment regimens for patients with arterial thrombotic APS-Sam Schulman
Main objective of the registry is to compare a VKA with therapeutic range, INR 2.0-3.0 plus low-dose aspirin (75-100 mg) with DAPT. The comparison will address efficacy and safety. The registry will also include patients treated with VKA alone and will serve as reference groups in comparison with VKA + low-dose aspirin and versus DAPT.

Current treatments practice in women with obstetric APS and women with obstetrical morbidity and “non-criteria” APS and adverse obstetrical outcomes: CORA international registry –Patricia Casais and Cristina Belizna
With this registry the investigators aim to evaluate management and outcomes of obstetric APS and to determine practice regarding women with adverse pregnancy outcomes who do not meet Sydney clinical or laboratory APS criteria. This is a joint project with the SSC Women’s Health Issues in Thrombosis and Hemostasis.

Survey on diagnosis and antithrombotic treatment in APS patients with ischaemic stroke, other brain ischaemic injury or arterial thromboembolism in other sites-Hannah Cohen
The main objective of this survey is to capture the views of experienced investigators with regard to their perception of the optimal approach to the antithrombotic treatment of patients with APS-associated ischaemic stroke, TIA or other brain ischaemic injury and associated cognitive impairment and the strategy for diagnosis of APS in these patients.

Update on International Registry of Thrombotic APS patients Treated With Direct Oral Anticoagulants-Stéphane Zuily
The registry follows the guidance document of the SSC LAC/aPL on use of DOAC in patients with thrombotic APS, that was published last year. The identification of APS patients treated with a DOAC and their enrolment in an international registry may inform future care. All cases of DOAC use in APS should be reported to the international registry supported by the ISTH. The registry is already started in France, and will be expanded by a RedCap registry through the ISTH-SSC.

Why aPS/PT and other second level aPL tests should be added to improve on the definition of APS-Vittorio Pengo
V. Pengo illustrates the added value of aPS/PT antibodies in APS. High risk thrombotic APS patients are those with triple positive profile, and aPS/PT are also present in these patients (tetra positive). aPS/PT antibodies might be a surrogate test for LAC and helpful in anticoagulated patients, to define the antibody profile where LAC testing is difficult. Also, in patients with incomplete antibody profiles (double or single positive for aPL) aPS/PT may contribute to the diagnosis.

An international, multi-centre study to validate the Taipan snake venom time as a lupus anticoagulant screening test with ecarin time as confirmatory test-Gary Moore
This multicenter study has been published as a communication from the ISTH-SSC LAC/aPL. Lupus anticoagulant (LA) assays are compromised in anticoagulated patients, and existing strategies to overcome the interferences have limitations. The prothrombin-activating Taipan snake venom time (TSVT) screening test and ecarin time (ET) confirmatory test are innately insensitive to vitamin K antagonists (VKA) and direct factor Xa inhibitors. Standardised TSVT/ET reagents for LA detection were validated in a multi-centre, multi-platform study. Six centres from four countries analysed patient samples with TSVT/ET. Anticoagulant spiking experiments were performed and samples containing potential interferences (i.e. direct thrombin inhibitors) were tested. Results were evaluated against locally derived cut-offs and imprecision was evaluated. Cut-offs were remarkably similar despite use of different analysers and donor populations. Coefficients of variation for TSVT and ET ratios were ≤5.0%. TSVT/ET exhibited high sensitivity, specificity, negative and positive predictive values. Interference was seen with direct thrombin inhibitors, unfractionated heparin and low molecular weight heparins, but not VKAs or direct factor Xa inhibitors. With this study, TSVT/ET are validated for LA detection in non-anticoagulated patients and those on VKAs or direct factor Xa inhibitors.

Qualitative classification of anticardiolipin and anti-beta2glycoprotein I antibodies – Katrien Devreese

The laboratory diagnosis of APS remains a challenge. LAC tests, as well as solid phase assays for aCL and aβ2GPI show methodological shortcomings, and the methodology is not standardized. For solid phase assays disagreement between different commercial assay kits and methods is observed. Illustrated by a sample of the ECAT Foundation (External quality Control program) differences in titer and semiquantitative classification is shown. Large inter-lab variability is observed for qualitative classification for aCL IgG, aCL IgM, aβ2GPI IgG, aβ2GPI IgM. The qualitative classification of aCL and aβ2GPI needs to be harmonized, since no clear rules are available how to do so. Different strategies can be used to identify thresholds for low-medium-high positivity. A clinical approach (ROC curve analysis) for establishing the threshold for low-moderate-high antibody positivity for automated systems results is in higher agreement with the ELISA based thresholds of 20-40-80. Titers in aPL differ between systems and therefore the ranges for classification into low-moderate-high applied for ELISA cannot be transferred to other platforms. Calculation of likelihood ratios confirms that 80 GPL for ELISA indicates the highest risk. Equally, for automated systems with adapted threshold, the LR for thrombotic and obstetric APS increased significantly in the moderate and high range. This model of defining the ranges for classification based on ROC curve analysis is not feasible for many labs. Alternative methods will be explored using standard materials.

Standardization of thrombin generation assays-Marisa Ninivaggi

Thrombin generation assay (TG) may be a useful tool in diagnosing patients with hyper- and hypocoagulability. Lack of standardization in performing the assays contributes largely to poor correlation between assays and study results. The current lack of standardization remains a major issue in the setting of TG, as illustrated in recent survey of the ISTH/SSC indicating differences in pre-, analytical and post-analytical factors amongst users. These factors may considerably affect the between-laboratory reproducibility of results. Based on the results of a survey (presented two years ago) and a current review of the literature, along with insights and strong consensus of key investigators in the field, a guidance for measurement of TG in a clinical setting is presented. Recommendations on blood drawing, handling, processing and sample storage, reagent concentration and source, analytical conditions on dilution of samples and temperature, calibration and replicate testing, calculation and interpretation of results and reference values are addressed to help in reducing the interlaboratory variation. These recommendations aim at harmonization between methods and laboratories to support the application of TG in patient diagnosis and management. These recommendations for the measurement of thrombin generation has been published last year.
A new projects will be started to compare thrombin generation (on distributed material) with an in-house protocol compared to the measurement with our standardized protocol, performed in laboratories worldwide.

**Update on the SKYLARK project (Successive follow up of antiphospholipid antibodies fluctuations in patients with clinical Sydney criteria for APS) and the NYMPHEA project (Antiphospholipid antibodies and lymphoma) - Cristina Belizna**

Nymphea: 5 international centers participate. Over 300 patients are registered now in the retrospective study. The online questionnaire is available on ISTH REDCAP. Results expected in 2022. Other new centers are welcome. SKYLARK: the retrospective register is ongoing. Online questionnaire is available on ISTH REDCAP. Other new centers are expected and welcome. For the prospective study 36 months: one year of inclusion and 2 years of follow up. 3 international centers (Spain, Serbia, France) agreed to participate.
Models of Thrombosis and Hemostasis

Chairman: Laura Gutiérrez

Co-Chairs: José A. Díaz, Margarethe Geiger, Peter Gross, Maxim Shaydakov, Beatrice Hechler, and Olivia R. Palmer.

ISTH 2021 SSC Meeting (Virtual)

17th July 2021 (13:30-15:30, Room 7)
Speakers pre-recorded their respective talks, and the session was streamed with the presence (live) of the chair, speakers (with the exception of Tetsuya Hara, who was not able to join the session) and moderators, who participated in the discussion as programmed. The audience was able to send or submit questions and comments through the available chat, and most of the questions were answered during the session streaming. Unanswered questions (i.e. when speaker was not available) were handled by the moderators, encouraging the participant to contact the speaker directly. The experience was outstanding and the IT support was perfect.

Session Program:
The program this year contained two parts, one covering selected pre-clinical models of platelet-related pathologic roles in different clinical backgrounds (in which external speakers participated), and the second one covering a joint project on Platelet Proteomics with the Platelet Physiology and Predictive/Diagnostic Variables SSCs, that we will pursue in the coming years.

Closing the first part, we gave time to one speaker (Dr. Rohith Jesudas) that had contacted our SSC enquiring for advice regarding the demand for diagnostic and prognostic models in hypermobile Ehlers-Danlos syndrome (directed to understand and better manage the bleeding presented in some patients; email 4th January 2021). Dr. Rohith Jesudas contacted us after the ISTH meeting (email 2nd August 2021) to thank us for the opportunity that the exposure at the ISTH meeting gave him, as he established a potential future collaboration with Dr. Bobby Lee (UNC, North Carolina, USA) who attended to the SSC session.

After an introduction from the Chairman, Laura Gutiérrez, the session as such started.
FIRST PART: Moderators, Peter Gross and Beatrice Hechler
1. In Vivo Imaging of Venous Thrombus and Pulmonary Embolism Using Novel Murine Venous Thromboembolism Model.
   Tetsuya Hara (Kobe, Japan).
2. Preclinical models of TRALI.
   Rick Kapur (Amsterdam, The Netherlands).
3. Preclinical models of platelet allo-immunization / refractoriness.
   Jacqueline Poston (Virginia, USA).
4. Bleeding in hypermobile Ehlers-Danlos syndrome: Demand for diagnostic and prognostic models to better understand this clinical presentation.
   Rohith Jesudas (Memphis, USA).

SECOND PART: Moderators, Margarethe Geiger and John-Bjarne Hansen (Predictive/Diagnostic Variables SSC Chairman).
5. State-of-the-art advances and cautionary insights in proteomics and systems biology studies of platelet phenotype and function.
   Joseph Aslan (Oregon, USA).
6. Round table: “Relevance of platelet/beyond platelet proteomics and the challenges encountered by young researchers”.
   Johan Heemskerk (Maastricht, The Netherlands), Ángel García (Santiago de Compostela, Spain), Patricia Maguire (Dublin, Ireland) and Patricia Martínez-Botía (Oviedo, Spain).

SSC Session Stats (See Appendix):
Participants: 464  Questions: 18

Publications:
Publications since last ISTH 2020:

- Injury measurements improve interpretation of thrombus formation data in the cremaster arteriole laser-induced injury model of thrombosis.
  Grover SP, Bendapudi PK, Yang M, Merrill-Skoloff G, Govindarajan V, Mitrophanov AY, Flaumenhaft R.
Publications in preparation:
- Standardization on selection and conduct of murine bleeding models.
  Brian Cooley. ISTH SSC grant. Dr. Cooley sent a manuscript to the SSC for evaluation, and the comments by SSC co-chairs was sent to him in November 2020. Laura Gutiérrez has sent him an email regarding the status of the manuscript review (email 1st September 2021). Dr. Cooley replied immediately notifying that he will send the revised version soon, and apologizing for his delay due to personal reasons.

Future publications planned:
- Platelet Proteomics, PLT depletion, TRALI pre-clinical models reviews on the state-of-the-art and identification of demands of standardization (see below current projects).

Past Projects:

Bleeding time (Final Report)
Project awarded by ISTH. Dr. Cooley’s work is at the end stage and his work. It was presented in Melbourne. Dr. Cooley did not complete the manuscript submission. Laura Gutiérrez has sent him an email regarding the status of the manuscript review (email 1st September 2021, see above).

The cremaster arteriole laser-injury model of thrombosis – standardization
This project was led by Dr. Steven Grover, PhD, from UNC. Dr. Grover sent the manuscript to be reviewed by our SSC. An initial review of it with 26 points was evaluated by the Animal, Cellular and Molecular Models SSC with feedback to be considered by the authors.
We are supporting our members working on standardizations on techniques/subjects under the umbrella of our SSC and endorsement of manuscripts will depend first on our revision and then passing on our recommendation to the board.
(Manuscript accepted in JTH, see above).

Current Projects:
All projects were presented at ISTH 2020 (Virtual). Below the project descriptions, which are all characterized by being executed in two phases: bibliographical (to assess the state-of-the-art, define demands and the experimental phase) and experimental (standardization).
Pre-clinical Models of Platelet Transfusion and Platelet Depletion

This project has been initiated as joint effort together with the Platelet Physiology SSC (Chairman, Marie Lordkipanidze and Co-Chair Justin Hamilton). We have initiated conversations, however, Justin Hamilton left the SSC and the project has been delayed. In brief, it was first suggested to elaborate a rigorous bibliographic review of platelet transfusion animal models and protocols, and platelet depletion animal models and protocols (and how they may be combined for specific studies). This information would be set on a concise review where potential applications, and standardization demands are set for future experimentally-driven projects.

We need to discuss about this project again with Marie Lordkipanidze, regarding the review writing, and also considering that we may have a manuscript for revision (from our group, Laura Gutiérrez), studying the impact on hematopoiesis by platelet depletion (as presented in the ISTH 2020 SSC Session). The plan is to arrange a meeting in September-October 2021 to set the milestones of the project.

Pre-clinical models of TRALI

Coordinator: Beatrice Hechler.
Participants: Olivia Palmer, Maxim Shaydakov, José Díaz and Laura Gutiérrez.
Potential collaboration with SSC PLT Immunology.

This project is a logical follow-up of the previous one, as was represented on the SSC meeting, with the talk by Rick Kapur.
Update: on the next SSC meeting, planned in September-October 2021.

Pre-clinical models of Pulmonary Embolism

Coordinator: Peter Gross.
Participants: Olivia Palmer, Maxim Shaydakov and José Díaz.

This project, proposed by Peter Gross, is at the core of the interests of our SSC. This was represented on the SSC meeting, with the talk by Tetsuya Hara.
Update: on the next SSC meeting, planned in September-October 2021.

Cellular Models of Megakaryopoiesis and Platelet Production, including co-culture and organoids

Coordinator: Beatrice Hechler.
Participants: Laura Gutiérrez.
Potential collaboration with SSC PLT Immunology, PLT Physiology and Vascular Biology.
We propose to describe and study current methods of primary cell culture, depending on species and tissue of origin, and cell lines, at different levels, including co-culture/flow and organoids. Update: on the next SSC meeting, planned in September-October 2021.

**Platelet Proteomics and Platelet manipulation**
Coordinator: Laura Gutiérrez.
Participants: Margarethe Geiger.
Potential collaboration with SSC PLT Physiology and Predictive/Diagnostic Variables.

We had several meetings with members of the other SSCs, which later expanded to a rich list of collaborators, some of them external to the SSCs.

These are the participants of the working group, which include senior and junior researchers:

Samuel Tassi-Yunga and Joseph Aslan, Paulina Szklanna and Patricia Maguire, Hervé Falet and Marie Lordkipanidze, Patricia Martínez-Botía and Laura Gutiérrez, Ángel García, Johan Heemskerk, Margarethe Geiger, Matthew Rondina, and John Bjarne Hansen.

We have started writing a comprehensive review of the state of the art of platelet proteomics (and beyond, i.e. microvesicles), where are the limitations of this approach and how and at which steps should standardization become an advantage to the field, and potential uses of platelet proteomics thereof, as biomarkers (predictive or diagnostic).

Update: the sections of the review have been assigned to writing groups. We will have a meeting in November-December with hopefully a sensible advance on the writing.

**Co-chair rotations:**
This year, our SSC has received 4 co-chair applications (Frederik Denorme, Alexander Brill, Steven Grover and Viviana Clavería), and, according to the schedule, three co-chairs are rotating-off (José Díaz, Margarethe Geiger and Maxim Shaydakov).

**CANDIDATE SELECTION PROCEDURE:**
The SSC chairs selected 3 out of the 4 candidates based on the sent curricular/interest documentation and thinking on the potential added value they could bring to the SSC.

All votes were added up and the 3 top rated candidates were selected.
- Frederik Denorme was selected as co-chair based on his expertise and interest on standardizing pre-clinical and \textit{in vitro} models of ischemic stroke.

- Steven Grover was selected based on her past contributions to the SSC on Vascular Biology and his expertise and interest on standardizing pre-clinical and \textit{in vitro} models of venous thrombosis.

- Valeria Clavería was selected as co-chair based on her expertise and interest on standardizing \textit{in vitro} models of platelet aggregation (high shear rate). She also had a positive evaluation after a scheduled interview with the chairman of the SSC, and sent a bio-sketch along. Her candidacy was supported by the rest of co-chairs.

We are convinced they will positively contribute to the SSC actions, and we will coordinate on the next meeting scheduled in September-October 2021, to which project they will enroll and strengthen, or whether they have project proposals to be considered.
**Popular questions**

@Rick Kapur - I may have missed it but, have you implemented actual platelet transfusion on your model?

Dr. Hara: Cool model. Do you know if factor XIII activity alters thrombus composition in this model?

Tetsuya - have you tracked whether the thrombi in your model resolve similarly to what is seen in humans and other models, e.g., with an increase in macrophage infiltration over time, along with the formation of new vascular channels? If so, how long does this take in your model?

Rick - does the TRALI in your model resolve? If so, how does the repair occur and over what time course? In LPS-induced models of ALI, for example, endothelial regeneration and junction re-annealing are seen in ~72-96hrs...

@Rick Kapur - do platelets then influence the gut microbiome and vice versa?

**Influential users**

- Laura Gutiérrez
- Blandine Maitre
- Colin Evans
- Grace M Thomas
- Patricia Maguire
1. Introduction

Jun Teruya, MD, DSc

Dr. Teruya gave introductory remarks of Plasma Coagulation Inhibitors subcommittee. Dr. Teruya will rotate off Chair of the subcommittee after this meeting and remain as a Co-Chair for one year. Dr. Vera Ignjatovic will be the Chair of the subcommittee after this meeting. Dr. Morishita will rotate off Co-Chair after this meeting. Sadly, Dr. Van Cott passed away in April this year. Therefore, 2 Co-Chair positions will be open.

Following is published SSC PCI communications and a research article funded by ISTH in 2020-21:
BRIEF REPORT

Racial differences in protein S Tokushima and two protein C variants as genetic risk factors for venous thromboembolism

Hiroko Tsuda MD, PhD1 | Kenta Noguchi PhD1 | Doyeon Oh MD, PhD2 | Zsuzsanna Bereczky MD, PhD3 | Lai H. Lee MD, PhD4 | Dongchon Kang MD, PhD5 | Luci M. S. Duus PhD6 | Maria das G. Carvalho PhD6 | Eriko Morishita MD, PhD7 | The SSC Subcommittee on Plasma Coagulation Inhibitors of the ISTH

2. In Memory of Professor Elizabeth (Betsy) Van Cott

Jun Teruya, MD, DSc

Dr. Van Cott became Co-Chair of SSC PCI in 2020. She was Medical Director of Coagulation at Massachusetts General Hospital, Harvard Medical School, Boston, USA. Dr. Van Cott was a world-renowned coagulation expert. Her clinical and academic impacts were broad and deep. She served multiple societies in her field. She was a president of NASCOLA (North American Specialized Coagulation Laboratory Association). She was an editor/associate editor of several journals. She published many academic papers and chapters and shaped current laboratory guidelines in coagulation. Especially she contributed to creating many guidelines. Dr. Van Cott loved her work, especially her training of her residents and
collaborations with her colleagues. This was recognized when she recently received the prestigious William Silen Lifetime Achievement Excellence in Mentoring Award from Harvard Medical School.

3. Antithrombin (AT) Deficiency Registry – An Update

Zsuzsanna Bereczky, MD

The aim of establishment of an international registry was to collect large amount of data from patients all over the world to help in answering the most important, still unanswered questions in this disease since clinical studies involving large number of patients and focusing on multiple clinical issues are hardly available and the idea of risk-differentiation according to subtypes leading to different therapeutic considerations is emerging. As an example, type II heparin binding site (IIHBS) AT deficiency is heterogeneous in terms of its clinical and laboratory behavior. In an in vitro study of plasma samples and in vitro expressed IIHBS mutants we have demonstrated differences in the characteristics of heparin binding; AT Padua had the weakest affinity to heparin according to the results of surface plasmon resonance studies, while AT Budapest 3 (ATBp3) had the strongest one among the mutants. In silico studies supported these findings, however in case of ATBp3 significant molecular instability was also found suggesting the presence of a quantitative component in its pathogenicity. In order to translate these biochemical findings into clinical language collection of more patients is required.

AT deficiency registry was established in 2020 by the Plasma Coagulation Inhibitors SSC with the major goals as follows:

- It allows a structured and standardized data collection suitable for statistical analysis
- It ensures the collection of high number of patients with the same AT deficiency subtype (mutation) and with the same additional risk factors
- Prospective data collection is possible to follow cases already introduced into the registry
- It helps in complex diagnosis of AT deficient patients (including molecular genetic studies) for those who do not have the possibility by connecting different hospitals to each other
- Data introduced into the registry is open for the scientific community, aggregated data can be exported from the registry continuously

The registry is supported by the RedCap Installation.

The presentation describes the registry and gives a point-to-point explanation of actions to be done for users. Most important steps are:

- The first action to be done, when joining to the registry is to register at this link: https://redcap.isth.org/surveys/?s=J4YA4E7AFN. By the registration of the institute/hospital, an accession username and password are sent to users via e-mail. This username serves as entry to the AT deficiency registry itself.
- In the main registry form, after clicking on “add/edit records” new patients’ data can be introduced.
• First, laboratory data of AT deficiency diagnosis, then genetic results are asked, where the mutation should be described according to the recommendations of the Human Genome Variation Society to avoid misleading conclusions.

• Clinical events are classified into three major groups: venous thrombotic symptoms, arterial events and pregnancy complications (if relevant). The registry is user-friendly and easy to follow. Plasma Coagulation Inhibitors SSC is asking the scientific community for help in widening our registry to reach the highest number of patients and to execute a successful project together. (In case of questions, please contact Dr. Zsuzsanna Bereczky, e-mail: zsbereczky@med.unideb.hu)

4. Beta Antithrombin, Measurement and Clinical Significance

Javier Corral, PhD

Antithrombin has two physiological glycoforms, alpha with 4 N-glycans, and beta lacking glycosylation at Asn165 with increased heparin affinity and reduced half-life in plasma. Despite being the minor form in plasma (10% of total circulating antithrombin) beta antithrombin has strong anticoagulant, anti-inflammatory, and antimicrobial activities, which support the specific analysis and quantification of this glycoform in different backgrounds through different available methods to understand its physiological and pathological relevance.

5. Tissue Factor Pathway Inhibitor (TFPI) in Bleeding Disorders

Alan Mast, MD

The role of elevated plasma TFPI in bleeding disorders was discussed in the context of its physiological locations and biochemical activities. TFPI is expressed in two isoforms in humans with TFPIβ localized to the endothelial surface and TFPIα within plasma and platelets. Recent data indicates that heparin releasable TFPIα is in the extracellular matrix rather than on the endothelium glycocalyx. A paper by Mehic and colleagues published this year found that among 620 patients with bleeding of unknown cause plasma TFPI was elevated compared to control groups and could contribute to the unexplained bleeding. However, none of the 620 patients had mutations in the FV B-domain that are associated with 5- to 20-fold elevated plasma TFPIα concentration. Three mutations have been identified in the FV-B-domain that produce a form of FV called FV-short. FV-short has high affinity for TFPIα and causes the autosomal dominant bleeding disorders, FV East Texas, FV Amsterdam, and FV Atlanta. Another family from Indiana in the USA has been found to have the FV East Texas mutation. Some members of this family have experienced chronic ulcerative mucositis and poor wound healing suggesting that there could be alterations in TFPIα within the extracellular matrix. Increased recognition of the contribution of elevated plasma TFPIα to bleeding disorders with measurement of plasma TFPIα and DNA sequencing of the FV B-domain is needed. As more patients with bleeding disorders associated with elevated TFPIα are recognized, we can define the pathophysiology underlying the symptoms observed.
6. Developmental Changes in the Protein C Structure

Vera Ignjatovic, PhD

Protein C plays a role in a number of biological functions including inhibition of coagulation, stimulation of fibrinolysis, inhibition of inflammation, and inhibition of apoptosis. Structurally, protein C is known to be highly post-translationally modified, including modifications such as hydroxylation, carboxylation, glycosylation, and phosphorylation, with the PROC gene associated with 370 known mutations. Protein C deficiency is associated with thromboembolic complications and the homozygous protein C deficiency, manifesting within hours of birth with life-threatening purpura fulminans requiring urgent protein C replacement, to prevent large- vessel thrombosis. Protein C concentrates purified from human plasma have been used since the 1990s, however, their use is based on the premise that protein C structure is comparable in children and adults. This presentation outlined the existing evidence for age-specific differences in protein C concentration, function, and structure and suggests that the current dosing strategies for protein C replacement in children need to be assessed with specific consideration of age-specific differences in the protein C structure.

7. Non-Factor V Leiden Activated Protein C Resistance

Gary Moore, PhD

Factor V Leiden (FVL) is responsible for >90% of hereditary activated protein C resistance (APCR) and international guidelines on laboratory testing necessarily concentrate on detection of FVL in the first instance. However, a variety of other, albeit rare, FV variants are known that confer resistance to activated protein C, some of which are clinically significant and warrant further investigation where FVL-negative APCR is encountered. Similarly, the multi-faceted phenomenon of acquired APCR can give rise to a phenotype-genotype discrepancy. FV variants such as FV Cambridge, FV Hong Kong and FV Arg486Lys exhibit mild in vitro APCR but clinically represent little or no thrombotic risk. Polymorphisms in the HR2 haplotype do not confer APCR themselves but increase thrombotic risk when present in compound heterozygotes with FVL. Conversely, thrombotic risk due to the APCR conferred by FV Nara, FV Bonn and FV Besançon is similar to or greater than that with FVL. Acquired APCR is commonly due to altered levels of certain coagulation factors or inhibitors or may be antibody mediated. It can be an independent risk factor for VTE and phenotypic detection is assay dependent. Elevated FVIII or FII are associated with acquired APCR and best detected with APTT-based assays without pre-dilution, whilst reduced free protein S or TFPI are the main determinants of APCR detected in thrombin generation assays. Acquired APCR via antibodies to protein C, protein S or FV is often associated with antiphospholipid syndrome.

8. Which Test to Choose: Activated Protein C Resistance First or Factor V Leiden Directly?

Jun Teruya, MD, DSc
Main reason of performing activated protein C (APC) resistance assay is screening for Factor V Leiden (FVL), which is one the most common hereditary thrombophilia risk factors among Caucasians. In a practical setting, there are two questions: 1. Should labs perform APC resistance assay first and then Factor V Leiden if it is positive? 2. Can acquired positive APC resistance assay be an independent risk factor for thrombophilia? As in all functional coagulation tests, there are a limitation and interference of the assay. They include different cut-off values of APC resistance assay among genders, use of anticoagulants especially direct oral anticoagulants, and presence of lupus anticoagulant. In terms of reagent cost, FVL assay is more costly than one assay of APC resistance assay. However, cost analysis should be carefully performed considering the volume of tests, hospital setting (having molecular diagnostic lab or not), or reference lab vs. hospital lab. There are 2 conditions that show discrepancy between phenotype (APC resistance assay) and genotype (FVL); stem cell transplant and liver transplant. In those cases, APC resistance assay needs to be performed. Congenital APC resistance without FVL can be a risk factor for thrombophilia although it is rare. The question of significance of acquired APC resistance needs to be investigated.

9. Updates on Using Recombinant Thrombomodulin for Disseminated Intravascular Coagulation

Eriko Morishita, MD

Thrombomodulin (TM) is an anticoagulant glycoprotein expressed on the surface of vascular endothelial cells. It is well known that the TM–protein C system converts thrombin from a procoagulant enzyme to an anticoagulant enzyme on vascular endothelial cells. Recombinant TM (rTM) consists of the extracellular portion of TM. Compared to natural TM, rTM has an approximately equivalent binding affinity for thrombin and cofactor activity for protein C activation. rTM is currently available only in Japan. rTM may be one of the important therapeutic approaches as an anticoagulant for DIC in sepsis, hematological malignancies, and solid tumors. However, further randomized, controlled trials with adequate patient numbers are required to better define the therapeutic role of rTM.
Minutes for SSC Platelet Physiology business session

Virtual meeting, Saturday, 17 July 2021 from 1:30 – 3:30 pm / 5:30 – 7:30 pm UTC

Chair: Marie Lordkipanidzé

Co-Chairs: Marie-Christine Alessi, Joseph Aslan, Marina Camera, Hervé Falet, Sofia Ramström, Matthew Rondina

Past Chair (invited): Paolo Gresele
Collaborator (invited): Maha Othman

The session was moderated by Hervé Falet (United States).

- Overview of the Platelet Physiology SSC and Projects

Marie Lordkipanidzé

The business session started with an introduction by Dr. Lordkipanidzé (Canada) who gave an overview of the mandate of the Platelet Physiology SSC, illustrated the Platelet Physiology subcommittee webpage within the ISTH site, including the options for interaction by registered members, and encouraged the audience to register. A call for application for the upcoming cycle (2021-2022) for a Co-Chair vacancy was made.

Publications of the last year were highlighted:

Upcoming publications were also presented:
- Standardization of flow cytometry for the assessment of inherited and acquired disorders of platelet number and function
- Led by Larry Frelinger and Jose Rivera Pozo
  - Under review in JTH
- Consensus/guidance on the methods for the study of platelet secretion
  - Led by Diego Mezzano
  - In preparation
- Guidance on the measurement of platelet dimensions: methods and clinical use
  - Led by Patrizia Norris
  - In preparation
- Conservation and shipment of platelets for platelet studies: a scoping review
  - Led by Marie Lordkipanidzé
  - In preparation

New and ongoing projects that could not be presented during the session were briefly updated:

- Immune Thrombocytopenia and Obstetric Neuraxial Anesthesia at Low Platelet Counts – An International Registry
  - Led by Maha Othman, Ann Kinga Malinowski, Nadine Shehata
    - In collaboration with Women’s Health issues in T&H SSC and the Platelet Immunology SSC
    - Call for participation was published, with particular outreach towards clinicians in the field of anesthesiology:

- Identification of Markers That Can Separate Procoagulant Platelets From Apoptotic Platelets
  - Led by Emma Josefsson & Sofia Ramström
    - In collaboration with Vascular Biology SSC
  - This new project was presented in the Vascular Biology SSC session by Emma Josefsson

- Platelet proteomics in health and disease
  - Led by Laura Gutierrez
    - In collaboration with Models of Thrombosis and Hemostasis SSC
    - Contributions from Joe Aslan and Hervé Falet from the Platelet Physiology SSC
  - This new project was presented in the Models of Thrombosis and Hemostasis SSC session by Joe Aslan as part of a roundtable discussion to delineate priorities
Ongoing collaborations with the other SSC of the ISTH were also highlighted:
- Genomics in Thrombosis and Haemostasis
- Women's Health Issues in T&H
- Vascular Biology
- Platelet Immunology
- Models of Thrombosis and Hemostasis

Ongoing projects were presented in more detail in this session (as described below):

- Evaluation of the ISTH Bleeding Assessment Tool (BAT) for the assessment of inherited platelet disorders: individual-level meta-analysis
  Paolo Gresele (Italy)

Dr. Gresele presented the preliminary results of an individual patient level meta-analysis of the studies assessing the utility of the ISTH BAT bleeding score for the clinical evaluation of patients with inherited platelet disorders (IPD). Individual patient data were obtained from 6 of the 7 published studies and cumulative data from the seventh, allowing to analyse data from a total of 1615 subjects, 678 of whom with IPD. Results showed that the ISTH-BAT is useful to discriminate IPD from healthy controls and inherited platelet function disorders from inherited thrombocytopenias with good accuracy. IPFD showed a higher number of haemorrhagic symptoms than inherited thrombocytopenias and vonWillebrand disease. Finally, the ISTH BAT correlated with results of platelet function tests.

- Biomarkers of in vivo platelet activation — a systematic review and meta-analysis
  Marina Camera (Italy)

Dr. Camera presented the results of a collaborative project between the Platelet Physiology SSC and the Vascular Biology SSC on Biomarkers of in vivo platelet activation: a systematic review and meta-analysis. The overall aim of the project was the identification of platelet activation markers predictive of cardiovascular events in order to improve the thrombotic risk stratification of patients with coronary artery disease. The analysis was focused on the soluble platelet activation markers in order to be easily measured by ELISA and thus potentially used in a wide manner all over the world. Not enough studies eligible for meta-analysis to identify markers of activation predictive of cardiovascular events were found. Thus the aim of the study was modified in order to verify whether any of the soluble marker is able to discriminate the pathological condition and/or its clinical presentation (acute versus stable coronary artery disease) from the healthy status. Out of 16 markers studied only 6 passed the selection criteria for meta-analysis and this was mainly due to a wide statistical heterogeneity i.e. differences in matrices, unit of measurement and clinical setting in terms of time of blood withdrawals at hospital admission. The results of this study
highlight the need of ad hoc designed longitudinal studies with comparable outcome and providing comparable informations in order to be used for analysis to implement the thrombotic risk stratification. In addition an individual patient level data approach could be considered in order to minimize the heterogeneity.

- A Multicentric Comparison of Platelet Aggregation Agonists Against NIBSC Standards: An Update
  Marie-Christine Alessi (France)

Dr. Alessi presented the first results of the ISTH-funded “Platelet Aggregation Standardization Project – PAPS Study”. This study aims to assess the interest of developing reference activators to standardize platelet aggregation. 28 laboratories participated in the study (25 from and 3 outside of Europe). Comparator reagents were distributed to each site. Each tested their own activators and the comparator on the same sample. 160 platelet-rich plasmas (PRPs) were tested. The results obtained with ADP were presented. They were not influenced by age, gender, and platelet count or investigator site. A general linear model showed the significant contribution of the source of the reagents to the variability of the maximal intensity of platelet aggregation. Three different ADP sources were more particularly assessed and confirm the interest of introducing an international comparator. While one of the reagents appeared to agree perfectly with the comparator, the other two showed differences, especially for low concentrations of ADP. In addition different ADP response profiles were observed with low and high responders. Overall these results indicate subtle differences in individual responses when comparing responses from in house and “standard” reagents indicating that the use of a standard reagent can help to harmonize results between laboratories.

- Standardizing Platelet Transcriptomics for Discovery, Diagnostics, and Therapeutics in the Thrombosis and Hemostasis Community (STRIDE) Study: An Update
  Matthew Rondina (United States)

Dr. Rondina summarized the progress made over the last year on the ISTH-SSC Large Project Grant supporting the STRIDE (Standardizing Platelet Transcriptomics for Discovery, Diagnostics, and Therapeutics in the Thrombosis and Hemostasis Community) Study. The goal of this study is to compare head-to-head two techniques for platelet isolation for transcriptomic studies (washed platelets and bead-depleted platelets). The STRIDE Study has completed harmonization and validation of protocols for platelet isolation from human samples, and distributed standardized kits to each enrollment site. Investigators begun enrollment of participants recruited at 6 sites across the globe, and will recruit approximately 50 male and female participants. Study enrollment is anticipated to be completed in late 2021, after which RNA-sequencing will be performed on all study samples.
Dr. Ramström and Dr. Jourdi presented a project launched in collaboration between the Platelet Physiology and Platelet Immunology SSCs that aims to generate an international expert consensus on relevant methods for the evaluation of platelet functions in patients with thrombocytopenia. Following a literature review, uncertainty in well-established methods, mainly light transmission aggregometry and flow cytometry (regarding pre-, post- and analytical aspects), and other potential novel methods of interest were identified. An inventory of key researchers who published in this field was also established. They will be invited to participate to expert panel discussions together with the interested ISTH members in order to elaborate a SSC communication draft that will be posted on the ISTH webpage for community review before finalizing a guidance document and publishing it during 2022. Researchers and professionals are strongly invited to participate to this project either by being part of the expert panel discussions or by sharing any item to be included in the guidance document. They can also ask to be notified when the draft paper is ready for review.

Dr. Ramström and Dr. Jourdi shared their email addresses (sofia.ramstrom@oru.se; georges.el.jerdi-jourdi@umontreal.ca; rsharma@versiti.org) and look forward to the input of each professional interested in this project.

PT-VWD is a rare autosomal dominant bleeding disorder; a functional defect in the platelet glycoprotein Ibα (receptor for VWF) resulting from a gain of function mutation in GP1BA gene. This defect creates a supersticky GPIbα protein that binds VWF and clears complex with resultant mucocutaneous bleeding, loss of HMWM and variable degree of thrombocytopenia. The disease is almost identical to 2B VWD; also functional defect but in VWF gene. The discrimination is critical as it directs treatment. Genetic analysis can confirm whether the mutation within VWF or GP1BA gene. Most cases of PT-VWD have started with a misdiagnosis as type 2B VWD. The international PT-VWD registry since 2007, has helped improve the awareness of the diseases and alerted the scientific community to the diagnostic dilemma and the role of genetic analysis for correct identification. A registry report was published in JTH in 2016. More recently and in collaboration 11 experts from 10 countries, and through RAND based approach we obtained a formal consensus among experts, an ISTH guidance document to guide appropriate diagnosis and management of patients with this disease was released late last year in JTH.

The issue of nomenclature in PT-VWD has been discussed on several occasions in literature. The current name may generate confusion with Von Willebrand Disease. It
does not accommodate the name of the defective protein or indicate the pathologic nature or the functional defect.

Additionally for mutation nomenclature, the use of old vs new numbering systems has been confusing. We wanted to re-visit the disease’ nomenclature and seek contribution from ISTH members and the scientific community on new proposal; particularly since PT-VWD has become a widely used term, before a standardized nomenclature can be established.

This is joint project from the PLT physiology, VWF and Genomics SSCs. We established a working group to explore issues around nomenclature, proposed possible names, sought input from experts in the field, and designed a survey to capture the ISTH members’ and scientific community’s views. We constructed 4 focused questions and will ask participants to rank their top three names from among the following list- rationale provided for each

1. Platelet type- Von Willebrand disease (PT-VWD)
2. Pseudo - Von Willebrand disease (Pseudo-VWD)
3. Platelet type- pseudo - Von Willebrand disease (PT-Pseudo VWD)
4. Platelet- 2B Von Willebrand disease (PLT- 2B VWD)
5. Platelet glycoprotein Ib alpha-Von Willebrand factor disorder (PLT GPIb alpha - VWF disorder)
6. Platelet glycoprotein Ib alpha gain-of-function disorder (GPIb alpha GoF - disorder)
7. Platelet GP1BA gain-of-function disorder (GP1BA GoF - disorder)
8. Bernard-Soulier gain-of-function (BS GoF)

The survey will be administered during or right after the congress. The survey will be open until August 31 2021. Data will be analyzed in September-October 2021 and reported.

In terms of attendance, there was a total of 286 attendees in the virtual session. Discussion from the audience was active and lively when interactions were solicited. Many participants emailed speakers with follow-up questions or expressed desire to participate in the SSC projects. There were technical issues with emails for recording and uploading talks in advance. However, all speakers were able to upload their talks and no technical glitches were present during the session. Within the constraints of the virtual meeting, the organization and virtual set-up were excellent. Each speaker kept to time, with the session running the expected 2 hours. Some ongoing projects could not be presented in this session due to time constraints.
Minutes from the virtual SSC on Predictive and Diagnostic Variables in Thrombotic Disease, July 17, 2021

The program was presented on a digital platform with prerecorded presentations and live poll questions followed by a live questions and answers session. A short introduction was given by the chair, Professor Hansen, with an overview of new project initiatives and ongoing projects in the SSC along with an encouragement to the audience to engage in ongoing and new projects in the SSC. Thereafter, Dr. de Wit presented an update on the project of standards for diagnosis of PE and DVT in cancer patients. The results of a systematic review and meta-analysis on the diagnostic accuracy of D-dimer and clinical probability estimation showed that D-dimer is highly sensitive with low to moderate specificity. Around one in 10 people with cancer can have DVT or PE excluded when combining low clinical probability with a negative D-dimer. Physicians across the world have also been interviewed on how they test people with cancer for VTE and found that most use imaging as the only test. Patients were also interviewed and they expressed deep trust in their physicians and did not mind having additional imaging.

Second, Dr. Morelli gave an update on risk factors and predictors of VTE in stroke. As a first initiative to improve prediction of venous thromboembolism (VTE) after ischemic stroke, a systematic review of demographics, clinical factors, and biomarkers for the occurrence of VTE in patients with ischemic stroke was performed. Of 4674 identified records, 26 studies met inclusion criteria and were included. Advancing age, female sex, measures of stroke severity, atrial fibrillation, cancer, history of VTE, elevated levels of D-dimer and C-reactive protein, and indices of dehydration were associated with VTE in multivariate models in two or more studies. The investigators conclude that this systematic review informs on several risk factors and predictors for the occurrence of VTE in patients with ischemic stroke. However, the majority of the included studies were of poor quality and had an overall high risk of bias. Dr. Morelli concluded that future research should focus on further identification and confirmation of risk factors which can be used to guide the development of risk prediction models and improve patient stratification.

Thereafter Dr. Klok discussed the need for standardization of managing the risk of bleeding during anticoagulant treatment for VTE. He argued that for patients with venous thromboembolism (VTE), prediction of bleeding is relevant throughout the course of treatment, although the means and goal of this prediction differ between the subsequent stages of treatment. Even in the absence of fully established risk prediction schemes and outcome studies using a prediction scheme for treatment decisions, the present evidence supports screening for and targeting of modifiable risk factors for major bleeding, as well as the application of decision rules to identify patients at low risk of bleeding complications, in whom long-term anticoagulant treatment is likely safe. In this lecture, Dr. Klok discussed preliminary results of a narrative review on biomarkers and risk assessment models for bleeding risk during anticoagulant treatment for VTE and gave an overview of the the SSC project to standardize the assessment of the bleeding risk of VTE patients.

Dr. de Jong presented the International Consortium for Health Outcomes Measurement in VTE: ICHOM-VTE initiative. In venous thromboembolism (VTE) care, binary outcomes such as bleeding, recurrence and mortality, are routinely used but do not always fully capture what is most important to patients. To further integrate the patient’s perspective when evaluating outcomes of care,
measurement of patient-relevant outcomes matters. The International Consortium for Health Outcomes Measurement (ICHOM) project on VTE was initiated with the aim to develop and validate a standard set of patient-relevant outcome measures for patients with deep vein thrombosis and/or pulmonary embolism for use in clinical care, to measure the effectiveness of treatment, support clinical decision-making and facilitate quality improvement. A diverse group of international VTE experts and patient representatives is convened to reach consensus via Delphi processes on outcomes and currently available outcome measures, that capture what matters most to VTE patients. Dr. de Jong expected the Standard Set to be finished at the end of 2021 or beginning of 2022 and that it would pave the way for a shift in focus to the value that care creates for patients, allowing for the introduction of value-based healthcare in VTE.

Next, Dr. Robert-Ebadi presented the establishment of an international core outcome set for clinical trials of interventions for venous thromboembolism – The VTE-COS project. The outcomes reported in VTE treatment studies focus mainly on efficacy and safety outcomes, such as VTE recurrence and bleeding, with great heterogeneity in outcome reporting as well as in outcome definitions and measurement. There is currently no formal consensus on which outcomes should be collected in VTE studies. The aim of the VTE-COS project is to define a Core Outcome Set (COS) of valid, homogeneous and relevant outcomes for use in clinical trials of interventions for VTE in adults, and then promote the COS internationally with researchers, knowledge users and other stakeholders. Dr. Robert-Ebadi showed that the project involved participants from all continents, and comprised mixed research methods to define “what to measure” (a systematic review has already been published, one-to one and focus groups interviews with patients and other stakeholders, Delphi surveys involving all stakeholder groups, and a final consensus meeting is planned), and “how to measure” by seeking validated tools.

Last Dr. Woller presented preliminary results from a systematic review of a new project initiative on biomarkers predictive of clinically overt VTE among hospitalized patients with COVID-19. There were 183 studies identified of which 124 were excluded upon title and abstract screening, and another 57 were excluded following full text review. Only two studies met inclusion criteria and data extraction demonstrated that only one biomarker, d-dimer, was found to be predictive of VTE among hospitalized patients with COVID-19. The SSC members determined the following. First, the quality and completeness of existing publications assessing biomarkers for predictiveness of hospital-associated VTE among COVID-19 patients have significant limitations. Second, well designed studies are necessary to assess what (if any) role exists to use biomarkers to predict VTE among hospitalized COVID patients, and finally, that presently inadequate evidence exists to advise use of biomarkers to direct care for VTE risk mitigation among hospitalized COVID patients.

The virtual meeting was well attended throughout the entire 2 hrs program and the prerecorded presentations were accompanied by constructive questions and discussions along with comments to some preliminary results of the poll questions.
Welcome, and overview of activities - **Chair of SSC: Maha Othman (Canada)**

Dr. Othman began by introducing the current committee co-chairs and thanked those who are rotating off the committee this year. Currently the committee includes Drs. Robert Sidonio (USA), Elvira Grandone (Italy), Dr. Rezan Abdul-Kadir, Dr. Anne Kinga Malinwoksi (Canada). Both Drs. Emmanuel Favaloro (Australia) and Patricia Casais (Argentina) are rotating off. She then reviewed the focus of the women’s SSC. She encouraged active participation in the committee’s by: participation in on-going projects, expression of interest and new ideas, and registering as members and joining our current 642. She also announced for the two vacant co-chair positions this year. Dr. Othman listed all current SSC projects, including the joint projects with other SSCs. Five new projects have been launched since last year. 7 new SSC publications were completed since ISTH 2020 were highlighted, all in JTH (except on in Br J of Anesthesia) and 2 from joint SSC projects. Publications since last ISTH were highlighted


- Malinowski AK, Othman M; International Society on Thrombosis and Haemostasis' Women's Health Issues in Thrombosis and Haemostasis SSC; Platelet Physiology SSC; Platelet Immunology SSC. Obstetric neuraxial anaesthesia in the setting of immune thrombocytopenia and low platelet counts: call to participate in an international registry. Br J Anaesth. 2021 Apr 27;S0007-0912(21)00212-9 [https://doi.org/10.1016/j.bja.2021.03.024](https://doi.org/10.1016/j.bja.2021.03.024).


Dr. Othman also highlighted the 2 guidance documents in progress. One is currently under review by the G&G committee: “Severe Combined Protein C deficiency” (proposal approved and manuscript under review) and the second: “Management of type 2B VWD in pregnancy”; the proposal is under review by G&G. Finally, she reviewed the SSC agenda and the ongoing projects and invited for active discussion and feedback to all topics.

Part I: Update on on-going SSC projects. Moderators: Ann Kinga Malinowski and Patricia Casais


Dr. Jevtic presented final update on a recently completed project regarding physician understanding and management of COVID-19 in pregnancy. This project focused on COVID-19 associated coagulopathy and complications in pregnancy. Our international survey featured 75 respondents with a completion rate of 34%. There was a diverse representation of subspecialties, including obstetrics and internal medicine. Physicians frequently monitored parameters including platelet count, C-reactive protein, D-dimer, and lymphocyte count. The most commonly identified abnormalities in COVID-19 associated coagulopathy were an elevated D-dimer, CRP, as well as thrombocytopenia. With increasing disease severity, physicians tended to prescribe higher anticoagulation doses with longer duration. Despite this, 7 pregnant patients with COVID-19 associated coagulopathy developed a thrombotic event – 4 were receiving standard prophylactic dosing, while 3 were receiving weight-based dosing. Ultimately, our recently published data demonstrate that there is significant heterogeneity in physician practices regarding COVID-19 and pregnancy, particularly in regard to anticoagulation management. There appears to be a significant thrombotic risk in those who develop concomitant coagulopathy that remains to be investigated. The manuscript from this work has just been published in JTH. https://onlinelibrary.wiley.com/doi/10.1111/jth.15462
Obstetrics and Gynaecological Outcomes of Women with Platelet Function Disorders

Speaker: Deborah Obeng-Tuudah (UK)

Dr. Obeng-Tuudah provided a brief background of the project, objectives and method for data collection. Obstetrics and Gynecological Outcomes of Women with Platelet Function Disorders. The presentations and management of women with known inherited platelet function. Disorders (IPFD) can be varied at times in the obstetrics and gynaecology settings due to the rarity of these conditions and the different types of platelet function disorders culminating into limited clinical experience. This study aims to study the presentations, complications and management regimes established in the many women with known platelet function disorders who have been referred to the tertiary haemophilia and thrombosis centre at Royal Free Hospital, London, United Kingdom. We also developed an ISTH SSC registry so that interested clinicians across the world can contribute and we can cumulate knowledge and experience across the world. Data from 38 women with a total of 52 pregnancies was analysed. We found that HMB is predominant presenting symptom in women with IPFD (53%), often starting from menarche and resulting in emergency department attendance. HMB can be difficult to treat medically, necessitating combination therapy and surgical management. Despite this bleeding disorder, the risk of miscarriage and threatened miscarriage (early pregnancy bleeding) was found to be similar to general population. The mode of delivery was mainly by NVD and caesarean section were obstetric indicated. Haemostatic cover reduced the risk of postpartum haemorrhage - Tranexamic acid can be used in all women and appears to be sufficient alone in mild to moderate platelet function disorders. If IPFD is not diagnosed, risk of PPH (EBL≥500mls) was over 80%. It is best practice to ensure uterine atony, trauma and retained products risks are minimised after labour. Close collaboration between Ob/Gyn, haematology and anaesthetic teams in patient care is of paramount importance in these women. More data is needed and clinicians are encouraged to contribute at: https://redcap.isth.org/surveys/?s=HTHE4TAMTM

WITEAM Study on Placenta-Mediated Pregnancy Complications and Thrombophilia

Speaker: Mariola Ortin (Spain)

Dr. Ortin reviewed the WITEAM, an observational, prospective, international and non interventional registry based study. This project started in 2017 by Dr. Amparo Santamaria; working group in the Spanish Society of Thrombosis and Haemostasis http://www.witeamproject.org who developed the project nationally. The project was then submitted and approved as an SSC international project. The questionnaire was developed and data were entered in the ISTH REDCap database. The registry has been available on ISTH website 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. Preliminary data from 32 cases/entries in three years. The small number of data prevented making meaningful conclusion at this point. Dr. Ortin, discussed the difficulties in patient recruitment and the limitation of the study including: lengthy questionnaire and the need for more promotion of the registry as possible reasons for low recruitment and proposed
COVID-19 and Pregnancy-A Kinga Malinowski (Canada)

Dr. Malinowski provided an overview and update on the current status of COVID-19 in pregnancy worldwide and the impact on maternal and fetal outcomes. Overall, 2-3 COVID waves and over 17 variants; some with greater transmissibility, increased symptom severity, higher resistance to therapies and diminished neutralization by antibodies. Symptoms in pregnancy are quite similar to symptoms outside of pregnancy and don’t vary by gestational age. The majority of patients have mild infection (85-90%); however, a proportion of patients will develop moderate to critical illness. In the first two waves, the risk factors for severe/critical illness were obesity, diabetes mellitus, hypertension, and older maternal age, as well as minority ethnicity status, with the majority of the severe infections occurring in the late second and third trimesters. Mortality and morbidity in pregnancy appeared to be increased in comparison to COVID-19 negative pregnant peers, with potentially five-fold higher rate of ICU admission, and three-fold longer ICU length of stay, as well as a 20-fold increased risk of death.

In Canada, in the first two waves, out of 25% of reproductive aged population who was COVID-19 positive, 1.2% were pregnant (in comparison to 1% in the UK and ~10% in the US). While the majority of women (93%) continued not to require hospitalization, compared to reproductive age COVID-19 positive non-pregnant peers, pregnant individuals had a four-fold higher incidence of hospitalization, 11-fold higher rate of ICU admission. Importantly, in contrast to the prior two waves, individuals with severe illness (hospitalized or admitted to the ICU) had no risk factors. From the UK experience of symptomatic pregnant women admitted for COVID-19 or other obstetric indications, the majority (87%) did not have respiratory compromise; however, in comparison to wave one, patients were more symptomatic, and there were higher rates of ICU admissions for severe illness.

There have been no COVID-19 associated teratogenicity reports. Though COVID-19 has been associated with low birth weight, with a systematic review including international data from 10,000 pregnancies noting a nine-fold increase in LBW over COVID-19-negative pregnant patients. In Canada, LBW has been noted in 10% of pregnant individuals with COVID-19, compared to a rate of 6% in the general population. Preterm Birth (PTB) has also increased, with international estimates between 17-20%, Canadian rates of 13% (an increase from 8%), and UKOSS estimates of 20%. Reassuringly, in the UKOSS data, only 20% needed NICU admission and only 0.5% resulted in a neonatal death. The majority of PTB occurred during the third trimester, at a mean gestational age of 34-35 weeks, with low risk of neonatal morbidity.

From the neonatal perspective, over 90% of infants born to COVID-19 positive mothers test negative for COVID-19. Of those infants who test positive, the majority is following
postnatal transmission (67%) as opposed to vertical transmission (30%). Most of the COVID-19 positive neonates have mild symptoms. Breastfeeding is not a risk factor for COVID-19 transmission and is encouraged.

Dr. Malinowski also discussed issues with COVID-19 vaccination in pregnancy.

**COVID-19 Coagulopathies & Pregnancy- COV-PREG-COAG Registry**

*Speaker: Sajida Kazi (UK)*

Dr. Kazi started by explaining that increased susceptibility to severe illnesses has been reported in the pregnant population, following infection with SARS-CoV-2 resulting in COVID-19. In the non-pregnant population, CAC with a thrombotic phenotype has been described in those with severe illness. Pregnancy is an inherently prothrombotic state, yet data on CAC in pregnancy remains scarce.

She then explained the objectives and method of data collection of the international registry (COV-PREG-COAG) developed by the Women’s SSC. The registry addresses COVID-19 associated coagulopathy (CAC) in pregnancy. The main objectives are: to examine coagulopathy and VTE events in COVID-19 affected pregnancies and their potential link to disease severity, to assess effects of COVID-19 related coagulopathy on maternal and fetal/neonatal outcomes, to study the hemostatic parameters in women with COVID-19, during each trimester of pregnancy and the postpartum period and to evaluate the use/effects of therapies such as LMWH, Steroids and other meds.

She the presented preliminary data. Based on the data from 340 COVID-19 affected pregnancies analyzed so far from the registry, over 80% of COVID-19 occurred in the third trimester and 55% of women were asymptomatic. Most common symptoms included cough (47%), fever (39%), shortness of breath (25%) and anosmia (17%). Comorbid pathologies were reported as follows: obesity (66%), respiratory illnesses (7.6%) and diabetes (2.4%). Among 336 women with complete information, 5% were hospitalized, 3.3% had severe disease, and 1.3% required ICU admission. CAC features included: prolonged PT/APTT (9.1%), thrombocytopenia (4.9%), elevated D-dimer (4.6%), hyper-fibrinogenemia (5%). 66% of women received postpartum thromboprophylaxis. There was one venous thromboembolic event and no maternal deaths. ISTH registry demonstrated that CAC and thrombotic events are infrequent in COVID-19 affected pregnancies. Further data is required before drawing definite conclusions. A careful and individualized VTE risk assessment should be performed taking into consideration other VTE risk factors to plan duration of LMWH after discharge. For those with a less severe condition and a short period of hospitalization, which did not result in delivery, 10 to 14 days of LMWH may be appropriate. For those with a severe disease, with very high D-dimer levels, particularly in the third trimester, this may mean continuation of LMWH throughout the rest of pregnancy and postpartum. For postpartum women, the duration of thromboprophylaxis may vary from 2 to 6 weeks, depending on other risk factors, mode of delivery, severity of COVID-19
infection, and duration of admission. The registry continues to recruit prospective or retrospective data from across the world. We encourage all physicians who care for COVID-19 in pregnancy to participate via ISTHREDCap tool available at https://redcap.isth.org/surveys/?s=4JPX9W98RH

Risks and Outcomes of Anticoagulation-Associated Abnormal Menstrual Bleeding in Patients VTE- The TEAM-VTE Study- Erik Klok (The Netherland)

Dr. Klok presented the preliminary results of the TEAM-VTE study, which was designed to establish the incidence of abnormal uterine bleeding in women in their fertile age, after initiation of anticoagulation for a first VTE diagnosis. Of the 98 female VTE patients (mean age: 34 years), 65% met at least one of the three predefined definitions of AUB (95% confidence interval (CI) 55-73%) during follow-up (PBAC score >100, PBAC score >150, or self-reported increased menstrual volume). New-onset AUB occurred in 60% (95% CI 47-71%). Notably, only 14% of patients with AUB received any treatment for menstrual bleeding. The prevalence of AUB decreased over time suggesting a spontaneous decrease in blood loss in each next menstrual period. Diagnostic work-up revealed abnormal transvaginal ultrasound occurring in 32% of patients with AUB and in 27% of patients with new-onset AUB. None of the patients were diagnosed with Von Willebrand disease. We expect that the full results of the study will become available by the end of 2021.

Part II: Joint SSC Projects & New Guidance-Moderator: Elvira Grandone

ISTH Guidance on Diagnosis and Management of SCPCD- Joint SSCs project- Adrian Minford (UK)

Dr. Minford presented the completed guidance document; coauthored by Leonardo R. Brandão, Maha Othman, Christopher Male, Rezan Abdul-Kadir, Paul Monagle, Andrew D Mumford, Dorothy Adcock, Björn Dahlbäck, Predrag Miljic, Maria T DeSancho, Jun Teruya. The document provides recommendations for the diagnosis and management of severe congenital protein C deficiency (SCPCD) and management of subsequent pregnancies. Chromogenic assay is preferred for measuring plasma protein C (PC) levels and repeat measurements, checking parents’ PC levels and genetic testing may be necessary to differentiate SCPCD from acquired causes of PC deficiency. Management of acute thrombotic events requires prompt treatment with intravenous PC concentrate or if unavailable, plasma. If available, PC concentrate, preferably by the subcutaneous route, is recommended for long term management. If there are cost restraints, a vitamin K antagonist (VKA) may enable lower doses of PC replacement. If PC concentrate is unavailable for continuous prophylaxis, long term management consists of a VKA (preferably with short term PC concentrate at the start of treatment) and protein C concentrate or plasma for thrombotic relapses. Since retinal and cerebral vessel thrombosis usually occur in late pregnancy, if the causal genetic mutation is known, prenatal diagnosis (PND) followed by early elective delivery (if the fetus has SCPCD) can potentially prevent blindness and neurodevelopmental handicap in
subsequent pregnancies. If the mutation is not known or if PND is declined, early elective delivery may still be offered provided a multidisciplinary team ensures proper neonatal management.

The ISTH guidance document drafted by the group is currently under review by the ISTH Guidance and Guidelines Committee and expected to be submitted to JTH soon.

**Global Registry of DIC and Pregnancy Speaker: Offer Erez (Israel)**

Dr. Erez gave an introduction on DIC in pregnancy and presented data from the registry. Obstetrical pathologies, resulting in disseminated intravascular coagulation (DIC), are one of the leading causes for maternal mortality worldwide. This is in spite of an adaptive physiologic mechanism that generates a physiologic prothrombotic state during gestation and the advanced medical and surgical hemostatic capabilities that have evolved during the past decades for controlling acute obstetrical blood loss. The international registry for DIC in pregnancy was established as a joint project of the DIC and Women Issues in Thrombosis and Hemostasis SSC's. The registry is a comprehensive survey of epidemiologic, clinical and laboratory data of cases of DIC in pregnancy. It currently has 113 entries of them 80 with complete datasets. One of the interesting findings we have thus far, is that the hemostatic parameters associated with development of DIC differ among the obstetrical complications. While DIC in women who had preeclampsia and HELLP syndrome was associated with low platelets count and fibrinogen concentration, in those who develop DIC following uterine atony or placenta accrete it was associated with prolongation of PT. Moreover, our findings emphasize the contribution of placental abruption as an underlying cause for DIC in pregnancy. The registry continues recruitment for another year

https://redcap.isth.org/surveys/?s=KFC8RN8XWC

**Part III: New Initiatives/Topics for Discussion- Moderators: Maha Othman and Rezan Abdul-Kadir**

**ISTH Common Data Elements in Postpartum Hemorrhage- Maha Othman (Canada)**

Dr. Othman presented this new initiative for discussion. This is a project on the development of Common Data Elements to aid in standardizing postpartum hemorrhage research. PPH is a leading cause of maternal death and a serious cause for maternal morbidity worldwide. PPH definitions remain inconsistent and reported research data varies across different studies. Some data may not be precise and there is also significant discrepancies in current PPH guidelines, thus potentially impacting maternal safety and well-being. Such variability often impedes the ability to conduct metanalysis or compare the outcomes of different studies, and also hinders the development of large studies. The first true effort was in 2018, through the development of a core outcome set for the prevention and treatment of PPH. Outcomes were identified through evaluation of systematic reviews of relevant studies yielding 160 outcomes from 121 RCTs on PPH prevention, 95 outcomes from 16 RCTs on treatment of PPH. Following further analysis, 35 and 31 outcomes for prevention and treatment of PPH were included in an international Delphi survey, culminating in a core
outcome set consisting of 9 outcomes for prevention and 12 for treatment of PPH, as minimum for every relevant PPH study. Further refinement in terms of definitions, measurement tools and also the corresponding variables for those proposed core outcomes will be useful and a consensus on such definitions and measurement tools for those and other outcomes identified through prior systematic reviews of PPH studies, will be vital. The objective of the study is to develop CDEs for worldwide adoption in clinical research addressing the prevention and treatment of PPH. The women SSC is encouraged by the ISTH’s prior experience and success in developing The ISTH Common Data Elements for VTE and will follow the same plan. The ISTH CDE for VTE was published in JTH last August. The plan for the current project is to construct a multidisciplinary team: Haem OB, MFM, OB Anesthesia, Midwifery specialists, patient representative and methodology expert. Representatives from international societies such as the International Federation of Gynecology and Obstetrics (FIGO), the World Health Organization (WHO), and the World Federation of Hemophilia (WFH) will be invited to provide feedback. Care will be taken to achieve representation based on gender, specific content expertise, and geographic location, including lower-resource settings. We will create the list of outcomes for which CDEs will be developed. Existing systematic reviews assessing outcome reporting in studies on prevention and treatment of PPH will be used together with documents outlining outcomes critical. General and relevant CDEs from previous ISTH CDE for VTE project will be used for decision making so as case report forms in previously utilized for research in PPH. The project is currently under review by ISTH Executive and await funding approval.

Women with Inherited Bleeding Disorders e-Learning Programme (Rezan Abdul-Kadir (UK))
Dr. Abdul-Kadir described this international multidisciplinary effort to design and disseminate an accredited educational programme to support physicians around the world in improving the diagnosis and management of bleeding disorders among girls and women. Health care professionals including general practitioners, obstetricians and gynaecologists, can play a crucial role in reducing the time to diagnosis of bleeding disorders in women and girls. The multidisciplinary team of experts, composed of experts who are actively involved in the field include adult, pediatric hematologists, OB/GYN specialist. The team has developed an accredited micro e-learning programme to suit the busy life of health care professionals. The goal is to increase awareness, knowledge, and understanding of bleeding disorders and their management among healthcare professionals who are the first to see signs and symptoms of bleeding disorders in women and girls. The programme consists of two short modules. Within each module there is a 2-minute animation video, a downloadable slide set and a flashcard. This is available at this link [https://www.hemostasisconnect.info](https://www.hemostasisconnect.info). The programme is also available on the ISTH academy.

In terms of attendance, there was a total of 711 attendees in the virtual session. We had no technical issues and the online session went very smooth with live discussion and we
were able to adhere to the 2h session time. For more information about our projects, please visit our website https://www.isth.org/members/group.aspx?id=100375