Standing Committees
Bleeding Assessment Tool Committee ...................................................... 2
Coagulation Standards Committee .......................................................... 3

Subcommittees
Animal, Cellular and Molecular Models .................................................. 5
Biorheology ................................................................................................. 8
Control of Anticoagulation ......................................................................... 13
Disseminated Intravascular Coagulation ..................................................... 16
Exogenous Hemostatic Factors .................................................................. No Meeting
Factor VIII, Factor IX and Rare Coagulation Disorders ............................. 18
Factor XI and the Contact System .............................................................. 43
Factor XIII and Fibrinogen .......................................................................... 45
Fibrinolysis .................................................................................................. 50
Hemostasis and Malignancy ....................................................................... 56
Lupus Anticoagulant/Phospholipid Dependent Antibodies .......................... 57
Pediatric and Neonatal Hemostasis and Thrombosis ................................. 60
Plasma Coagulation Inhibitors ................................................................... 63
Platelet Immunology ................................................................................... 68
Platelet Physiology ..................................................................................... 71
Predictive Hemostatic Variables ............................................................... 73
Vascular Biology ........................................................................................ 76
Von Willebrand Factor ............................................................................... 82
Women’s Health Issues in Thrombosis and Hemostasis ............................. 90
Working Groups
Working Group on Genomics
ISTH-BAT Standing Committee – Business Meeting
24 June 2014

Business Meeting Minutes

Attending: F. Rodeghiero, B. Coller, A.K. Chan, R. Kadir, S. Eichinger, A. Tosetto

Excused: P. Harrison, W. Ageno, P. Gresele, A. Federici, P. James, J. DiPaola and D. Lillicrap

Francesco Rodeghiero opens the meeting by briefly summarizing the aim and scope of the SC for convenience of the new members. After that, he presents the main critical problem concerning a more widespread use of the ISTH-BAT. Indeed few (or none) of the researchers interested in currently ongoing studies are willing to obtain from their IRBs (or re-discuss) an approval to enter data specifically in the ISTH-BAT repository. Despite the process needed to obtain credentials on the ISTH-BAT websites has been made much straightforward and transparent, some improvements still need to be done.

Barry Coller summarizes the current efforts at Rockefeller University (RU) to improve usability. There is a need to create a database of officially approved studies by the BAT SC, as some Centres having already data inside the repository never applied for approval. He is also trying to review with the RU IRB if agreements between Principal Investigators and RU may be changed – ideally for retrospective studies it could be just fine for investigators to get a consent from study participants and their IRB to store clinical data, whatever the location of the repository and without a specific reference to the BAT-RU repository website. In this way, having a new consent form would not longer be required. He however stresses the need to renew the agreement between PIs and RU every year.

Barry Coller also recognizes that there are some open problems, that are currently being addressed by the new software developer Yupu Liang, particularly the algorithm for automatic score calculation and the wording reporting the ISTH-BAT and the EU MCMMDM-1 VWD study scores.

It is finally decided that Francesco Rodeghiero / Alberto Tosetto will coordinate with Barry Coller / Yupu Liang to keep the development of the ISTH BAT-R active and as smooth as possible for the end-user.

By Alberto Tosetto, 4 July 2014
Review of Lot #4 (A Hubbard)

Lot #4 of the SSC/ISTH Secondary Coagulation Standard commenced dispatch in April 2012 at a cost of £4.00 (GBP) per vial. Between June 2013 and end May 2014 a total of 11,156 vials were issued. Manufacturers in Europe received a total of 8,800 vials and External Quality Assurance schemes (UK NEQAS; College of American Pathologists, USA; Study Group on Thrombosis and Haemostasis, France) received a total of 1,356 vials. Information on the availability of Lot #4 was distributed to 150 manufacturers in May 2014 and the possible impact on use of the standard will be monitored. The fourth time-point for the stability testing of Lot #4 was completed in January/February 2014. Estimates of the residual relative potency for the four analytes (FV, FVII, FVIII, antithrombin) from the two testing laboratories (NIBSC, Royal Hallamshire Hospital, Sheffield) were similar. Mean predicted degradation rates were below 0.1% per year for vials stored at -20°C for all analytes indicating that Lot #4 is extremely stable and this supports the assigned expiry date of "end December 2020". Test were also performed for Fibrinogen by Clauss assay, but there were assay anomalies for the vials stored at higher temperatures (+20 and +37°C) and these results could not be used to generate a predicted degradation rate. However, estimates for fibrinogen in vials stored at +4°C for 4 years were similar to the vials stored at -20°C indicative of good stability.

Experience of EQA schemes with Lot #4

UK NEQAS (S Kitchen)

Four vials of SSC Lot #4 were used for "trouble-shooting" purposes between July 2013 and June 2014 relating to assay issues for FVIII, FIX, Protein C and Protein S. SSC Lot #4 has also been included as an anonymous sample in one survey (WFH IEQAS) which covered testing for FVIII, FIX and VWF. There was good agreement between the median values from the study and assigned values for FVIII, FIX, VWF:Ag, VWF:RCo and VWF:CB, with a maximum deviation of only 7%. Regarding tests for VWF activity, there has been an increase in the number of laboratories using latex based assays; a survey in May 2014 indicated around two-thirds of laboratories had moved from conventional VWF:RCo methods to the new tests. The overall median result for Lot #4 from these tests (IL and Siemens kits combined) was considerably higher than the results from conventional methods. However, the Siemens kit was found to produce values similar to conventional methods for Lot #4 and VWD patient samples whereas the IL kits were associated with increased values. This prompted discussion on the need to assign values for these new methods to the International Standard or to Lot #4 since, at present, the assigned values are derived from conventional VWF:RCo methods. It was agreed that the kit manufacturers will be asked for details of their calibration procedure.
SSC Lot #4 was issued in two surveys during 2013 – one for FVIII and VWF and a second for thrombophilia testing. Mean estimates for FVIII:C ranged from 80 – 90 IU/dl in comparison to the assigned value of 88 IU/dl. Results using the Biodata method for VWF:RCo recovered a mean value (89.5 IU/dl) close to the assigned value (84 IU/dl) whereas the Siemens BC method produced a negative bias with mean of 66.5 IU/dl. There are an increasing number of laboratories using the VWF:GP1b methods and a mean value from 12 laboratories using the Hemosil method (87.4 IU/dl) was close to the assigned value for VWF:RCo. This finding was in contrast to the UK NEQAS results which indicated higher values for the IL kit. It was possible that there might have been changes to the kit calibration procedure between the UK NEQAS and CAP surveys. Mean estimates of VWF:Ag using the Stago Liatest or Hemosil methods were close to the assigned value of 116 IU/dl whereas there was a negative bias with 6 laboratories using the Siemens method. Mean estimates for the thrombophilia associated analytes (Antithrombin activity and antigen, Protein C activity and antigen, Protein S activity and antigen) were close to the assigned values. Overall the survey results support the assigned values and stability of Lot #4.

Lot #4 and the JCTLM database (E Gray)

Previous lots of the Secondary Standard have been accepted on to the JCTLM database as internationally recognized reference materials. The process for application to the database has changed since Lot #3 was accepted and there are now strict requirements for commutability studies. Undertaking commutability studies for all 21 analytes is not a realistic option for Lot #4 and it is therefore unlikely that the standard will be accepted on to the database.

There were 15 attendees.
Animal, Cellular and Molecular Models

Chairman: Susan Smyth (United States)

Co-chairmen: Cecile Denis (France), Tom Knudsen (Denmark), Toshiyuki Miyata (Japan), Leslie Parise (United States), Denise Sabatino (United States)

Thursday, 26 June (8:45-12:45)

1. Top rated abstract: Early gene expression biomarkers sensitive to microvolume blood in the joint. N. Hakobyan.

This abstract identified gene signatures that were sensitive to the presence of small volumes of blood in the knee joint of mice, in an effort to identify markers specific for joint bleeding.

2. Mouse model of thrombosis induced by inferior vena cava stenosis. J. Geddings.

This presentation reviewed a recent subcommittee-endorsed publication on the strengths and weaknesses of suture-induced venous thrombosis in small rodents.


This presentation described models associated with inhibitor development and required aspects of the immune response.


Using the ZFN technique, the authors generated Factor XIII-deficient rats. Extensive characterization suggested several similarities with hemophilia A in humans.


This presentation described ARFI as a non-invasive and sensitive method for assessing bleeding.


This presentation reviewed animal models of defects in myosin heavy chain9. Species similarities and differences were noted.

This presentation summarized the literature with respect to the effect of natural and pharmacological inhibitors of coagulation on atherosclerosis. For any given drug, most of effects were similar in the models, however, subtle differences between models were noted.

8. The need for rat models of thrombosis. L. Parise.

This presentation demonstrated an important signaling pathway present in human platelets but lacking in mouse platelets. It presents in rat platelets sets the foundation for the importance for developing and standardizing rat models of thrombosis.


This presentation described the state of the field with respect to animal models of VT.

Business Meeting of the Animal, Cellular, and Molecular Models Subcommittee

A. Recent publications

   Under review with J Thromb Haemost
3. Influence of genetic background on bleeding phenotype in the tail-tip bleeding model and recommendations for standardization.
   Approved by SSC.

B. Suggestions for other SSC communications

1. Animal models to validate therapies for antidotes for anticoagulant therapy (approved at SSC 2013)
2. Consensus/guideline statement for animal models of venous thrombosis use.

C. Topics for Educational session in 2015:

1. The Pre-analytical conditions in "Point of care assays” for platelet function, platelet inhibitors, anticoagulation for ventricular assist devices, ECMO, and cardiopulmonary bypass. This could include VerifyNOW, activated clotting times (ACT), thromboelastography (TEG), and thrombin generation assays among others.
2. The current role of nonhuman primates in atherosclerosis, thrombosis studies and for safety and efficacy studies during drug development
3. A review and update of the previously published consensus and guidelines for platelet function studies and coagulation assays in mice.1-3
4. Guidelines for the "basic package” for characterization of animal models of hemophilia
5. The committee also discussed the proposal for devoting the session to a symposium on Animal Models of Venous Thrombosis with the goals of creating a consensus/guideline for animal models of VT use, developing a subgroup with the capacity to evaluate new animal models of VT (or modifications on existing ones) to be considered for application in the field, and establishing a mechanism for annual/biannual reporting to the Animal Models SSC.
Opening Comments, Keith Neeves, USA, "Update on ongoing subcommittee projects” 8:00-8:20

The mission and mandate of the Biorheology Subcommittee was presented. Results from our recently completed project on “Flow-dependent thrombin and fibrin generation” was summarized with a focus on recommendations for integrating immobilized TF into flow assays. Details and supplemental material can be found in recently SSC Communication [Neeves et al., JTH, 12 (2014)]. Our new project on scaling in hemorheology was presented in the context of defining nomenclature and utility of using scaling approaches to comparing in vitro and in vivo experiments.

Jonathan Cowman, Royal College of Surgeons, Ireland, "Platelets from Premature Neonates Have Increased Platelet Affinity for von Willebrand Factor (VWF) Under Arterial Shear Compared to Platelets from Term Neonates” 8:20-8:40

Platelet function of premature and full-term infants was measured by rolling on VWF in custom parallel plate flow chambers at 1500 1/s that uses <100 µL of whole blood. Platelet rolling and translocation were measured by custom platelet tracking software at 30 fps. There was a significant increase in the number of platelets, translocation and rolling distance in premature infants. Premature infants had higher GP1b expression. Questions: What dye was used to label platelets? DiOC6. Did you look at MPV/RBC size/deformation? No differences in MPV/RBC.

Session 1. Thrombosis, circulating tumor cells and metastasis

Nigel Mackman, University North Carolina, USA “Circulating tumor-derived microparticles and thrombosis,” 8:40-9:00

Tumor cells release tissue factor (TF)+microvesicles (MV) that may contribute to venous thrombosis in cancer patients. An assay to measure levels of MV TF activity in plasma was presented (Khorana, JTH, 2008; Blood, 2008). It was found that tumors released TF+MVs into the circulation of mice and these MVs enhanced thrombosis in different models. In human studies elevated levels of MV TF activity preceded venous thrombosis in pancreatic cancer patients but not in other types of cancer. These studies suggest that MV TF activity may be a useful biomarker to identify pancreatic cancer patients that are at risk for thrombosis. Understanding the mechanism by with tumor-derived TF+ MV enhance thrombosis may reveal targets for novel anticoagulant therapy. Questions: Lack of correlation between TF+MV and
thrombosis in cancer? TF+MV are one of many parameters in cancer associated thrombosis and will only play a role when TF+MV can overcome endogenous anticoagulation mechanisms.

Ali Amirkhosravi, Florida Hospital, USA, "The role of tissue factor in cancer-associated coagulation activation and metastasis,” 9:00-9:20

Cancer cell-associated tissue factor has been shown to strongly enhance metastatic potential. In addition, tissue factor can be released from tumor cells in association with microparticles, and these tumor cell-derived TF-bearing particles can enter the circulation and activate coagulation. In the first part of this presentation, mechanisms by which tumor cell TF procoagulant activity promotes experimental metastasis were discussed. In the second part, data regarding physical and procoagulant characteristics (both in vitro and in vivo) of tumor-derived TF was presented. Questions: What isoform of TF is found in the non-sedimented fraction? Unknown, possibly exodomain of TF. Further discussion centered on potential issues in targeting TF systemically.

Michael King, Cornell University, USA, "TRAIL-coated leukocytes that kill cancer cells in the circulation,” 9:20-9:40

Metastasis through the bloodstream contributes to poor prognosis in many types of cancer. Mounting evidence implicates selectin-based adhesive interactions between cancer cells and the blood vessel wall as facilitating this process, in a manner similar to leukocyte trafficking during inflammation. In this talk, King described a unique approach to target and kill colon and prostate cancer cells in the blood that causes circulating leukocytes to present the cancer-specific TNF-related apoptosis inducing ligand (TRAIL) on their surface along with E-selectin adhesion receptor (Mitchell et al., PNAS, 2014). This approach, demonstrated in vitro with human blood and also in mice, mimics the cytotoxic activity of natural killer cells and increases the surface area available for delivery of the receptor-mediated signal. The resulting "unnatural killer cells” hold promise as an effective means to neutralize circulating tumor cells that enter blood with the potential to form new metastases. They are currently testing the technology in an orthotopic model of spontaneous prostate cancer metastasis. Questions: Do cancer cells marginate like leukocytes? That is the hypothesis since they have size and stiffness that is similar to leukocytes, likely in an environment (near wall region) that is rich in leukocytes and that is why the effect of the TRAIL is enhanced under shear flow.

Owen McCarty, Oregon Health Sciences University, USA, "Procoagulant phenotype of circulating tumor cells,” 9:40-10:00

The identification, isolation, and characterization of circulating tumor cells (CTCs) promises to enhance our understanding of the evolution of cancer in humans. CTCs provide a window into the hematogenous, or "fluid phase," of cancer, underlying the metastatic transition in which a locally contained tumor spreads to other locations in the body through the bloodstream. With the development of sensitive and specific CTC identification and isolation methodologies, the role of CTCs in clinical diagnostics, disease surveillance, and the physical basis of metastasis continues to be established. This talk focused on the quantification of the basic biophysical properties of CTCs and the use of these metrics to understand the hematogenous dissemination of these
enigmatic cells. Questions: Discussion regarding thrombin generation on individual and clusters of CTCs. Are CTCs dead/apoptotic, especially in clusters? Have stained with annexin V in some cases but staining protocol not particularly compatible with CTC isolation/identification methods.

Session 2. Biomechanics in hemostasis and thrombosis
Moderator: Judith Cosemans

Wilbur Lam, Georgia Tech/Emory, USA, "Platelets and the mechanical microenvironment," 10:20-10:40

During hemostatic and thrombotic processes, platelets are subjected to a myriad of biochemical and biophysical signals and cues. As clot formation ensues, platelets interact with polymerizing fibrin scaffolds, exposing platelets to a large range of mechanical microenvironments. Whether platelets can sense microenvironmental mechanical properties, such as substrate stiffness, and transduce those cues into differential biological signals is largely unknown, however. To that end, Dr. Lam showed observations that as platelets mechanosense the stiffness of the underlying fibrin/fibrinogen substrate, increasing substrate stiffness leads to increased platelet adhesion, spreading, and contraction. Importantly, adhesion on stiffer substrates also leads to higher levels of platelet activation, as measured by integrin αIIbβ3 activation, α-granule secretion, and procoagulant activity. Mechanistically, it was determined that Rac1 and actomyosin activity mediate substrate stiffness-dependent platelet adhesion, spreading, and activation to different degrees. This capability of platelets to mechanosense microenvironmental cues in a growing thrombus or hemostatic plug and then mechanotransduce those cues into differential levels of platelet adhesion, spreading, and activation provides some biophysical insight into the underlying mechanisms of platelet aggregation and platelet activation heterogeneity during thrombus formation. Questions: Discussion focused on correlation between these studies and classical clot retraction as well as the ability of unactivated platelets to bind to fibrinogen (vs fibrin).

Martin Guthold, Wake Forest University, USA, "The mechanical properties of single fibrin fibers," 10:40-11:00

In the past, working with purified fibrinogen, Guthold and colleagues have determined the mechanical properties of single fibrin fibers, such as their extraordinary extensibility and elasticity, moduli and relaxation times (e.g. Liu, W., et al. (2010) J. Thrombosis and Haemostasis 8, 1030-1036.; Liu, W., et al. (2006), Science 313, 634). Recently, they started to investigate the more complex and more physiologically relevant fibrin fibers formed from plasma clots, in an effort to find relationships between single fibrin fiber mechanical properties and diseases. They have now determined the mechanical properties of single fibrin fiber of individuals who have cardiovascular disease (CVD), diabetes, or who have undergone an acute bout of strenuous exercise. It was found that fibrin fibers from old individuals with CVD are much more stretchable (~1.5 times), elastic (~1.4 times) and much stiffer (higher modulus) than those from healthy people. Moreover, they found that acute exercise also has a significant effect on fibrin fiber mechanical properties; fibrin fiber extensibility decreases significantly after
exercise. Diabetes does not have a significant effect on single fibrin fiber mechanical properties. However, in the diabetes data, and subsequently in all other samples, they saw a startling correlation between fiber diameter and fiber stiffness: Fibrin fiber modulus decreases as the diameter of the fiber increases. For most samples, the modulus varied as R-1.3 to R-1.6, except for older individuals with cardiovascular disease, where the modulus varied as R-1.0. Guthold proposed a model in which the density of fibrin fibers varies: fibrin fibers have a dense core, and a less dense periphery.

2. Liu, W., et al. (2006) "Fibrin Fibers have Extraordinary Extensibility and Elasticity" Science 313, 634

Session 3. In vitro flow assays: Methods and emerging concepts
Moderator: Owen McCarty

Michael Lawrence, University of Virginia, USA, "Adherent monocyte promotion of mural microthrombus formation in flow via platelet sequestration." 11:00-11:20

Venous thrombosis is often a consequence of peripheral vascular inflammation, particularly in large collecting veins. In an inflammatory milieu, endothelial cells express adhesion molecules that are capable of attracting both leukocytes and platelets. In the case of platelets, rolling on the endothelium via both P-selectin and PSGL-1 ligands has been proposed as a pathway for vascular thrombus formation. We hypothesize that co-adherent monocytes provide an additional mechanism for amplification of platelet recruitment to the vascular wall due to their high density of platelet adhesion ligands. In a parallel plate flow chamber model of inflamed vasculature, endothelial cells (HUVEC) activated with the cytokine IL-1 were able to accumulate resting and TRAP-treated platelets via previously adherent monocytes in greater numbers (3 to 10-fold) than without the presence of monocytes at wall shear stresses ranging between 0.03 – 0.8 Pa. Accumulation of platelets on monocytes was predominately mediated by P-selectin/P-selectin glycoprotein ligand-1 (PSGL-1) above 0.1 Pa wall shear stress. At lower flows platelet accumulation depended on both PSGL-1 and GPIIb/IIIa. Neutrophils were only 45% as effective as monocytes in recruitment of flowing platelets even though using the same adhesion receptor mechanisms. Endothelial-adherent monocytes and neutrophils may therefore contribute in nucleating platelet binding to inflamed endothelium under venous flow conditions. Questions: Have you measured differences in morphology between monocytes and neutrophils (e.g. spreading)? Have not measured directly, but likely neutrophils will spread and present filopodia that will perturb the flow field, and thus platelet adhesion, in a different way than monocytes.

Mitsuhiko Sugimoto, Nara Medical University, Japan, "Evaluation of soluble or immobilized tissue factor in von Willebrand factor-dependent thrombus formation under flow conditions,” 11:20-11:40

Although tissue factor (TF) is up-regulated upon vessel wall damage and plays a pivotal role in thrombus formation, its functional relevance under physiologic blood flow conditions is poorly understood. Using an in vitro perfusion chamber system, Sugimoto and colleagues have studied
the relevant role of TF in thrombus formation mediated by von Willebrand factor (VWF), a distinctive flow-dependent thrombogenic surface, under flow conditions with varying shear rates. Human recombinant TF (Innovin) were co-coated with purified VWF onto a glass plate to prepare ‘surface-immobilized TF/VWF complex’. Recalcified citrated whole blood was perfused over a VWF-surface in the presence or absence of surface-immobilized TF. Mural thrombi formed on VWF-surface were double-stained with fluorescently labeled anti-fibrin and anti-fibrinogen antibodies. Fibrin generation was evaluated as a ratio of fibrin relative to fibrinogen fluorescence within mural thrombi. In addition to the enhancing effects on fibrin generation, immobilized TF significantly up-regulated VWF-dependent platelet adhesion and aggregation under high shear rate conditions, although soluble TF showed no effects in this regard. These results suggest a synergistic functional link between immobilized TF and VWF in mural thrombus formation under high shear rate conditions. Questions: Does TF bind to VWF? Unknown.

**Judith Cosemans, Maastricht University, Netherlands, "Multi-parameter assessment of thrombus formation: Identifying platelet function defects,” 11:40-12:00**

Assays measuring platelet aggregation (thrombus formation) at arterial shear rate mostly use collagen as only platelet-adhesive surface. Here Cosemans and colleagues report about a multi-surface and multi-parameter flow assay to characterize thrombus formation in whole blood from healthy subjects and patients with platelet function deficiencies. A systematic comparison was made of 52 adhesive surfaces with components activating the main platelet adhesive receptors, and of 8 output parameters reflecting distinct stages of thrombus formation. Three types of thrombus formation can be identified with a predicted hierarchy of the following receptors: glycoprotein (GP)VI, CLEC-2 > GPIb > α6β1, αIIbβ3 > α2β1 > CD36, α5β1, αvβ3. Application with patient blood reveals distinct abnormalities in thrombus formation in patients of severe combined immune deficiency, Glanzmann’s thrombasthenia, Hermansky-Pudlak syndrome, May-Hegglin anomaly or gray platelet syndrome. Cosemans proposes that this test may be useful for the diagnosis of patients with suspected bleeding disorders or a pro-thrombotic tendency. Study will appear in Nature Communications. Questions: Discussion focused on the type of ligands used for some receptors (CLEC-2, α6β1).
Control of Anticoagulation

Chairman: Walter Ageno (Italy)
Co-Chairmen: Rebecca Beyth (US), Benilde Cosmi (Italy), Mark Crowther (Canada), Ismail Elalamy (France), Elaine Hylek (USA), Pieter Kamphuisen (the Netherlands), Peter Verhamme (Belgium), Henry Watson (UK)

Session 1: Wednesday, 25 June (8:00-12:00)
Session 2: Thursday, 26 June (8:45-12:45)

The program of the meeting was divided in two parts. The first part was dedicated to laboratory issues with the direct oral anticoagulants and to the presentation of current and future SSC registries and standardization activities, and was held on the 25th of June. The second part, held on the 26th of June, included a joint symposium between the SSC Control of Anticoagulation and the Working Group on Perioperative Thrombosis and Hemostasis and a session on the reversal of anticoagulant drugs.

The first part of the meeting was introduced by a brief summary of the subcommittee mandate by the SSC chair, Walter Ageno, and was followed by the presentation of the top rated abstract in Control of Anticoagulation. The abstract entitled: Reversal of Enoxaparin-Induced Anticoagulation in Healthy Subjects by Andexanet Alfa, an Antidote for Direct and Indirect FXa Inhibitors – A phase 2 randomized, double blind, placebo-controlled trial was presented by Dr. Mark Crowther.

The first session of the morning, entitled Laboratory Issues with the New Oral Anticoagulants, was chaired by Dr. Ismail Elalamy and Dr. Rebecca Beyth. The first speaker, Dr. Jovan Antovic, summarized available tests to measure plasma concentration and activity of the direct oral anticoagulants and discussed problems related to inter-individual variability in responses. Dr. Antovic presented the results of a number of studies carried out at his institution and compared his findings with those of previously published studies, concluding that only HTI and ECA for dabigatran and anti-Xa assays for the direct factor-Xa inhibitors should be used when strictly necessary. Dr. Ismail Elalamy discussed the clinical utility of drug testing, given that no correlation between test results and clinical outcomes has yet been conclusively proven, as also stressed by Dr. Antovic. He suggested that trough levels should be used to detect insufficient drug concentration or excessive accumulation, but again warned on the lack of clinically validated cut-off levels. Dr. Elalamy proposed the use of specific and drug-dedicated laboratory tests before invasive procedures or when excessive accumulation is suspected, but recommended a wait and see approach only in asymptomatic patients. Dr. Elaine Gray presented the results of an international collaborative study that involved 19 centers from 9 countries and assessed different methods of measurement for dabigatran. The results of this SSC project showed higher intra- and inter-individual variations for lower drug concentrations than for higher drug concentrations and that direct thrombin inhibitors specific methods were more quantitative and accurate than aPTT and PT based assays. Dr. Mark Crowther discussed the effects of the of the direct oral anticoagulants on thrombophilia testing and highlighted the fact that all these drugs have a major impact on most available tests, suggesting the need to performing these tests only
when anticoagulant drugs have been discontinued. Dr. Crowther also stressed the need for a correct use of thrombophilia testing. Finally, Dr. Job Harenberg presented the results of multicentre, international collaborative study on the determination of dabigatran, rivaroxaban, and apixaban from plasma, serum, and urine samples of patients on treatment with the oral direct thrombin and factor Xa inhibitors. In particular, Dr. Harenberg stressed the finding of a high sensitivity and specificity, accuracy, negative and positive predictive values (all >95%) of drug testing in urine samples by point of care methods, a finding that can be clinically useful to detect the presence of the drug in the emergency setting.

The second session of the morning, entitled Update on Current SSC Registries and Standardization Activities and New Proposals, was chaired by Dr. Elaine Hylek and Dr. Benilde Cosmi. The first speaker, Dr. Sam Schulman, presented an update of the SSC registry entitled Recurrent venous thromboembolism in anticoagulated patients with cancer. The study is finally completing enrolment with 198 included patients of the targeted sample of 200 patients. Dr. Schulman presented some preliminary results of the study. Dr. Saskia Middeldorp presented a new proposal for a registry on patients who become pregnant while treated with the direct oral anticoagulant drugs. Dr. Middeldorp described a few cases that occurred in phase III randomized controlled trials with rivaroxaban and edoxaban and highlighted the need to learn more about the management of these patients and about pregnancy outcomes. This information can only be collected by means of an international, multicentre registry and the call for participating centers is open. The registry will be endorsed by this SSC. Subsequently, Dr. Walter Ageno presented an update of the registry on patients with splanchnic vein thrombosis. This study, which enrolled more than 600 patients from 33 centers in 12 countries, has recently completed the 2-year follow-up and the final analysis of the long-term outcomes is ongoing. Meanwhile, baseline results have been recently published. Dr. Ageno also proposed a new registry aimed to collect information on the management of major bleeding events and on recurrent thromboembolic events in patients treated with the direct oral anticoagulants. The registry has received SSC endorsement and is ready to start. A number of investigators from different countries in the world have already accepted to participate. A minimum sample of 100 events is anticipated. Dr. Ton van den Besselaar presented a proposal for a standardization project for the replacement of the current international standards for thromboplastin. The call for participating laboratories is open and the results are expected to be presented at the next SSC meeting and then submitted to WHO for approval. Finally, Dr. Scott Kaatz reported on an ongoing project for the standardization of the definition of clinically relevant, non-major bleeding in clinical trials, including randomized controlled trials and observational studies. Dr. Kaatz acknowledged the high variability in defining a bleeding event as relevant, and reported on a careful literature review that summarized previously used definitions. The work on a consensus on a standardized definition will be shortly finalized.

The second part of the meeting started with the joint symposium on the Management of patients on novel oral anticoagulants or with coronary stents who need surgery, chaired by Dr. Walter Ageno and Dr. Jerrold Levy. The first speaker was Dr. James Douketis, who presented the results of a study on the management of dabigatran in patients undergoing invasive procedures. Dr. Douketis highlighted that a substantial proportion of patients on anticoagulant drugs require invasive procedures each year and that therapeutic decisions involve different specialists. He then reviewed evidence from substudies of phase III RCTs with dabigatran and from recent
registries or management studies. Dr. Pierre Albaladejo presented a collaborative prospective project involving different specialists from different French speaking countries. The project is addressing the management of major bleeding and urgent surgical procedures with the use of specific guidelines that the group produced. In particular, Dr. Albaladejo presented the results from the cohort of patients with major bleeding. Subsequently, Dr. Summer Syed presented the results of a study measuring platelet inhibition in post-PCI patients with major adverse outcomes after undergoing non-cardiac surgery. Dr. Syed reported that up to 16% of patients with recent PCI undergo non cardiac surgery within 1 year from the procedure. Because the management of antiplatelet drugs is crucial in these patients, a study assessing risk factors for major adverse cardiovascular events after surgery was carried out with the measurement of platelet inhibition before and after surgery using TEG-platelet mapping assay. Dr. Syed reported high rates of major adverse cardiovascular events and of major bleeding events in this high risk population, without finding a correlation with the degree of platelet inhibition. Finally, Dr. Alex Spyropoulos reviewed available evidence from phase III studies on periprocedural management of patients treated with the direct oral anticoagulants and discussed the unanswered clinical questions that still need to be addressed. To start filling this knowledge gap, Dr. Spyropoulos proposed a registry aimed to capture management strategies and outcomes in this setting, the registry will ask endorsement of this SSC.

The second part of the meeting entitled Reversal of old and new anticoagulant drugs was chaired by Dr. Mark Crowther and Dr. Pieter Kamphuisen. In this session, Dr. Marc Samama extensively reviewed available strategies for warfarin reversal in bleeding patients or in case of urgent procedures. Dr. Joanne van Ryn presented the results of recent studies assessing the efficacy and safety of an antibody fragment for reversal of dabigatran, Idarucizumab, and described ongoing and future projects. Likewise, Dr. Mark Crowther reviewed current evidence with Andexanet alpha for reversal of factor Xa inhibitors and presented the design of ongoing studies. After general discussion, Dr. Sam Schulman reported on two ongoing twin studies, FRED and UPRATE, on the management of bleeding in patients receiving direct anticoagulants. Dr. Edelgard Lindhoff-Last presented the RADOA-registry also collecting information on bleeding patients and on patients requiring emergency interventions treated with direct anticoagulant drugs or vitamin K antagonists. The study has just started enrolment in Germany. Finally, Dr. Nakisa Khorsand presented a proposal for the standardization of the definition of clinical outcome for patients treated for anticoagulant related bleeding. Dr. Khorsand proposed to perform a systematic review of the literature, to subsequently distribute a questionnaire to ISTH members and physicians involved in the treatment of oral anticoagulant associated major bleeding; and, finally, to obtain a consensus among key opinion leaders on the definition of clinical outcome based on the results of the systematic review and on the opinion of interviewed physicians. The project is expected to be completed in 2015.
Disseminated Intravascular Coagulation

Chairman: Jecko Thachil (UK)
Co-Chairmen: Marcello Di Nisio (Italy), Satoshi Gando (Japan), Takashi Ito (Japan), Shinichiro Kurosawa (USA), Jorn Nielsen (Denmark), Hideo Wada (Japan)

Monday, 23 June (14:15-18:15)

The meeting commenced with the following proposals

- To obtain more interest in the DIC survey set up by Marcello DiNisio. This would be hoped to be the first step towards an international DIC registry
- To encourage more work on DIC in non-septic and non-trauma setting; especially in obstetrics, paediatrics and possibly malignancies
- To identify interest in using thromboelastometry in the diagnosis and management of DIC

In the first session, we had the best abstract in the section presented by Dr. Miguel Cruz from the USA. He explained the role of the A2 Domain of von Willebrand Factor in inhibiting microvascular thrombosis. He presented the results of some elegant experiments where the particular domain was identified to bind to Fibrin and thus prevent any clot formation in mice models. This was hoped to reduce mortality in endotoxemia-Induced Disseminated Intravascular Coagulation and proposes to be a possible therapeutic modality in the future.

Next, we had a very inspiring talk from Professor Niranjan Kissoon about the problems of sepsis management in developing countries. Mortality in young children from sepsis remains very high in these settings despite advances in therapeutics in the developed world. Critical care provision had to commence at the closest place to the patient, like the parents and health workers. Also the important role of coagulation activation and endothelial dysfunction in these conditions contributing to the mortality was stressed. These mechanisms may contribute in varying spectrum to the differing pathogenesis of the commonest killers, malaria, dengue, and bacterial sepsis. Prof. Kissoon persuaded us to focus on understanding the differences in this spectrum and thus aid in improving the management of such dreaded diseases.

Dr. Bernd Jilma from Vienna, Austria, then enlightened us on the interesting dynamics of Von Willebrand factor and platelets in the pathogenesis of DIC. He updated the literature on the potential role of ADAMTS-13 in identifying specific groups of DIC, wherein recombinant ADAMTS-13 may prove useful. He also suggested the possibility of using this marker in combination with Von Willebrand factor propeptide in the diagnosis of DIC. We were fortunate to have Dr. Jilma also explain some similarities between malaria and DIC.

Dr. Simon Abrams from Liverpool, UK presented the elegant work where histones were linked to endothelial dysfunction both in vitro and in vivo. He explained how in-house anti-histone antibodies could block it and thus cause survival of mice injected with histones. Further work from him demonstrated how C-reactive protein (CRP) could bind to histone and prevent its
deleterious effects. This was critical in the clinical setting and explains the acute phase reactant response which he studied in patients with trauma. He noticed that the late rise in CRP prevented poor outcome in patients with high levels of histones.

Lastly, Professor Toshiaki Iba updated us on the role of anticoagulant therapy in DIC. He started off with some work he had performed on neutrophil extracellular traps and its role in DIC. He also explained the role of antithrombin and its current use in patients with DIC in Japan. The dose difference and selection of patients were crucial. Further work is being carried out in this regard.
Factor VIII, Factor IX & Rare Coagulation Disorders

Chairman: Flora Peyvandi (Italy)
Co-Chairmen: Michael Makris (UK), Danijela Mikovic (Serbia), Steven Pipe (USA), Elena Santagostino (Italy), Midori Shima (Japan), Guy Young (USA)

Session 1: Wednesday, 25 June (8:00-12:00)
Session 2: Thursday, 26 June (8:45-12:45)

BUSINESS SESSION, FIRST PART
June, 25th 2013
Dr. Peyvandi opened the session welcoming the audience. Dr Peyvandi opened the SSC business session by thanking all co-chairs of this subcommittee for the work done in the last year.

She reported about the state of the art of the SSC:
1. Recommendations published as Official SSC Communications on JTH
   - Consensus definitions in rare bleeding disorders (Peyvandi et al, JTH 2012:10)
   - Pharmacokinetics (Bjorkman and Collins, JTH 2013:11)
   - Potency labelling of clotting factor concentrates (Hubbard et al, JTH 2013;11:988-9)
     - Standardization of methods for performing the clot wave form analysis (Shima et al, JTH 2013;11:1417-20)
   - Standardization of methods for performing the thromboelastogram (Chitlur et al, JTH 2014;12:103-6)

2. Consensus definitions in hemophilia (Chair: V. Blanchette) – 2009/2011. Recommendation accepted as Official SSC Communications to JTH
3. Clinical trial design for hemophilia (Chair: D. DiMichele) – 2011/2013. Recommendation will be soon submitted as Official SSC Communications to JTH

Dr. Peyvandi presented the on-going projects:
- The definition of mild Hemophilia A (Chair: M. Makris) – 2012/2014
- Factor V deficiency, clinical heterogeneity and treatment (Chair: D. Mikovic) – 2013/2015
- Standardization and quality management of genetic assays for diagnosis of hemophilia (Chair: V. Jenkins) – 2013/2015
- Prophylaxis in patients with inhibitors (Chair: C. Escuriola) – 2013/2015
- Prophylaxis in patients without inhibitors (Chair: V. Blanchette) – 2013/2015
- Consensus definitions and recommendations for immune tolerance induction (ITI) in hemophilia with inhibitors (Chair: E. Santagostino) – 2013/2015
- Standardization of anti-FVIII inhibitor assays (Chair: K. Moertens) – 2013/2015
- IRS-PTPs: inhibitor reporting standardization in previously treated patient (Chair: A. Iorio) – 2013/2015
• Standardisation of post-registration surveillance (Chair: F. Peyvandi) – 2014/2016
• Evaluation of hemostatic efficacy of novel FVIII/FIX concentrates and FVIII-inhibitor by-passing agents (Chairs: A. Lawrie and A. Tripodi) – temporarily stopped

CLINICAL TRIALS DESIGN AND SURVEILLANCE
Session Chairpersons: Donna Di Michele (USA) and Frits R. Rosendaal (The Netherlands)

Report on the SSC project: Definitions in Hemophilia. Victor Blanchette (Canada) on behalf of the ISTH SSC on Factor VIII, IX & Rare Coagulation Disorders Project Group on Consensus definitions in hemophilia (Nigel Key, MD, Rolf Ljung, MD, Marilyn Manco-Johnson, MD, Marijke van den Berg, MD and Alok Srivastava, MD)

Evaluation of the safety and efficacy of novel hemostatic agents and of different prophylaxis regimens in individuals with inherited bleeding disorders (focus the hemophilias) requires consistency in definitions of commonly used end points. The mandate of the Project Group was to perform a critical review of relevant information and to prepare definitions of: classification; inhibitors; regular replacement therapy (prophylaxis); bleeding (and re-bleeding) into joints and muscles; target joints; and response to therapy including surgical haemostasis. Reaching a consensus proved to be more challenging for some definitions such as the cut-off between normal and a positive inhibitor level for which a value of ≥ 0.6 Nijmegen-Bethesda Units was considered a positive test result. Inhibitors were considered transient if they disappeared within six months of first appearance despite antigenic re-challenge. Another contentious area is the consensus definition of primary prophylaxis. The current proposed definition is "regular continuous” replacement therapy started in the absence of documented joint disease as determined by physical examination and/or imaging studies, and before both the second clinically evident joint bleed and age 3 years. Finally, the chosen definition of a joint bleed, a commonly used end point in clinical trials of new factor concentrates or prophylaxis regimens, was "an unusual sensation ("aura") in the joint, in combination with any of the following: increasing swelling or warmth of the skin over the joint; increasing pain; progressive loss of range of motion, or difficulty in using the limb as compared to baseline. In infants and young children reluctance to use the limb alone may be indicative of a joint/muscle bleed…” Response to treatment of joint/muscle bleeds should be assessed at defined times following infusion of clotting factor concentrates; in the case of surgery assessment of response should be performed by a surgeon/ anesthetist involved with the procedure. The proposed definitions have been accepted for publication by the Journal of Thrombosis and Haemostasis.

Report on the SSC project: Clinical trial design for hemophilia. Donna Di Michele (USA)

The mandate for the Project Group on Clinical Trials for New Products in Hemophilia (CTPG) arose not only from pragmatic concerns about the feasibility of populating and conducting multiple simultaneous new clotting factor trials, but also from the perspective of harmonization of these requirements based on common principles. This led to the question of whether innovative and evidence-based approaches to trial simplification might increase feasibility without compromising assessment of product safety and efficacy. Based on this rationale, the CTPG was tasked with exploring alternative approaches for the pre- and post-licensure study of so called ‘me-too’ and novel biologic replacement products for hemophilia A and B. The group was constituted on the basis of member expertise in clinical care and
investigation, immunology, clinical trial design and statistics, and included regulatory science representatives from the Food and Drug Administration (FDA), USA, and the European Medicinal Agency (EMA). Input from critical stakeholders was solicited at international and FVIII/IX SSC Subcommittee meetings; from the relevant industry; and from selected experts in clinical trial design methodology. The CTPG ultimately narrowed the project scope to encompass recommendations for an alternative approach to statistical analysis and the clinical design and statistical analysis of pre-authorization trials for both ‘me-too’ and novel factor VIII (FVIII) concentrates in previously treated patents (PTPs). This approach is rooted in the combined agency regulatory goals for pre-licensure studies, and incorporates both current immunological theories of neoantigenicity and consensus clinical efficacy endpoint definitions. Innovative approaches to the clinical design of new product safety (immunogenicity) trials were considered and based on the known epidemiology and immunology of FVIII inhibitor development in congenital hemophilia A. Recognizing that the optimal design of pre-authorization clinical trials remains hampered by poor understanding of the precise nature of the interactions between the therapeutic FVIII products and the recipient’s immune system, the CTPG recommended a systematic and harmonized collection of clinical and biological data from subjects entering pre- and post-authorization new product studies. The CTPG also advocated for future exploration of 1) the feasibility of international harmonized post-authorization studies using existing national and international database infrastructure and consensus standardized minimum datasets, and 2) eventual EMA/FDA harmonization in critical areas such as the evolving landscape of inhibitor assays and the disparate approaches to product authorization in children.

Too many PUPs studies, not enough PUPs. Guy Young (USA)
From the mid-90s to the late 2000s, few new medications/studies were developed for patients with hemophilia. Recently, however, with the advent of modified factor products as well as renewed interest in studying the immunology of inhibitor development, several new PUP trials are underway or will soon be underway. With the recent approval of rFIXFc and the imminent approval of rFVIIIIFc as well as other modified agents in various stages of clinical development, there will suddenly be a need for many PUPs to participate in clinical trials as part of post-licensing agreements. In addition, investigator-initiated PUP trials are ongoing (HIPS and SIPPET) and additional such studies will be developed (INHIBIT study pending funding). Keeping in mind that the total number of PUPs born in the USA and Europe per year is 400 for FVIII and 100 for FIX and 500 and 125, respectively, and considering that many of these PUPs won’t end up at sites that open the trial, many of these patients will be needed to participate in clinical trials. This creates a variety of concerns for patients, investigators, and sponsors. For patients, the main concerns are the safety of participating in trials with novel modified factors for which the risk for inhibitor development (and perhaps other adverse events that may be unique to PUPs) is unknown. For investigators, the main concerns will be the time commitment required to participate in trials as well as ethical concerns in enrolling PUPs in trials with new agents. In addition, investigators may feel pressured by sponsors to participate in trials or worse may pressure patients to participate given financial incentives and other considerations. Furthermore, investigators who open more than one PUP trial face the prospect of deciding which patients should participate in which trials. For sponsors, the major concern will be identifying enough sites and subjects to complete the studies in a timely fashion. There are no easy solutions to any
of these issues and an ongoing dialog between the scientific community and the sponsors is the best way to minimize potential problems.

**New SSC project: IRS-PTPs: inhibitor reporting standardization in previously treated patient.** Alfonso Iorio (Canada) on behalf of the ISTH SSC on Factor VIII, IX & Rare Coagulation Disorders Project Group (Bernardi F, Lillicrap D, Lip G, Makris M, Peyvandi F, Rosendaal F)

Background: The development of inhibitors in previously treated hemophilia A patients (PTPs, i.e. patients having received =>150 days of treatment with factor VIII) is a rare event, estimated at about 3 per 1000 patient years of observation. For this reason, the ISTH SSC identified PTPs as the most suitable population for assessing antigenicity of new factor VIII concentrates. Inhibitor rates in PTPs have subsequently being used to perform direct 3, 4 or indirect 1, 5 comparisons of the immunogenicity of different factor concentrates. Critical yet missing pieces in the picture are the understandings of the potential triggers for inhibitor development and their subsequent natural history. Indeed, inhibitors may occur in PTPs in absence of switching.

Aims: 1) To define a checklist of patient and inhibitor characteristics to be included (either in the main text or as online-only appendix) when submitting for publication studies about the incidence of inhibitors in PTPs. 2) To produce a SSC recommendation to authors and journal editors to adopt the checklist.

Methods: We performed a systematic review of the evidence to assess: a) which details are usually reported or not reported about published inhibitor cases b) which guidelines do exists informing the optimal reporting of clinical cases and c) which indications for reporting clinical cases are provided by the instructions for the authors of major journals. The results of the systematic review will inform the preparation of a draft checklist and recommendation, which will be refined in a Delphi process involving of hemophilia experts.


**New SSC project: Standardization of post-registration surveillance.** Flora Peyvandi (Italy)

In the recent years, many promising strategies are emerging to enhance especially the half-life of therapeutic proteins. These bioengineering strategies have been employed to manufacture novel coagulation factors that prolong the bioavailability with increased potency and resistance to inactivation and potentially reduced immunogenicity. These novel drugs have the potential to dramatically transform the treatment of hemophilia by substantially reducing the frequency of injections. Promising data from the pre-authorization phase of clinical trials have been presented. Nevertheless, a prolonged period of surveillance will be required to collect more accurate data on the safety and efficacy and additional data to ensure consistency in the long-term of novel drugs.
Moreover, the available data on the novel long acting drugs are limited by the relatively few number of patients enrolled in the pre-authorization phase, and for this reason, large-scale post-marketing pharmacovigilance studies are necessary to gain sufficient numbers of patients for statistically valid assessments. A project group assembled by the Factor VIII, Factor IX and Rare Coagulation Disorders SSC Subcommittee aims to establishing the requirements for an international harmonized post-authorization studies using existing national and international database infrastructure and consensus standardized minimum datasets. These data would complement limited pre-authorization data on product safety and haemostatic efficacy, as well as contribute to a greater understanding of immunogenicity and the potential implementation of novel more sensitive antibody detection assays. As a first step the project group decided to revise the available template forms used by ATHN, CHESS, EUHASS and UKHCDO to collect data on safety and to ask regulatory agencies recommendations on what type of data should be collected to optimize the quality of the information, especially for long-acting products. This working group will have three/four conference calls during 2014/2015 and will meet during next ASH and/or ISTH meetings.

DISCUSSION
Discussion regarding exposure day definition for longer acting product. Not so much information: we need to redefine better both the exposure days and number of infusion. Regarding post-registration surveillance, Steven Pipe stated that post-infusion efficacy requires attention and dedicated studies. Moreover it is crucial to think about who is going to collect these data: it would be preferable to have an independent body.

SAFETY AND EFFICACY EVALUATION OF NOVEL DRUGS
Session Chairpersons: Guy Young (USA) and Flora Peyvandi (Italy)
A variety of new medications including factor replacement therapies with extended half-lives and non-replacement pro-hemostatic drugs are either already approved or in the pipeline. In the coming years, it is likely that clinicians will have a broad array of pro-hemostatic drugs to choose from to manage patients with hemophilia. There are two critical issues with respect to laboratory testing that have surfaced. First, the issue of potency labeling of the new products is crucial to ensure that what the amount of factor stated on the label is actually what is in the bottle, and second is the issue of therapeutic monitoring of the infused products in patients for PK assessments and surgery.
During this session, there were 3 sets of talks given. The first were by laboratory scientists. Dr. Hubbard presented the ISTH SSC recommendations for potency labeling of novel factor therapeutics. This was followed by a presentation by Elaine Gray from NIBSC demonstrating the high degree of variability of laboratory results from a field study evaluating various new factor products with the many different aPTT reagents for one-stage clotting assays as well as chromogenic assays. It was clear that depending on the assay used for specific products, the CV% are often very high suggesting that results of such tests for specific products must be interpreted with caution. The final talk of this portion of the session was from Dr. Rosen from Rossix labs demonstrating a possible mechanism for why one specific novel agent, N9-GP, provides such poor results with the one-stage clotting assay. The next set of talks were given by representatives from 5 companies with novel products either recently approved or in the pipeline. The presentations were given by Dr. Turecek (Baxter), Dr. Muller (Bayer), Dr. Sommer (Biogen Idec), Dr. Bensen-Kennedy (CSL Behring) and Dr. Ezban (Novo Nordisk). Briefly, what is clear
is that the various new drugs all present some problems with respect to therapeutic monitoring with standard one-stage clotting assays. While some of the products can be reliably assayed by some of the reagent/coagulometer combinations, some assays will overestimate and some will underestimate the amount of factor present. Strategies to overcome these issues include using a product-specific laboratory standard, recommending specific reagents/instruments to assay specific products, using chromogenic assays which overcomes the problems with one-stage clotting assays, or introducing a correction factor. What is clear is that laboratory monitoring of new products will be complex and many issues need to be addressed and resolved in the coming years. In the last part of the session, two talks from our regulatory colleagues, Annelise Hilger from EMA and Mark Weinstein from FDA provided the view from the perspective of the regulators.

Do we need to change the SSC recommendations for potency labeling with arrival of long acting products? Anthony Hubbard (UK)

The recommendations on potency labelling were published last year and since then the interest from regulators and manufacturers has been very encouraging. This was obvious during a workshop on the characterisation of new clotting factor concentrates held at the European Medicines Agency when almost every manufacturer of novel FVIII and FIX products reported their approach to potency labelling by reference to the published decision tree. The central theme of the recommendations was to describe the necessary product characterisation on which the decision for potency labelling should be based. This guidance remains relevant, however, it is now possible to comment further on some aspects:

1) Decision on the method for potency labelling:
It appears that all novel FVIII and FIX products, under development at present, can provide valid assays relative to the WHO IS Concentrates and manufacturers are intending to label using International Units (IU). However, some products display potency discrepancies not only between the chromogenic and clotting methods but also within methods, for instance, when different APTT reagents are used in the clotting method. In this situation, where several valid but different relative potencies are possible, it may be necessary to provide more guidance on the choice of method, including the reagent, for labelling. An important consideration in this choice should be maintaining the equivalence of the IU between existing licensed products and the novel products which would ideally be administered using a similar IU/kg dosage albeit with different frequency for the "longer-lasting" therapeutics. Large differences in the recommended IU/kg dosage between established products and the novel products could lead to confusion and undermine the credibility of the IU. The choice of potency labelling method should therefore take into account potency comparisons with existing products in vitro, using the same methods and reagents, as well as information on comparative haemostatic efficacy from in vivo studies.

2) Importance of the manufacturer's in house product standard:
Definition of the route for IU labelling of some novel products, relative to the WHO IS Concentrate, may need to specify not only the method used (e.g. clotting or chromogenic) but also the specific reagents (e.g. APTT reagent). This may mean that the link of the product to the WHO IS Concentrate can be affected by the reliability and consistency of reagents beyond the control of the product manufacturer. In these situations the consistency of product potency labelling will rely more heavily on the manufacturer's in house product standard and greater emphasis on its stability and replacement strategy will be necessary.

3) Post-infusion testing:
The recommendation that manufacturers should provide guidance to clinical laboratories on post-infusion testing remains valid. The recovery of products where the measured potency is sensitive to the type of APTT reagent might not be suitably monitored when local clotting methods use different APTT reagents. Although logistically challenging, it might be necessary for clinical laboratories to adopt specific laboratory test procedures for some products post-infusion, for instance, the use of recommended reagents or product-specific standards.

**Report of the new generation FIX collaborative NIBSC study. Announcement of the replacement FIX standard study.** *Elaine Gray (UK)*

Seventeen laboratories participated in a study to investigate the comparability of recombinant and new generation factor IX products with the WHO 4th International Standard (IS) for FIX concentrate, the WHO 4th IS for FII, VII, IX, X, Plasma and the NIBSC recombinant FIX reference preparation. Samples included in the study were a plasma-derived product, three recombinant FIX products and three long-acting products. Assay methods included in the study were one-stage clotting assays using three common APTT reagents (APTT-SP, Actin FS and SynthAFax) two common chromogenic assay kits (ROX and Hyphen) and, in addition, laboratories’ own routine APTT reagent (n=12). All samples can be assayed validly against either the 4th IS FIX concentrate, the 4th IS Factors II, VII, IX, X, Plasma or the NIBSC recombinant FIX reference preparation. Samples included in the study were a plasma-derived product, three recombinant FIX products and three long-acting products. Assay methods included in the study were one-stage clotting assays using three common APTT reagents (APTT-SP, Actin FS and SynthAFax) two common chromogenic assay kits (ROX and Hyphen) and, in addition, laboratories’ own routine APTT reagent (n=12). All samples can be assayed validly against either the 4th IS FIX concentrate, the 4th IS Factors II, VII, IX, X, Plasma or the NIBSC recombinant FIX reference preparation. Intra- and inter-laboratory assay variability was low for the plasma derived and the recombinant products for all methods against the three different reference standards. For the long-acting products, despite low intra-laboratory variation, inter-laboratory variability was high. Assay discrepancies were observed between the clotting assays using different APTT reagents and also between clotting and chromogenic assays for the recombinant and the long-acting products when assayed against the concentrate or plasma IS. When the recombinant products were assayed against the recombinant reference preparation, assay discrepancies were reduced. Based on the results of this study, it is proposed that an International Standard for Recombinant FIX be established to support potency labelling of recombinant FIX products and candidates will be evaluated in the forthcoming collaborative study for the replacement of the current International Standard for plasma-derived FIX Concentrate.

**Activation kinetics of FIX by specific aPTT reagent explain discrepancies observed in one-stage assay for N9-GP.** *Rosén P, Bryngelhed P, Rosén S (Rossix AB)*

In order to investigate the cause of the large discrepancies observed in FIX potency determination of long-life rFIX preparations with one-stage (OS) methods depending on the aPTT reagent used, a study was performed with N9-GP, BeneFix and the 4th IS FIX Conc where subsampling was made in OS methods both during contact activation and after addition of calcium ions. Generated FXIa and FIXa was determined with sensitive chromogenic methods. Actin FS, APTT SP and SynthAFax were used as aPTT reagents. No difference in FXIa generation vs time was observed for the three FIX preparations within any given aPTT reagent. However, the actual level of generated FXIa with Actin FS was only about 2/3 vs APTT SP and SynthAFax. FIXa generation vs time was similar for the three FIX preparations with both SynthAFax and the chromogenic Rox Factor IX kit. The large discrepancy was instead caused by large differences in FIXa generation when using APTT SP and Actin FS on analysis of N9-GP. FIXa generation was impaired with Actin FS and, importantly, high levels of FIXa were generated with APTT SP already before addition of calcium ions. Our results identify the cause
of the FIX potency discrepancy and demonstrate that different aPTT reagents may have a strong impact on FIXa generation.

**Activity measurement of BAX 855, PEGylated recombinant full-length human FVIII**

*Peter Turecek (Baxter)*

Baxter has developed BAX 855, a PEGylated recombinant Factor VIII product, which is currently in late stage clinical development. BAX 855 is based on the parent full length rFVIII molecule in ADVATE® produced from CHO cells. BAX 855 is chemically modified by conjugation with a proprietary stable PEG reagent from Nektar Therapeutics using comparable conjugation technology as had been successfully employed in other marketed and licensed PEGylated drug products. Baxter has decided to use the one-stage clotting assay for its BAX 855 product. The reason is that both clotting and chromogenic activity values for BAX 855 are better aligned than for BDD-rFVIII or other modified FVIII products which had been previously in clinical use or are in clinical development and where discrepancies between one-stage clotting and chromogenic values caused severe issues for patients [1-4]. The potency determination for BAX 855 uses Actin FSL which is the same activator reagent as also used for the one-stage clotting assay used for potency determination of ADVATE®. By using this specific set-up of the one-stage clotting assay good agreement with the chromogenic assay is achieved. The other assay parameters are also comparable to the one-stage clotting assay used for potency assignment for ADVATE® (automated coagulation analyzer BCS/XP, immunodepleted FVIII deficient plasma freeze dried, same incubation time and assay set-up, same in-house secondary standard for rFVIII). Using the one-stage clotting assay for potency assignment makes the determination of recovery in patients reliable as in clinical practice only the one-stage clotting assay is used. Preliminary results from the phase 1 study on BAX 855 showed that the mean incremental recovery (IU dL-1/IU kg-1) measured with the one-stage clotting assay was in the same range as for the study comparator FVIII product ADVATE®.

According to EMA guideline CHMP/BPWP/144533/2009 on clinical investigation of recombinant and human plasma derived FVIII products "preferably, the same assay should be used for analysis of the product and the patient’s plasma”. At the same time the guideline recognizes that potency assignment for FVIII products has to be performed with the chromogenic assay as requested by European Pharmacopoeia monograph 1643, human coagulation factor VIII (rDNA). In contrast in clinical practice the one-stage clotting assay had remained for the last 50+ years as the preferred method to determine FVIII plasma levels. Therefore Baxter assumed that a new FVIII product should also be determined for potency by the same assay as used in clinical practice.

References:

2. Gruppo et al., Haemophilia. 2004 Sep;10(5):449-51
3. Hubbard et al., Thromb Haemost. 2003 Dec;90(6):1088-93
4. Leong et al., Poster presented at the XXIII Congress of the ISTH, Kyoto, Japan, 2011

**BAY 94-9027; Evolution and Significance of Potency Assays used in the PROTECT VIII trial and recommendations for future clinical monitoring.**

*Nikolaus Mueller+*, *Yvonne Katterle*, *Lilley Leong*, *Derek Sim*, *Lisa Regan*, *Prasad Mathew*, *Georg Lemm*
BAY 94-9027 is B-domain deleted rFVIII with site-specific pegylation to reduce the clearance of FVIII. BAY 94-9027 has a half-life of approximately 19h in a phase I study, and is currently being investigated in adult and pediatric patients with Hemophilia A. The potency of BAY 94-9027 in the final container is determined by chromogenic substrate assay (CSA) employing a product standard which is calibrated against the WHO 8th IS. BAY 94-9027, sucrose formulated rFVIII, and plasma-derived WHO 8th IS perform identically (superimposable calibration curves) in the CSA, indicating that this method is the most accurate for BAY 94-9027 assay.

Similar FVIII activity for BAY 949027 is attained using a One-Stage aPTT assay with ellagic acid activators and with some silica activators, e.g. HemosIL SythaSil kit (IL), Pathromtin kit (Siemens). These results indicate that OS assays using ellagic acid activators and selected silica activators are acceptable alternatives to CSA. Both, CSA and OS have been validated to quantify BAY 94-9027 in human plasma samples. The CSA was calibrated using recombinant BAY 94-9027 release standard diluted in a FVIII deficient human plasma pool. A low (1.25 – 50 IU/dL) and a normal level (3 – 200 IU/dL) working range have been established. Accuracy and precision of the low level method ranged between 92.8 to 98.2% and 14.6 to 20.7 %, respectively, whereas for the normal level method, the values were 101 to 108% and 6.01 to 12.0%, respectively. An ellagic acid based OS method was validated in the range of 1.5 to 80 IU/dL using Siemens Behring Standard Human Plasma diluted with FVIII deficient human plasma pool as calibrator. Accuracy and precision ranged between 131 to 136% and 3.38 to 6.90%, respectively. Results from ongoing work with the OS method based on selected silica activators will be presented. Conclusion: Bay 94-9027 can be best assayed by the chromogenic method. For the OS method, both ellagic acid and some silica-based activators give comparable results to the chromogenic assay and qualify as well.

**Assays for Therapeutic Monitoring of ELOCTATE™ and ALPROLIX™. Jurg Sommer (Biogen Idec)**

ELOCTATE, a recombinant B-domain deleted factor VIII Fc fusion product (rFVIIIFc) has comparable specific activity to native FVIII on a molar basis and no reagent or instrument dependent discrepancies were identified in the one-stage clotting assay. A blinded field study conducted in 30 clinical hemostasis laboratories furthermore showed that ELOCTATE could be measured as accurately as Advate® in spiked plasma samples by a variety of one-stage clotting assays calibrated against common FVIII plasma standards. The chromogenic FVIII activity of ELOCTATE measured in 11 of these laboratories using 5 different commercial kits was on average just 10 to 20% higher than the one-stage activity. The hemostatic activity of ELOCTATE was shown to be comparable to that of Advate in subjects that were evaluated by thrombin generation and whole blood clotting assays during the phase 3 clinical study. Combined with the clinical efficacy results, these studies indicate that any of the existing one-stage or chromogenic assay methods may be used for monitoring rFVIIIFc activity in patients (without the need for a product specific standard) and, as is the case with currently marketed FVIII products, the activity determined by these methods is a suitable surrogate marker for in vivo efficacy of ELOCTATE.

In a phase 3 clinical trial, a prophylactic regimen of ALPROLIX™ designed to achieve trough levels of 1 to 3% FIX activity in individual subjects resulted in annualized bleeding rates (ABRs) that were consistent with the expected bleeding rates based on current FIX products. Global hemostasis assays performed during the clinical study also showed that ALPROLIX, while
longer-acting, provided an ex vivo hemostatic activity per unit of plasma FIX activity that was comparable to the comparator rFIX product (BeneFIX®). Meanwhile, a field study involving 30 clinical hemostasis laboratories demonstrated significant assay variability for both ALPROLIX and BeneFIX, though ALPROLIX had somewhat higher aPTT reagent dependent variability than BeneFIX. Despite the observed assay discrepancies, 80% of laboratories were able to measure a 0.8 IU/mL ALPROLIX sample within ± 30% accuracy. Laboratories using a kaolin based aPTT reagent were a notable exception, since they underestimated ALPROLIX activity by as much as 50%. Overall, the majority of laboratories were able to measure ALPROLIX with reasonable accuracy using their existing one stage clotting assays and without using a product specific standard. ALPROLIX activity by the one-stage clotting assay will thus be largely representative of its in vivo hemostatic activity. To address potential concerns about the accuracy of locally performed clotting assays, Biogen Idec has established a designated ALPROLIX and ELOCTATE Reference Laboratory and provides a rFIXFc Laboratory Sample Kit for research use.

rVIII-SingleChain and rIX-FP. Debra Bensen-Kennedy (CSL Behring)
rVIII-SingleChain is a single-chain recombinant factor VIII molecule being developed by CSL Behring (CSLB) for prophylaxis, prevention, treatment of bleeding episodes and surgical prophylaxis in patients with hemophilia A. Unlike other B domain deleted recombinant FVIII molecules, rVIII-SingleChain is a single polypeptide chain, B domain truncated recombinant factor VIII. Since 1994, the chromogenic substrate (CS) assay has been the reference method of the European Pharmacopoeia for the assignment of factor VIII concentrate potency [Mikaelsson 2001]. CSLB uses the CS assay for potency assignment and regards the method to be of greatest value due to the evidence from other modified FVIII products that alterations of the B domain of FVIII can influence one-stage (OS) assay results. For rVIII-SingleChain, both the OS and the CS FVIII assays deliver parallel and linear results against the WHO International Reference Standard in terms of assay performance. As expected and in line with other recombinant and B domain deleted FVIII products, rVIII-SingleChain potency assignment results in a discrepancy between the two assay formats. In order to ensure the appropriateness of the method used to assign potency, a tiered approach was taken to determine which of the FVIII activity assays represent the true in vivo functionality of rVIII-SingleChain. CSLB’s approach was in line with recommendations from FVIII and FIX Subcommittee of the SSC of the ISTH [Hubbard 2013]. CSLB has demonstrated that results obtained in pre-clinical, PK and clinical studies utilizing the CS assay method are reflective of true potency. With respect to clinical monitoring, CSLB acknowledges that the OS assay remains the preferred method. For this reason, the development program was designed to test FVIII:C with both assay methods. Results obtained in clinical PK data were linear across the full range of values and consistent with what was observed in pre-clinical studies. The linear relationship between the results of CS and OS assays offers the ability to effectively guide clinicians utilizing OS methodology to interpret FVIII:C results and remain in alignment with CS testing results and potency assignment. This will lead to a simplified approach to calculate and target critical activity levels when treating patients.

rIX-FP, a recombinant fusion protein linking coagulation factor IX with albumin, is being developed for prophylaxis, prevention, treatment of bleeding episodes and surgical prophylaxis in patients with hemophilia B. rIX-FP is a recombinant protein generated by the genetic fusion of the two cDNAs of human albumin to human coagulation factor IX to prolong the terminal half-life. Potency is assigned utilizing the OS clotting assay, in line with all other currently available
FIX products. CSLB regards the OS clotting assay to be of greatest value for potency assignment and has demonstrated that results obtained in pre-clinical, PK and clinical studies support utilizing the OS method. Clinical trial data utilizing paired patient samples measuring FIX:C in both local and central laboratories will also support recommendations for clinical monitoring.

**Factor VIII and FIX long acting products: which assay should be used to evaluate their efficacy? Mirella Ezban (NovoNordisk)**

The introductions of new longer half-life products for replacement therapy have the potential to improve the clinical outcome and reduce the burden of disease in hemophilia. The one stage clot assay is primarily used for post-infusion monitoring. The assay is a biologic assay with inherent variation. Variation with existing factor products is considerable. This existing variability and complexity is being increased with the new longer half-life products. N8-GP and N9-GP are GlycoPEGylated recombinant FVIII and FIX molecules in late stage clinical development. The PEG moiety interferes with some aPTT reagents used in the one-stage clot assay. 1,4 Careful assessment of the accuracy and precision of the assays for potency labeling and therapeutic monitoring is therefore necessary to support the evaluation of the efficacy and safety of these new long acting factor products.

Potency assignment for both N8-GP and N9-GP follow the SSC recommendations and the European Pharmacopeia (Ph.Eur) and the two molecules are calibrated against the respective WHO standards. The process for selecting a valid and accurate potency assay for each of the molecules will be described. N9-GP potency is assigned with the one stage clot assay using the aPTT reagent SynthAFax; the validity of which is supported by results obtained by other biological assays showing minimal PEG inference such as chromogenic assays and thrombin generation assays. A similar approach has been followed for N8-GP, however, a chromogenic assay has been used for potency assignment according to the Ph. Eur. The extent to which GlycoPEGylation interferes with post infusion monitoring has been analyzed in selected expert laboratories using spiked hemophilia plasma samples. For N8-GP acceptable values were obtained with chromogenic assays and with the most commonly used aPTT reagents. For N9-GP chromogenic assays gave accurate results but only a limited number of aPTT reagents could be used. The clear question to be addressed is how to assure patient safety and to minimize complexity in laboratory work flow while providing patients the choice of treatment which may reduce the burden of their treatments and improve quality of life. Expansion of the use of chromogenic assays may be the common choice for factor activity monitoring of new long-acting factor products.

References:

1. Viuff et al Haemophilia 2011; 17: 695-702
5. Holm PK, et al. ISTH 2013, Amsterdam, NL. Poster PB 3.49-1
Report on the 2013 EMA-EDQM Workshop on "Characterization of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples". Anneliese Hilger (EMA)

The Workshop was held on 28-29 November 2013 at the European Medicines Agency (EMA) and organised jointly by EMA (with Biologics Working Party and Blood Products Working Party support) and the European Directorate for the Quality of Medicines and HealthCare (EDQM).

The workshop discussed potency assays used for product labelling and testing of patient’s post-infusion samples for new clotting factor VIII and IX concentrates. A number of new recombinant products are in the late stages of development and it was felt that a more harmonised approach to assigning potency to these clotting factor concentrates was required. The objectives of the workshop were to provide a point of reference when making future decisions for licensing or changes to the relevant European Pharmacopoeia texts.

Manufacturers of new products are undertaking a thorough characterization of the performance of new products in different assay systems as recommended by ISTH. For all products in development, valid assays versus the international concentrate standard were obtained and potency was expressed in IU. The decision tree defined in the ISTH recommendations is considered useful in many cases. However, for some products, valid assays could be obtained with both chromogenic and one-stage clotting assays but these different assays give very different potency values. Furthermore, for a number of the products, valid one-stage clotting assays give very different potency values depending on the APTT reagent used, especially for some long-acting products. Therefore, correlation with biological activity from in vitro, non-clinical and clinical efficacy studies is important in selecting the appropriate assay. Precise methods are needed for potency labelling of products (80-120%), whereas for clinical diagnosis sensitivity is important e.g. down to <1% and for clinical monitoring it is needed to cover dosing and trough levels. Clinicians wish to have one assay to be used to assign product potency and which correlates with patient factor levels. Different assays could lead to discrepant product plasma values measured post-infusion. A thorough characterization of Factor VIII and FIX products according to ISTH recommendation needs to be performed to develop the most appropriate assay for potency labelling. In addition, adequate measures to avoid any missdosing or potential problems regarding monitoring of patient plasma samples need to be proposed by the manufacturer.

FDA’s Current Considerations on Assays for Potency Determination of Long Acting Clotting Factor Concentrates. Mark Weinstein (FDA)

There are multiple issues to consider when evaluating the potency of long acting clotting factor products. These include the fact that the one stage clotting assay (OC) along with a plasma reference standard are used by most laboratories worldwide to monitor FVIII/FIX activity, whereas the labeled product potency may be determined using a chromogenic assay (CA). The validity of a particular method of potency assignment is based on its correlation with efficacy in clinical trials, and no one set of reagents or standards has been suitable to measure potency of all products. Recent studies have shown that activity results for a given product can vary greatly depending on the reagents and reference standards used, particularly for FIX products, and more so for the OC than for the CA. Manufacturers need reference standards and validated assays to ensure product potency, consistency of manufacturing and the stability of
products over time. Regulatory agencies have not reached consensus on the best approaches to potency determinations.

Given these considerations, US licensed manufacturers have followed a paradigm of assaying new FVIII and FIX products using both OC and CA tests (as appropriate for the product) to calibrate an internal reference standard against an International Concentrate Standard; assigning potency to a new product against the internal standard; determining levels of the product in plasma samples against the internal reference standard; and determining levels of the product in plasma samples against a plasma standard. However, for some products, an OC may simply not be possible, raising issues for product labeling. With the diversity of new products and the potential of obtaining differing potency results depending on choice of assay and assay conditions, additional effort is needed to optimize potency labeling for end users. In situations where disparities are expected to exist between activity levels determined in clinical labs (generally using OC assays on plasma) and labeled product potencies (determined by a non-equivalent method), the user community needs to be educated how best to interpret the assay results. Typically, such information would be conveyed by the manufacturer.

DISCUSSION
In this session there was an important and fruitful discussion regarding which assay should be used for the longer acting product post infusion laboratory testing among scientists, physicians, manufacturers and regulators (FDA and EMEA). All bodies declared to be knowledgeable regarding the assay issues for the longer acting products. It was clear from all presentations that chromogenic assay could be a more suitable assay for PEGylated products, particularly for B-domain deleted ones. Both regulators seem to be collaborative and “flexible” for any additional assays (including chromogenic assay if there would be evidence regarding its advantage to the one-stage clotting assay).

BUSINESS SESSION, SECOND PART
June, 26th 2013

TOP-RATED ABSTRACT
In vivo selection of genetically manipulated platelets corrects murine hemophilic phenotype and induces immune tolerance even using a low multiplicity of infection for transduction.
Qizhen Shi
Objective: Our previous studies have demonstrated that lentivirus-mediated platelet-specific (2bF8) gene therapy can restore hemostasis in hemophilia A (HA) mice with or without inhibitors. In this study, we aimed to enhance platelet-FVIII (Plt-F8) expression while minimizing potential toxicities. Methods: A novel lentiviral vector (LV), which harbors dual genes, the 2bF8 gene and a drug-resistance gene, the MGMTP140K cassette, was constructed. Plt-F8 expression in HA mice was introduced by bone marrow (BM) transduction and syngeneic transplantation. After BM reconstitution, the recipients were treated with BG/BCNU monthly for 3 or 4 times. Animals were analyzed by PCR, qPCR, FVIII:C assays, and inhibitor assays. Phenotypic correction was assessed by tail clipping tests and ROTEM analysis. Results: When an MOI (multiplicity of infection) of 1 was used for transduction, Plt-F8 expression in recipients was only 0.22±0.15 mU/108 platelets before the drug treatment, but remarkably increased to 4.33 ± 5.48 mU/108 platelets (n=16) after BG/BCNU treatments, which is 2.89-fold higher than the data obtained from our regular 2bF8LV with an MOI of 10. 2bF8 proviral DNA
was barely detectable (0.01±0.02 copies/cell) before chemoselection, but it increased to 0.42 ±
plusmn;0.15 copies/cell after BG/BCNU treatments. Fifteen of 16 treated animals survived tail
clipping. Blood loss and whole blood clotting time were normalized in the treated recipients.
When an MOI of 10 was used, Plt-F8 expression was enhanced from 1.63±0.36 mU/108 platelets
to 14.18±5.39 mU/108 platelets after 3 BG/BCNU treatments. Notably, no anti-FVIII antibodies
were detected in the treated animals even after exogenous rhFVIII challenge.
Conclusion: We have established a powerful in vivo selective system that allows us to enhance
the therapeutic efficacy of 2F8 gene therapy and induce immune tolerance in hemophilia A
mice.
DISCUSSION was on how this could help in clinical trials.

CLINICAL AND LABORATORY EVALUATION
Session Chairpersons: Steven Pipe (USA) and Kathelijn Fischer (The Netherlands)
In patients with hemophilia, the residual levels of clotting factor (FVIII or FIX) are considered as
the most important prognostic factors. Based on that, hemophilia patients are classified into the
severe, moderate and mild categories, despite several observations suggesting that this may be
actually insufficient. For instance, about 10-20% of patients classified as having severe
hemophilia A do not have major bleeding complications.
On the other hand, the classification of patients with mild hemophilia is not entirely clear.
Classification is based on laboratory testing, but 30% of patients with mild hemophilia exhibit
discrepant FVIII:C levels depending on whether they are measured by the one- stage or
chromogenic assay. In addition, patients with FVIII/IX levels >40% are not clas
sified as having
hemophilia, but they may bleed.

Report on the SSC project: Bleeding score in hemophilia: a prognostic tool for clinical
outcome. Maria Elisa Mancuso (Italy)
Despite the lack of knowledge, on the relation between bleeding symptoms and FVIII levels,
treatment strategies are often implemented, sometimes pre-emptively, only on the basis of
residual factor level (ie., prophylaxis vs. on demand therapies). Appreciation of the true
prognostic value of residual factor level is therefore a major, clinically relevant, task to be
undertaken by the SSC-ISTH Working Group on Clinical outcome evaluation.
With this as background the Working Group (WG), together with 18 physicians expert in the
field established, has initiated a 4 rounds Delphi method, to establish consensus-based clinical
criteria to define the degree of severity of hemophilia independently from residual FVIII/FIX
levels. The group came up with 4 major and 1 minor criteria and established that the presence of
2 major criteria was the minimum requisite to define a severe disease. A specific score was
assigned to each criterion. The next step for the Working Group will be to propose a validation
study of the predictive value of residual FVIII/FIX activity measured at diagnosis as compared
with the definition of the severity of the disease done by using the aforementioned criteria.

Report on the SSC project: The definition of mild hemophilia A. Michael Makris (UK)
The WG is examining the possibility of allowing the diagnosis with a higher FVIII:C level in the
presence of a family history or pathogenic mutation. It is also investigating the possibility of
raising the 0.40iu/ml for all, if there is sufficient published evidence for bleeding above this
level.
A further important issue is that the ISTH definition does not specify the type of FVIII:C assay to be used in the definition of hemophilia. The WG will propose that both 1-stage and chromogenic assays are performed on all mild hemophilic patients at diagnosis and that the ratios are reported as 1-stage over chromogenic. Ratios of <0.5 or >2.0 indicate significant discrepancy.

The third area being addressed by the WG is the issue of inhibitors in patients with mild hemophilia A. The WG feels that all mild hemophilic patients should have their genetic defect identified as this can aid in the management if a high inhibitor risk mutation is present. All mild hemophilic patients exposed to FVIII concentrate should have a FVIII inhibitor level measured with the Bethesda assay six weeks after exposure.

**DISCUSSION**

In the follow-up of the bleeding score project the Project group proposed to do a validation study to evaluate the score. The proposal is to include a minimum of 20 randomly selected patients born 1990-2010, irrespective of hemophilia type or severity. The outcome of the score would be correlated to FVIII/IX levels. It was discussed that especially the moderate patients would be an interesting group, but this group may require central laboratory evaluation of the baseline FVIII/IX level. Several participants discussed selection bias, as patients with a more severe phenotype would be started on prophylaxis sooner than those with a milder phenotype. Regarding the patients with FVIII/IX levels >= 40%, it was pointed out that we have no tools to distinguish bleeders from non-bleeders. The suggestion was to actively look for other laboratory abnormalities that may explain bleeding in these patients. The committee feels that genetic analysis is also important.

**RECOMMENDATIONS ON HEMOPHILIA TREATMENT**

*Session Chairpersons: Elena Santagostino (Italy) and Alok Srivastava (India)*

Dr Srivastava had apologized for not being present due to personal reasons.

**Report on the SSC project: Consensus definitions and recommendations for immune tolerance induction (ITI) in hemophilia with inhibitors. Elena Santagostino (Italy)**

Consensus definitions of clinical and laboratory endpoints in the different subsets of patients undergoing ITI are required because these would allow more reliable data analyses and comparisons between studies, furthermore, prompting the initiation of international prospective surveys able to collect more homogeneous data during a prolonged follow-up period. The aim of this project is to provide definitions of ITI outcome standardizing the methodologies for the assessment of treatment endpoints. An additional aim is to establish harmonized strategies for ITI therapy in inhibitor patients belonging to different and well defined prognostic groups. Based on a literature review, several issues have been identified for discussion and deliberations within the working group.

Critical issues include:

- ITI outcome defined on the basis of strict pharmacokinetic criteria not always reflect the clinical benefit obtained with ITI;
- the need for repeated pharmacokinetic assessment and inhibitor testing after a wash-out period makes these procedures very demanding;
- inhibitor testing by the modified Nijmegen-Bethesda assay is relatively insensitive to very low titers;
- FVIII pharmacokinetic measurements in the presence of low-titer inhibitors is a challenge with the available methodology.
- the definitions of good- and bad-risk patients based on historical and pre-ITI titers are more easily applied to young patients because this information may not be available in older patients.
- very heterogeneous patients are grouped together under the "umbrella” of bad-risk patients.

The current presentation will briefly discuss these issues.

**DISCUSSION**

There were many positive reactions to the proposals made by the Project Group. In addition, the group was asked to address the issue of when to discontinue ITI as well as consider the bleeding phenotype during ITI in some of the definitions (e.g. for partial response).

Guy Young highlighted the importance of achieving an agreement on a certain ITI duration (many treatment courses have been prolonged for years without any clear benefit for the patient). Elena Santagostino commented that this is one of the issue to be defined, however, different treatment durations may be needed for different prognostic groups of patients.

Victor Blanchette highlighted the need for standardized procedures for PK testing; his question was on the potential use of population-based PK models. Elena Santagostino answered that these models are not applicable to the heterogeneous group of patients with inhibitors.

Massimo Morfini raised the issue of the lower limit of normal FVIII half-life, to date fixed at 6 hours: this is too short in his opinion. He also disagreed on having a measurable through level of FVIII as a criteria for tolerance; in his opinion other PK parameters such as FVIII clearance should be considered.

Alfonso Iorio highlighted the clinical relevance of bleeding during ITI; this should be included as a criteria to guide treatment choice.

**Report on the SSC project: Prophylaxis in hemophilic patients without inhibitor.** Victor Blanchette (Canada) on behalf of Peter Collins, Kathelijn Fischer, Margareth Ozelo, Alok Srivastava and Guy Young.

The mandate of the Project Group is to prepare an evidence-based Report of long-term prophylaxis in persons with hemophilia A and B who are inhibitor negative. Topics to be considered are: 1) when and how to start prophylaxis; 2) strategies for adjusting prophylaxis regimens in different patient cohorts e.g. children, adolescents and adults; 3) outcome measures relevant to long-term prophylaxis aimed to prevent/delay progression of bleed-related arthropathy; 4) potential impact of prophylaxis on adverse outcomes other than joint damage e.g. intracranial hemorrhage, inhibitor formation; 5) adherence to prophylaxis and factors that influence adherence; and 6) potential impact of long-acting factor VIII/IX concentrates on prophylaxis regimens in the future. A formal literature search has been initiated that will start from the point of the Cochrane Review " Clotting factor concentrates given to prevent bleeding and bleeding related complications in people with hemophilia A and B " [Iorio A et al. The Cochrane Library, Issue 9, 2011]. An exact search strategy to that used in the Cochrane Review will be used to identify all additional relevant literature on prophylaxis for person with
hemophilia from 2010 to the current time. Members of the Project Group will review all relevant material identified by the Cochrane search and the additional search to extract the information on the various aspects of prophylaxis. External experts, including health care providers, Agencies involved with decisions refunding of clotting factor concentrates and patient groups will be invited to provide commentary on the draft Report from the Project Group before it is finalized.

DISCUSSION
As the mandate of the SSC was to provide clinical guidance on various aspects of prophylaxis, a formal search according to the rules of evidence based medicine generation may have to be reported separately. It was discussed that the Project Group should avoid duplication the work of the Cochrane collaboration. The question when and how to initiate prophylaxis in young boys with severe hemophilia was considered as the clinically most important question. Kathelijn Fischer asked the audience to make proposals for research questions. Flora Peyvandi clarified that this is not the aim of SSC, she prompted the group to work on definitions as initially agreed upon.

BY-PASSING AGENTS
Session Chairpersons: Midori Shima (Japan) and Yesim Dargaud (France)

FVIII / FIX SSC session on by-passing agents comprised five presentation on the two major topics i.e. development of new treatment strategies in patients with inhibitors and monitoring of by-passing agents.

Several publications reported the potential interest of the global haemostasis assays including thrombin generation assay, thromboelastography and clot waveform analysis for the monitoring of by-passing therapy. Standardization of the assays is ongoing; recommendations were already published on the use of thromboelastography and clot waveform analysis. A manuscript will be submitted to the J Thromb Haemost on standardization of thrombin generation measurement for hemophilia. Lyon Hemophilia Center plans to organize a multicenter clinical study in 2015, evaluating thrombin generation assay for individualizing by-passing therapy in patients needing elective surgeries. Potential investigator centers will be invited to participate. A recent study organized at the Hemophilia Center of Milan reported that under the conditions used in this study, TGA was not able to predict either the hemostatic response to different by-passing agents used at different doses nor the risk of bleeding complications. With regard to clot waveform analysis, it has been recently shown that using a new reagent combining ellagic acid and tissue factor might be of interest for the monitoring of by-passing agents. Thrombin generation assay and clot waveform analyses were also suggested as potential monitoring methods for ACE910 which is a recombinant humanized anti-factor IXa × factor X bispecific monoclonal antibody mimicking a cofactor function of factor VIIIa. Differently from FVIII, ACE910 does not require the activation process. In addition, there is a substantial difference in the inactivation, in the binding affinity to FIX(a) and in the capability of binding to phospholipid. Therefore, global haemostasis assays that are not directly influenced by the difference of the activation process would be of interest for the monitoring of this new therapeutical approach.

The use of RNA interference (RNAi) to target the natural anticoagulant antithrombin as a strategy to rebalance the hemostatic system and improve thrombin generation, and therefore hemostasis, in hemophilia was reported as a new therapeutical approach for hemophilia with
inhibitors. ALN-AT3, a subcutaneously administered RNAi therapeutic targeting AT, is currently being developed for the treatment of hemophilia. Promising results were obtained in hemophilia mice models and non-human primates, with no significant adverse events reported. The potential for subcutaneous route of administration, infrequent dosing, and applicability to persons with hemophilia who have inhibitors, support the continued development of ALN-AT3.

Global assays for the evaluation of the efficacy of by-passing agents. Yesim Dargaud (France)

Haemostasis and its abnormalities have been traditionally assessed by plasma clotting times. While factor assays based on these tests have defined the different coagulation disorders and are useful for diagnosis, they also have several limitations. Currently, there are tests that can quantitatively assess the overall haemostatic potential of blood. The process of thrombin generation and fibrin clot formation can be captured with greater sensitivity and completeness by assays that measure global haemostasis. These include i) the thromboelastography, ii) the clot waveform analysis and iii) thrombin generation assays using different instrument systems.

There are several publications in the field of thromboelastography and hereditary or acquired bleeding disorders. Case reports and small case series have addressed the usefulness of thromboelastography in the management of bleeding or monitoring of treatment with bypassing agents. Recently, recommendations of the SSC working group on the standardization of thromboelastography measurements in hemophilia were published. The utility of clot waveform analysis to define qualitative and quantitative differences at levels of FVIII:C less than 0.01 IU/mL has been previously reported, raising the possibility that the correlation observed, between the laboratory definition of severity and the clinical phenotype, could be improved by this approach. A recent publication suggested the interest of the assay for monitoring by-passing therapy. Recommendations of the SSC working party were recently published with regard to the standardization issues of the assay.

Several groups reported a relatively good correlation between thrombin generation assays and the bleeding tendency of patients with hemophilia but has not been used to classify the severity of the disease so far. One of the greatest impact of thrombin generation assays in the field of hemophilia has been in the monitoring of by-passing agents. The ex vivo monitoring that would reflect achievement of haemostasis in vivo still needs further studies, though several attempts have already been initiated. In this respect, the thrombin generation assay might be used to predict the differential response to recombinant FVIIa and Feiba tested prior to in vivo administration, and might provide further insight into the optimal dose of therapy pre- and postoperatively. If one considers that there probably is a level of thrombin generated that predicts clinical efficacy, this assay could be used for monitoring of bypassing agents and for optimizing the infusion schedule by tailoring doses and frequency of injections individually for each single patient. However, correlation of the TGA parameters with in vivo clinical response needs to be further established if we believe that this assay may represent a surrogate marker for monitoring bypassing therapies. In order to establish the usefulness of this test in multicenter clinical studies, a standardized methodology needs to be developed. This is being carried out through a working group established by the SSC. The manuscript is currently in revision by the members of the working group and will be submitted for publication in July 2014. A multicenter clinical study evaluating TGA for the monitoring of by-passing therapy coordinated by Lyon Hemophilia Center will be organized in 2015.
**Thrombin generation in Hemophilia treated-patients. Maria Elisa Mancuso (Italy)**

Background and aims: FVIII activity measurement is the mainstay of laboratory monitoring during surgery in patients with hemophilia A (HA) without inhibitors. No routine coagulation monitoring is available for hemophilic patients with inhibitors treated with by-passing agents. Global coagulation tests as thrombin generation assay have been considered has potential candidate to to predict hemostatic response in those with inhibitors treated with by-passing agents. The aim of this study was to evaluate if TGA is able to predict the hemostatic and clinical response to by-passing agents in patients with HA and inhibitors.

Materials and Methods: TGA was assessed in platelet-rich (PRP) and platelet-poor (PPP) plasma with the addition of corn trypsin inhibitor (CTI) in 16 patients with severe HA and high-responding inhibitors aged 5-59 yrs (median: 39). Blood samples were collected in 2 different settings: in a non-bleeding state after a single infusion of one by-passing agent (either aPCC or rFVIIa) in order to assess a kind of PK after by-passing agent administration and during major orthopaedic surgery. Thirteen patients underwent the assessment in a non-bleeding state and 10 during surgery, being 7 those patients in whom TGA was measured in both conditions. PK assessment was performed after a standard 90-120 mcg/kg dose of rFVIIa, a high 270 mcg/kg dose of rFVIIa and/or a 80 IU/kg dose of aPCC. Blood samples were drawn at baseline, 30 minutes and 3 (if rFVIIa) or 6 (if aPCC) hours. During surgery TGA was assessed once daily prior and 30 minutes after by-passing agents injection starting from the pre-operative bolus and for at least 4 consecutive post-operative days. Haemostatic treatment to cover surgical procedures was established irrespective of TGA measurements. Results: In the non-bleeding state assessment TGA increased after each by-passing agents injection however it was not able to discriminate any difference between the two drugs and between the different doses of rFVIIa. Similarly, in the surgical setting TGA did not reveal different responses related to the type of drug, the dose used and/or the occurrence of bleeding complications (n=5). Moreover, a lack of response of the TGA curve was observed during the post-operative period irrespective of treatment regimen modifications in all patients. Conclusions: our results indicate that TGA is not a suitable tool to monitor hemostatic response during surgery in patients with hemophilia and inhibitors treated with by-passing agents. In fact, TGA was not able to predict either the hemostatic response to different by-passing agents used at different doses nor the risk of bleeding complications.

**DISCUSSION**

In this session, there were two groups presenting opposite results on the use of thrombin generation assay. This clearly indicates that the use of thrombin generation assay requires standardization. To this regard Dr. Dargaud started discussion on the importance of pre-analytical conditions for global haemostasis assays. Questions on blood sampling, plasma preparation and the use of CTI were addressed. The educational DVD showing the key pre-analytical steps, generated by Lyon Hemophilia Center is available for clinical studies in the frame of the standardization of TGA.

**Application of clot waveform analysis to hemostatic monitoring of bypassing therapy. Keiji Nogami (Japan)**

Background: Assays to determine the optimal hemostatic effects of bypassing therapy in hemophilia A (HA) patients with inhibitors are difficult to compare. Clot waveform analysis
CWA, based on the continuous monitoring of routine coagulation parameters (PT/aPTT), offers a useful method for assessing global clotting function.

Objective: We investigated the technique of CWA for the hemostatic monitoring of bypassing therapy in HA patients with inhibitors.

Methods and Results: Ellagic acid (Elg), tissue factor (TF), or both (Elg/TF) were used as trigger reagents in CWA. The standard parameters; clot time (CT), maximum coagulation velocity (|min1|), and acceleration (|min2|) were recorded. An optimal monitoring was defined as (i) a significant difference in these parameters between plasmas from HA patients with inhibitors and normal plasmas, and (ii) a significant improvement in these indices in HA patients with inhibitors after bypassing therapy. Experiments in vitro demonstrated that there were significant differences between plasmas from HA patients with inhibitors and normal plasmas with various triggers, in the order Elg > Elg/TF >> TF. Addition of therapeutically achievable concentrations of bypassing agents, however, showed significant improvements in the different parameters only with Elg/TF, suggesting that this reagent provided the most appropriate assay. A total of 20 plasmas from HA patients with inhibitors in which bypassing agents were infused were evaluated ex-vivo by Elg/TF-CWA. The postinfusion parameters CT and |min2| reflected clinical effects, and were close to normal levels. Furthermore, Elg/TF-CWA facilitated quantitative evaluation of perioperative hemostatic management of bypassing therapy in HA patients with inhibitors.

Conclusions: CWA is a promising method for the quantitative monitoring of bypassing therapy during routine automated clotting assays with a modified trigger reagent comprising a well-balanced mixture of Elg and TF.

Monitoring the hemostatic efficacy and safety of new therapeutic drugs (what assays should be used?)

Restoring hemostatic balance in hemophilia by RNAi targeted silencing of antithrombin.

Benny Sorensen (Alnylam)

Introduction: Hemophilia A or B are congenital bleeding disorders caused by dysfunctional propagation of thrombin generation due to deficiency in factors VIII or IX in the presence of normal levels of anticoagulants resulting in an imbalance of the hemostatic system toward a bleeding phenotype. We are currently investigating the use of RNA interference (RNAi) to target the natural anticoagulant antithrombin (AT) as a strategy to rebalance the hemostatic system and improve thrombin generation, and therefore hemostasis, in hemophilia. ALN-AT3, a subcutaneously administered RNAi therapeutic targeting AT, is currently being developed for the treatment of hemophilia.

Material and methods: Preclinical studies in hemophilia mouse models have investigated the ability of ALN-AT3 to silence AT and thereby correct thrombin generation as measured by Calibrated Automated Thrombin (CAT) generation assay, restore hemostatic plug formation in real-time laser injury clot formation visualization, and control traumatic bleeding in a saphenous vein bleeding model. Preclinical studies were also conducted in vehicle and ALN-AT3 treated non-human primates followed by infusion of high-dose anti-Factor VIII antibody to induce inhibitor hemophilia A and measurement of thrombin generation. The association between AT reduction and factor VIII and IX equivalence was assessed by in vitro thrombin generation studies using human hemophilia A and B plasma samples. Finally, a phase 1 clinical study in healthy volunteers and severe/moderate hemophilia A or B patients has been initiated. Part A in healthy volunteers has been completed and Part B in hemophilia patients is ongoing.
Results: ALN-AT3 treatment targeting residual AT levels of 20-40% in hemophilia A and B mouse models increased thrombin generation, restored real-time localized hemostatic plug formation in the laser-injury model comparable to treatment with full-length recombinant factor VIII. ALN-AT3 controlled traumatic bleeding in the saphenous vein model with an increase in number of hemostatic events equivalent to that achieved with infusion of 25IU/kg full-length recombinant factor VIII. ALN-AT3 treatment targeting 20% residual AT levels normalized thrombin generation in non-human primates with induced high titer inhibitor hemophilia A. In vitro titration studies with factor VIII or IX in human hemophilia A or B plasma as well as decreasing AT showed that targeting residual AT levels of 40-60% is equivalent to factor VIII or IX trough levels ranging from 10-15%. In Part A of the Phase 1 study, healthy volunteer subjects received a single subcutaneous dose of ALN-AT3 and, per protocol, the maximum allowable level of AT knockdown was set at 40%. Initial results show that a single, low subcutaneous dose of ALN-AT3 at 0.03 mg/kg resulted in an up to 28-32% knockdown of AT at nadir that was statistically significant relative to placebo (p < 0.01 by ANOVA). This led to a statistically significant (p < 0.01) increase in peak thrombin generation, that was temporally associated and consistent with the degree of AT knockdown. ALN-AT3 was found to be well tolerated with no significant adverse events reported.

Conclusion: Collectively, these data suggest that the use of a novel RNAi therapeutic targeting AT is a promising approach for restoring hemostatic balance in hemophilia, and potentially, other bleeding disorders. Further, the potential for subcutaneous route of administration, infrequent dosing, and applicability to persons with hemophilia who have inhibitors, support the continued development of ALN-AT3.

Monitoring the hemostatic efficacy and safety of new therapeutic drugs (what assays should be used?)

ACE910, anti-FIXa/X bispecific antibody. Takehisa Kitazawa (Chugai Pharmaceutical Co, Japan)

ACE910 is a recombinant humanized anti-factor IXa (FIXa) x factor X (FX) bispecific monoclonal antibody that places these two factors into spatially appropriate positions and mimics a cofactor function of factor VIIIa (FVIIIa). We previously demonstrated that ACE910 had an enough hemostatic activity even against on-going bleeds and completely prevented spontaneous joint bleeds in non-human primate models of acquired hemophilia A. In the phase I clinical study, ACE910 exhibited a long half-life of approximately 1 month and a good bioavailability with subcutaneous dosing in healthy volunteer subjects.

The cofactor activity of ACE910 in plasma samples is principally measurable by the assays ever used for measuring factor VIII (FVIII) activity and/or effect, because ACE910 is a FVIIIa-mimetic. (Due to the high species-specificity of ACE910, the assays must include both human-origin factor IX (FIX)/FIXa and human-origin FX.) In terms of comparing ACE910 activity with FVIII activity, however, each assay demonstrates a different correlation between them. For example, ACE910 at 300 nM exhibited peak height of thrombin generation equivalent to 10 U/dL of FVIII did in vitro, while it shortened APTT beyond 100 U/dL of FVIII did. We consider that such inconsistency should attribute to the different properties between two molecules. Differently from FVIII, ACE910 does not require the activation process. In addition, there is a substantial difference in the inactivation, in the binding affinity to FIX(a) and in the capability of binding to phospholipid.
We are now on the way to elucidating how such differences affect ACE910’s clinical hemostatic efficacy and thus on the way to identifying the most appropriate assay and/or assay condition for ACE910 in terms of the FVIII-relative hemostatic activity. Although I recognize that the data are not necessarily sufficient at the present, for starters I’d like to discuss here the possibility of the clot waveform analysis and thrombin generation assay that are not directly influenced by the difference of the activation process, referring to in vivo animal hemostatic data.

**DISCUSSION**
The rationale for the use of a mixture of ellagic acid and tissue factor as the optimal reagent for the monitoring of by-passing therapy by CWA was discussed. For the monitoring of ACE910, FVIII activity assays or global haemostasis assays might be the more adapted laboratory tools, investigations are currently ongoing in Dr Shima laboratory.

**RARE COAGULATION DISORDERS**
*Session Chairpersons: Danijela Mikovic (Serbia) and Michael Makris (UK)*

**Rare coagulation disorders resource room. Amy Shapiro (USA)**
*Dr Shapiro had apologized for not being present due to personal reasons and the presentation was made by Dr. Flora Peyvandi.*

Introduction/background: Very rare bleeding and clotting disorders exist worldwide, yet knowledge of these conditions and their management is often suboptimal. The majority of healthcare professionals have little experience treating these disorders, with limited resources for their diagnosis and treatment. Moreover, as rare coagulation disorders represent a small potential commercial market, few, if any, specific therapies exist for these conditions. As a result, affected individuals often face delayed diagnosis, insufficient laboratory evaluation, and limited treatment options. The Rare Coagulation Disorders Resource Room (www.rarecoagulationdisorders.org) was created in 2013 to address these healthcare gaps. This dynamic, open-access website was developed through a collaboration of the international RBDD Registry (http://www.rbdd.org/), the Indiana Hemophilia & Thrombosis Center (www.ihtc.org), and the Rare Coagulation Disorders Working Group of the National Hemophilia Foundation (NHF), a group appointed by NHF’s Medical and Scientific Advisory Council.

**Aim**
The Rare Coagulation Disorders Resource Room aims to provide first-line educational resources for both healthcare providers and individuals with very rare coagulation disorders. The website represents an important step in a global initiative to enhance ongoing research and registry efforts and foster collaboration among a growing international network of care providers. The ultimate goal of the website is to improve the health and quality of lives of individuals with these rare disorders through improved awareness, diagnosis, and increased knowledge of the clinical manifestations and sequelae of these disorders.

**Methods:** The website content was written by leading experts in coagulation disorders and represents revised updates of initial articles published in a 2008 supplement of Haemophilia. The content has been formatted to provide current and searchable information including the basic science, clinical management, available laboratory and genetic testing, clinical trials, and global research initiatives for very rare and heterogeneous coagulation disorders.

**Results:** The website currently hosts content on 12 rare bleeding disorders* with an additional disorder (FXIII deficiency) to be added in 2014. Since its launch in October 2013, the website
has garnered almost 12,000 page views in approximately 3,000 sessions, with new visitors accounting for 76% of sessions. Visitors viewed an average of 4 pages per session over an average 3.5 minutes, with 86% of sessions from a desktop computer. The top 5 countries for website visitors were the United States (57%), the United Kingdom (5%), France (3.7%), Canada (3%), and India (3%). Most of the visitors (34%) reached the website through an organic search, while 33% came directly to the site, 31% through referral, and 2.6% through social media. Visitors who came through referrals stayed longer on the website compared to visitors through an organic search (4 minutes, 6 seconds vs. 3 minutes, 8 seconds) and viewed more pages per session (4.80 vs. 3.45). The top websites for direct referrals were hemophilia.org (41.80%), rbdd.org (20.40%), and isth.org (9.42%). The 5 most commonly viewed disorders were platelet function defects (17% of pageviews), prothrombin deficiency (11.5%), fibrinogen deficiencies (11.2%), PAI-1 deficiency (11%), and plasminogen deficiency (10.2%). The website has been supported through a variety of organizations such as links posted on EUHANET (Haemophilia Central) and on the ISTH website in the resource section; an announcement by the National Hemophilia Foundation in their eNotes on 11/5/13 and on their website, and through their newsletter HemAware in 2014. The Hemophilia and Thrombosis Research Society sent an e-blast to their membership and posted a link on their website; OrphaNews Europe provided an announcement in their newsletter, and the World Federation of Haemophilia posted a short blurb on their RBD webpage and in an e-blast.

Summary/Future plans: The Rare Coagulation Disorders Resource Room serves as a platform for expanded global education and fosters data collection and international collaboration on clinical trial design. The Resource Room also serves as a repository for information on registries and provides valuable education on rare bleeding disorders. In the future, the website will include content on clotting disorders and patient education materials.


**EAHAD mutation databases on Factor VII and Factor XI.** D. Hampshire, C. A. Ludlam, G. Kemball-Cook, A. Cairo, K. Gomez, A. Goodeve, J. McVey, S. Perkins, F. Peyvandi and P. Rallapalli on behalf of European Association for Haemophilia and Allied Disorders.

Dr Ludlam had apologized for not being present due to personal reasons and only a summary has been presented.

Readily accessible databases of sequence variation in coagulation factor genes have, over the past 20 years, been an important source of information for clinicians and researchers in haemostasis, e.g. HAMSteRS for factor VIII and the VWF database. Recently an initiative by the European Association for Haemophilia and Allied Disorders (EAHAD) has led to the updating of databases for factors VIII and IX along with the addition of much additional protein structural information and sequence data for many animal species. This, along with the well-established VWF database, is available through the EAHAD database portal (www.eahad-db.org). A strength of these databases is that they also include phenotypic information in relation to each reported mutation, e.g. history of inhibitors and bleeding severity. This EAHAD database initiative is now in the process of making widely available equivalent genetic and phenotypic
variation data for factors VII and XI. To achieve this the Steering Committee is collaborating with the Rare Bleeding Disorders Database and other databases for these two clotting factors to bring together and update these data to make them readily accessible. It is anticipated that this part of the project will be completed before the end of 2014. When the update has been completed these data will also be available through the EAHAD database portal. Correspondence and enquiries about the EAHAD Coagulation Factor Variant Database project should be addressed to Christopher Ludlam (CAL@Ludlam.org.uk), Geoffrey Kemball-Cook (Geoffrey@kemball-cook.co.uk) or Dan Hampshire (D.Hampshire@Sheffield.ac.uk).

**Report on the SSC project: Factor V deficiency, clinical heterogeneity and treatment.**

*Danijela Mikovic (Serbia), Roberta Palla, Marzia Menegatti, Flora Peyvandi (Italy)*

Factor V (FV) deficiency represents 8-10% of all rare bleeding disorders (RBDs). Severity of symptoms is variable and correlates poorly with laboratory phenotype. Although the most common symptoms are prolonged bleeding after trauma and mucosal bleeding, major spontaneous bleeding like haematoma, hemarthrosis, gastrointestinal, central nervous system and umbilical cord bleeding episodes occur in a significant number of patients who need replacement therapy. Replacement therapy of FV can be administered only through fresh frozen plasma, preferably virus-inactivated, since FV concentrates are not available and FV is not present in cryoprecipitate or prothrombin complex concentrates.

The aim of the project is to achieve common definitions on different aspects of the FV deficiency using longitudinal harmonized data collection system. As the first step focused and concise questionnaire was filled up by each participating centre to collect basic data regarding number and structure of the group of patients with FV deficiency. Blood samples will be collected according to protocol to measure the levels of FV clotting factor in blood and to obtain DNA for mutation detection. Variables that will be prospectively followed include characteristics of bleeding episodes, type and intensity of treatment, treatment results and complications using existing web-application in "Prospective evaluation of the intensity of bleeding episodes in patients with coagulation factors deficiency" (PRO-RBDD) project.

So far, 11 centers from 9 countries were enrolled. Data on 47 patients were entered. Among them there are 23 males and 24 females. Twenty-five patients are less than 18 years old while 22 are adult patients. There are 8 patients with FV activity <1IU/dl, 17 patients with FV activity between 1-10 IU/dl and 22 patients with FV activity >10 IU/dl. Data related to the availability of phenotype and genotype laboratory investigations as well as treatment possibilities in each center were entered. FV deficiency project will provide essential information on the course and optimal management of FV deficiency, allow design of new clinical trials and foster the development of specific treatment products.

**Factor X concentrate. Registration of novel drugs for RBDs. Peter Feldman (Coagulation Factors Research & Development, Bio Products Laboratory – BPL)**

The development of a factor X concentrate for patients with factor X deficiency exemplifies the difficulties in registering such drugs for rare bleeding disorders. Orphan Drug Designations offer pre-registration regulatory assistance and subsequent market exclusivity. These benefits help to recover the development costs, which are the same as for any drug, but recoverable only from sales to a much smaller patient cohort. The clinical trial in this patient population required a single protocol which satisfied different regulatory agencies. Even
with parallel US and European protocol assistance, different expectations required time-consuming iterations to achieve consensus.

Other clinical challenges to the registration programme have been:

- identification and enrollment of eligible patients for the clinical study, with intrusive and lengthy sampling proving a disincentive;
- prospective establishment of investigator sites for a separate surgery study, because the need, location and timing for surgeries could not be predicted;
- European paediatric clinical trial requirements delaying the registration and availability of product, even for the adult population.

Licensing authorities must follow legally-binding regulations; if these regulations were amended, access to treatment could be facilitated. Adults and children in this patient group receive personalised care, individually modulated by dose recovery and efficacy. The requirement for lengthy clinical trials of dubious statistical power could be waived, in favour of marketing authorisation based on risk-benefit assessment. This is particularly appropriate for coagulation factor proteins which have a well-understood physiology. Such an assessment could address: safety; indicative pharmacokinetics; broad indications in adults and children; and enhanced post-registration monitoring of clinical outcomes.

**DISCUSSION**

There was a question about the frequency of updating the rare coagulation disorders resource room and duplication with other websites. This was anticipated by the authors who were due to meet to discuss the issue in Milwaukee but had to postpone their meeting due to Dr Shapiro’s absence. An update plan will be developed.

After the EAHAD database presentation a question arose about whether the original FVIII and IX databases would remain live and the answer was no as these are now superseded by the new database. It was also pointed out that these are not the only FVIII and IX mutation databases.

**Note by Christopher Ludlam:** People who attempt to access Hamsters at its usual address of [http://hadb.org.uk/](http://hadb.org.uk/) will find a notice giving directions to the updated VIII database directly and via the [www.EAHAD-DB.org](http://www.EAHAD-DB.org) portal but also there is the option to continue to access Hamsters through the web site. The plan is to close Hamsters at the end of 2014.

Mark Soucie from CDC reported that they also support databases for these two factors; it was suggested that the two groups work together to avoid duplication of effort. Moreover, there was a question whether the EAHAD group could be interested in genotyping patients affected with RBDs. Flora Peyvandi answered that at this stage the group works on already available data.

There was a question about efficacy of rFVIIa in factor V deficiency. Although the factor V database collects data on this treatment this is simply about the fact that this treatment was used and no data on its efficacy is available.

Dr Feldman presented on the challenges of bringing a new product for a rare bleeding disorder to market and was asked about the development costs. He indicated that so far BPL has spent 5-10 million UK pounds ($8-16million) on their new factor X concentrate program and they are hoping to get it licensed in the USA within months rather than years.
Factor XI and the Contact System

Chairman: Jonas Emsley (UK)
Co-Chairmen: Jose Govers-Riemslag (the Netherlands), Christine Mannhalter (Austria), Joost Meijers (Netherlands), James Morrissey (USA), Thomas Renne (Sweden), Ophira Salomon (Israel)

Thursday, 26 June (8:45-12:45)

Owen McCarty – (Portland, USA) FXI activation and the virulence of infectious agents - The presentation of the cleavage of TFPI by factor XIa further defining the nature of the pathway leading to thrombosis as opposed to hemostasis.

Ricky Travers – (Urbana, USA) Visualizing polyP in thrombi and using novel anti-polyP compounds to stop thrombosis. This showed imaging of polyP emerging from cells and described data on compounds capable of binding to polyP and inhibiting thrombosis with minimal bleeding risk.

Thomas Renné – (Karolinska Stockholm, Sweden) Factor XII function in thrombosis and hemostasis This was a description of the use of an antibody 3F7, which bound to the FXII protease domain. It was shown to thromboprotection in extracorporeal circulation without increasing bleeding risk.

Evi Stavrou – (Cleveland, USA) Contact Activation Murine KOs: Unexpected Findings When Characterizing Mechanisms Related to Thrombosis Protection. This talk described contact system independent functions of plasma kallikrein.

Coen Maas – (Utrecht, the Netherlands) Plasmin triggers proteolytic Factor XII activation on endothelial cells, which is accelerated by the lysine substitution T328K that causes type III angioedema. This presentation detailed a new mechanism for the activation of mutant FXII.

Judith Cosemans – (Maastricht, the Netherlands) Factor XII regulates the pathological process of thrombus formation on ruptured plaques. This talk described novel in vivo functions of FXII for plaque-driven thrombosis and show FXII to contribute to thrombus stability.

Elaine Gray – (NIBSC, Potters Bar, UK) An International Standard for Activated Factor XI (FXIa). A standard was presented on FXIa in response to problems with immunoglobulin preparations being linked to thrombosis due to contact protein contamination in purification of immunoglobulins. This described an indirect assay involving FIX and FX and a chromogenic readout of FXa production. Different laboratories utilised this technique and were able to show very similar results in quantifying FXIa. The SSC board recommended the adoption of this standard. As a comment at the meeting it was mentioned that immunoglobulin preparations should all be tested with this standard together with FXI replacement therapy.

Theme: Inhibitors of FXI and the contact system: Safer anticoagulation
**Umesh Desai** – *(Richmond, USA)* Allosteric inhibitors of FXIa. This described compounds which allosterically inhibit FXIa activity by binding to the anion binding exosite in the protease domain. The first part described the discovery and characterisation which was published and subsequent part of th

**Jon Kennisten** – *(Burlington, USA)* – Discovery and characterization of a highly specific antibody inhibitor of plasma kallikrein. This talk described a crystal structure of an inhibitory antibody Fab fragment bound to the protease domain of kallikrein. This antibody is in clinical trials for the treatment of edema.

**Rebecca Smock** – *(Seattle, USA)* - Inhibiting the contact pathway with RNA aptamers . This was a very elegant presentation and in the first part published data was described showing nucleic acid aptamers which bind to FXII and inhibit its activity. The pattern of inhibition inhibited contact activation as measured by FXII activity varied based on which activator is used. In the second part of the talk new data aptamers which inhibit FXI

**Vladimir Kolyadko** – *(Russia)* Characterisation and development of factor XIIa inhibitors for assaying tissue factor triggered coagulation. TOP ABSTRACT. This presentation described the effect of corn trypsin inhibitor CTI on various assays. Open discussion

**Joost Meijers – José Govers-Riemslag (the Netherlands)** Polyphosphates and activation of the contact system. Is the contact system involved in both arterial and venous thrombosis? Joost Meijers, Amsterdam, and José Govers-Riemslag, Maastricht, introduced a new addition to the SSC session with an open discussion. The audience was allowed to vote on several questions with controversial opinions that dealt with two topics :
1). Polyphosphates and activation of the contact system, and
2). Is the contact system involved in both arterial and venous thrombosis? With active audience participation, the lively discussion reviewed relevance of platelet polyphosphates for activation of the contact system ex vivo and in vivo. The discussion on the relevance of the contact system for thrombosis gave the consensus that murine data support roles of factors XI and XII in both venous and arterial thrombosis, but that the roles of the factors in especially venous thrombosis in humans needs to be further investigated.
Factor XIII and Fibrinogen

Chairman: Helen Philippou (UK)
Co-Chairmen: Zsuzsa Bagoly (Hungary), Vytautas Ivaskevicius (Germany), Marlien Pieters (South Africa), Sanj Raut (UK), Verena Schroeder (Swizerland), Alisa Wolberg (USA)

Monday, 23 June (14:15-18:15)

14.15 Top rated abstract - Identification of Sequence Variations in Fibrinogen Genes Causing Haemorrhagic or Thrombotic Diathesis.
 Mario von Depka (Germany)
This study examined 122 fibrinogen gene variants from 96 patients, making this amongst the largest cohort studies of variants on the fibrinogen gene. New mutations were identified.

14.15-14.45 Standardisation of Turbidity Measurements as Part of the Standardisation Process of the Fibrin Structure Measurement Techniques
 Marlien Pieters (South Africa)
A new project was proposed to standardise the method of turbidity to assess fibrin structure in plasma samples. It is acknowledged that there is great variability in lab to lab protocols and that it is therefore difficult to make comparison of data generated from different labs. The aim is to establish a single protocol that can be employed in ~10 labs to ascertain laboratory variations using the same protocol. A pilot study published as an official SSC communication was published in 2012 in JTH using the permeation method. The aim now is to standardise the turbidity method as this is more frequently used in many labs. The clinical relevance of why we should initiate this project was discussed. Weisel et al., Blood 2013 wrote a brief report entitled "Information about clotting, especially in the early stages that determine clot properties and the gel point, has clinical relevance, because the gel point is used diagnostically and disorders of clotting of fibrinolysis accompany or cause many pathologic conditions, including MI and stroke." There have been many clinical studies that have also suggested clot structure is important to both venous and arterial thrombotic diseases. The audience agreed that there was a clinical relevance to standardisation of the methodology. There was good discussion on how to standardise the reagents and we also discussed addition of tPA to the method to also observe fibrinolysis. General concepts were decided and it also seemed appropriate to include the Fibrinolysis SSC as a joint project with the FXIII and Fibrinogen SSC. Final conditions for the assay were to be discussed after the meeting between Philippou, Pieters and Mutch. Pieters was to take initial steps in optimising conditions and recruit potential labs wishing to take part. Funding would be applied for to support reagent and shipping costs.

14.45- 15.00 Assessment of Clauss Assays in Measurement of Fibrinogen in Therapeutic Concentrates
 Sanj Raut (NIBSC, United Kingdom)
Thrombin Clottable Fibrinogen assays are used by Manufacturers (of Fibrinogen Concentrate/Fibrin Sealant Products) and Regulatory Laboratories for Lot Release. Clot removal (CLOTr) method is the recommended European Pharmacopoeia method and this method was used to establish the current 2nd International Standard for Fibrinogen Concentrate (09/242). However, this method is slow, cumbersome, time consuming & labour intensive; not at all ideal as a high throughput assay. There is therefore a need for a more simple/automated alternative method.

Clauss assay is one alternative functional assay used routinely for measuring fibrinogen in plasma and is based on time for fibrin to clot. High concentrations of thrombin are used (typically 100 IU/ml) to ensure clotting times are independent of thrombin concentration over a wide range of fibrinogen levels. Fibrinogen samples are incubated with thrombin and calcium at 37°C, and time taken to clot is compared vs reference standard. Most laboratories have an automated Clauss method for measuring plasma (method based on photo optical end point determination).

This study assessed whether Clauss assay would be suitable for measuring fibrinogen in therapeutic concentrates. This assay was modified & optimised for measuring fibrinogen concentrates (i.e. valid assays with standard vs test system & using parallel-line analysis). Data from Clauss assays (measuring Fibrinogen concentrates) using mechanical end-point instrumentation (e.g. KC4) was found to be comparable to data from CLOTr method and Absolute Methods (Kjeldahl & Clot Weight). However, significantly higher potencies were obtained from Clauss assays using photo-optical instrumentation or when attempting to automate the assay (e.g. using ACL Top analysers) & using specific commercial kits. This was still the case when pre-diluting Fibrinogen concentrates in Fibrinogen Deficient plasma (in order to mimic assays of plasma) to help standardise turbidity issues.

The study concluded that Clauss assays may be suitable for measurement of Fibrinogen Concentrates using mechanical end-point instrumentation (e.g. KC4), but may NOT be suitable for measurement of Fibrinogen Concentrates using photo-optical instrumentation. Further work is on-going.

15:00 -15:15 FXIII-B Standardisation Update and Change of Free FXIII-B in a FXIII-A Deficient Patient on Recombinant FXIII-A2 Therapy

Éva Katona Hungary

Dr Eva Katona, University of Debrecen, Hungary, was unable to attend the meeting due to unforeseen circumstances. It was therefore not possible for her to give an update on the ELISA methods for measuring free and bound FXIII-B for our on-going SSC project. At short notice Dr Verena Schroeder, University of Bern, Switzerland, gave a short summary on the background and current state of this project. As soon as reliable ELISA methods are developed, the official project proposal will be submitted to ISTH/SSC headquarters and WHO/ECBS, followed by a collaborative study to determine free and total FXIII-B plasma levels in the WHO FXIII plasma standard.
15:00-15:15 Importance of the Activation Peptide for FXIII Expression Helena Handrkova Switzerland
Verena Schroeder Switzerland Hans P. Kohler Switzerland
In order to understand the role of activation peptide of FXIII (AP-FXIII), FXIII variants were cloned with progressive deletions from N-terminus and expressed in a mammalian cell line. It was found that the N-terminal part of AP-FXIII is important for the expression, stability and dimerisation of FXIII. As the most important residues, the motif 8FGG11R was identified.

15.30-15:45 The Effect of Factor XIII and Other Regulators of Fibrinolysis on the Outcome of Thrombolysis in Ischemic Stroke Patients
132 consecutive ischemic stroke patients who underwent thrombolytic therapy by tPA were investigated, blood samples were taken on three occasions: before thrombolysis, immediately after the administration of tPA and 24 hours after the therapy. FXIII levels and major polymorphisms of both subunits were determined from the blood samples. FXIII levels were significantly higher in acute ischemic stroke patients on admission as compared to 302 healthy controls. FXIII levels decreased during thrombolysis. Low levels of FXIII 24 hours after thrombolysis were associated with short- and long-term mortality, but were not associated with therapy-associated intracranial hemorrhage. The outcome of thrombolysis was not influenced by any of the investigated FXIII polymorphisms.

16.05 – 16.20 Recommendations for Criterion and Algorism of Laboratory Tests for Autoimmune Hemorrhaphilia Due to Anti-Factor XIII/13 Antibodies
Akitada Ichinose Japan | Hans P. Kohler Switzerland | László Muszbek Hungary | Helen Philippou United Kingdom On behalf of the Factor XIII and Fibrinogen SSC Subcommittee of the ISTH
Coagulation Factor XIII is a plasma pro-transglutaminase, which stabilizes fibrin clots by crosslinking fibrin monomers, and fibrin and other proteins, and thus plays an important role in hemostasis. Autoimmune hemorrhaphilia due to anti-Factor XIII/13 (FXIII/13) has been on the increase in the 21st century. As of March 2014, a total of 79 AHXIII/13 cases have been diagnosed in the world. In order to raise the awareness about AHXIII/13 not to overlook it as well as to save its patients’ lives a consensus for "criteria and algorism of laboratory tests for AHXIII/13” was discussed. It was concluded that AHXIII/13 is not an acquired isolated defect of the FXIII molecule itself but a disturbance caused by its autoantibodies. AHXIII/13 cases are diagnosed by a combination of a severe decrease in FXIII/13 activity and the presence of anti-FXIII/13 autoantibodies, among patients with unexplained hemorrhages. Since patients with this
disease manifest life-threatening bleeding symptoms, prompt diagnosis and treatment are essential to save their lives.

16:20-16:35 Modification of the ISTH/SSC Bleeding Assessment Tool ver. 2010 and Its Field Test for Japanese Patients with Acquired Hemorrhaphilia due to Anti-F13 Autoantibodies
Akitada Ichinose Japan | Mayumi Sugiura-Ogasawara Japan | Alberto Tosetto Italy | Francesco Rodeghiero Italy | Paula James Canada
Professor Ichinose discussed a revised bleeding assessment tool for the assessment that can lead to the diagnosis of acquired hemorrhaphilia.

16.35 – 16.50 FXIII and the Pathogenesis of Inflammatory Arthritis
Matthew Flick USA
Dr Flick presented work that investigated the hypothesis that the coagulation transglutaminase, factor XIII (FXIII), drives arthritis pathogenesis by promoting local inflammatory and tissue degradative/remodeling events. Using a combination of in vivo and in vitro approaches, it was reported that FXIIIA appears to support arthritis pathogenesis by multiple distinct mechanisms. The data support the notion that FXIIIA drives pathways linked to local inflammation, which are likely fibrinogen-dependent and pathways directing the regulation of local osteoclast differentiation and function, which appear to be fibrinogen-independent.

16.50 – 17.05 Alloantibodies Formed During Replacement Therapy in FXIII Deficient Patients
Georges-Etienne Rivard Canada
A well characterised case study was presented showing the development of an alloantibody against FXIII during prophylaxis that resulted in a fatal outcome. It was discussed that such antibodies should be searched for at pre-dose FXIII determinations. In this case the alloantibody tightly bound to FXIII-A and exerted a combined inhibitory effect on the cleavage of activation peptide by thrombin, on the Ca2+ induced activation of FXIII-A2, and on the activity of FXIII-A2. The inhibition of Ca2+ induced conformational change was proven to be the dominant effect.

17.05 – 17.20 Fibrin Accumulation Secondary to Loss of Plasmin-Mediated Fibrinolysis Drives Inflammatory Osteoporosis?
Jonathan Schoenecker, USA.
There was a slight change in the programme and Dr Schoenecker was able to present the work instead of Richard Allen who was originally going to stand in for Dr Schoenecker. Interesting links between the role of the fibrinolytic system and the development of inflammatory osteoporosis where discussed, providing insight into novel mechanisms.
17.20 – 17.35 Factor XIII as a Determinant of Thrombosis
Alisa Wolberg USA
Dr. Wolberg’s presentation summarized their recent findings on a newly-recognized role for factor XIII activity in venous thrombosis. Dr. Wolberg’s talk, based largely on a recent publication from her laboratory (Aleman et al. 2014 J Clin Invest, in press), showed data supporting the finding that factor XIII activity mediates red blood cell retention in venous thrombi in a mouse model of thrombosis and in human samples in vitro. Dr. Wolberg’s data also demonstrated the importance of fibrinogen residues gamma390-396 for this function; this effect appears to result from reduced binding of factor XIII to fibrinogen, and consequently, delayed factor XIII activation and delayed fibrin crosslinking. These exciting findings are the first identification of a specific activity (factor XIIIa) that mediates red blood cell presence in venous thrombi and suggest factor XIII(a) may be a novel target for reducing venous thrombosis.

17.35 – 17.50 The likely Role of Plasma Protransgutaminase (Factor XIII) in Cross-linking Alpha 2-antiplasmin to Circulating Fibrinogen
Michael Mossesson USA
Professor Mossesson’s presentation regarded Fibrinogen-bound alpha-2-antiplasmin (Fgn-bound a2AP) in the context that the amount of a2AP covalently linked to circulating fibrinogen probably plays an important role in regulating the fibrinolytic potential of blood. To examine this, an ELISA for measuring Fgn-bound a2AP in the circulation was developed, and determined the level of Fgn-bound a2AP in 200 healthy subject plasmas. What remains to be done is to measure the fibrinolytic potential of these plasmas and compare these values to the levels of Fgn-bound a2AP."

17.50 – 18.15 SSC Discussion and Project Updates
Dr Philippou opened the discussion to the audience regarding the development of a project to standardise the assessment of fibrin structure and its stability in plasma samples using turbidometric assays as initiated by Marlien Pieters’s presentation. General viewpoints on the criteria to be assessed and the development of the assays were discussed. It was concluded that Dr Philippou would extend this project to the Fibrinolysis subcommittee and that Drs Philippou, Mutch and Pieters would discuss the optimisation of the assay, with Dr Pieters taking initial steps to optimise the assay in her laboratory and that the project will be co-lead with the NIBSC (Dr Sanj Raut and Dr Craig Thellwell). Funding would be applied for to perform a standardisation study using at least 7 international laboratories using reagents form NIBSC. Due to Dr Katona not being able to attend the meeting it was agreed that the follow-up of the development of the ELISAs for the assessment of both total and free FXIII-B subunit would occur after the meeting to progress the on-going SSC project.
Fibrinolysis

Chairman: Nicola Mutch (UK)
Co-Chairmen: Jonathan Foley (Canada), Ann Gils (Belgium), Paul Kim (Canada), Craig Thelwell (UK), Shirley Uitte De Willige (the Netherlands), Waander Van Heerde (the Netherlands)

Wednesday, 25 June (8:00-12:00)

Co-Chairs not in attendance: Shirley Uitte de Willige (Netherlands), Ann Gils (Belgium)

08:00-08:15 Differential Effect of Dabigatran, Rivaroxiban and Apixaban on Thrombomodulin Mediated Activation of Protein C and Thrombin Activated Fibrinolysis Inhibitor (TAFI) May Impact Their Safety and Efficacy Profiles, Debra Hoppensteadt (USA)

Objectives: The new oral anticoagulant agents Dabigatran, Rivaroxiban and Apixaban target thrombin and factor Xa to mediate their anticoagulant effects respectively. However significant differences have been reported in their pharmacodynamics actions. This study was designed to investigate the effects of active forms of Dabigatran, Rivaroxiban and apixaban on the activation of protein C and TAFI by thrombin-thrombomodulin complex.

Methods: The active form of Dabigatran was synthesized. While Rivaroxiban and Apixaban were extracted from commercially available tablets. Both agents were dissolved in appropriate solution matrices at a stock concentration of 100 µg/ml. Thrombin-thrombomodulin mediated activation of protein C and TAFI were measured using specific chromogenic substrate based methods at a concentration of 0–10 µg/ml in different matrices. The activation of Protein C and TAFI were measured using mass spectrometric and immunoblotting methods.

Results: Dabigatran produced a strong inhibition of the generation of both the activated protein C and TAFI (IC50 < 1.0 µg/ml) whereas Rivaroxiban and apixaban did not produce any inhibition of the activation of either of these proteases. Dabigatran also inhibited the amidolytic actions of thrombin-thrombomodulin complex whereas Rivaroxiban and apixaban did not produce any effect. Neither Dabigatran nor Rivaroxiban produced a direct inhibition of activated protein C or TAFI at concentrations of up to 10 µg/ml. Mass spectrometric and immunoblotting methods showed that Dabigatran blocked the activation of both TAFI and Activation C by thrombin-thrombomodulin complex whereas rivaroxaban and apixaban did not.

Conclusion: The persistent inhibition of thrombin and its regulatory effects by Dabigatran may differentiate its pharmacologic profile from Rivaroxiban and apixaban. Since thrombin plays several regulatory functions, its persistent inhibition may compromise hemostatic regulation by this agent.

08:15-08:40 Plasmin dependent thrombin generation enhancement, Waander Van Heerde (The Netherlands)
This presentation focused on the influence of fibrinolysis on the coagulation pathway. Initially the use of global assays, was discussed, as presented at the SSC meeting in Amsterdam. New information on the role of plasmin enhanced thrombin generation was discussed, as obtained using the Nijmegen Hemostasis Assay (NHA). The NHA is an assay that simultaneously measures thrombin and plasmin generation in one single well. By doing so, interaction between coagulation and fibrinolysis can be studied (for example TAFI activity) and vice versa. The importance of a combined assay was illustrated by an example in which the phenotypic abnormalities with respect to bleeding risk was explained by measuring plasma of severe hemophilic patients and patients with deficiencies in fibrinolytic inhibitors. Based on this data a revised hemostatic balance was suggested in which fibrinolysis play an important role.

08:40-09:10 Fibrinolysis when there is no fibrin? The role of t-PA and plasmin in the brain, Robert Medcalf (Australia)

The first part of this presentation covered the role of the fibrinolytic system in the central nervous system. Much effort was devoted to not only highlighting the fact that t-PA, either alone or via plasmin, influence many aspects of neurobiology, but does so in an environment where there is no fibrin at all under normal circumstances. Particular emphasis was devoted to describing the role of t-PA/plasmin in promoting memory development, mood and anxiety, neurotransmission and glutamate signalling. Another important area covered was the role of t-PA/plasmin at influencing blood brain barrier permeability and glucose uptake and how under some circumstances brain t-PA could be either deleterious or neuroprotective.

Optimal formation of plasmin by t-PA requires a co-factor and in the circulation, this is provided by fibrin. Hence fibrin serves as both a co-factor and as a substrate for plasmin formation. The latter part of this presentation illustrated the fact that other proteins can provide essential co-factor activity for t-PA effectively substituting for fibrin. For the most part, these proteins are misfolded or aggregated proteins. A clear example of this occurs when cells become necrotic following injury or stress where they undergo a unique intracellular aggregation event recently referred to as "Nucleocytoplasmic coagulation". This process generates a structure on the protein surface that not only facilitates lysine-dependent binding of plasminogen but also provides the essential co-factor activity for t-PA. The plasmin formed on the surface of these misfolded proteins then become substrates for plasmin and are cleared, in a manner indistinguishable to the removal of fibrin. As the removal of necrotic cells is known to involve the innate immune response, it appears likely that plasmin formation on the surface of misfolded proteins within necrotic cells may also promote the phagocytic arm of the immune response.

In summary, this presentation emphasised the fact that fibrinolysis as a process is not restricted to the removal of fibrin deposits in the circulation. Indeed, the fibrinolytic system could be renamed to better reflect its broader role in physiology.

09:10-09:40 The relationship between the fibrinolytic system and S. pyogenes virulence, Frank Castellino (USA)
The basis of F. Castellino's presentation involved a description of the properties of S. pyogenes (GAS) and especially the regulation of virulence factors that provide the tissue tropicity and extent of virulence for these bacteria. The focus was on the manner in which the host plasminogen-plasmin-fibrinogen system functions in virulence and how certain invasive strains of GAS can hijack the host plasminogen system to circumvent the innate immune response of the host.

09:40-10:10 The mechanism of gamma'-Fg resistance to lysis, Paul Kim (Canada)

Fibrin (Fn) clots formed from γ'-fibrinogen (γ'-Fg), a variant with an elongated γ-chain, are resistant to lysis compared with clots formed from the predominant γA-Fg; a finding previously attributed to differences in clot structure due to delayed thrombin-mediated fibrinopeptide (FP) B release or impaired cross-linking by factor XIIIa. We investigated whether slower lysis of γ'-Fn reflects delayed plasminogen (Pg) binding and/or activation by tissue plasminogen activator (tPA), reduced plamin-mediated proteolysis of γ'-Fn, and/or altered cross-linking. Clots formed from γ'-Fg lysed more slowly than those formed from γA-Fg when lysis was initiated with tPA/Pg when FPA and FPB were both released, but not when lysis was initiated with plasmin, or when only FPA was released. Pg bound to γ'-Fn with an association rate constant 22% lower than that to γA-Fn and the lag time for initiation of Pg activation by tPA was longer with γ'-Fn than with γA-Fn. Once initiated, however, Pg activation kinetics were similar. Factor XIIIa had similar effects on clots formed from both Fg isoforms. Therefore, slower lysis of γ'-Fn clots reflects delayed FPB release, which results in delayed binding and activation of Pg. When clots were formed from Fg mixtures containing more than 20% γ'-Fg, the upper limit of the normal level, the delay in lysis was magnified. These data suggest that circulating levels of γ'-Fg modulate the susceptibility of clots to lysis by slowing Pg activation by tPA, and provide another example of the intimate connections between coagulation and fibrinolysis.

10:10-10:30 Coffee Break

10:30-11:00 Visualizing plasmin on cell surfaces, Tetsu Urano (Japan)

Vascular endothelial cells (VECs) contribute to keep the patency of vasculature by expressing anti-coagulatory and pro-fibrinolytic activities. Tissue-type plasminogen activator (tPA), an enzyme which catalyzes the initial step of fibrinolysis, is secreted from VECs as an active form and directly enhances fibrinolytic potential. Recently, we succeeded to visualize its secretory dynamics in GFP-tagged tPA (tPA-GFP) expressing VECs using total internal reflection fluorescence microscopy. tPA-GFP appeared to have a unique secretory dynamics and to remain on the cell surface after exocytosis from its secretory granules. Studies using mutants of tPA-GFP suggested that the binding to the cell surface was heavy-chain- as well as catalytic activity-dependent. The retained active tPA-GFP on cell-surface effectively activated plasminogen, which accelerated further accumulation of plasminogen on cell surface as well as intercellular/matrix adhesive area, and effectively lysed fibrin network formed on VECs. PA inhibitor-1 (PAI-1) facilitated dissociation of cell surface-retained tPA-GFP by forming a high molecular weight complex, and suppressed the expression of fibrinolytic activity. Thus, PAI-1 appeared to control fibrinolytic activity not only in plasma but also on the surface of VECs. We also analyzed micro-thrombus formation and its lysis on laser-induced injury site of VECs using
intra-vital confocal microscopy. These processes appeared to be well controlled by spatiotemporal regulatory system of platelets activation as well as the activation of coagulation cascade. The involvement of fibrinolytic system from the very early phase of thrombus formation was also proved by a time-dependent accumulation of Glu-plasminogen in an LBS-dependent- and plasmin activity-dependent-manner in the center of the thrombus where platelets are fully activated to expose phosphatidylserine and fibrin is formed. Exogenously infused human tPA facilitated the Glu-plg accumulation, which preceded the effective lysis of the microthrombi.

11:00-11:30 Following lysis in real-time – lessons from confocal microscopy, Nicola Mutch, (UK)

This presentation describes the development of models to study fibrinolysis. Current models take into account different parameters such as endogenous protein concentration, cellular contribution, fibrin structure and flow. Issues arise in defining a model which takes into account all of these parameters. In this presentation we describe recent work from our laboratory that combine flow, cellular contribution, endogenous protein concentrations and the ability to monitor changes at the ultrastructural level by confocal microscopy in terms of fibrinolysis. Using these models we elucidate dramatic differences in the ability of tPA and uPA to lyse thrombi. We analysed plasminogen binding in thrombi under flow conditions by perfusing recalcified citrated whole blood (1000 s-1) over collagen/tissue factor spots and monitored by fluorescent confocal microscopy. Platelets labelled with DIOC6 and/or fluorescently labelled fibrinogen (OG488 or Alexa fluor 647) were incorporated during thrombus formation. Dylight 633-plasminogen primarily localised with fibrin(ogen) bound to the platelet surface. Addition of hirudin to prevent fibrin formation significantly reduced plasminogen binding. Lysis was visualised by including the plasminogen activators (PA), tPA and uPA, during thrombus formation. A dose-dependent effect of both PA was observed, with lower concentrations being more efficient than uPA. Fibrin directly associated with platelets was more resistant to lysis. These results indicate that platelet-bound plasminogen is primarily associated with fibrin and consistent with this thrombus lysis under flow is predominantly mediated by fibrin-associated plasmin(ogen) but a small pool is directly associated with the platelet surface. Abrogating secondary generation by inclusion of heparin dramatically enhances lysis in whole blood thrombi illustrating the constant balance between these two opposing systems in thrombus stability.

11:30-12:00 The WHO 4th International Standard for Plasmin and an update on D-Dimer standard development, Craig Thelwell (UK)

C. Thelwell (NIBSC) presented an update on WHO International Standards (IS) projects, including the completed study to calibrate the WHO 4th IS for Plasmin; introducing a new study to replace the WHO 3rd IS for streptokinase, and an update on the development of a D-Dimer standard, presented on behalf of Colin Longstaff and the D-Dimer project team.

The WHO 4th International Standard for Plasmin
The current WHO 3rd IS for plasmin (97/536) is used to standardise plasmin and plasminogen potency measurement for commercial products and for research, and stocks are now low and a replacement is needed. Future demand for the IS may increase due to renewed interest in plasmin as a direct acting thrombolytic drug, and is currently being investigated in clinical trials. A collaborative study was organised to calibrate a replacement using a candidate material donated by a plasmin manufacturer. This was a therapeutic grade plasmin preparation derived from human plasma. The bulk plasmin was reformulated and freeze dried into sealed ampoules (4303 ampoules were available from the fill) and coded 13/206. Study participants (16 laboratories representing 12 different countries) were provided with ampoules of 13/206 (as coded duplicates samples A and B) and the 3rd IS (sample S) and requested to perform four independent assays including multiple doses and replicate measurements. Of the 16 laboratories 15 completed the study and returned results, which were analysed using a parallel line analysis. An overall unweighted geometric mean (GM) potency of 8.0 IU was calculated for 13/206 based on the unweighted GM of individual laboratories’ results, with a geometric coefficient of variation (GCV) of 7.8 %. This potency was based on chromogenic assays, for consistency with the calibration of the 3rd IS, with a range of chromogenic substrates represented and no reagent bias observed. Three laboratories also performed fibrinolytic assays which were statistically valid, but too few to calculate a potency or to draw conclusions on the relationship between chromogenic and fibrinolytic methods. This potency value was proposed and endorsed by the study participants and an expert review panel nominated by the Fibrinolysis SSC Subcommittee Chair, and will be submitted to the WHO ECBS for establishment in 2014. A molar concentration of 1.5 µM was calculated using active-site titration at NIBSC and it is proposed to include this in the IFU (instructions for use) for information only.

**WHO 4th International Standard for Streptokinase**

Stocks of the WHO 3rd IS for streptokinase (00/464) are low and a replacement is needed. Streptokinase remains the most widely used thrombolytic drug worldwide, especially in developing countries, and is the most popular thrombolytic standard with 280 ampoules distributed each year (average 2011-2013). It is proposed to calibrate a replacement relative to the 3rd IS (in IU) using chromogenic and fibrin based assay methods, and the project has been endorsed by the WHO ECBS. Laboratories interested in being involved in the study were encouraged to make contact, with the study planned for 2015.

**Update on SSC Subcommittee Project on D-dimer Standard Development 2014**

An SSC Subcommittee Project is working on developing a new WHO IS for D-Dimer. A batch of plasma from multiple pools, containing high levels of D-dimer was provided by the Royal Hallamshire hospital (Sheffield, UK), and has been freeze dried (SS-258). Ampoules of SS-258 were stored at elevated temperatures and assayed after various times using a variety of methods. A greater loss of activity was found than might be expected from an antigen, and one possible explanation for this loss is aggregation. Large fibrin degradation products (FDP) have been shown to aggregate, which appear to form cross-beta structures, staining positive with thioflavin T. To investigate this FDP preps were made by mixing fibrinogen, thrombin, FXIIIa, plasminogen and activator. To get a good mixture of FDP sizes and structures tPA and uPA were used as activators with both glu- and lys- plasminogen. Samples were taken
throughout the lysis and pooled, and freeze dried with albumin and either trehalose or maltose (SS-405). Ampoules of SS-405 were stored at elevated temperatures and accelerated degradation assays indicated that trehalose was good at stabilising D-dimer antigen levels, but maltose was not. Looking at thioflavin T fluorescence trehalose appears to prevent the formation of cross beta structures. A second FDP fill (SS-444) was done with several different formulations: FDP + trehalose, FDP + sucrose, FDP in plasma +/- trehalose. Assays on these samples show poor recovery of D-dimer (approx. 20%) with no added protein, and both plasma and trehalose were required to prevent loss. A first phase collaborative study is proposed including samples of SS-258 (patient plasma with D-dimer), FDP with albumin and trehalose, FDP in plasma with trehalose, and possibly with patient samples (to assess commutability). The results will inform the design of a definitive study to establish an IS.

12.00 Closing remarks by Nicola J Mutch

The audience are invited to register membership of the committee on the SSC-fibrinolysis website. There is an open call for new projects for the subcommittee to develop as well as to participate in existing projects. We also discussed the possibility of using other social media, such as facebook or research gate to communicate interests of the group. The ISTH meeting in Toronto, Canada was advertised.
Hemostasis and Malignancy

Chairman: Alok A. Khorana (USA)
Co-Chairmen: Marc Carrier (Canada), Howard Liebman (USA), Nigel Mackman (USA), Ingrid Pabinger (Austria), Joseph Palumbo (USA), Jeffrey Zwicker (USA)

Tuesday, 24 June (14:00-18:00)

Attendance: Alok A. Khorana (USA), Marc Carrier (Canada), Howard Liebman (USA), Nigel Mackman (USA), Ingrid Pabinger (Austria), Joseph Palumbo (USA) and Jeffrey Zwicker (USA)

Invited Speakers: Bo Zhang (abstract), Jeffrey Zwicker, Alok A. Khorana, Howard Liebman, Marc Carrier, Ingrid Pabinger, Nigel Mackman, Brian Cooley, Marcello Di Nisio

There were 10 presentations, including guidance documents, updating subcommittee activity and discussing new proposals.

The first portion of this SSC session, co-chaired by Drs Pabinger and Khorana, focused on activity by the Subcommittee in generating new data and developing guidance statements. Dr. Zwicker provided an update of recently published data regarding thromboprophylaxis in hospitalized cancer patients. Dr. Khorana discussed a draft guidance statement on prevention of VTE in medical cancer outpatients. Dr. Liebman discussed potential roles for direct oral anticoagulants in prevention of cancer-associated thrombosis and Dr. Carrier discussed their role in treatment.

The second portion of this SSC session, co-chaired by Drs Palumbo and Mackman, provided updates of SSC activity on clinical and translational projects. Dr. Pabinger provided new information from the ongoing Vienna CATS registry, regarding the role of interleukins. Dr. Mackman proposed a TF standardization project and invited collaborators. Dr. Cooley discussed mouse models of cancer-associated thrombosis. Finally, multiple investigators provided brief updates on the larger ongoing clinical trials in the field of cancer-associated VTE including the CATCH trial of treatment of VTE with tinzaparin (Khorana), the PHACS trial evaluating the use of dalteparin as prophylaxis in cancer outpatients at high risk for VTE (Khorana), the SOME and PERIOP trials (Carrier), long-term treatment of VTE after 6 months the LONGHEVA trial now converted to a registry (Kamphuisen), Dr. DiNisio presented data from a registry of incidental VTE. In this multicenter, prospective study, patients with unsuspected pulmonary embolism are followed up for to one year to assess the incidence of recurrent symptomatic VTE, bleeding, and mortality. The status of the study was presented with an update of the number of centers involved and patients included.

The meeting was adjourned at 18:00.
Lupus Anticoagulant/Phospholipid Dependent Antibodies

Chairman: Bas de Laat (the Netherlands)
Co-Chairmen: Tatusya Atsumi (Japan), Maria Laura Bertolaccini (United Kingdom), Katrien Devreese (Belgium), Thomas Ortel (USA), Rolf Urbanus (The Netherlands), Denis Wahl (France)

Monday, 23 June (14:15-18:15)

This year the SSC subcommittee will focus on the establishment of standards for the solid phase assays, the anti-β2-glycoprotein (β2GPI) assays and the anti-cardiolipin assays. In the introduction Dr. de Laat pointed out that these standards are needed not only for comparison of results between different assays or different centers. These standards are also needed to move the field forward as many basic science studies, executed to find a possible mechanism to explain the occurrence of thrombosis and pregnancy morbidity, use different types of "antiphospholipid antibodies".

In order to give some guidelines to manufacturers of solid phase assays, Dr. Devreese headed the project out of the SSC to come with recommendations. These recommendations for aCL and aβ2GPI assays intend to ameliorate the performance of these solid-phase assays. In solid phase assays (mostly enzyme-linked immunosorbent assays (ELISA), although also newer automated platforms) several factors contribute to variability in pre-, post- and analytical conditions. In the concise report, published in JTH as an official SSC communication, more detailed information is provided on some issues aiming to reach more standardisation. Patient selection, blood collection, choice of assays, performance characteristics, interferences, duplicate versus single testing, standards and calibration, results expression, cut-off values calculation, and results interpretation are addressed.

The next speaker, Dr. Kelchtermans, provided evidence that future standards have to take into account that some epitopes on β2GPI can be cryptic. Substantial evidence exist that domain I of β2GPI I contains an immunodominant, cryptic, pathogenic epitope exposed only in the open conformation of β2GPI. Dr. Kelchtermans showed that there is a high variability of the exposure of this thrombosis-related epitope in commercially available anti-β2GPI assays, resulting in the under-diagnosis of the anti-phospholipid syndrome and explaining at least part of the inter-assay variability. Currently used assays should be optimized to ensure satisfactory expression of this epitope and avoid false negative results. She suggested that antibodies, described by the group of the Laat et al, recognizing this epitope should be included in the panel of standards. Dr. Bertolaccini was the next speaker on this topic and showed an initiative lead by Prof. P.L. Meroni and Dr. R. Willis (Prof. Pierangeli’s lab) to implement standards derived from patient plasma and a monoclonal antibody (HCAL). Using the aforementioned patient standards she showed in agreement with Dr. Kelchtermans differences between assays of different manufacturers. They both agreed on the need to exchange standards to test for epitope specificity.
Dr. Moore presented the first CLSI guideline on lupus anticoagulant (LA) detection that was published in April 2014. There are many commonalities with the recent ISTH SSC and BCSH guidelines but also some important contrasts that were highlighted. A preliminary coagulation screen employing a LA-unresponsive APTT is considered integral to the LA detection process. Preferred first-line LA screening tests are dRVVT and LA-responsive APTT, although other assays such as KCT, dPT and TSVT are not excluded as additions or adjuncts providing that due recognition is given to performance characteristics. Testing order is re-prioritised as screen, confirm and then mixing test, to reduce false-negatives arising from the dilution effect. Normalised ratios should be derived from the reference interval mean as denominator. A question was raised about false-positive screening tests when employing +2SD not 99th percentile. The CLSI guideline indicates that it is the screen, confirm and (mix) composite that secures diagnostic interpretation. Non-LA abnormalities or outliers will not generate the screen and confirm discordance diagnostic of LA.

Dr. Gray presented the final results of the collaborative study to evaluate 3 plasma samples as the 1st International Reference Panel for LA. The candidates were prepared by titration of LA negative and LA positive patient plasmas to obtain a negative (12/148), a moderate positive (12/150) and a strong positive (12/152) LA samples. Results from DRVVT, APTT, SCT, dPT, KCT, ASLA and TSVT confirmed the LA status of these 3 plasmas. The participants and experts from the SSC endorsed with the proposal and recommend the establishment of this reference panel to the ECBS/WHO.

Dr. Urbanus presented his work on the possible interference of Vitamin K antagonists (VKA) on accurate detection of LA due to their prolonging effects on clotting times. He investigated whether VKA influence LA test results and found that LA can be reliably assessed in LA-positive patients with an INR>2.5. VKA did not cause a false positive LA test outcome in LA negative samples with an INR>3.0. However, VKA might cause false negative LA test results in weakly positive samples. The SSC recommends performing mixing experiments for LA assessment in samples with an INR between 1.5 and 3.0. Mixing did not influence LA-test results obtained with dRVVT reagents, but increased the strength of LA detected with SCT reagents. The mixing procedure itself, however, caused false negative test results in weakly positive samples. He therefore concluded that LA can be accurately assessed in samples with an INR>2.5 and mixing tests are not necessary. Since these results are based on a small number of patients and only two different LA reagents, he proposed a multicentre study to assess the effect of VKA treatment on LA detection and the effect of mixing studies thereon. This project will be placed on the SSC website.

Dr. Pengo proposed another study including oral Xa inhibitors. Triple positivity (LAC+, aCL+, aβ2GPI+, same isotype) in APS is associated with thromboembolic events and severe pregnancy morbidity. Triple positive APS patients are at high risk of recurrent thromboembolic events. As compared with aspirin, VKA therapy significantly reduces thromboembolic recurrences, although it might prove insufficient in some cases. The new oral anticoagulant rivaroxaban, an inhibitor of factor Xa, is at least as effective and safe as warfarin in preventing venous and arterial thromboembolism and significantly reduces cerebral bleeding. Rivaroxaban does not need laboratory control thus being very much appreciated by the young population of patients with APS. At variance with other new anticoagulants it is administered once daily favoring
patient’s compliance. Dr. Pengo has therefore organized a prospective, randomized clinical Trial comparing Rivaroxaban vs warfarin in high risk patients with thrombotic AntiPhospholipid Syndrome (TRAPS) (European clinical trials database 2013-004575-13). This is a randomized, open label, non-inferiority, prospective, multicenter, non-profit study. Twenty Italian centers will start enrollment on July-September 2014. Centers from foreign countries will start at the end of this year.

Dr. Wahl presented his work on anti-domain I antibodies. There have been reports describing that the presence of anti-domain I antibodies better correlates with thrombosis than antibodies directed against other epitopes on β2GPI, though all of these were retrospective. Dr. Wahl executed a prospective study including over 100 patients. They were able to show that the detection of anti-domain I antibodies displayed clinical significance.

Dr. Atsumi presented his work on phosphatidylserine-dependent anti-prothrombin antibodies (aPS/PT). Worldwide scientists on APS agreed, in the International Congress on Antiphospholipid Antibodies as well as SSC, that aPS/PT would potentially contribute for a better recognition of APS Therefore, a retrospective and cross-sectional international multicentre study was designed to determine the value of aPS/PT for the diagnosis of APS. The first cohort involved eight centres from 7 countries, showing 61% sensitivity and 86% specificity of IgG and/or IgM aPS/PT for APS diagnosis. To confirm those data, a second cohort was collected from 5 countries and the results are coming soon. Jacob Rand and Lucia Wolgast of Montefiore Medical Center reported on ‘Annexin A5 Resistance in Different Patient Populations.” They applied this novel mechanistic assay, previously studied in small groups of highly selected patients to the "real world” setting of a major inner city U.S. hospital population, to ~ 1,000 patients. They reported that: annexin A5 anticoagulant ratios (A5R), do not vary significantly between males and females or among different age groups or ancestries. Reduced A5R levels were detected in about half of aPL-positive patients with thrombosis, and in ~17% of aPL-negative patients with thrombosis, but only in ~6% of apparently healthy subjects. Finally, there was a correlation between multipositivity for aPL assays and reduction of A5R levels, a finding that was confirmed in a blinded study of prospectively obtained coded samples provided by Dr. Vittorio Pengo of University of Padua. A correlation with clinical outcomes will be analyzed in the coming weeks.
**Pediatric and Neonatal Hemostasis and Thrombosis**

**Chair:** Anthony Chan (Canada)

**Co-Chairmen:** Mariana Bonduel (Argentina), Leonardo Brandao (Canada), Elizabeth Chalmers (UK), Neil Goldenberg (USA), Paolo Siminoi (Italy), Heleen van Ommen (the Netherlands)

Monday, 23 June (14:15-18:15)

The focus of the Subcommittee is to address issues in the area of thrombosis and hemostasis in children and neonates by developing clinical standards for evaluation, foster international collaboration in research and clinical trials, establish/maintain registries and to generate, publish and distribute reports and recommendations relating to patient care for the pediatric population.

Overall, work within the Subcommittee is done through the Working Groups lead by one of the Co-chairs.

1. **Position Papers:**

Two position papers has been submitted:

1. Recommendations for the development of a dedicated paediatric anticoagulation service. (Lead by Paul Monagle working with Fiona Newall)
2. Recommendations for the assessment of non-extremity venous thromboembolism outcomes. (Lead by Anthony Chan working with Madhvi Rajpurka)

One position paper is at an advance stage of preparation and will be submitted within the next few months:

2. **Guidance Papers:**

Two guidance papers have been submitted and are under revision.

The guidance papers are:

1. Venous thromboembolism in children: Considerations for thrombophilia testing. [Update 2012] (Lead by Ulrike Nowak-Gottl)
2. The use of inferior vena cava filters (IVCF) in children. (Lead by Anthony Chan working with Suzan Williams)

3. **Administration:**
One Co-chair (Dr. Paolo Simioni) will step down. Dr. Shoshana Revel-Vilk is nominated to the co-chair position. The nomination is based on their past contribution to work of the subcommittee and to avoid country or continent over-representation. Recommendation: I recommend that the SSC make a decision on new Co-chair(s) and appoint a new Chair at the meeting so that transition of leadership can be done smoothly.

4. Ongoing Projects:

Below is the list of ongoing projects with the intention of developing a position statement or a guidance paper (to be determined by the Working Group lead by the Co-chair):

1. Diagnostic criteria for thrombosis in children. (Responsible Co-chair: Leonardo Brandao) Progress Presented at SSC meeting
2. Antiplatelet therapy in children. (Responsible Co-chair: to be determined)
3. HIT in children. (Responsible person: Guy Young)
4. Antithrombin replacement in heparin therapy. (Responsible Co-chair: Elizabeth Chalmers)
5. Management of coagulopathy in liver disease. (Responsible person: Paul Monagle working with Maria Magnusson) Progress Presented at SSC meeting
6. Liver and renal biopsies in coagulopathic patients. (Responsible person: Paul Monagle working with Maria Magnusson)
7. VTE prophylaxis. (Responsible co-chair: Neil Goldenberg) Progress Presented at SSC meeting
8. Interpretations of coagulation inhibitors in the context of developmental hemostasis. (Responsible co-chair: Anthony Chan)
9. Congenital Severe Purpura Fulminan Registry. (Responsible person: Vicky Price, Adrian Minford) Progress Presented at SSC meeting
10. Management of pulmonary embolism. (Responsible co-chair: Neil Goldenberg)
11. Management of arterial thrombosis. (Responsible co-chair: Neil Goldenberg working with Manuela Albisetti) Progress Presented at SSC meeting

Two new projects have been proposed:

1. Antithrombin Registry with a focus on genotype and phenotype correlation. (Responsible person: Riten Kumar)
2. Standardized approach in treatment of Neonatal Thrombosis. (Responsible co-chair: Heleen van Ommen)

Collaborations with other Subcommittees (DIC and Women’s Health Issue) have been discussed.

We plan to make use of the ISTH SSC website for communicating with ISTH members beyond the Co-chairs such that ISTH members can be more engaged with the work of SSC. At the
moment, there are 39 registered members for the pediatric/neonatal hemostasis and thrombosis subcommittee.

Anthony K. C. Chan  
Chair, Pediatric/Neonatal Hemostasis and Thrombosis Subcommittee
Plasma Coagulation Inhibitors

**Chairman:** Richard Marlar (USA)
**Co-Chairmen:** Elisabetta Castoldi (the Netherlands), Steven Kitchen (UK), Jun Teruya (USA), Hiroko Tsuda (Japan)

Tuesday, 24 June (14:00-18:00)

**Session #1: Welcome and Abstract Presentation**

**Welcome and Introduction**
Speaker: Richard Marlar (USA)

**Top Rated Abstract**
Speaker: Vera Korneeva (Russia Federation): *Corn trypsin inhibitor non-loop regions are required for the specific inhibition of factor XIIa*

V. Korneeva presented her research based on her abstract dealing with the molecular structure responsible for the molecular mechanism of corn trypsin inhibitor inhibition of the coagulation enzyme, factor XIIa.

**Session #2: Molecular Genetics**
Chairman: Jun Teruya (USA)

**Update and Maintenance of the Antithrombin, Protein C and Protein S Mutation Databases**
Speaker: Richard Marlar for Elisabetta Castoldi (The Netherlands)

In 2011, the Plasma Coagulation Inhibitor Subcommittee proposed developing and maintaining a locus-specific genetic mutation database for Antithrombin, protein C and protein S. However in the last three years only three people volunteered to help develop and maintain the database. This project would be a long term (continuous) undertaking requiring experienced curators and a significant time commitment to check the literature, clinical presentations and probably require long term funding. Since the SSC has a Working Party on Genomics, the Plasma Coagulation Inhibitor Subcommittee recommended contacting the Working Group on Genomics to transfer this project to this group. Initial contact was made with Willem Ouwehand of the Working Group on Genomics.

**Subcommittee Project Update: Racial differences in genetic risk factors for venous thromboembolism** Speaker: Hiroko Tsuda (Japan)

Background: Factor V Leiden and prothrombin G20210A, well-known hereditary thrombophilia in Caucasians, are not found in Asian, Africans and Australoid. In contrast, protein S (PS) and protein C (PC) deficiencies are much more prevalent among Asians than non-Asians. PS
Tokushima (K155E, K196E in HGVS nomenclature) and two PC gene mutations, PROC c.565C>T and PROC c.574_576del, all three representing type II deficiency, are identified as hereditary thrombophilia in Japanese and Chinese, respectively. In order to elucidate the racial differences in genetic risk factor for VTE, the worldwide distribution of these three mutations is investigated.

Update: 1) We build up a global network of professional contacts, which includes Asian and European countries and USA, in collaboration with the members of JSTH-SSC, APSTH, and ISTH-SSC. 2) After the ethical approval, VTE patients and healthy individuals are recruited through the use of standardized protocol and registry sheets. Racial groups are divided into white (Caucasians), Hispanic, black, East Asian, South Asian, Other Asian, or Other. 3) Analysis of plasma and DNA samples are mainly performed in Japan. The total PS assay system (Tsuda T. et al. Blood Coag Fibrinolysis. 23: 56-63, 2012) has been improved, achieving a high sensitivity for screening of PS Tokushima by evaluating the specific activity of the APC cofactor function of plasma PS.

Session #3: New Proteins
Chairman: Steve Kitchen (UK) & Hiroko Tsuda (Japan)

Tissue Factor Pathway Inhibitor- General Aspects
Speaker: Paul Ellery (USA)

Tissue factor pathway inhibitor (TFPI) inhibits factor VIIa and factor Xa to limit thrombin generation. It is produced by the endothelium of the microvasculature and platelets. Two isoforms, TFPIα and TFPIβ, arise from alternative splicing. While both isoforms inhibit factor VIIa and factor Xa, they have different C-termini which give them distinct anticoagulant activities. TFPIα is a soluble protein. Protein S is a co-factor for TFPIα that enhances its inhibition of factor Xa by localizing it to a membrane surface. TFPIα also tightly binds to an acidic region of the factor V B-domain providing a key exosite interaction that allows physiological inhibition of early forms of prothrombinase. TFPIβ is GPI-anchored at the endothelial surface, where it is optimally located to inhibit TF-mediated processes. Platelet TFPI is exclusively TFPIα. Platelet TFPIα modulates hemostasis in mice with hemophilia and limits thrombus development following murine vascular injury. Plasma TFPI is heterogeneous and exists in the full-length (TFPIα) and several C-terminally truncated forms that associate with plasma lipoproteins. It has been measured in numerous disease states. Low levels weakly correlate with thrombosis risk. Patients with factor V East Texas have 10-fold elevated plasma TFPIα, which is thought to be responsible for the moderately severe bleeding diathesis associated with this disorder. Plasma TFPI is affected by a number of variables, including gender, age, plasma LDL, glucose, factor V and protein S concentrations, as well as oral contraceptive use. Measurement of platelet TFPI may be a more informative hemostatic/thrombotic indicator in disease states because it is affected by fewer variables than plasma TFPI.

Tissue Factor Pathway Inhibitor- Clinical Assays
Speaker: Dorothy Adcock (USA)
A number of manufacturers produce TFPI assay kits to measure free TFPI, Total TFPI or TFPI activity. All are labeled as research use only in the U.S. TFPI is a complex glycoprotein that exists as two primary isoforms (these vary in composition) that can be found in multiple locations, specifically, free in plasma, bound to lipoprotein in plasma, bound to cell surfaces, as well as intracellular pools. TFPI in the plasma includes a full length (total) glycoprotein as well as truncated forms of varied sizes which are largely bound to lipoprotein. Antigen kits are based on quantitative sandwich enzyme immunoassay methodology. The capture and detection antibodies are proprietary and are not standardized between kits. These kits therefore likely measure at least slightly different plasma TFPI populations based on the epitopes of the capture and detection antibodies. Activity assays measure essentially only free TFPI and not TFPI bound to cell surfaces and therefore measures only a fraction of TFPI’s intravascular anticoagulant potential. These assays are based on TFPI’s ability to inhibit the catalytic activity of TF/FVIIa and do not measure TFPI’s ability to inhibit prothrombinase. Plasma TFPI activity therefore is not a reliable indicator of intravascular TFPI anticoagulant activity. Calibrators for both antigen and activity assays consist of either normal plasma or recombinant TFPI and there is no TFPI standard. Finally, both TFPI antigen and activity assays are likely affected by pre-analytic variables as platelet activation with sample procurement and processing may cause release of TFPI alpha and there is potentially an impact on levels of TFPI alpha related to the length of tourniquet when drawing the sample. Following processing, the post centrifugation platelet count may also affect levels of TFPI alpha in those samples that have undergone a freeze-thaw cycle.

**Tissue Factor Pathway Inhibitor- Clinical Relevance**
Speaker: Jun Teruya (USA)

Total and free TFPI were measured for patients on ECMO who were receiving unfractionated heparin therapy. Heparin increases TFPI level. It is not clearly understood the mechanism, but some articles suggest heparin not only releases TFPI from endothelial cells, but also stimulates synthesis of TFPI in endothelial cells. Our data showed continuous increase of TFPI during ECMO therapy, suggesting heparin may be stimulating to synthesize more TFPI. The response of TFPI upon heparin administration is different between patients >1 year old and < 1 year old. It was concluded that TFPI also plays a major role for heparin anticoagulation.

**Session #4: Laboratory Testing**
Chairman: Steve Kitchen (UK) & Richard Marlar (USA)

Subcommittee Project Update: Protein S activity assays
Speaker: Ian Jennings (UK)

External Quality Assessment data from multiple EQA programs has repeatedly demonstrated that protein S activity assays have significant statistical and clinical differences depending on the different assay methods, reagents and manufacturers. This occurs in normal samples and protein S deficient samples. The Plasma Coagulation Inhibitor Subcommittee is investigating the cause and extent of the discrepancies between these reagents and methods. A proposal for this study has been approved. The delay has been the inability to obtain appropriate samples for shipment to multiple laboratories for testing; however acquiring plasma has been reinstated at the
NEQAS site. This project will restart in the next few months and samples will be sent to participating laboratories. Preliminary results are expected to be presented at the next SSC meeting.

Planning for Preparing Guideline Manuscripts  
Speaker: Richard Marlar for Piet Meijer (The Netherlands)

The Plasma Coagulation Inhibitor Subcommittee has proposed the development and submission of four manuscripts for publication in JTH detailing assay performance, acceptable methodology and significant pitfalls based on published literature (evidence based). The four manuscript topics are:

- Antithrombin, Coordinator: P. Meijer (The Netherlands)  
- Protein C, Coordinator: S. Kitchen (UK)  
- Protein S, Coordinator: R. Marlar (USA)  
- APC-Resistance, Coordinators: D. Adcock (USA) and G. Moore (UK)

During the committee meeting, a request was made for volunteers to help with preparation of the manuscripts. Briefly, the manuscripts will contain: a short description of the component, the assay principles, review of the available clinical assay methods, pitfalls and problems, pre- and post-analytical issues and recommendations for testing methods. Submission for publication is expected by the next SSC meeting.

Session #5: Discussion and Future Directions  
Chairman: Richard Marlar (USA)

Planned activities for the 2015 Subcommittee Meetings  
Speaker: Richard Marlar (USA)

For the 2015 Meeting of the ISTH-SSC Subcommittee on Plasma Coagulation Inhibitors, the committee proposed the following topics.

1. Resolution of the locus-specific Antithrombin, protein C and protein S databases
2. Progress report and preliminary data on the racial differences project.
3. Progress report and preliminary data on the protein S activity assays project.
4. Presentation of the near-final drafts on the four proposed manuscripts on clinical assays for antithrombin, protein C, protein S and APC-Resistance.
5. Proposed status of TFPI as an important inhibitor of coagulation.

Future and long term goals for Subcommittee  
Speaker: Richard Marlar (USA)
For future studies, the Plasma Coagulation Inhibitor committee will consider TFPI, other plasma coagulation inhibitors, other components of plasma inhibitor systems. As well, the committee will evaluate the roles of plasma coagulation inhibitors in the development of thrombophilia.
Platelet Immunology

Chairman: Yves Gruel (France)
Co-Chairmen: Donald Arnold (Canada), Tamam Bakchoul (Germany), Sentot Santoso (Germany), Yoshiaki Tomiyama (Japan), Chris Ward (Australia)

Wednesday, 25 June (8:00-12:00)

Part 1. Top Rated abstract

The effects of different B-cell activating factor receptors on lymphocyte function and secretion of cytokines in immune thrombocytopenia. Presenting author: Yanan Min (China)

Unfortunately, the author apparently did not attend the meeting and this presentation was therefore cancelled.

Part 2. Drug-induced and autoimmune thrombocytopenia

1- During this session, the usefulness of the measurement of immature platelet fraction (IPF) by a Sysmex autoanalyzer for the diagnosis of primary ITP was discussed by Y Tomyiama (Japan). The preliminary results revealed that XN-1000 showed better CV to measure IPF.

2- T Bakchoul (coauthor D Arnold) also discussed the methodological aspects related to the detection of drug-dependent antibodies. Several questions remain to be further defined such as the number of platelets, and the concentrations of drug to be used for testing. In any case, a confirmation by another laboratory is requested to ensure the responsibility of a specific drug in DITP.

3- On the other hand, T Bakchoul (coauthor J Fuhrmann) also discussed the optimal conditions to be used when evaluating the pathogenic effect of a platelet antibody using a NOD.SCID mice model. The methods for isolate and inject the platelets as well as the conditions of injection of the antibody have been briefly discussed.

4- Finally, A Pecci from Italy gave a lecture on the diagnosis of inherited thrombocytopenia and recalled that many affected patients are often misdiagnosed as having ITP. In this regard, he therefore outlined how essential is the questionnaire looking at a familial history of bleeding and low platelet count and the careful analysis of platelet size and morphology.

Part 3. : Alloimmune thrombocytopenia

The key messages delivered during this session were:

1- The diagnosis of NAIT is confirmed when a genetic paternal/maternal incompatibility for a Human Platelet Antigen is identified and a corresponding antibody is detected in the maternal serum.
2- HPA typing should be hold using at least two methods: phenotyping and genotyping or genotyping using at least tow different primers two avoid mistyping due two new variants in HPA.

3- Reference laboratories are able to diagnose only about 30% of all referred suspected cases of NAIT. The following reasons for this diagnostic gap should be taken into consideration: 1) maternal immunization against low frequency HPA antigens; 2) Low avidity antibodies ; 3) some "conventional” antibodies (such as HPA-3, -15, -2) are difficult to detect, and 4) HLA antibodies may cause some cases of NAIT.

4- Modified Epitope-specific monoclonal antibodies represent a promising therapeutic approach in the management of FNAIT. While safety has already proven in human, protective efficiency still need to be verified in clinical trail.

Part 4. : Heparin-induced thrombocytopenia

The key messages delivered during this session were:

1- The team of Y Gruel (France) has developed 5B9, a new monoclonal antibody (MoAb) to PF4/heparin complexes with a human Fc fragment that fully mimics human HIT antibodies. A comparative evaluation of this antibody with 5A1, another MoAb developed in Japan and that is also a potential standard for HIT diagnosis assays showed that 5B9 was the only one to activate platelet in a heparin-dependent manner.

2- The performances of flow cytometric analysis of platelet activation for the diagnosis of HIT has also been evaluated in France in a large cohort of patients (B Tardy). Combined with and IgG-specific assay, this approach appeared as sensitive and specific than SRA.

3- The use of Platelet Microparticle Generation Assay for the diagnosis of HIT has also been discussed by V Minet (Belgium)

4- Finally, C Ward presented an update on the practical use of whole blood impedance assay for HIT diagnosis, but this procedure has to be prospectively evaluated.

Final discussion and perspectives

Based on the presentations and the discussion of this SSC meeting, the following projects are planned for the next future.

The first is to prepare an official communication of the SSC related to the laboratory testing in patients with suspected drug-induced thrombocytopenia (DITP).

The second project is about to be finalized by Tamam Bakchoul (Greifswald, Germany) and will propose recommendations on the use of NOD/SCID mouse as a model for studying the pathogenesis of immune thrombocytopenia.
The third ongoing project under the coordination of Chris Ward (Sydney, Australia) aims to propose a standardized procedure about the use of the whole blood impedance aggregometry (using the Multiplate analyzer) for the diagnosis of HIT.

Finally, Y Gruel will propose in the next week a study coordinated by the SSC aiming to evaluate the use of 5B9 as a standard in HIT immunoassays.
The meeting began with a 10 minute presentation and 5 minutes discussion by the top rated abstract in Platelet Physiology – Dr. Satoshi Nishimuar from Japan presented his work on Morphological Distinction Unravels Mechanisms of Platelet Biogenesis from Bone Marrow Megakaryocytes.

The SSC business session then began at 14:15 pm. Paul Harrison (UK) briefly outlined the new mission statements of the ISTH SSC and the platelet physiology SSC and followed this with an update of current ongoing and future projects. The LTA guideline that was finished and agreed upon in Cairo in 2010 is now published as a JTH paper in the June 2013 issue: Cattaneo et al, Recommendations for the Standardization of Light Transmission Aggregometry: A Consensus of the Working Party from the Platelet Physiology Subcommittee of SSC/ISTH.J Thromb Haemost. 2013 Apr 10. doi: 10.1111/jth.12231. The detailed survey (from 202 laboratories) of laboratory practice for the diagnosis of inherited platelet disorders coordinated by Paolo Gresele is now accepted for publication in JTH and available online: Gresele et al, Diagnosis of suspected inherited platelet function disorders: results of a worldwide survey DOI: 10.1111/jth.12650. The guidelines for the diagnosis of inherited platelet disorders have also just been submitted to JTH although Paul reported that this needs to be resubmitted with 6 authors instead of 10 but we are currently in discussions with the journal and the SSC chairman about this. Diego Mezzano and Andrew Mumford have also drafted and edited an excellent review and long due article on "Platelet Release Assays” that will shortly be submitted to JTH for peer review with the future aim for the SSC to produce a new Guideline/position statement. Other projects include the Diagnostic Utility of the ISTH BAT for platelet function disorders (in collaboration with the ISTH BAT working party) and the SSC is also working on a Guideline/position statement on monitoring P2Y12 inhibition. We are also monitoring developments on the Evaluation of methods for measuring the platelet transcriptome (in collaboration with the thrombogenomics working party) for a possible future project.

Francesco Rodeghiero (Italy) then gave a brief presentation on the history and evolution of the ISTH Bleeding Assessment Tool (BAT) and its potential role for platelet function disorders. Paul then presented an overview of the proposed study on a retrospective multicentre study on the BAT for adults with platelet function defects. The ISTH Bleeding Assessment Tool (ISTH-BAT) is a consensus-based, publicly available tool designed to evaluate the severity of bleeding symptoms, primarily in patients with congenital disorders of haemostasis (see Rodeghiero et al, JTH 2010:8: 2063–2065). The tool includes a standardized questionnaire and a 0-4 bleeding score for each symptom that is used to compute an overall bleeding score. Based on the ISTH-BAT, a web-based instrument has been developed by Barry Coller at the Rockefeller University,
NY, USA, named Bleeding Assessment Tool Repository (BAT-R). The database is designed to collect and store data regarding bleeding symptoms in humans, providing a user-friendly, web-accessible platform, encouraging uniformity in the standardization and collection of bleeding histories. Individual patient data from each centre can be entered, the BAT calculated in a standardized format and data downloaded from the website in excel format. Data can also be shared and compared with the existing database. Paolo Gresele (Italy) then followed this with the final presentation of the working party for the diagnosis of inherited platelet disorders including a detailed diagnostic algorithm. Paolo then announced a new SSC retrospective study on the validation of this new diagnostic algorithm. Invitations to participate will be announced to members and on the website and newsletter.

In the second session Maha Othman (Canada) gave an updated overview of Platelet Type VWD and announced a new SSC study "Prospective evaluation of bleeding phenotype in PT-VWD to support evidence based diagnosis and management". Loredana Bury (Italy) gave a related presentation on new insights into Megakaryopoiesis and Proplatelet Formation in PT-VWD. Andrew Mumford (UK) then concluded the first session on GPCR Structure/Function variants in platelets before the coffee break.

The final session was devoted to a debate on anti-P2Y12 monitoring with 3 excellent speakers – Udaya Tantry (USA) on "Why should Anti-P2Y12 therapy be monitored", Bernd Jilma on "Why studies on tailored antiplatelet therapy failed so far" and Marco Cattaneo on "Anti-P2Y12 therapy should not be monitored”. Each speaker answered individual questions about their topics before being invited back onto stage for a general discussion at the end. The slides presented and the lively discussion will form the basis of a future SSC position statement/guideline to be drafted by the end of 2014. Paul Harrison concluded the session and announced that after 3 years he is to stand down as chairman and that the new chairman of the SSC will now be Paolo Gresele.

Paul thanked everyone especially previous chairman and co-chairs and the current SSC for all their hard work and support.
Welcome and Introduction of co-chairpersons

Paul Kyrle (PK) welcomed the participants also on behalf of the co-chairs. PK thanked James Douketis for chairing the Subcommittee in the previous years. PK presented the mandate of the subcommittee and the on-going subcommittee projects. PK invited interested colleagues to participate in the work of the group and gave a brief overview on the upcoming presentations.

Part I: Predictive Variables in Arterial Disease

Chairman: A. Spyropoulus

Prediction of Recurrent Thrombosis in Arterial disease – is it possible?

Shinya Goto summarized the pathomechanisms leading to arterial plaque formation and plaque rupture. He then presented the data of REACH, a multinational registry of approx. 68,000 patients with arterial disease. The objective of REACH is to identify clinical risk factors of recurrent arterial thromboembolism and to construct a risk score for recurrence prediction. Important risk factors of recurrence turned out to be advanced age, diabetes, no. of affected vascular beds, smoking, a recent cardiovascular event and heart failure. Aspirin and statin use were associated with a lower recurrence risk. On the basis of the corresponding HR for recurrent arterial thrombosis, a risk score was constructed and internally validated. Patients in the highest risk category had a recurrence risk as high as 60%.

Pharmacogenetics or Phenotyping as predictors of Stent Thrombosis

Bernd Jilma discussed the role of pharmacogenetic and platelet function testing in order to identify patients at high risk of stent thrombosis. There is evidence from many studies that such patients can be pinpointed, but most studies show a benefit neither from an increase in the clopidogrel dose nor from switching to newer anti-platelet drugs. There are 2 issues that need to be considered. 1) mainly "low-risk" patients were included in these studies and 2) the thrombogenicity of modern stents is very low thereby substantially reducing the event rate. It is thus more than questionable that future studies, which should be carried out in high-risk patients only will show a benefit of pharmacogenetic and platelet function testing.

Prediction of perioperative stroke in patients with or without atrial fibrillation: Do the CHADS and CHA2DS2- Vasc scores help?
James Douketis showed unpublished preliminary data indicating that the above-mentioned scores are helpful in identifying patients at risk of postoperative stroke regardless of the presence or absence of atrial fibrillation. The most important risk factors for postoperative thromboembolism turned out to be advanced age followed by diabetes mellitus and atrial fibrillation.

Part II: Predictive Variables in Venous Disease

After a break of 15 minutes the selected top rated abstract entitled "D-Dimer for the diagnosis of symptomatic upper extremity deep vein thrombosis" was presented by Benilde Cosmi from Italy. She showed that a diagnostic strategy consisting of measuring of D-Dimer and ultrasonography is effective and that the 3-months incidence of upper extremity thrombosis within 3 months is well under 1% in individuals who tested negative on D-Dimer and ultrasonography.

Role of D Dimer in predicting recurrent venous thromboembolism - an update

Clive Kearon reviewed the role of D Dimer measuring to predict VTE recurrence and concluded that regarding the duration of anticoagulation measurement of D Dimer is only meaningful for individual (rather than general patient) counselling. For instance women with an unprovoked proximal DVT or PE with a high D Dimer may opt for extended anticoagulation or men with an unprovoked proximal DVT or PE and a normal D Dimer may opt for a limited time period of anticoagulant treatment.

Predicting VTE in cancer patients – is it clinically relevant?

Marc Carrier first discussed the role of clinical characteristics and biomarkers to predict VTE occurrence. He then eluded on risk prediction models, in particular the Khorana score, which separates high-risk patients from patients with a moderate or low risk of cancer associated VTE. The score should be used to counsel patients at high risk of VTE as regards the signs and symptoms of VTE and also to provide pharmacological prophylaxis to selected high-risk patients. An interventional trial investigating the efficacy of thromboprophylaxis in high-risk cancer patients has recently completed recruitment.

In which patients should we use aspirin for VTE prevention?

Marc Samama addressed the role of aspirin in primary postsurgical thromboprophylaxis and in the prevention of recurrent VTE. He showed the data of older studies demonstrating an advantage of aspirin over placebo in orthopaedic surgery. He also eluded on current guidelines in which the recommendations to use aspirin in orthopaedic patients are weighed differently. As regards VTE recurrence aspirin is less effective than anticoagulants but confers a lower risk of clinically relevant bleeding.

Alex Spyropoulus dicussed the IMPROVE risk assessment model (RAM) to stratify medical patients into high and low VTE risk categories. This prediction tool has recently been validated by the use of 2 large registries. A web-based risk calculator is available.
Maura Marcucci eluded on the methodological problems encountered during RAM validations. The Vienna Prediction Model was used as an example. The most important issue is that the absolute event risk differs between cohorts making an accurate risk assessment in an individual patient almost impossible.

Lisbeth Eischer presented the study design and very limited data of the VALID study, a prospective cohort study to externally validate the Vienna Prediction Model.

June 25, 2014

Meeting of the SSC Working Project on D Dimer and other predictors of recurrent VTE

Start: 13:15

Chair: J. Douketis
Attendees: A. Iorio, B. Cosmi, M. Marcucci, PA Kyrle

The working group has recently provided data to a group of scientists from Birmingham (at the last meeting represented by Dr. David Fitzmaurice). There was full agreement among attendees that a health care system analysis using the database of the working group is most welcomed. It was also agreed on a construction of a RAM together with a validation by the use of large studies or registries (MEGA, RIETE). There was also full agreement that the construction of a new RAM without validation is not sensible as already 2 RAMS which were constructed using (parts of) the aforementioned database exist, one of them (Vienna Prediction Model), the other the DASH score. Serious concerns were expressed regarding a possible duplication of already published data. These concerns will be conveyed to the Birmingham researchers.

The Vienna Prediction Model was recently validated by the use of the aforementioned database. The attendees of the meeting discussed the upcoming submission of the manuscript.
Vascular Biology

Chairman: Rienk Nieuwland (the Netherlands)
Co-Chairmen: Francoise Dignat-George (France), Elizabeth Gardiner (Australia), Anna Randi (UK), Florence Toti (France)

Thursday, 26 June (8:45-12:45)

Shedded proteins and receptors (Chair: Elizabeth Gardiner, Australia)

Background and latest developments of metalloproteolytic shedding in vascular biology.
Bruce Walcheck, University of Minnesota, USA

Walcheck gave an outstanding overview of the A Disintegrin and Metalloproteinase (ADAM) family in the context of vascular biology. He focused on shedding/release of chemokines and Fc receptors from human leukocytes, which have important implications in regulation of the immune response. The Fc receptor CD16 is present on essentially all CD56(dim) peripheral blood natural killer (NK) cells. Upon recognition of antibody-coated cells, CD16 delivers a potent signal to NK cells, which in turn eliminate targets through direct killing and cytokine production. He discussed regulation of CD16 surface expression after NK cell activation. Cytokine activation and target cell stimulation led to marked decreases in CD16 expression. Activation of CD56(dim) NK cells by cross-linking CD16 with antibodies resulted in a loss of CD16 and CD62L, which correlated with increased interferon-γ production. His research group demonstrated that ADAM17 is expressed by NK cells, and its selective inhibition abrogated CD16 and CD62L shedding, and led to enhanced interferon-γ production, especially when triggering was delivered through CD16. Fc-induced production of cytokines by NK cells exposed to rituximab-coated B cell targets was also enhanced by ADAM17 inhibition. This supports an important role for targeting ADAM17 to prevent CD16 shedding and improve the efficacy of therapeutic antibodies. Over-activation of ADAM17 in NK cells may be detrimental to their effector functions by down-regulating surface expression of CD16 and CD62L.

Platelet receptor metalloproteolytic regulation and impact on platelet age and function.
Emma Josefsson, Walter and Eliza Hall Institute, Melbourne, Australia

Josefsson gave an excellent update on mechanisms relating to loss of glycoprotein Ib (GPIb), the platelet receptor for von Willebrand Factor (VWF), which is cleaved by ADAM17 during activation. Platelet concentrates for transfusion must be stored at temperatures above 15oC as chilling leads to rapid clearance of platelets in humans after transfusion. She described the mechanism that causes GPIb receptors to cluster on blood platelets at cold temperatures. Hepatic macrophage β2-integrin binding to β-N-acetylglucosamine (β-GlcNAc) residues on the GPIb-α chain leads to rapid clearance of acutely chilled platelets after transfusion. Although capping β-GlcNAc moieties by galactosylation prevents clearance of short-term-cooled platelets, this strategy is ineffective after prolonged refrigeration. Prolonged refrigeration increased the density and concentration of exposed galactose residues on platelets such that hepatocytes, through Ashwell-Morell receptor binding, become increasingly involved in platelet removal. ADAM17 mediates shedding of significant amounts of GPIb-α in transfusion bags of platelets. Macrophages rapidly removed a large fraction of transfused platelets independent of their storage
Survival of transfused platelets could be improved by treating bags with inhibitors of ADAM17. By minimizing ADAM-mediated receptor shedding in platelet transfusion bags or in human blood circulation in thrombocytopenic patients with platelet production defects, platelet survival could be enhanced.

Discussion on clinical aspects of receptor shedding. Chris Ward, Kolling Research Institute and Royal North Shore Hospital, Sydney, Australia

Ward gave a timely and important update on the utility of platelet shed proteins as biomarkers of human hematological diseases. Importantly he suggested the criteria that a prospective biomarker should conform to, in order to be useful in clinical diagnostic situations. Specifically, a biomarker should be

1. platelet specific
2. readily measurable
3. demonstrate a specific increase in response to a disease situation
4. levels should change upon commencement of successful therapy or change in disease status.

He then outlined some of the challenges faced by clinicians in making a correct diagnosis of hemolytic uremic syndrome versus thrombotic thrombocytopenic purpura and indicated some encouraging recent data regarding the utility of soluble GPVI as a biomarker of TTP that reflects the increased platelet activation and clearance.

Circulating Endothelial Progenitor Cells (Chair: Anna Randi, UK)

Background and latest developments in isolation of circulating endothelial progenitor cells. Reinhold Medina, Centre for Experimental Medicine, Queen’s University Belfast, UK

Dr Medina provided a comprehensive and clear review of the field of Endothelial progenitor cells (EPCs), which suffers from confusion in nomenclature and methods. The first reports that circulating cells, identified as progenitor for the endothelial lineage, had the capacity to repair and regenerate damaged blood vessels, were met with great enthusiasm. However, these early EPC were identified using assays that overlapped with cells of the hematopoietic lineage. Major disputes and controversy in the field damaged the "credibility" of these cells, with some even doubting their existence. Over the past decade, a consensus view has finally been reached. The term EPC in the literature has been used to identify essentially two types of cells: one from myeloid origin and another from a yet unknown origin. Both have a role in tissue regeneration by promoting the repair of blood vessels and aiding in the re-perfusion of ischemic areas; however only the latter can be considered a "true" endothelial progenitor or EPC. True EPC can be isolated from whole blood, and expanded in culture to generate colonies with robust clonal proliferative potential, endothelial markers and ability to form de novo vessels in vivo. These cells have been called with various names, most commonly endothelial colony forming cells (ECFC) or blood outgrowth endothelial cells (BOEC). EPCs are very rare, probably accounting for only ~0.01% of circulating cells. Their origin remains uncertain; they are currently thought to derive from hematopoietic stem cells in the bone marrow as well as vascular stem cell niches in the vessel wall. Pre-clinical and clinical investigations evaluating the therapeutic potential of cells labelled as EPCs have produced variable results. This may be, at least in part, due to the use
of different populations, mainly including cells of the myeloid lineage. Circulating "EPC"s have also been proposed as useful cell biomarkers of disease. Dr. Medina recommended that the scientific community should define the nomenclature and characteristics of EPC, and standardize markers and methods across laboratories, to achieve a uniform terminology to identify cells and their various sub-types.

**Isolation of blood outgrowth endothelial cells. Anna Randi, National Heart and Lung Institute, Vascular Sciences, Imperial College London, UK**

Randi focused on the population of "true" endothelial progenitor cells, also called blood outgrowth endothelial cells (BOEC). These cells can be isolated from ~50 ml whole blood; they have been also isolated from cord blood and bone marrow. These cells have been used to investigate EC function in patients; to generate iPS cells; for gene & drug delivery; for tissue engineering. A number of protocols have been published which are similar in the basic steps and in the robustness of the procedure, which is successful in 70-75% of isolations. Randi listed key papers which report BOEC isolation methods:

- Blood 2004;104 :2752–2760;

She then listed key methodological points:

- Time from blood sample collection to processing;
- Time of colonies appearance;
- Number of colonies / ml;
- Colonies expansion.

After showing some examples of isolation and characterization, Prof. Randi pointed out some outstanding method development issues, namely the isolation from frozen samples, crucial to be able to expand patients’ studies, and optimization of cell culture conditions for cell therapy applications. Conclusions from her presentation were:

- To isolate BOEC for cell biology studies, current methods are adequate
- Experience in endothelial cell culture required
- Standardization for consistency and reproducibility
- Optimization is required for isolation from Frozen samples
- Modifications to the standard methods should be introduced for cell therapy applications

**Flow cytometry techniques to detect circulating endothelial progenitor cells. Florence Sabatier, Aix Marseille University, Marseille, France**

Sabatier showed that flow cytometry (FCM) is an alternative to colony forming assays that allows a direct counting of endothelial progenitor cells (EPC) suitable for clinical assessment of vascular repair capacity. However, FMC analysis of EPC remains a technical challenge. First, the very low level of EPC in blood requires the implementation of various recommendations for rare events detection. Main technical aspects are the need for a pre-
enrichment step, the strict exclusion of non-viable cells, debris and non-nucleated cells that may generate false positive events, and adapt strategies for defining positivity of labeling of rare events.

Second, due to the absence of specific marker, EPC phenotype is derived from the presence of a combination of antigens that attest for stemness or progenitor state and endothelial engagement, along with markers allowing identification of EPC subtypes. Indeed, the identity of the heterogeneous population of cells covered by the term "EPC" has been clarified recently based on a clear distinction between vasculogenic EPC of non haematopoietic origin (the true EPC subset) and haematopoietic cells with angiogenic activity. EPC have to be detected as viable CD34+/CD45- expressing endothelial antigens such as CD146 or CD31. While such phenotype is clearly identified in cord blood cells, it is near undetectable in peripheral blood from healthy subject. The clinical usefulness of this EPC subset remains to be demonstrated. By contrast, hematopoietic progenitor cells with angiogenic potential can be detected using recently optimized FCM protocols. These cells are viable, CD34+/CD45dim with forward scatter and side scatter characteristics similar to lymphocyte and expressing KDR. Based on this phenotype, the previous biomarker value assigned to CD34+/KDR+ cells is currently being confirmed in cardiovascular risk patients. Thus, the recent advances in the field of EPC biology now translate in a more consensual terminology and optimized FCM protocols. Detection of hematopoietic progenitors with angiogenic capacity seems a good compromise between sensitivity, specificity and applicability in clinical practice. Improving technical performance of FCM for rare cells analysis may provide better sensitivity for detection of non-hematopoietic endothelial progenitors. Altogether, these advances pave the way for improved standardization.

**Microparticles (Chair: Françoise Dignat-George, France)**

**Isolation and characterization of platelet-derived vesicles. Pia Siljander, Hensinki University, Helsinki, Finland**

The participation of platelets in physiological functions other than haemostasis and thrombosis is also reflected by the formation of platelet-derived extracellular vesicles (EVs) under various conditions and agonists. These EVs are considered to participate in the processes mediated by platelets and therefore these EV should be carefully characterized to better understand platelet function.

Siljanders research group has optimized a platelet-derived EV isolation protocol from leukocyte-free platelets, and characterized both thrombin and collagen co-induced EVs and LPS-induced EVs and compared these to Ca2+ ionophore -induced EVs and time-matched controls. In addition to the quantitative differences in EV numbers depending on the activating condition, also qualitative (proteomics) and the quantitative protein content of the EV subpopulations (microparticles and exosomes) were observed under the various conditions. Measured by nanoparticle tracking analysis and transmission electron microscopy, about 90% of the vesicles are < 500 nm and 65-80% were < 250 nm. In addition, she observed that the protocol of separating microparticles from exosomes by a centrifugation at 20,000 x g for 40 minutes does not yield homogenously sized EV (sub)populations, but rather overlapping vesicle pools, indicating that the platelet-derived EVs are extremely heterogeneous. Due to the activation-dependent differences in the protein-vesicle ratios of the various EVs, it was concluded that protein content of EVs should not be used as a means for sample standardization. Finally, the use of Ca2+ -ionophore as an EV-inducer is discouraged because the resulting EVs greatly differ
from those induced by physiologically relevant agonists. Taken together, the presented results promote further optimization of platelet-derived EV isolation and additional characterization and functional studies.

**Measurements, applications, and impact of the refractive index of extracellular vesicles.**

Edwin van der Pol, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Vesicles are often studied by methods that detect light scattering, such as flow cytometry and nanoparticle tracking analysis (NTA). The amount of light scattered by a vesicle depends on its size and refractive index (RI). Consequently, knowledge of the RI is required to determine the vesicle size and provide insight in the smallest detectable vesicles. We have measured the diameter and light scattering of vesicles and beads by tracking their Brownian motion with NTA (NS500, Nanosight Ltd). We analytically described the relation between the diameter, RI, and light scattering of beads using Mie theory to determine the RI of vesicles from cell-free human urine. The mean refractive index of urine vesicles was 1.37, which is in agreement with preliminary results from others. Edwin used the most recent knowledge on the RI of vesicles to discuss recent achievements and insights in the optical detection of vesicles, including Swarm detection, flow cytometry calibration, and the detection limit of NTA and Raman microspectroscopy. Furthermore, he hypothesized that the RI may provide a novel label-free parameter to distinguish vesicles from protein aggregates. This work was funded by the European Metrology Research Programme (EMRP) under the joint research project HLT02 (Metves). The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.

**Standardization (Chair: Rienk Nieuwland, the Netherlands)**

**Overview of Microparticle standardization.** Romaric Lacroix, Aix Marseille Université, Marseille, France

Lacroix summarized the key steps achieved by the Scientific Subcommittee on Vascular Biology towards standardizing microparticle (MP) analysis. About pre-analytics, a protocol has been proposed and validated in a multi-center study. Such consensual protocol significantly reduces the variability of platelet and erythrocytes-derived MP measurement. About analytics, a collaborative workshop has also been organized to define the inter-laboratory reproducibility of MP counts using flow cytometry (FCM). The bead-based calibration system proved to be useful to allow instrument qualification and monitoring. However, differential behavior among FCM sub-types still impedes standardization for MP enumeration. Consequently, a modified strategy has been proposed to overcome this issue and has been evaluated in a multi-center study including 61 flow cytometers of 14 different types. The new bead-based strategy is accessible on most commercially available instruments. As a result, no significant variability was observed between instrument types measuring PMPs with different optical systems. Finally, provided that instrument intrinsic behaviors for size-related measurements are taken into account, beads can be used as an efficient standardization tool for MPs. Lacroix also stressed on the need of educational efforts which have to be associated with the MP-related guidelines. These standardization achievements represent an important step towards the use of MPs as biomarker in clinical practice.
Standardization of vesicle detection by flow cytometry through vesicle size approximation.
Frank Coumans, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
Coulmans presented the basic theory behind the standardization effort based on a Mie model of the scatter to size relationship. Coumans and van der Pol developed a software application to determine the Mie relationship for most commercial flow cytometers based on a measurement of a vial of beads. The draft protocol is finished, and looks well throughout. Samples of selected (METVES; www.metves.eu) reference materials and biological samples have been shipped to the 32 participating sites worldwide (Europe, US, Australia, etc.). Coumans and van der Pol ran an internal test of their protocol in the AMC. For the 4 different flow cytometers, the tested Mie approach functioned better than a bead-based approach similar to the Megamix approach. The lively discussion after the presentation focused on the choice of refractive index, and on the absence of a pan-vesicle marker that would aid triggering on fluorescence.
von Willebrand Factor

Chairman: Jorge Di Paola (USA)

Co-Chairmen: Sandra Haberichter (USA), Daniel Hampshire (UK), Paula James (Canada), Koichi Kokame (Japan), Johanna Kremer Hovinga (Switzerland), Alberto Tosetto (Italy)

Session 1: Monday, 23 June (14:15-18:15)

Session 2: Tuesday, 24 June (14:00-18:00)

Day 1, June 23rd

Welcome and Introduction of best scoring abstract in VWF/ADAMTS13 category

Jorge Di Paola, MD, USA

Mathew Auton, PhD from Mayo Clinic was the winner of the abstract competition for the category VWF/VWD/ADAMTS13. He presented the missfolding of the A1 domain and its influence on the pathogenesis of VWD. Through conformational metrics he determines the missfolding and stability that these mutations confer to the A1 domain and their effect on platelet rolling velocity, also comparing to VWF:RCo/Ag ratio.

Update on ISTH/BAT Standing Committee (in collaboration with VWF SubCommittee)

Introduction

Dr. Rodeghiero, Italy, presented data from PubMed and Google scholar about the use of BAT in the literature. Specifically on how many papers have been published with this particular tool. He also presented the Rockefeller University website and how any investigator can access and input deidentified data.

Use of the ISTH-BAT to record bleeding symptoms of normal individuals

Barry Coller, USA. Self-administered Bleeding Assessment Tool” and ”Normal range of bleeding scores for the ISTH-BAT: adult and pediatric data from the Merging Project. Dr. Coller emphasized the administrative issues regarding the input of data. Then he went ahead and presented the use of the BAT on a total of 140 healthy individuals, with females tending to have higher BAT score. Age does not appear to have an impact on BAT scores. However there are some differences in bleeding scores among centers (Canada and USA) particularly for gum bleeding. It is not clear what causes the difference, but overall the BAT scores are highly reproducible.
Paula James, Canada. Data from The Merging Project determining the normal range for the adult and pediatric normal range of bleeding scores for the ISTH-BAT was presented. Additionally, preliminary data from two Self-BAT studies was shown. The Merging project has 1368 individuals represented. Normal range in males is 0-3, and in females is higher 0-5. No differences were seen in children. The Self-BAT is a questionnaire converted to lay language and there was a high correlation coefficient between this one and the ISTH-BAT. Screening tool in females appears to have higher sensitivity than in males.

Alberto Tosetto, Italy. Presented the concept of a study for patients with mild bleeding disorders The Rebel Study (Retrospective Evaluation of Bleeding Etiology). The study will be retrospective with a plan enrollment of 250 cases and 250 controls. They will save plasma and DNA samples and then search for genetic variants and biomarkers.

Augusto Federici, Italy. Presented the performance of ISTH-BAT in type 3 VWD patients within the 3Winters-IP Project. Interestingly there is significant discrepancy between BAT scores among patients with this disease, which traditionally has been associated with severe bleeding. The study is ongoing.

Paul Harrison, UK. Presented the concept of a retrospective multicenter study on adults with a platelet function defect diagnosed by a senior physician according to the SSC guideline. The study is designed for adults. Criteria for inclusion will be mucocutaneous bleeding with normal PT, aPTT, VWF and will compare platelet disorders with VWD Type 1 and healthy individuals. The study opening will be announced soon.

Session 1 VWF/ADAMTS 13 Assays

Chairs: Sandra Haberichter and Johanna Kremer Hovinga

Standardization of VWF Activity Assays

Imre Bodo, Hungary. Presented data on the standardization of the VWF:RCo assay. VWD type 1 and 2 samples with known levels and mutations were blinded, frozen and distributed among 7 different laboratories. In general, all assays were found to be reliable and comparable.

Specifically four commercially available assays were compared to the classic platelet-based, Ristocetin-triggered assay by eight laboratories using 95 well-characterized samples.

In summary, judging from the current incomplete analysis, the assays all performed similar, with few examples of differential assay behavior. The clinically relevant discrepancies include the misclassification of the tested (commercially not available) ELISA tests to detect the two included type 2B patients, and the expected false positive results by all but one assays using Ristocetin when analyzing the p.P1467S polymorphism (unexpectedly, the AccuStar assay did not give the expected false positive result). Also, the antibody assay gave higher results for p.V1665E, but this difference did not lead to misclassification.
The SSC subcommittee approved the following nomenclature for the VWF activity assays assessing the ability of VWF to bind GPIb on platelets (while the name of collagen binding - VWF:CB remains unchanged): Platelet-based assay:

VWF:RCo

Assays using recombinant GPIb, where the reaction is triggered by Ristocetin:

VWF:GPIbR

Assays using spontaneous binding to recombinant GPIb with a gain-of-function mutation:

VWF:GPIbM

Assays using binding to a monoclonal antibody directed to the A1 domain:

VWF:Ab

**WHO 1st International Standard ADAMTS13, Plasma**

*Anthony R. Hubbard, UK.* Presented a collaborative study involving 32 laboratories which main goal was to assign values for ADAMTS13 function and antigen to the proposed WHO 1st IS ADAMTS13 plasma (coded 12/252). Value assignment was based on assays relative to local pooled normal plasma preparations arbitrarily assigned 1.0 unit per ml. Most laboratories used either a FRET assay (n=18) or an activity ELISA (n=9) to measure ADAMTS13 function and all laboratories used ELISA for antigen measurement. Combination of all results gave overall means of 0.91 units/ml and 0.92 units/ml for function and antigen respectively. Inter-laboratory variability was low considering that each laboratory used a different local plasma pool with GCVs of 12% and 16% for function and antigen respectively. All of the participants have agreed with the proposal to assign these consensus mean values to the proposed WHO 1st IS. The proposed WHO 1st IS will be submitted for formal establishment by WHO in October this year. An accelerated degradation study has indicated that the proposed WHO 1st IS is extremely stable with predicted loss of function and antigen below 0.1 % per year for ampoules stored at -20°C.

Two samples (C & D) from a patient with acquired ADAMTS13 deficiency due to an inhibitory autoantibody were also included in the study. The level of ADAMTS13 in Sample C was below the limit of detection for assays of function in many cases (21/32) and calculated estimates were only possible in 11 laboratories. However, 31/32 results were consistent with a severe deficiency below 0.1 units/ml. Patient Sample D contained a higher level of ADAMTS13 than Sample C and only 8/32 data sets from the function assays were not amenable to quantification. The overall mean estimate for function in Sample D was 0.15 units/ml with 23/24 laboratories agreeing levels below 0.3 units/ml. Ratios of function to antigen for Samples C and D were greatly reduced at 0.11 and 0.24 respectively compared to normal plasma (0.99). This finding together with the large inter-laboratory variability of estimates for the patient samples is most probably related to the presence of circulating ADAMTS13-antibody complexes. The availability of a common reference material (proposed WHO IS) could help to identify the
methodological issues responsible for this variability. Assays of recombinant ADAMTS13 (Sample E) indicated valid comparison of dose-response relationships with normal plasma (proposed WHO IS) but large inter-laboratory of estimates for both function and antigen. This could indicate that the proposed WHO 1st IS is unsuitable for the measurement of recombinant ADAMTS13.

**New ADAMTS 13 activity assay**

*Joshua Muia, USA.* Presented the results of his research (working with Dr. Evan Sadler), highlighting opportunities to improve ADAMTS13 assays, and then shared recent data comparing FRETS-rVWF71 with two commercially available ADAMTS13 assay kits (highly comparable). He finished the talk by discussing briefly the development of ADAMTS13/VWF Fluorolisa, a method that will permit simultaneous assay of ADAMTS13 antigen and activity using a single instrument.

**Collagen Assays**

*Veronica Flood, USA.* VWF collagen interactions occur with a number of vascular collagens. Using plasma samples from the Zimmerman Program, she compared binding of VWF to collagens 3, 4, and 6 as well as ristocetin cofactor activity. There was no difference between collagen 4 and 6 binding; both demonstrated reduction in type 1 and 2M VWD subjects with A1 domain sequence variations. Collagen 3 binding defects occurred in types 2A and 2B VWD and corresponded to loss of high molecular weight multimers. Although collagen 4 and 6 were highly correlated, neither correlated with collagen 3 and none of the collagens correlated with ristocetin cofactor activity.

**Multimer Assays**

*Sandra Haberichter, USA.* Presented data on the quantification of multimer analysis. VWF multimer analysis is qualitative, and therefore a subjective assessment open to interpretation. To address this shortcoming a quantitative method for multimer analysis was developed that incorporates performing densitometry, determining area-under-the-curve, and calculating the percentage of low-molecular weight (LMW), intermediate-molecular weight (IMW) and high molecular weight (HMW) multimers. Quantitative analysis revealed subtle loss of HMW multimers in a subset of type 1 patients. Type 2B patients had a loss of HMW and IMW multimers. Type 2A subjects were divided into those with loss of HMW and decreased IMW (similar to type 2B subjects) and those with a virtually complete loss of HMW and IMW. Quantitative analysis of VWF multimer patterns more clearly distinguishes patients with various subtypes of VWD than subjective analysis and provides an objective measure of VWF structure.

**Session 2**

**The Life Cycle of Von Willebrand Factor**

**Chairs:** *Paula James and Alberto Tosetto*
Blood Outgrowth Endothelial Cells

Paula James, Canada. BOEC are an accessible source of vascular endothelium and an emerging cellular model for studying VWD. Data from normal individuals and Type 3 VWD patients were presented. Several BOEC were analyzed from different VWD types and patients with chronic kidney disease. Several immunofluorescence figures were shown from VWD type 3 patients that demonstrated a diffuse pattern as previously seen in cell culture models.

VWF Strings

Jose Lopez, USA. Dr. Lopez presented new technology for the fabrication of microvessels. He showed how endothelial cells will become confluent and will adopt the shape of the vessel and secrete long (even cm) VWF strings. Discussion ensued regarding the potential for this model to study not only VWF production and behavior but also angiogenesis. Data on ApoA1 (a component of HDL) as a stabilizer of VWF was also presented.

Platelet VWF

Walter Kahr, Canada. Dr. Kahr showed his studies on platelet VWF. Gray Platelet syndrome mice (NBEAL2 -/-) have normal VWF but no alpha granules. The VWF in megakaryocytes of NBEAL2 -/- is distributed on the outside of the cell in what it appears to be adherent to the cell membrane. This could be explained by VWF not being packed in alpha granules and therefore "leaking" out of the cell.

Clearance of VWF

David Lillicrap, Canada. Dr. Lillicrap presented data on the role of CLEC4M and Stabilin2 on the variability of VWF levels. These genetic variants had been discovered through the CHARGE study and this is one of the first biology experiments that confirm a role on the VWF secretion and clearance process.

Peter Lenting, France. LRP1 has recently been identified as a shear stress-dependent clearance receptor for VWF. It appears that various regions in the VWF protein (D'D3, A1 and D4 domains) contain an interactive site for LRP1. In addition, several mutations (VWD-type 1 R1205H and type 2B V1316 mutation) in these regions relief the shear stress-dependency of the LRP1 interaction, providing a rationale for the increased clearance of these mutants. Thus, LRP1 contributes not only to the basal clearance of VWF, but also seems involved in the enhanced clearance of various VWF mutants that are characterized by a reduced circulatory half-life.

Day 2, June 24th

Session 3

Genetics, Genomics and Population Studies

Chairs: Daniel Hampshire and Koichi Kokame
Effect of age on VWF levels

*Frank Leebeek, The Netherlands.* In the general population VWF levels are highly variable. Age seems to be one of the most important factors determining VWF levels considering recent data from large population-based studies in elderly individuals. During the presentation Dr. Leebeek focused on the effect of age on VWF levels in patients diagnosed with VWD. It is expected that with increasing age VWF levels will also increase in patients with VWD, especially in type 1 patients, which may lead to a change in bleeding pattern. This may also have implications for treatment of these patients. The results of our study in elderly individuals included in the Willebrand in the Netherlands (WiN) study, a large cohort study in over 800 patients with VWD, were presented and VWF levels do change in VWD type 1 but not in VWD type 2.

Genetic variants of ADAMTS13 in aHUS

*Vahid Afshar-Kharghan, USA.* Atypical hemolytic uremic syndrome (aHUS) is associated with complement dysregulation. They have studied 30 patients with aHUS and found that the partial deficiency of ADAMTS13 (<60% activity) is a relatively common finding among these patients. More recently this group discovered that VWF acts as a cofactor for Factor I-mediated complement inhibition. The cofactor activity of VWF depends on its multimeric size, with shorter multimers having the maximum cofactor activity and ultra large (UL)VWF multimers having none. As a result, coexisting ADAMTS13 deficiency might reduce the threshold for thrombotic microangiopathy in patient with complement dysregulation, but also can enhance complement activity by generating larger size VWF multimers. Their results are also consistent with recent studies reporting complement activation in thrombotic thrombocytopenia purpura.

Genetic variants in VWF that influence the diagnosis of VWD type 1

*Jorge Di Paola, USA.* Von Willebrand disease (VWD) type 1 is characterized by incomplete penetrance and variable expressivity. Mild bleeding is common in the general population and many patients are diagnosed with VWD type 1 despite having normal or near normal VWF levels. This group hypothesized that common variants in VWF may influence VWF levels and impact the diagnosis of VWD type 1. Tagging SNPs throughout VWF (n=94) were typed in a multigenerational Amish pedigree (n=445). Significant associations between VWF:RCo and twelve of the SNPs were identified. Association analysis in Caucasian controls from the ZPMCB-VWD cohort (n=150) replicated associations for nine of the eleven SNPs selected for follow-up. Further replication analysis verified that two SNPs (rs11064003 and rs2239162) are associated with VWF:Ag in the GABC cohort (n=934) and six SNPs (rs11612370, rs11612384, rs216330, rs11064003, rs2231962, and rs216293) are associated with VWF:Ag in the TSS cohort (n=2,232). Comparison of the minor allele frequencies of all eleven VWF tagging SNPs between the ZPMCB-VWD Control and ZPMCB-VWD Type 1 (n=310) cohorts showed that the minor allele of rs7964554 (lower VWF:Ag) was more common, whereas the minor alleles of rs11612370, rs11612384, rs11064003, and rs2239162 (all higher VWF:Ag) were less common in VWD type 1 cases indicating that these variants may act as modifiers of diseases.

Association and Linkage for VWF and VWFpp levels
Karl Desch, USA. Dr. Desch focused his presentation on data recently published in PNAS describing modifying loci for VWF levels. He then went ahead and presented new data on linkage in his 2 large cohorts for VWF propeptide and for VWFpp/VWF:Ag ratio.

Report on VWD projects

Report of VWF Database

Dan Hampshire, UK. Databases of sequence variation in coagulation factor genes are an important source of information for clinicians and researchers working in the field of haemostasis, e.g. the VWF database (VWFdb). A strength of several of these databases, including VWFdb, is that they report all known sequence variation and include phenotypic information in relation to reported variants, e.g. coagulation factors levels, history of inhibitors and bleeding severity. VWFdb is now part of a European Association for Haemophilia and Allied Disorders (EAHAD) initiative aimed at bringing together coagulation factor databases under a single front-end portal (www.eahad-db.org) and updating these databases with additional protein structural information and inter-species sequence conservation data.

Report of the EU Project

Alberto Tosetto, Italy. The EU group is still active but has had problems with grant funding for their projects. They have found that genetic hybridization techniques have been useful to detect some deletions not detected by Sanger sequencing.

Report of the Canadian Project

Paula James, Canada. Proposal of a study of angiodysplasia in VWD. No cases of AD reported in VWD type 2N. New study in Canada will recruit patients with AD in Canada, to understand the natural course of this disease.

Report of the Belgian-Czech Project

Alain Gadisseur, Belgium.

Belgian-Czech cooperation in the BRNO-VWD Study: Update

The BRNO-VWD Study is a family-based analysis of VWD in the Moravia area of the Czech Republic performed by the University Hospital Brno (Czech Republic) and the Antwerp University Hospital (Belgium). Blood samples were collected from VWD patients (proband) together with at least one affected sibling or parent. Blood was collected from 205 patients from 95 families with suspected VWD. Updated results of the laboratory work were given. The distribution of different subtypes of VWD was as follows: VWD type 1 in 61% of the families and type 2 in 35% (type 2A 23%, type 2B 5%, and type 2M 7%). Molecular analysis is still ongoing with currently 63/95 families fully analysed and the remainder partially with mostly exons 14, 51, 15 and 51 to do. So far, 39 mutations in the VWF gene have been found, of which 12 are new to the ISTH VWD database and awaiting gene expression studies. MLPA analysis is
currently running. Clinical and laboratory data will be merged when VWF gene sequencing has been completed in all patients.

Belgian multicenter study into von Willebrand Disease (B-Will)

The first results were presented of the Belgian multicenter study into von Willebrand Disease (B-Will), which is based on the Brno-VWD study in design. Currently, 86 patients representing 68 families have been included, with a type distribution similar to the Brno-VWD study. So far, 17 mutations have been found, of which 5 are new to the ISTH VWD database and awaiting gene expression studies. The mutations found so far show little similarity to the mutations found in the Brno-VWD study.

Glycosylation of VWF: functional implications

Chairs: Imre Bodo and Jill Johnsen

ABO is a complex modifier of VWF

Jill Johnsen, USA. ABO blood group is a strong inherited modifier of VWF level, with blood group O individuals generally exhibiting lower VWF levels than non-O individuals. However, ABO is a genetically and biochemically complex carbohydrate blood group system, and there is evidence for distinct effects on VWF between ABO blood types. Thus, a higher resolution ABO system approach than "O" vs. "non-O" is warranted when considering ABO as a modifier of VWF.

Glycosylation of platelet VWF and resistance to ADAMTS13 proteolysis

James O’Donnell, Ireland. Dr. O’Donnell presented data on platelet VWF and post translational modifications. Platelet VWF has ultralarge VWF multimers. Glycosylation is different between plasma VWF and platelet VWF. AB not expressed in platelets. H antigen is present in platelets. Sialylation is also reduced in platelets VWF. Platelet VWF is resistant to ADAMTS 13 and that appears to be dependent on N glycan distribution. This perhaps is physiologically relevant so you have "local" hemostasis when platelets get activated.

O-linked glycans in VWF and interactions with ADAMTS13 and GPIba

Agata Nowak, UK and Tom McKinnon, UK VWF contains 10 O-linked glycosylation sites. 8 of these flanking the A1 domain, with four O-linked glycans present in each of the proline rich linker regions between the D3 & A1 domains and A1 & A2 domains. In the present study we demonstrate that the O-linked glycans N-terminal to the A1 domain regulate binding to platelets by controlling access to the GPIb binding site.
Welcome and introduction of co-chairmen

Rezan A Kadir (UK) welcomed all participants on behalf of the co-chairmen. She provided an overview of current SSC collaborative work and encouraged attendees and SSC members to become involved with future projects.

Marc Blondon (US) presented the first Top Rated Abstract that was a population-based case-control study to assess the association between newborn birth weight and maternal venous thromboembolism in the postpartum period. This study confirmed a substantially increased risk (RR 3.0) of venous thromboembolism (VTE) in mothers with low birth weight newborns even when adjusting for other cofounders. This important finding could be incorporated into VTE assessment in the postnatal period.

Educational activities

Session Chairmen: Rezan A Kadir, Sabine Eichinger

Rezan A Kadir (UK) presented an overview and future perspectives of the educational activities of the SSC. She described the historical evolution of Women’s Health Issues in Thrombosis and Haemostasis (WHITH) meetings and provided details of the upcoming meeting in Berlin in February 2015.

Update on International Registries and SSC Initiated Projects

Saskia Middeldorp (the Netherlands) (on behalf of Menno Huisman who was unable to attend the meeting) presented the progress of diagnostic studies of suspected DVT and PE during pregnancy. She described the outcome of studies (EDVIGE and PEP) that assessed the safety of compression ultrasonography versus CTPA in pregnant women. She also described the goal and progress of two multicentre outcomes studies ‘CLOT-3’ and ‘LEFT rule’ that assess diagnostic strategies to rule out VTE events in pregnant patients. She invited attendees to become recruiting centres for these studies.

Elvira Grandone (Italy) presented an update the OTTILIA register, an observational study on antithrombotic prevention in thrombophilia and pregnancy loss. In addition she presented a prospective observational study that assesses thrombophilia in patients with recurrent failures in assisted reproductive techniques. She invited attendees to participate in this registry.
*Claudia Chi (Singapore)* presented an update on Mirena intra-uterine system (IUS) and HMB in women with inherited bleeding disorders. She outlined the rationale for collecting ongoing data on Mirena use (i.e. treatment efficacy, expulsion rates, use of adjuvant tranexamic acid) in women with inherited bleeding disorders (IBD). She invited attendees to participate with data collection through the ISTH SSC webpage.

*Claire Phillip (USA)* presented an update on the Menorrhagia Working Group of the SSC. She described the new ISTH SSC bleeding assessment tool and how the sections on menorrhagia and PPH have evolved. She explained how the ISTH SSC BAT requires assessment to demonstrate its validity and usefulness for screening in women presenting with heavy menstrual bleeding (HMB) and PPH.

**Thrombosis**

*Session chairmen: Andra James (USA), Claudia Chi (Singapore)*

*Guillarmina Giradi (UK)* presented her work on antiphospholipid syndrome (APLS) in pregnancy. She outlined the contribution of experimental models and how they enhance our understanding of APLS and how pathological mechanisms lead to adverse pregnancy outcomes. She then described the translational research work: a case of a woman with previous severe pre-eclampsia treated with pravastatin during pregnancy.

*Saskia Middeldorp (the Netherlands)* presented recommendations for thromboprophylaxis in pregnancy with emphasis on the postpartum period. She presented data on the absolute risk of VTE in women with or without personal or family history of VTE/thrombophilia and the risk reduction if given thromboprophylaxis. She presented the unresolved issues of VTE prophylaxis in pregnancy and emphasised how current practice is based on weak (grade 2c) evidence. She then invited attendees to participate in the ‘High-Low study’, a prospective randomised trial of intermediate and low dose low molecular weight heparin use in pregnancy.

*Hannah Cohen (UK)* presented ten cases of uterine vein thrombosis diagnosed with transvaginal ultrasound. She described the risk factors (including thrombophilia) and the time for resolution in each case. She questioned the clinical significance of these cases and whether there was an associated increased risk of proximal extension, pulmonary embolism or pelvic vein insufficiency, although emphasised that this was currently unknown.

**Haemostasis**

*Session chairmen: Rochelle Winikoff (Canada), Ida Martinelli (Italy)*

*Peter Kouides (USA)* presented an overview of von Willebrand Disease (VWD) in women with a focus on diagnostic challenges (women being under and over-diagnosed) and unresolved treatment issues (TA versus DDAVP, and underuse of Mirena IUS). He promoted the need for studies that investigated women for a haemostatic abnormality (VWD or platelet function disorder) in women with uterine abnormalities or anovulatory bleeding. He proposed a step-wise
approach for lab testing, incorporating the bleeding score, in women with heavy menstrual bleeding (HMB).

_Meera Chiltur (USA)_ presented an overview of haemostasis in the newborn and the difference aetiologies of neonatal intracranial haemorrhage (NIH). She outlined coagulation disorders that were implicated in NIH including haemophilia, neonatal alloimmune thrombocytopenia, vitamin K deficiency and rare bleeding disorders.

_Rezan A Kadir (UK)_ presented data from a systematic review on cranial bleeding in newborns with haemophilia. Symptomatic intracranial haemorrhage within haemophilia neonates was 11 and 4 times higher with assisted vaginal delivery and spontaneous vaginal delivery, respectively compared to caesarean delivery. She invited attendees to collaborate with a prospective MRI screening study.

**PPH – Evaluating the Evidence**

_Session chairmen: Claire McLintock (New Zealand), Rezan A Kadir (UK)**

_Sophie Susen (France)_ presented the evidence for tranexamic acid use in postpartum haemorrhage with results from two randomised trials and a meta-analysis. She highlighted the main findings from a French study that used high dose tranexamic acid which included i) a reduction in total blood loss, ii) a decreased proportion of severe PPH, iii) a reduced bleeding time in the tranexamic acid group. She then described the larger trials of tranexamic use in non-obstetric bleeding patients and provided details of the WOMAN (WOrld Maternal Antifibrinolytic Trial) trial that is ongoing.

_Peter Collins (UK)_ presented the evidence that fibrinogen is a useful biomarker for prediction of severe PPH. He explained how a Fibtem (taken with rotational thromboelastometry) at 5 minutes shows good correlation with clauss fibrinogen level and is more rapidly accessible. He then provided an overview of the fibrinogen trials in obstetric haemorrhage to date, of which the results are awaited.

_Takao Kobayashi (Japan)_ described the main causes of severe obstetric haemorrhage. He then presented the results of a systematic review that included 64 publications on the use of rFVIIa use in PPH. Most publications reported a good response following rFVIIa use in PPH. He then described an open label RCT of rFVIIa use in PPH which showed a reduction in the need for interventional procedures. Caution is required with its use due to the potential increased risk in VTE found in the treatment group. Future prospective studies are required to evaluate the safety of rVIIa use in PPH.

_Susan Halimeh (Germany)_ presented the use of rotational thromboelastometry to guide transfusion therapy in PPH.
Working Group on Genomics in Hemostasis

Chairmen: Anne Goodeve (UK, excused), Willem H Ouwehand (UK), Pieter Reitsma (the Netherlands)

Monday, 23 June (14:15-18:15)

**Session 1 – Chair – Willem H Ouwehand, Cambridge, UK**
Professor Willem H Ouwehand opened the session thanking all the people attending the ThromboGenomics Working Group on Genomics.

**Speaker: Professor Kathleen Freson – Belgium**
**Title: Human Phenotype Ontology Terms: Does it works?**
The underlying principles and the use of Human Phenotype Ontology (HPO) terms for phenotype clustering in patients with bleeding and platelet disorders was outlined. A key difference between HPO and other bleeding phenotype ontologies is the ability to also code pathobiologies outside the narrow remit of bleeding and platelet disorders. It is hoped that this will provide additional power in gene discovery programmes.

**Speaker: Dr Anne Kelly – UK**
**Title: Application of HPO to bleeding and platelet disorders**
The HPO coding allows standardised recording of detailed clinical phenotype. A logic clustering code was developed and applied to 716 HPO-coded cases with bleeding and platelet disorders. Some preliminary evidence was presented that the automated clustering of cases may facilitate gene discovery

**Speaker: Dr Daniel Hampshire – UK**
**Title: The coagulation factor variant database**
An update on the combined European CoagDB portal to provide access to variant databases for all coagulation factors. There has been excellent progress with the F8 database and it is hoped that efforts on F7 and F11 will be completed by Dec 2014

**Speaker: Dr Daniel Bellissimo – USA**
**Title: The role of the curator for the ThromboGenomics database**
One of the aims of the ThromboGenomics project is to develop a sustainable and high quality curated database of gene annotations including clinically relevant DNA sequence variants for rare inherited bleeding and platelet disorders (BPD). The importance of a stable reference sequence, of gene annotation at Locus Reference Genomic (LRG), and the curator’s responsibilities of the interpretation of sequence variants have been discussed. Integration of BPD gene sequence variants with these of other control databases (1000 Genomes, NHLBI-ESP, UK10K, GEL100K, etc.) is seen as a critical development in cleaning up historical erroneous data entries.

**Session 2 - Chair: Pieter Reitsma, Leiden, the Netherlands**

**Speaker: Dr Ji Wu – USA**
**Title: Discover with Confidence: NimbleGen Target Enrichment Technology for next generation sequencing**
The NimbleGen target enrichment protocol and design, in use for the ThromboGenomics samples, have been presented.

**Speaker: Dr Ilenia Simeoni – UK**

**Title: A NGS Diagnostic Platform for inherited bleeding and platelet disorders**
An update on the three main activities of the ThromboGenomics project: 1) Development of a next generation sequencing (NGS) platform 2) gene curation and 3) database development for stable references of sequence variations were presented. Good progress has been made on all three activities and it is expected that the validation of the NGS platform will have been completed by June 2015.

**Speaker: Professor Willem H Ouwehand in substitution of Dr Augusto Rendon – UK**

**Title: Genomic medicine in the care path of patients with bleeding and platelet disorders**
Discussed the advances of genomic medicine to discover new genes and new variants to elucidate the mechanism of unresolved bleeding and platelet disorders. Whole genome sequencing is now available at low cost and provides better coverage than whole exome sequencing in the coding ("exome") space. The importance of data sharing has also been highlighted

**Speaker: Dr Jill Johnsen – USA**

**Title: NGS approaches in familial ITP**
Results of a whole exome sequencing study on two pedigrees with familial ITP were presented. No conclusive results could be drawn and further cases will require analysis to identify the genetic architecture of this rare disorder.

**Speaker: Dr Pieter Reitsma – The Netherlands**

**Title: Resequencing of 100 cases with Familial Thrombophilia**
Results of the targeted resequencing of 100 cases with familial thrombophilia were presented.

**Final Remarks**

**Progress**
The ThromboGenomics (TG) project is now in the final stage of the validation of the next generation sequencing platform. Significant improvements have been achieved for the establishment of a stable database where sequence variations can be stored. The global TG network continues to expand including new collaborators, curators, clinicians and researchers from all around the world. Similarly exciting advances have been made with the European CoagDB portal and as both databases are "open access" there seems to be a great opportunity to fuse the knowledge of these two databases. It was generally accepted that the largest challenge was the accurate curation of variant databases.

**New chairman**
Willem H Ouwehand mentioned at the opening and closing of the meeting that the Genomics Working Group requires at least one new Chairman. One of the two founder Chairman of
ThromboGenomics Dr Thomas Kunicki (USA) has stood down from his post because of retirement. It was emphasised that since the other three Chairs are from Europe that it was essential to have representation from other continents. Members and attendees of ISTH with an interest to be appointed to one of the two vacant posts were invited to submit their CV before Friday 11th July by email to Dr Ilenia Simeoni at is250@cam.ac.uk or Prof Willem H Ouwehand at who1000@cam.ac.uk.

**Transition to SSC Subcommittee**
Willem H Ouwehand attended the ISTH SSC meeting and was informed that ThromboGenomics could apply for SSC subcommittee status. A relatively "light” application process is to be followed to achieve this possible transition.

Dr Ilenia Simeoni PhD  
Scientific Coordinator ThromboGenomics  
Cambridge Translational Genomics Laboratory  
Cambridge, UK  
July 2014.  
Website: [www.thrombogenomics.org.uk](http://www.thrombogenomics.org.uk)