56th Annual SSC Meeting of the International Society on Thrombosis and Haemostasis

May 22 – 25, 2010 | Cairo, Egypt

56th Annual Scientific and Standardization Committee Meeting

Cairo, Egypt
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Animal Models

24 May 2010
Cairo, Egypt

Chairman: Timothy Nichols (US)
Co-chairmen: Edward Conway (CA), Shaun Coughlin (US), Jay Degen (US), Nigel Mackman (US), Eva-Maria Muchitsch (AT), Susan Smyth (US), Hugo Ten Cate (NL), Hartmut Weiler (US)

1. Standardization of the Murine Tail Bleeding Model – Eva-Maria Muchitsch. A short manuscript has been approved for submission. Dr. Muchitsch reviewed the status of the project and proposed a standardized study between labs. The possibility of partial funding was discussed.

2. Models of clotting in Murine Atherosclerosis – Dr. Hugo ten Cate reviewed the strengths and weaknesses of murine models of atherosclerosis with emphasis on thrombosis complicating atherosclerosis.

3. Mouse Models of von Willebrand Disease – Dr. David Lillicrap reviewed the status of creating mouse models of human VWD using transgene expression. Drs. Lillicrap, Cecille Denis, and Peter Lenting are working on this in their respective laboratories. A short paper was suggested to identify the key issues in creating murine models of human VWD with this approach.

4. Sensitivity of the Whole Blood Clotting Time to F.IX in Canine hemophilia B– Dr. Tim Nichols presented this project as a candidate for the short manuscript.

5. Ferric chloride arterial thrombosis standardization – Dr. Nigel Mackman was not able to attend but anticipates submitting a short manuscript. Dr. Tim Nichols gave a brief update on the status of this project.

6. Plans for Animal Models Subcommittee Meeting at XXIII ISTH 2011 Kyoto Japan
   a. New member – Dr. Yasuo Ikeda, Chairman of the Council, The Japanese Society on Thrombosis and Hemostasis, and President of XXIII Congress of ISTH Kyoto, recommended Dr. Toshiyuki Miyata for membership on the Animal Models Subcommittee.
   b. An educational and working reports meeting will be organized during the coming months.
Chairman: Johan Heemskerk (NL)
Co-chairmen: Lawrence Brass (US), Thomas Diacovo (US), Shaun Jackson (AU), Mike King (US), Armin Reininger (DE), Jaap-Jan Zwaginga (NL)

The subcommittee session in Cairo (2010) was less well attended than in the previous years, with 60 (before break) to 40 (after break) attendants. Since the meeting in Boston (2009) major movements forward have been made to overview and standardize key issues regarding blood rheological processes of potential clinical relevance.

**Session 1. Thrombus formation: novel methods and standardization**
(session chair: A. Reininger, T. Diacovo)

Chairman J. Heemskerk (Maastricht, the Netherlands) opened the meeting with a short overview of the subcommittee activities regarding standardization of biorheological assays in relation to today's presentations. The first session mostly concerned issues studied in the special project *Measurement of thrombus formation under flow ex vivo and in vivo*. A series of three presentations was given on innovative flow devices. First, C. Jones (Reading, United Kingdom) presented a novel way of time-resolved measurement of thrombus formation on a microfluid biochip. By extensive analysis of multiple parameters of three-dimensional fluorescence images of stacks of fluorescent-labelled platelets (using Slidebook or ImageJ software), array type of information was obtained on the activation state of platelets in a thrombus. M. Roest (Utrecht, the Netherlands) explained the status of the recent worldwide inventory to the use of flow devices for measurement of thrombus formation. Further, he demonstrated the clinical potential for flow devices in measurement of thrombus formation. This group used a perfusion assay in the absence of fluorescence for the measurement of platelet deposition on a collagen surface that was sufficiently reproducible, if temperature, anticoagulation, the storage of blood and the collagen surface were well controlled. The flow assay appeared to be sensitive to deficiencies of GPIIb/IIIa, GPIb, P2Y12 blockage and ADAMTS13. It was remarked that such flow assays also rely on other factors, such as platelet and red cell count and construction/size of the flow device. Subsequently, K. Neeves (Golden, CO, USA) showed a novel high throughput assay of platelet adhesion using newly constructed microfluidic devices. In the assay platelets (DIOC6 label) and fibrinogen (labelled fibrinogen) were stained, and thrombus formation from whole blood was measured at high-shear flow rate using a multichannel flow chamber. A comparison was made between collagen surfaces consisting of (difficult to control numbers of) fibres and surface-polymerized microfibrils. This microfluidic device is used for measurements of the clinical phenotypes of VWD. In a modified version, the device is combined with micropatterns of thrombogenic surface to obtain a high throughput flow chamber.
Comments were made on the reproducibility of these experiments upon multiple blood drawings from a single donor.

To discuss the importance of coagulation, A. Reininger (Munich, Germany) showed recent results designed to measure fibrin formation and thrombus stability under flow. It was demonstrated that spread platelets are the firm base of a solid thrombus, while thrombin/fibrin is needed for thrombus stabilization. In real-time imaging studies to the build up of thrombi, fibrin fibres started to be formed in the vicinity of platelet after about 5 min. There was radial outgrowth of fibrin from platelet aggregate. This was also observed in stagnation-point flow devices. The conclusion was that a surface of activated platelets foster the coagulation process, generating fibrin fibres making a thrombus resistant to embolization. On behalf of C. Denis (Paris, France), P. Lentink discussed the possibilities and limitations in the standardization of in vivo thrombosis studies in mouse. The results were presented of a worldwide survew to methods and procedures used in different laboratories to measure thrombus formation in in vivo mouse models. Mostly used are the FeCl₃ injury models, while the photochemical and laser injury models are somewhat less popular. In general, most labs are satisfied with their injury models and find these sufficiently reproducible. Only the laser models are not easy to use. Skills of operator appear to be important for the use of all injury models. In most laboratories, the type of vessel used for injury depends on the research question. Main parameters to consider in reproducibility for the various models. The results of the questionnaire allow to make a list of parameters that can be standardized, and a list of parameters that are difficult to standardize. A report on the outcome of this questionnaire will be written.

This session was followed by a short break.

Session 2. Blood flow-dependent processes in vitro and in vivo: novel concepts
(session chair: M. King, J. Heemskerk)

The second session started with two presentations on behalf of the working party Practical biophysics of cellular bond characteristics mediated by flow. An overview paper of the results of this working party is in preparation as a subcommittee report. M. King (Rochester, MN, USA) gave a presentation entitled, Lessons learned from the measurement of leukocyte adhesion receptor kinetics. Tether formation appears to be a crucial step in the selectin-mediated slow rolling of leukocytes over endothelial cells. Cell displacement can be described as a function of the transient tether and microvillous tether formation at various applied shear stresses. Estimation of the lever arm mediated by the tether is important in the quantification of the bond formation and the catch-bond kinetics. In general, the Bell model of firm/transient adhesion and no adhesion can be used to determine flow-dependent molecular interactions. Evidence was provided that tethering via CXCR1 interactions to immobilized IL8 is another way of flow dependent bond formation. T. Diacovo (New York, NY, USA) continued the presentation by discussing the platelet adhesion kinetics: from mathematical modelling to in vivo relevance. The speaker presented data showing why adhesive receptors from different glycoprotein families can have a similar function
in flow-dependent adhesion. In particular, the kinetics of flow-dependent interaction of PSGL1 with P-selectin and those of GPIb complex with VWF were discussed. High temporal resolution microscopy in combination with a flow chamber was used to determine the kinetic properties of receptor-ligand bonds use of to precisely measure flow dependent tether bond (smallest unit of adhesion observable) interactions. As will be discussed in the paper, bond kinetics can then be measured by application of the Bell model. The importance of the developed kinetic mathematic models for the in vivo situation was tested by high temporal measurements using a laser-induced mouse vascular injury model, comparing the adhesion of injected mouse and human platelets.

W. Nesbitt (Melbourne, Australia) gave an overview of the laboratory work on microfluidic assays to study local disturbances in blood rheology on platelet adhesion and aggregation. The effects of the presence of platelet aggregates on laminar flow and hemodynamics in vivo and in vitro were discussed, under precisely defined stenotic shear conditions. Microfluidic chips were used comprising of multiple microchannels coated with different adhesive proteins. In stenotic channels, the determining factors (including microgradients) that affect the size and build up of a thrombus were determined. In the last part of the presentation, mechanisms where shown how the hemodynamics can effect platelet aggregation.

Reports were given originating from the working party Standardization of thrombogenic surfaces in flow. M. Kuijpers (Maastricht, the Netherlands) showed data demonstrating the reproducible measurement of flow-dependent thrombus formation on an atherosclerotic plaque surface both in vivo and in vitro. In both types of assays, consistent effects were obtained of reversible inhibition of platelet P2Y12 receptors. The consequence was markedly unstable thrombus formation and increased embolus shedding, also under conditions of controlled coagulation. On plaque material, the interaction of platelet activation with coagulation was apparent from the (ADP/P2Y12-dependent) formation of fibrin fibres, originating from platelets. The presented methods provide good possibilities for standardized flow-dependent methods in the presence of coagulation. The last presentation was given by M. Sugimoto (Nara, Japan) on the role of ADAMTS13 in flow-dependent thrombus formation. It was shown that in both human and mouse blood blocking or absence of ADAMTS13 provoked a strongly increased thrombus formation at high shear rate. Parallel-plate flow chamber assays appeared to be well suitable to detect the effect of acute VWF multimer cleavage by ADAMTS13. By fluorescence imaging, the distribution of VWF and ADAMTS13 activity in a thrombus could be visualized. The importance of ADAMTS13 for clinically relevant in vivo thrombus formation was deduced from studies using an in vivo model of middle cerebral artery (MCA) occlusion and laser Doppler flow measurement of thrombus formation. Furthermore, injection of recombinant human ADAMTS13 reduced the increased brain infarction observed in ADAMTS13 deficient mice with this MCO model.

The meeting was closed by thanking all speakers and participants.
Note. A presentation on future plans of the working party *Survey of flow-determined modulation of molecular processes in thrombosis and haemostasis with emphasis on VWD* was given in a session of the VWF Subcommittee.
Control of Anticoagulation

23 May 2010
Egypt, Cairo

Chairman: Trevors Baglin (UK)
Co-chairmen: Walter Ageno (IT), Job Harenberg (DE), Clive Kearon (CA), John Olson (US), Gualtiero Palareti (IT), Sam Schulman (CA), Antonius Van Den Besselaar (NL)

Introduction and update on activities:

Current Active SSC Registries

1) Recurrent venous thromboembolism in anticoagulated patients with cancer

Sam Schulman, Anna Falanga
Start July 2006, completion 2010

2) Splanchnic vein thrombosis

Walter Ageno, Francisco Dentali, Sam Schulman
Start July 2008, completion 2010

3) Cerebral vein thrombosis

Walter Ageno
Start July 2008, completion 2010

Planned new SSC Registries / Working Parties

1) Proposal - A working party to delineate methodology/endpoints/harmonization of present and future studies on perioperative AC management and bridging therapy

Alex Spyropoulos

Current SSC Standardization Projects

Revision of statistical procedures in ISI calibration
AMHP van den Besselaar

Performance requirements of Point-of-Care monitors
AMHP van den Besselaar
Standardization of platelet thrombography
Trevor Baglin

**Planned SSC Standardization Projects**

1) **Standardization of tests to evaluate effect of factor Xa inhibitors**

Job Harenberg
Start 2010

**SSC Publications in Past Year**

1) Definition of major bleeding in clinical investigations of antihemostatic medicinal products in surgical patients - Schulman S, Angerås U, Bergqvist D, Eriksson B, Lassen MR, Fisher W; Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis - JTH 2010;8:202

2) Definition of post-thrombotic syndrome of the leg for use in clinical investigations: a recommendation for standardization - Kahn, Partsch, Vedantham, Prandoni, Kearon Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis - JTH 2009;7:879

3) Recommendations on biosimilar low-molecular-weight heparins - Harenberg, Kakkar, Bergqvist, Barrowcliffe, Casu, Fareed, Mismetti, Ofosu, Raake, Samama, Schulman Subcommittee on Control of Anticoagulation of the SSC of the ISTH - JTH 2009;7:1218


**Planned SSC publications**

1) Reporting prothrombin time results as International Normalised Ratios for patients with chronic liver disease - Tripodi, Baglin, Robert, Kitchen, Lisman, Trotter - accepted for publication in JTH - April 2010

2) Risk of recurrent venous thromboembolism after stopping treatment in cohort studies: recommendation for acceptable rates and standardizing reporting - Kearon, Iorio, Palareti - submitted to JTH as official recommendation May 2010
3) International Collaborative Study for the calibration of a proposed international standard for thromboplastin, human, plain - Tripodi, Chantarangkul, van den Besselaar, Witteveen, Hubbard - submitted to JTH as official recommendation April 2010

4) Standardisation of platelet thrombography - for submission to JTH as official recommendation May 2010

Additional SSC Special Projects

None

Educational Activities

None scheduled

Items requiring 2010 SSC Approval (standards or publications)

None

Session 1
Chairs T BAGLIN & TON VAN DEN BESSELAAR

Reviseion of WHO Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy
Ton Van Den Besselaar

The need for 2 or 3 international reference preparations and procedures for replacement and calibration were summarised. Real time stability studies and statistical procedures were reviewed specifically. An option for stability testing by freezing aliquots (at -20 and -150) of RBT/05 and rTF was proposed for commencement in 2010. Items presented in detail were:

1) ISI calibration samples with an INR between 1.5 and 4.5 by both reference and test thromboplastin should be selected to prevent cumulative bias over subsequent calibrations;

2) criteria for outlier detection and exclusion to reduce intra- and interlaboratory variation of the ISI;

3) necessity for ISI model to be linear.

A manuscript describing all the issues presented will be submitted to the SSC with a view to modifying WHO guidelines.

(Manuscript anticipated 2010).
REGISTRY: Recurrent venous thromboembolism in anticoagulated patients with cancer
Sam Schulman

An update of the registry was presented. 120 patients have been recruited from 13 centres. The target was originally 200 patients from 50 centres with at least 2 patients per centre. The purpose of the registry is to review current practices and form a basis for future interventional studies.

Reporting Prothrombin Time Results as International Normalised Ratios for Patients with Chronic Liver Disease
Armando Tripodi

Inappropriate use of the INR\textsubscript{VKA} in the MELD system of organ allocation for liver transplantation was explained. Importantly in the MELD system the INR has been identified as the most important parameter. Methods of alternative ISI calibration were presented. A simple interim pragmatic solution was presented; namely that for a specified thromboplastin the ISI\textsubscript{VKA} can be used when the difference between ISA\textsubscript{VKA} and ISI\textsubscript{LIVER} has been evaluated and the difference in values is less than 10%. An SSC recommendation has been accepted for publication in JTH.

UK NEQAS Anticoagulant dosing exercise
Ian Jennings

The results of a paper exercise of external quality assessment of oral anticoagulant dosing (warfarin) using 3 patient scenarios was presented. The results indicated a wide range of adopted target INRs (and ranges), frequent use of manual dosing (i.e. not using computerised decision support models) and resultant inappropriate dosing. The approach could be used locally by centres or regions to identify irregular dosing and hence to standardise dosing.

Intra-individual variation of INR and analytical quality requirements
Ton Van Den Besselaar

Traditional laboratory measurements of INR have an imprecision of 2% but imprecision is higher with some point-of-care (POC) devices. An explanation of the relationship of biological and analytical variation on total variation and the definition of optimum and desirable analytical variation was presented. An ongoing project of determination of biological variation and POC specific analytical variation was presented and a request for volunteer participants was made. Potential participants should contact Dr van den Besselaar directly.

The influence of the direct f Xa inhibitor rivaroxaban on thrombin generation assays in patients with thrombophilia
Thomas Siegemund
The results of measurement of the effect of rivaroxaban spiking of plasma samples on a chromogenic thrombin generation assay and calibrated automated thrombography were presented. The assays performed significantly differently indicating the need to recognised that thrombin generation assays are not necessarily interchangeable and a requirement for standardisation.

*Thromboprophylaxis after knee arthroscopy and lower leg immobilization with LMWH - monitoring and endpoints*
Suzanne Cannegieter

The need for data on risk benefit in terms of 1) harm to patients, and 2) cost, was presented in the context of two planned randomised studies with recruitment of 3000 patients over 2 years in 5 centres in the Netherlands was presented. The aim of the studies was to determine the net gain or loss. The importance of measuring 'orthopaedic outcomes' such as joint haematoma, joint infection and loosening was highlighted during the discussion.

*Rivaroxaban and apixaban - age, weight, gender and renal function on rivaroxaban pharmacokinetics*
Michael Rud Lassen

No presentation was available.

*A working party to delineate methodology/endpoints/harmonization of present and future studies on perioperative AC management and bridging therapy*
Alex Spyropoulos

A proposal for a working party was supported.

**Action 1**: Pending approval at the SSC business meeting Dr Spyropoulos will convene a writing group (maximum of 6 members) and will aim to submit a manuscript for approval as an SSC recommendation by the autumn of 2010.

**SESSION 2**
Chairs T Baglin & Walter Ageno

*Update on the development of generic low molecular weight heparins and adherence to recommendations of scientific organizations*
Job Harenberg

Biosimilar LMWHs are being used in some countries but none yet approved for use in North America or Europe. Data was presented that showed whilst average APTT or anti-Xa responses were comparable the variability of response between individuals can be significantly greater for a biosimilar LMWH. This has important implications for patient safety and product efficacy. An SSC on biosimilar low-molecular-weight heparins was published by the subcommittee in 2010 (see publications).
Report of the working party on standardization of methods to determine direct factor Xa inhibitors
Job Harenberg & Michel Samama

A protocol has been distributed to 15 potential participant laboratories. It is intended that work will begin soon with a report prepared in preparation for the 57th SSC in 2011. Professor Samama presented data on measurement of rivaroxaban by PT and proposed the use of plasma calibrants for standardisation and data on a specific ant-Xa assay used to quantify the concentration of drug in plasma.

Differential Effects of Various Anticoagulants as measured by Thromboelastography.
Guy Young

Data showing differential effects of UFH, LMWH and DTIs on thromboelastograph parameters as compared with APTT and anti-Xa activity was presented. The need to develop an understanding between the different tests and clinical correlates was emphasized. Data on the effect of rVIIa was also presented again demonstrating differential effects on different assays.

Thrombin generation in orthopaedic patients receiving rivaroxaban
Sam Machin

Data on the effect of LMWH (fragmin) as compared with rivaroxaban on thrombin generation after orthopaedic surgery was presented. In keeping with know relative T1/2 a greater effect of rivaroxaban was evident 24 hours after surgery (16 to 20 hours after drug) as compared with LMWH. In addition the effect 3 to 4 hours after drug was variable with LMWH but in comparison relatively consistent with rivaroxaban.

Long term control of anticoagulation in patients with thrombosis in unusual sites

Cerebral vein thrombosis registry.
Walter Ageno

An update of the registry was presented. 326 patients have been recruited from 33 centres. 225 patients had portal vein thrombosis. The target was originally 500 patients. The purpose of the registry is to review current practices and form a basis for future interventional studies.

Thrombosis in unusual sites.
Francesco Dentali

An update of the registry was presented. 587 patients have been recruited from 24 centres. Mean follow up is 4.3 years with a median duration of anticoagulant therapy of 12 months. Therefore, the majority of follow up time is when patients are not receiving
anticoagulant therapy and the recurrent CVT rate is only 2.8% but with a 3.7% VTE incident rate at another site.

*New prospective data on CVT and OAT.*
Ida Martinelli

No presentation was available.

**New oral Anticoagulants** – An open discussion identified issues to inform the work agenda of the SSC.

Key issues identified were:

1) the need to determine the effect of the new oral anticoagulants on coagulation tests; both those that might be used to specifically quantify the anticoagulant effect of a drug and also the effect on routine tests currently in use. The reasons for this are that there will be i) a requirement for a validated test in specific situations and ii) that routine tests such as the PT and APTT will continue to be used and patients will increasingly be taking these drugs. Abnormal results will be observed and it is necessary to know how these tests perform in relation to the new drugs, i.e. what will a test result mean. **A Working Party to define the effects of new anticoagulants on haemostasis assays** was proposed. The Chairman will consider the membership of this Working Party and facilitate formulation of a proposed work programme.

**Action 1:** to assemble the working party by Autumn 2010 and thereafter define a work programme to be presented at the 57th SSC in 2011.

**Action 2:** Chairman to establish contact with drug manufacturers to identify i) work being undertaken to address effect of drugs on assays that manufacturers are aware of, and ii) consider ways of collaborative working to address effect of drugs on assays.

2) In specific issues such as obesity, renal impairment, episodes of overanticoagulation, emergency surgery, reversal therapy it will be necessary to be able to measure the anticoagulant effect of a new drug and from this understand the associated antithrombotic effect and bleeding tendency. Guidance on this will need to relate to the assay work described in 1).

**Action 3:** Chairman to consider the process for production of clinical guidance in parallel with methodology and establish a **Working Group on monitoring and measuring new anticoagulant Drugs** for this purpose (see also Action 4).

3) In paediatric patients monitoring will be required and again this will need to relate to the assay work described in 1).

**Action 4:** Chairman to communicate with Perinatal/Paediatric Subcommittee to seek involvement in the Working Group to oversee the results from the working party.
4) the need for education of non-experts on the use and effects of new anticoagulant drugs and clinical management of patients in elective and emergency situations.

Action 5: **Working Group on monitoring and measuring new anticoagulant Drugs** to include professional education (not patient information) in their agenda particularly with consideration as to how the ISTH will contribute to this task.

**Planning of future SSC activities**

No additional activities were suggested from the floor. The chairman closed the meeting with thanks to all speakers and participants and informed the participants of:

1) The new format for SSC recommendations with a limit of 6 writing group members and submission to JTH of manuscripts of no more than 2000 words with additional information available on the ISTH website.

2) The proposal being considered at the ISTH Council meeting that ISTH develops a process to produce clinical guidance as distinct from SSC recommendations produced by Subcommittees.
I. Diagnosis and Treatment

Dr. Hideo Wada presented several of the accomplishments of the previous chairman, including the formation of a working group, establishment of overt DIC diagnostic criteria, construction of a template of non-overt DIC diagnostic criteria, collaborations with fibrinolysis, hemostasis and malignancy, plasma coagulation inhibitors, and control of anticoagulation, among others, and the proposal of new markers. Thereafter, Dr. Wada said that he hoped to continue to collaborate and establish the non-overt DIC criteria with several modifications. Furthermore, Dr. Wada also plans to investigate other associations such as critical care medicine to improve the poor survival rates in patients with DIC.

Dr. Cheng-Hock Toh’s report entitled "British Society for standards in Hematology Guideline in DIC" emphasized that thrombin formation was important in DIC patients due to sepsis, and that the ISTH DIC score was well correlated with mortality. The APTT waveform was also found to be correlated with the ISTH DIC score. Dr. Toh introduced several recommendations as guidelines: Blood products support in the patients with less than 50,000/μl of platelet count is recommended (Grade C, evidence level IV). The administration of FFP in the patients with low levels of fibrinogen is recommended (C, IV). The prophylaxis of VTE with low molecular weight heparin in the patients with a high risk in the ICU is recommended (A, Ib). The administration of antithrombin (AT) is not recommended (A, Ib) but the administration of activated protein C (APC) is recommended (A, Ib).

Dr. Hideo Wada introduced the Japanese guidelines regarding DIC treatment. These guidelines focused on septic DIC and had 6 recommendation levels (consensus, A, B1, B2, C and D). These recommendations were demonstrated for each type (asymptomatic DIC, DIC with severe bleeding, and DIC with organ failure). The treatment of the underlying disease and blood transfusion in severe bleeding cases were consensus, anticoagulation therapy for recommendation A, and antifibrinolytic and fibrinolytic therapies were recommended for D.

The difference between these guidelines was discussed; synthetic protease inhibitor, recommendation level for AT and antifibrinolytic therapy, etc. Dr. Toh suggested that making this guideline universal will aid in the establishment of international guidelines. The role of platelets in DIC was also discussed.
II. Educational session

Dr. Ikuro Maruyama spoke on the topic, “Pathogenetic role of alarmin, HMGB-1 in DIC”. A high mobility group B-1 (HMGB-1) is released from the nucleus in necrotic cells. HMGB-1 activated TF expression in monocytes through the NFκB/p53 pathway. RAGE is the receptor for HMGB-1 and induces inflammation and alteration in cell morphology. Dr. Maruyama emphasized HMGB-1 is an important inducer of septic shock, DIC and MOF. The importance of HMGB-1 in sepsis was demonstrated in a mouse model, and the plasma levels of HMGB-1 correlated well with the DIC score, SOFA score and overall mortality. HMGB-1 acts as a local adjuvant for immune defence, and circulating HMGB-1 causes death. Thrombomodulin (TM) regulates HMGB-1 by binding to HMGB-1 on N terminal EGF like D domain. It is reported that LPS also binds on the N-terminal EGF like D domain.

Dr. Jecko Thachil’s talk was entitled “Pathophysiological consideration in sepsis-induced DIC”. Thrombocytopenia is often observed in sepsis. Dr. Thacil reviewed the causes of thrombocytopenia in sepsis. 1. Bacterial-platelet interaction, 2. VWF multimers and fibrinogen interaction; ADAMTS13, IL8, TNF, IL-6, thrombin, plasmin and granulocyte- derived elastase affect the cleavage of VWF multimers, 3. Increased attachment to the endothelium, 4. microangiopathic hemolysis (thrombosis).

Dr. Pantep Angchaisuksiri spoke about “DIC due to tropical disease”, and reviewed mainly malaria and dengue infection. Malaria is the most important parasitic diseases and malaria patients show several symptoms. The incidence of bleeding in severe malaria varies from 10% to 25%. P.falciparum can cause DIC with vascular endothelial cell damage, showing increased VWF propeptide (VWFpp) and decreased ADAMTS13. Anti-malarial agents are recommended and exchange transfusion shows benefit in patients with parasitemia >30% and severe systemic complications. Dengue virus causes Dengue hemorrhagic fever. Aedgs aegypti is the principal mosquito vector in virus transmission. In dengue hemorrhagic fever, activation of endothelial cells (increased VWFpp and TM, decreased ADAMTS 13) and DIC induces coagulopathy (increased TAT and D-dimer).

III. Standardization issues

Dr. Jorn Dalsgaard Nielsen reported on “Considerations on the use of the ISTH non-overt DIC scoring system” and reviewed previous reports of overt DIC and non-overt DIC scoring system on platelet counts, PT and D-dimer and antithrombin. Thereafter, he presented the reformed non-overt DIC scoring system. He will begin a prospective study to evaluate this new scoring system.

Dr. Doyeun Oh reported on the “Evaluation of modified non-overt DIC criteria on the prediction of the poor-outcome in patients with sepsis”, and prospectively studied the modified non-overt DIC criteria to detect the early-phase DIC from January 1, 2004 to December 30, 2006. In 136 patients, 76, 17 and 42 patients were diagnosed as non-
DIC, non-overt DIC and overt DIC, respectively. Adding AT/PC reduced the patient mortality from 47.1% to 25.0%. Including organ failure score in the diagnostic evaluation also improved the ROC analysis on the non-overt DIC criteria.

Dr Jecko Thachil reported on the “Working group on Clt WF analysis”. Biphasic APTT reflects Ca$^{2+}$ and the CRP complex. He will analyze APTT wave form in patients with sepsis with a new MDA analyser using the “Plate turbidity assay”.

Dr Hyun Kyung Kim reported on the “Accuracy of platelet counting by the automated hematologic analyzers in disseminated intravascular coagulation: The potential effects of platelet activation on platelet measurement”. She evaluated the 5 methods of automated hematologic analyzers for platelet counting. An immune method was well correlated with the reference (IRM). Platelet activation was frequently associated with severe coagulopathy.

Dr. Hideo Wada presented a report entitled: “Prospective study in Japan” He prospectively examined three different diagnostic criteria for DIC as established by the Japanese Ministry of Health and Welfare (JMHW), the International Society of Thrombosis and Haemostasis (ISTH) and the Japanese Association for Acute Medicine (JAAM) in 686 patients suspected of having DIC. Results were as follows: DIC is related to mortality. Sepsis and trauma associated with DIC have a poor outcome. JAAM DIC diagnostic criteria were sensitive to poor outcome, while the ISTH overt DIC diagnostic criteria were specific for poor patient outcome. Modified non-overt-DIC diagnostic criteria may be useful for the early stages of DIC.

Future studies for possible working groups;

1) Trauma
2) Standardization of D dimers
3) Reforming the non-overt DIC criteria
4) Recommendation the DIC treatments
The meeting started at 15:00 hours on the 24th of May, 2010 with the Chairman’s welcome to over 100 participants. He thanked the co-chairs, Christine Lee and Jean-Marie Saint-Remy, who had finished their terms, for their contributions to the work of the subcommittee and introduced the new co-chairs, Jan Astermark, Kathelijn Fischer and Leonard Valentino. Drs. Astermark and Tuddenham were unable to attend the meeting this year. He also explained the structure of the program this year and mentioned the working parties that were created to address specific issues.

Completed/Submitted reports and recommendations – A. Srivastava (India)

There were no reports to present this year but the participants in the meeting were reminded of the SSC rule that all official communications from SSC activities must be submitted first to JTH through the subcommittee chair who will pass it on to the SSC chair for onward transmission to JTH, once found suitable.

Section -1: Rare Bleeding Disorders (RBDs)
Chairpersons: Flora Peyvandi (Italy) and Alok Srivastava (India)

European Network of Rare Bleeding Disorders (ENRBDs) – Flora Peyvandi (Italy)

The EN-RBD database was established and funded by the DG SANCO, European Community in 2007. Till now, 13 European partners have collected data on 576 patients affected with different type of RBDs. The enrolled patients were classified based on residual coagulant activity in plasma as severe (<1%, except FXIII with <5% and fibrinogen with <10mg/dl), moderate (1-10%, except FXIII with 5-10% and fibrinogen with 10-50mg/dl) and mild (>10% or fibrinogen with >50mg/dl). 17% of patients were classified as severe, 22% moderate and 61% mild based on this criteria. 73% of patients with severe deficiency and only 13% with mild deficiency had at least one important bleeding episode such as CNS, GI, umbilical cord bleedings, haematoma or haemarthrosis (p<0.000001). Mucocutaneous bleedings such as epistaxis, oral cavity, cutaneous and menorrhagia were constantly present in all groups of patients with different laboratory phenotypes. Afibrinogenemia, severe FXIII and FX deficiency resulted to be the most severe type of RBDs, with 100% of patients who experienced at least one major bleeding, and no patient remained asymptomatic. Severe FXI deficiency was the least severe type of RBD, and approximately 40% of patients remained asymptomatic. Patients carrying more than 10% of coagulant activity or 10 mg/dl of...
fibrinogen in plasma had significantly less major bleeding symptoms (p<0.000001). Age of diagnosis seemed to correlate well with the laboratory phenotype only in patients affected with fibrinogen, FX and FXIII deficiency; patients affected with severe deficiencies had usually an early onset of bleeding: median age 1.7 years (0-12.8) for fibrinogen, immediately after birth (0-0.5) for FX and 5.1 (1.3-18.5) for FXIII. These analyses clearly indicate that RBDs should not be studied as a whole group, and forthcoming studies should focus on the evaluation of specific aspects of each disorder. The results of the EN-RBD database could finally help to define the classification of the clinical severity of RBDs based on their laboratory phenotype.

**Rare Bleeding Disorders: The World Federation of Hemophilia database – Paula Bolton Maggs (UK) on behalf of the Data and Demographics committee**

The World Federation of Hemophilia conducts a global survey about people with bleeding disorders each year (since 1998) by questionnaire to the national member organizations (NMOs). These are patient organizations in more than 100 countries whose ability to collect data is variable, but they are advised to co-ordinate with their doctors and governments. Initially focused on haemophilia and von Willebrand disease, since 2004 data has been collected on the rare bleeding disorders and each year we modify the questions and seek more information. There are of course caveats about the data. Some countries will only identify severely affected individuals who present with bleeding, whereas others may pick up asymptomatic heterozygotes. Some haemophilia societies will have limited access to patients attending one or a few major cities in their country whereas others have national registries which cover 75-100% of the population. In 2008, 21,510 patients with rare bleeding disorders were recorded from 108 countries (61 have national registries of some kind) representing 91% of the world population. However some countries are only reporting from a small proportion of their total population. Coverage is better in Europe and in countries with higher economies. Fewer data are available for African countries and those in South East Asia. Although overall data for the world, for Europe in the RBDD registry and in the UKHCDO registry shows similar percentages of the rare disorders, there is some variation in reported prevalence in different parts of the world reflecting different cultures. Surprisingly perhaps for autosomal disorders, the ratio of women to men did not show an excess of women for most of the disorders.

**Bleeding scores in RBD – R. Palla (Italy) (presented by Flora Peyvandi)**

The bleeding score (BS) is a quantitative approach previously used to assess the severity of bleeding history in patients referred for mild bleeding disorders such as type 1 of von Willebrand disease, showing not to be suitable to individuate severe bleeders. Therefore a BS was designed to define the individual hemorrhagic risk of patients affected with rare bleeding disorders (RBDs). Each bleeding episode was assigned one point; additional points (up to a maximum of three) were assigned for nature of the bleeding symptom (spontaneous vs. posttraumatic), size/localization, frequency, prophylaxis and type of treatment, which were predefined for each bleeding episode. The sum of all these points represented the global bleeding severity of the investigated
subject. An analysis of the BS was carried out on 492 patients affected with different type of RBDs, enrolled in the EN-RBD project, funded by the European community (DG Sanco), aimed at developing a network of European treatment centers dealing with RBDs. Patients were classified based on residual coagulant activity in plasma as severe (<1%, except FXIII with <5% and fibrinogen with <10mg/dl), moderate (1-10%, except FXIII with 5-10% and fibrinogen with 10-50mg/dl) and mild (>10% or fibrinogen with >50mg/dl). In afibrinogenemia, severe FX and FXIII deficiency, a significant difference was observed among these three groups of patients (median test: p=0.01). However, in FXI deficiency the BS values were similar in all patients carrying different laboratory phenotype severities (p=0.4), assigning a BS value of 0 even in patients with severe phenotype, indicating a lower risk of bleeding manifestations. The BS is an useful tool to select severe patients from milder only in afibrinogenemia, FX and FXIII, but did not allow us to distinguish phenotype severities in other type of deficiencies such as FV, FVII and FXI.

Monitoring hemostasis during surgery in FXI deficiency – K. Gomez (UK)

In the UK, standard treatment for severe factor XI (FXI) deficiency is with plasma derived FXI concentrates. However, there is an increasing need to reduce exposure to plasma derived products. We report the use and efficacy of low dose recombinant factor VIIa (rFVIIa, NovoSeven®), to cover surgery (caesarean section, cholecystectomy and abdominoplasty) in four female patients (FXI:C 2-4 IU/dL, aged 32-51 years) who wished to avoid exposure to plasma. Our aim was to find the optimal dose of rFVIIa by in vitro spiking of patient samples and to correlate this with the response to rFVIIa in vivo. Prior to surgery, venous blood was collected into sodium citrate with corn trypsin inhibitor and spiked with 0.25 – 1.0 µg/mL rFVIIa in-vitro, equivalent to a 15-70 µg/kg dose of rFVIIa in vivo. Analysis using thromboelastometry and thrombin generation assays, triggered with tissue factor, showed that the thrombin generation assay was insufficiently sensitive to the haemostatic defect in these patients. A concentration of 0.5µg/mL was as effective as 1.0µg/mL rFVIIa in normalising thromboelastometry in-vitro in all four patients. Therefore, patients received 30-40µg/kg rFVIIa at 2-4 hourly intervals with tranexamic acid 1g 6 hourly. Post treatment samples were taken at 10-240 minutes and showed initial normalisation of thromboelastometry with gradual return to baseline between 2-4 hours. In conclusion, low dose rFVIIa therapy was successfully used in four patients with severe FXI deficiency undergoing surgery to prevent bleeding and can be monitored using thromboelastometry.

Definitions in rare bleeding disorders – Report of the working group. F. Peyvandi (Italy), P. Bolton Maggs (UK), C. Negier (France), A. Shapiro (USA), D. Dimechele (USA), A. Tripoli (Italy), A. Srivastava (India), C. Lee (UK).

Over the last few years significant volume of data has been collected through various registries regarding the number of patients with RBD around the world. From a scientific and clinical perspective, it is important now to understand the bleeding profile of these patients and correlate them with their factor levels. This is the first level of analysis needed to begin to define the severity of these diseases. Such a classification will be
important not only for the clinical management of these patients but also for their inclusion in clinical trials for product for treatment. The WP is fortunate to have access to the EN-RBD registry data that has very carefully collected and analyzed the factor levels with clinical bleeding in these patients. This will form the basis for the initial assignment of disease severity of these patients. The WP will also strive to obtain similar data from other sources to complement what is available through the EN-RBD registry and attempt to strengthen the assignments of severity. These recommendations will then be circulated for peer comments before presentation at the next meeting of the subcommittee.

Section 2. Clinical issues- I (Assay / EQA / Phenotype)

Chairs: C. Negrier (France) and A. Srivastava (India)

Standardization of the Thrombin Generation Test: Report of the working party – C. Negrier (France), Y. Dargaud (France), H.C. Hemker (Netherlands), T. Lecompte (France), R. Luddinton (UK), A. Wolberg (USA)

The aim for the Working Party was to propose a methodology that may be considered to be the recommended way to perform the thrombin generation test so as to bring standardization in studies performed using this method and the literature. The WP recognized that several variations of the method to perform the thrombin generation test exist in the literature with regard to reagents, instruments and interpretation. However, there is a common feeling that the most used so far and the one on which most work has been done is the CAT assay (Hemker et al.). The WP however recommends that a thorough description of the method should be done in any paper on the subject.

Literature published in 2009/2010 has been carefully followed and some lab data and information exchanged. Particularly, a minimisation of the variability of the assay has been carried out among 5 European centers and the results published in Thromb Res 2010. Though the group recognized that further activity should be carried out in this direction, and particularly on the use of a reference plasma as well as the possible influence of the type of phospholipid membrane and the type of tissue factor used, it was also felt that the results from the various labs using the CAT assay and the measured variability of the assay were good enough to try to translate some application of TGA into clinical research. In this regard, 3 further steps were suggested: 1. Use of a common method in 10 centres (5 NA, 5 EU) as a validation exercise for evaluating single clotting factor deficiency (FVIII, FIX, FVII, FXI...) and try to correlate with the bleeding phenotype (if score available). 2. Replication exercise in 10 centres (5 NA, 5 EU) on the use of the pilot study done in Lyon, using TGT in surgery carried out in patient s with inhibitors receiving bypassing agents (NovoSeven®, FEIBA®). 3. The first two steps should be completed before the method can be considered to be fully validated and recommended for wider use along an educational video made for this purpose. Finally, it will need to be evaluated in clinical settings such as prophylactic factor replacement therapy.
The mandate of the thrombelastography / thromboelastometry (TEG/ROTEM) working party (WP) is to develop methodologies that may be considered to be the recommended way to perform TEG / ROTEM within the area of haemophilia and rare bleeding disorders (RBD). Furthermore, the WP would also list criteria and challenges to bring standardization in studies performed using these methods. Several variations of the method to perform the TEG / ROTEM within the area of Haemophilia and RBD exist in the literature with regard to reagents, instruments and interpretation. Since Q4 2010, the TEG / ROTEM working party has conducted i) a review of published literature (case reports excluded), ii) emphasized similarities and differences of assay methods, and iii) set up a list of proposals on how to achieve standardization of TEG/ROTEM within the area of Haemophilia / RBD. Two different whole blood assay approaches have been investigated: 1) low tissue factor (dilution of Innovin) activation and 2) kaolin activation. In total, 9 studies have been detected using various dilutions of Innovin for activation (including 109 patients), whereas only 2 studies have been conducted using kaolin (including 19 patients). Comparison of study results showed pronounced differences as exemplified by a clotting times varying with a ratio of up to 2.29 in studies conducted with TF 1:17000 and a ratio of up to 6.60 in studies conducted using kaolin. Furthermore, only weak association of CT and level of TF dilution was demonstrated. Despite inter-laboratory variation, single centre studies using low TF for activation have shown good correlation with the clinical phenotype. Clinical data with kaolin are so far sparse. The TEG / ROTEM WP has come to the following preliminary conclusions: i) Whole blood TEG / ROTEM can be performed with activation with minute amounts of tissue factor, ii) Dilution of Innovin causes considerably variable results, iii) Batch to batch variation of Innovin seems to constitute a serious threat / challenge to standardization of TEG / ROTEM within the area of Haemophilia / RBD, iv) There seem to be different methodologies for calculating dynamic parameters, v) Unfortunately, it is still rarely standard practice to use corn trypsin inhibitor to abolish artificial contact activation. Furthermore, with regard to the use of kaolin, the following preliminary conclusions are suggested: i) whole blood TEG / ROTEM can be performed with activation with kaolin, ii) Reproducibility requires further investigation, iii) difference between patients with and without inhibitors requires further investigation, iv) from a practical point of view, this method seems easier than dilution of Innovin. This TEG / ROTEM WP has set itself the following tasks to suggesting standardization of the methodology for this test: 1. Explore reliable source of tissue factor: a. Antigen level and activity; b. Validation and standardization; c. Secure delivery and distribution (e.g. collaboration with diagnostic industries). Once this is done, there will be need to evaluate the proposed method at the single institution level followed by multi-institutional studies. The WP would also suggest larger studies using Kaolin as activator in order to validate reproducibility and demonstrate a clinical correlation.
Standardization of clot waveform analysis: Report of the working party – M. Shima (Japan), J. Thachil (UK), S C Nair (India), A. Srivastava (India).

Clot waveform analysis (WA) is a new technique for assessment of clotting function using automated coagulometer basically performing clinical laboratory coagulation assays using a variable wavelength photo-optical detection system. During the performance of routine clotting assay such as activated partial thromboplastin time (aPTT) and prothrombin time (PT), it is possible to figure a clot waveform reflecting whole blood clotting process by continuous measurement of the changes in either transmittance or light scatter. The resultant photo-optical data profiles are called waveforms caused by their sigmoid patterns. Since the continuous measurement can be done by many type of automated coagulometers currently in use, the advantageous point of the WA is the potential for wide applicability to many laboratories in which such instruments are available. Furthermore, very conveniently, WA can be performed at the same time as regular routine assays. The third useful point is that the wave form can be mathematically processed by a software to derive several quantitative parameters such as coagulation velocity and acceleration. To date various reports describing about the utility of WA have been reported. WA has been shown to be useful for the assessment of overall clotting function in various hemorrhagic disease such as haemophilia and for monitoring of hemostatic treatment. Furthermore, it is also useful for early identification of DIC associated with sepsis by abnormal biphasic waveform. However, there is need to establish the optimal condition for WA in order for it to be to applied more widely in a uniform manner. The working party of WA launched to standardize the method of WA using current available automated clotting machine such as the various ACL systems, since MDA-II is no more available. Furthermore, attempts are being made to establish the tissue factor (TF) triggered WA measurement system reflecting current concept of cell based coagulation. For this purpose, there is need to test the concentration of TF and to consider the addition of corn trypsin inhibitor (CTI).

Could global hemostasis tests be used to tailor treatment in hemophilia?
J.P. Antovic (Sweden), D. Mikovic (Serbia), M. Holmström (Sweden), N.M.H. Soutari (Sweden), P.Elfvinge (Sweden), A.Antovic (Sweden)

The evaluation of two global hemostasis tests: endogenous thrombin potential (ETP) and overall hemostatic potential (OHP) before and 30 minutes after intravenous injection of FVIII-concentrate in hemophilia A patients treated prophylactically and on-demand was described. A significant correlation (r=0.79) between both ETP and OHP and FVIII was observed. Using ETP and OHP, it was possible to discriminate different severity of hemophilia and normal values (329.2±12.2, 199.1±32.5, 71.3±9.9, 373.7±10.7 for mild, moderate, severe hemophilia A and normal values of FVIII respectively for ETP) and (7.1±0.6, 1.9±0.7, 0.4±0.1 and 9.8±0.6 for mild, moderate, severe hemophilia A and normal values of FVIII respectively for OHP). All parameters differed between patients with different clinical severity (determined by the number of bleedings per year (> or <= 3) as severe or mild respectively) (0.006±0.0005 and 0.11±0.02, 83.1± 12.9 and 256.2±32.1 and 0.4±0.1 and 2.9±0.6 for FVIII, ETP and OHP respectively) but the variation was much wider with ETP and OHP than with FVIII.
results. Both ETP and OHP increased after intravenous injection of FVIII-concentrate (from 77.4±14.9 to 346.0±13.8 and from 0.3±0.2 to 8.9±0.5 in patients treated on-demand and from 171.6±28.9 to 309.9±13.0 and from 3.4±0.9 to 8.4±0.8 in patients treated prophylactically). In spite of higher basal values of both ETP and OHP in patients treated prophylactically and much higher post treatment FVIII level (0.59±0.08) in comparison to the level in patients treated on-demand (0.31±0.02), that difference after the treatment was not observed either for ETP or for OHP with ETP being even borderline, but not significantly, higher in the on-demand group (p=0.44). In spite of the general agreement over the FVIII level, both ETP and OHP better correlate with clinical status and response to the therapy in some individual patients. ETP and OHP are potential options for evaluating the severity of hemophilia A and tailoring the treatment to the individual needs of patients.

This part of the meeting closed at 7:00 pm on the 24th of May, 2010.

This part of the meeting started at 9:00 am on the 25th of May, 2010.

Section 3. Clinical issues- II (Inhibitors / Prophylaxis / Novel Therapies). Chairs: C. Hay (UK) and K. Fischer (The Netherlands)

Definitions in hemophilia – Report of the working party. V. Blanchette (Canada), A. Srivastava (India), N. Key (USA), M. Soucie (USA), H. M. Van den Berg (Netherlands), R. Ljung (Sweden), M. Manco Johnson (USA), A. Gringeri (Italy).

About 10 years ago, the SSC FVIII / IX subcommittee provided some definitions of the classification of the severity of hemophilia based on factor VIII coagulant activity. (Thromb Haemost 2001; 85:560) This paper also included the definitions of the low and high responders for functionally active neutralizing antibodies based on the Bethesda assay. There is need now to provide further definitions that are relevant to current needs of clinical practice and evaluation of new therapeutic agents. These include the following: the possibility of defining severity based on bleeding rather than factor level alone or a combination; defining what is a significant inhibitor, what is transient and what is persistent; what is a joint bleed and a target joint, what are the different types of prophylaxis, what is adequate response when treating acute hemarthroses, a common end point in the assessment of new products, what is successful immune tolerance induction. To address these issues, the working party will review literature and suggest definitions on which we will seek consensus using standard methods of doing so. The issues being considered for definition include the following: severity of hemophilia, inhibitors in hemophilia, joint bleeds and issues related to that, types of prophylactic factor replacement therapy, adequate response to factor replacement. This work has been initiated and is expected to be completed over the next 6-9 months, followed by peer review and finalization.
Pharmacokinetic evaluation FVIII / IX for clinical practice in hemophilia: Report of the working party. P. Collins (UK), K. Fischer (Netherlands), E. Tuddenham (UK), Blanchette V (Canada), Björkman S (Sweden)

Knowledge of an individual patient’s pharmacokinetic (PK) response to factor VIII is likely to improve clinical outcomes and cost effectiveness of treatment in patients with haemophilia A, especially in the context of prophylactic treatment. Measuring PK according to ISTH guidelines for new concentrates requires a washout and multiple blood samples over a 48 hour period. This is difficult to do in routine practice especially in young children. Using a population PK model and Bayesian analysis PK can be measured on 1-3 blood samples with almost as much accuracy as with a full sampling procedure. No washout is required and, for a patient taking prophylaxis in the morning, PK can be measured by taking blood samples in the afternoon after the infusion and the following morning and afternoon. The timing and the dose of the infusion (the preceding 2 infusions) and time of the blood samples needs to be known accurately but the exact timing is not important. Population PK models and Bayesian analysis has been used for many years and has a proven record for therapeutic drug monitoring of cyclosporine, aminoglycosides and digoxin. A number of population PK models for factor VIII have been published. Proof of principle that this methodology can be used for factor VIII PK has been published and measurement at even one time point has been shown to be much better than weight based dosing in the context of prophylaxis. This methodology can be used to more accurately target any trough level during prophylaxis that a clinician decides is appropriate for an individual patient. A prospective clinical trial is being developed to test the validity of the methodology and its effect on bleed rate in clinical practice.

International ITI study: Report of Interim analysis – D. DiMichele (USA), C. Hay (UK)

The International ITI study is a multicenter prospective trial randomizing to low (50 IU/kg 3x weekly) and high (200 IU/kg daily) dose FVIII regimens in good-risk severe hemophilia A inhibitor subjects (age <8 years, peak historical titer 5-200 BU/mL, inhibitor present <24 months, and pre-ITI titer <10 BU/mL). ITI success is defined by: negative (<0.6 BU/mL) titer; recovery ≥66% expected, and half-life ≥6h within 33 months. 33 subjects from 27 countries were recruited prior to 12/11/09. Subject demographics did not differ between treatment arms (age, titer, use of plasma-derived concentrate). 62/116 randomized subjects reached a study endpoint (success/partial response/failure). ITI success rates did not differ between treatment arms: 76% of pts reaching a study end-point became tolerant and but only 57% using an intention to treat analysis; p=ns. Median time to achieve negative titer and normal recovery was significantly (□50%) shorter with high dose (p=0.002 and 0.001, respectively). Efficacy was unaffected by infection. Time to first catheter infection did not differ between arms. Low-dose subjects experienced significantly more frequent bleeding at all stages of ITI and also during post-ITI prophylaxis (HR 2.56, p 0.000067). Approximately 90% of these bleeds occurred in the 1st phase of ITI (before the Bethesda titre became –ve). No difference in efficacy could be demonstrated between the two treatment arms though
tolerance was achieved more slowly by low-dose subjects. The study was terminated because a significantly higher bleeding rate was observed in the low-dose arm and because it was judged to have insufficient power to prove therapeutic equivalence.

Genetic predictors of inhibitor development. J. Astermark (Sweden), J. Oldenburg (Germany)

We report on results from an association study using a candidate gene panel on 680 subjects including 331 inhibitor patients from 3 different multi-center cohorts: the Hemophilia Inhibitor Genetics Study (HIGS), the Malmö International Brother Study (MIBS) and the Hemophilia Growth and Development Study (HGDS). Analysing the haplotypes H3 of the F8 gene did not result in an increased inhibitor risk (OR 0.65). Thus FVIII polymorphisms and haplotype mismatch were not associated with increased risk of inhibitors, as it has been reported in the literature. Applying the investigation of 14,626 SNPs that are distributed all over the genome 121 SNPs were associated with inhibitor formation. The most significant candidates were Mitogen Activated Protein Kinase 9 (MAPK9, OR=2.5) which plays a key role in the regulation of lymphocyte activation and proliferation and the Glycoprotein V (CD36, OR= 0.6) a Membrane glycoprotein on a variety of cells including platelets, monocytes, macrophages and endothelial cells. A third candidate was the Receptor Type Tyrosine-protein Phosphatase R (RTTPPR, OR=0.5), a member of the protein tyrosine phosphatase (PTP) family. The same cohorts support earlier findings that the HLA Class II haplotype DQB*0602/ DQB1*1501 seems to be associated with a higher risk of inhibitor development.

Regulatory perspective on clinical development of FVIII / IX concentrates: FDA – N. Jain (USA)

The new/next generation FVIII/FIX products are bioengineered recombinant products that have been modified to potentially improve rFVIII/FIX biosynthesis, functional activity half life and immnnmogenicity properties of the FVIII/FIX molecules. Currently, from a regulatory standpoint, the evaluation of safety, efficacy and adequate quality control of these products remain a challenge and raises more questions then answers. The one stage and chromogenic assays are standard potency assays that are used to measure the FVIII/FIX activity in clinical samples and to label the vial of the product. Can these assays be used to measure the potency of the new/next generation products with altered functional properties? It is possible that a potency assay specific for the product type may need to be developed and validated. The question of assigned unitage to express FVIII/FIX activity will also need to be addressed. Parameters to establish safety in pre-licensure clinical trials remains the biggest challenge. The use of currently established parameters (exposure days) and statistical approach to evaluate immunogenicity may need modification. For the use of a different potency assay, unitage and dosing schedule an extensive education programs for both patients and treaters may need to be considered. In conclusions, the safety and efficacy of these new/next generation products will be evaluated on the currently established criteria until further information is available to guide their assessment in other ways.
EU Regulatory perspective on clinical development of FVIII / IX concentrates: EMA – A. Hilger (Germany)

In the European Union the clinical trial design for recombinant and plasma-derived Factor VIII/Factor IX products is based on the requirements of specific Notes for Guidance on the Clinical Investigation of FVIII and FIX. The drafting and discussion of these publicly available documents is in the responsibility of the Blood Product Working Party (BPWP) located at the European Medicines Agency. Following new European legal requirements (e.g. paediatric regulation) and progress on scientific knowledge these NFGs are currently under discussion. During the external consultation phase contributions of interested and concerned parties e.g. patient organisations, hemophilia treaters, industry, academia and regulators are received and will be taken into account. Main critical issue within the drafting process is the implementation of studies in children. Especially timing of these studies and investigation in specific patient cohorts (e.g. PUPs) are of particular interest.

Section 4. Standardization issues
Chairs: J. Oldenburg (Germany) and L. Valentino (USA)

New clotting factor concentrates – Biosimilars or biosuperiors? How are they to be evaluated for clinical use? L. Valentino (USA)

The management of patients with severe hemophilia requires replacement of the deficient clotting factor: factor VIII (FVIII) for hemophilia A and factor IX (FIX) for hemophilia B. Although the infectious risks associated with factor replacement therapy have been largely eliminated through enhanced donor screening and rigorous viral testing, inactivation, and elimination techniques for human plasma-derived concentrates and the introduction of recombinant FVIII and FIX products, other problems remain. The development of alloantibodies that inhibit clotting factor activity continues to complicate treatment, affecting up to one-third of hemophilia A patients and about 5% of hemophilia B patients with severe disease. Furthermore, the implementation of and adherence to FVIII or FIX prophylaxis, considered standard of care for persons with severe hemophilia, is hampered by high cost and the need for frequent intravenous infusions. Current research is focused on developing less immunogenic and longer-acting FVIII and FIX drugs that uniquely promote hemostasis, are more affordable, and do not require intravenous administration. This article reviews the emerging therapeutic agents that are expected to revolutionize hemophilia care within the next 3 to 5 years.

Evaluating new concentrates – What are the issues? M. Lee (USA)

Recently there have been directives from regulatory authorities, in particular the European Medical Agency (EMEA), regarding the design of clinical trials with Factor VIII and IX. Not surprisingly, the biggest issues concern the conduct of pharmacokinetic (PK) evaluations and inhibitor risk determination, the primary concerns in assessing the viability of new coagulation factor concentrates. The SSC has on previous occasions codified the design of PK studies in adults (Lee, Morfini, et al 2001), but has left the
issue of pediatric PK evaluation to be determined. This is particularly salient given the recent EMEA directive on pediatric studies indicating a 7 time point design. Given the difficulties in blood draws in this population and the recent paper from Bjorkman et al (JTH, 2010), it is suggested that a smaller experiment will still allow for the determination of recovery, modified AUC and elimination rate/half-life. Insofar as inhibitor risk determination is concerned, the primary issue is sample size. Regulatory authorities have indicated a minimum number which are based on either logistic feasibility or rigid statistical calculation. The latter is based on an approach requiring a limitation on the upper bound of a confidence interval for the true risk. However, this method has been previously shown to have poor power to accept a low risk preparation (Lee and Roth, 2006). A preferable alternative is to use a Bayesian approach that incorporates prior risk data in order to achieve a prediction interval to which a high probability can be assigned with acceptable power. The main impediment here is determining the prior distribution of risk, particularly when a new preparation is involved. It is proposed that data from all like products be pooled and the Bayesian methodology be adopted as an SSC accepted approach.

**Evaluating new factor concentrates: Regulatory perspectives of the EMA – A. Hilger (Germany)**

Numerous new factor concentrates are currently under development with different biotechnological approaches e.g. to extend plasma-half life in order to reduce the frequency of treatment. In the EU the clinical development program for all medicinal products has among other conditions to comply with paediatric requirements as laid down in Regulation (EC/1901/2006). Accordingly, prior to clinical investigation in children a paediatric investigation plan needs to be approved by the Paediatric Committee (PDCO) located at the European Medicines Agency. Currently European Pharmacopoeia monographs are available for plasma-derived Factor VIII /Factor IX and recombinant Factor VIII. Since specific monographs for novel products are not yet in place, the existing monographs should be taking into account. In view of the complex and variable physico-chemical, biological and functional characteristics of factor concentrates, it will not be acceptable to submit a reduced clinical dossier when claiming similarity to a reference medicinal product. As a result, applications for such similar products will still need to satisfy the safety and efficacy requirements described in the EU-BPWP clinical guidelines for “new products”. However, applicants do have the possibility to discuss particular deviations from guidelines within the scope of an EU-Scientific Advice procedure. In general, same quality, efficacy and safety evaluation standards apply for marketing authorisation of new factor concentrates within the EU.

**Evaluating new factor concentrates: Regulatory perspectives of the FDA – M. Weinstein (USA)**

A new factor concentrate is defined here as a recombinant plasma protein analogue that has been deliberately modified or associated with other entities, to result in a product with novel properties compared to those of the native plasma protein. Novel properties may include different pharmacokinetics, pharmacodynamics, specific activity,
immunogenicity or efficacy compared to the native protein. The FDA expectations for the licensing of these “new” factor concentrates rest on the same principles as for conventional analogues: that the manufacturer ensure the safety, purity and potency of the concentrate. Among safety considerations to ensure that benefits outweigh risks are an absence of risk from adventitious agents, and acceptable immunogenicity. Efficacy is typically demonstrated by manufacturers through studies providing in vitro potency, pharmacokinetic and phamacodynamic data, and adequate and well-controlled clinical trials in relevant populations. The study findings are translated into appropriate labeling information for dosing amount and frequency. Potency is established by linking assay methods and standards to a defined biological effect, and is maintained by ensuring product stability. Manufacturers establish purity by demonstrating clearance of adventitious agents, developing an acceptable impurity profile, having a validated consistent manufacturing process, and characterizing the physiochemical properties of the product. A comprehensive understanding of the biophysical characteristics of a “new” factor allows a manufacturer to design and control the manufacturing process, conduct developmental studies, assess potential post marketing changes in manufacturing, and ensure product quality during development, at approval, and over the post-approval lifecycle of the product. Product characterization typically involves state-of-the-art analytical methods to assess numerous physical parameters (e.g. primary, secondary, tertiary, quaternary structure; glycosylation, aggregates, etc.); stability studies; in vitro and preclinical studies; and bioassays to assess the occurrence of neutralizing antibodies in non-clinical and clinical studies. Issues related to the use of how these characterization studies might apply to pegylated recombinant coagulation factors were discussed. Similar considerations could be used in the study of fusion proteins (e.g., recombinant analogues fused with an Fc fragment). The new factor concentrates also present challenges to potency determination and assignment. Potency tests should measure the specific ability or capacity of the product to achieve a defined biological effect. Does each new form require its own reference standard? What assay best reflects clinical outcome and is accessible in a clinical testing laboratory? When and how should methodology be changed? A possible approach would be to collect data during the product development stage and determine an acceptable dosage regimen based on analytical results and clinical outcome. In the case of new FVIII products, this could involve the collection of data using both the one stage clotting assay (OC) and the chromogenic substrate assay (CS); the correlation of potency determined by the sponsor to that derived from clinical labs that do not have the sponsor’s internal reference material (traceable to an international standard); and the use of a correction factor to reconcile the difference in results between the OC and CS methods. However, with some new products, the unitage may have to be defined differently, including the possible dosing by protein content (mass) instead of activity. Recommendations for studies in support of regulatory applications are made on a case-by-case basis, considering previous experience with similar products, and situations. FDA works with other institutions and industry to resolve issues related to the use of new technologies in the development of new products. The findings and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or
Completed and new standardization projects on Standards – A. Hubbard (UK)

Replacement WHO International Standards for the estimation of factor VIII in plasma and concentrates were established by WHO in October 2009. The WHO 6th IS Factor VIII/VWF Plasma (07/316) was assigned values for 5 analytes (FVIII:C, FVIII:antigen, VWF:antigen, VWF:RCo, VWF:CB). There was very good agreement between the mean estimates for FVIII:C by the one-stage clotting method (0.67 IU/ampoule; n=31) and the chromogenic method (0.70 IU/ampoule; n=20). The WHO 8th IS Factor VIII Concentrate (07/350) was assigned a value for FVIII:C by assay relative to the WHO 7th IS FVIII Concentrate. Excellent agreement in the mean estimates by both one-stage clotting and chromogenic methods has allowed a single potency of 9.4 IU/ampoule to be assigned. Two ongoing studies for the replacement WHO 4th IS Factors II, VII, IX, X, Plasma and the WHO 2nd IS VWF Concentrate are scheduled for completion this year with establishment by WHO in October 2010. New projects for the replacement of the WHO IS Factors II and X Concentrate and the WHO IS Factor VII Concentrate will begin in 2010 with scheduled completion in 2012.

4th International standard for blood coagulation factors II, VII, IX and X (Plasma) – E. Gray (UK).

Twenty-nine laboratories from 14 different countries participated in a collaborative study to value assign the proposed 4th International Standard for Blood Coagulation Factors II, VII, IX and X, Plasma (09/172). Local normal pooled plasmas were included to provide information on any drift of the “plasma” unit. The intra-laboratory variability was low. Over 75% of the laboratories obtained potency estimates that had intra-laboratory geometric coefficients of variation (GCV) of less than 5% when the proposed standards were assayed against the 3rd International Standard, indicating that the participants performed assays for factors II, VII, IX and X reproducibly and with high precision. For all four factors, inter-laboratory variability was low for estimates of the proposed 4th International Standard against the 3rd International Standard (GCV <3%), and higher against the local plasma pools (GCV 5 - 11%). There were no major differences in potency estimates related to the use of different reagents and methods and the potency estimates from chromogenic and clotting assays were therefore combined to give the overall mean potencies. Potency estimates calculated relative to the local normal plasma pools were 1%, 6%, 6% and 5% lower for FII, FVII, FIX and FX respectively when compared to potencies calculated relative to the 3rd International Standard and the differences in the estimates were significant for FVII, FIX and FX. The differences in the potency estimates could not be attributed to degradation of the 3rd International Standard, but the variability of the plasma pools could be an important contributing factor. It is therefore recommended that the proposed 4th International Standard for Blood Coagulation Factors II, VII, IX and X, Plasma (09/172) be assigned with potencies for functional activity, calculated relative to the 3rd International Standard for Blood.
Coagulation Factors II, VII, IX and X, Plasma: 0.89, 0.99, 0.86 and 0.89 IU/ampoule for FII, FVII, FIX and FX respectively.

FVIII inhibitor standard – K. Mertens (Netherlands)

The development of a FVIII inhibitor standard was initiated at the 2003 Subcommittee Meeting, and subsequently endorsed by WHO and EMEA. The initial study, which included 22 laboratories and 5 candidate materials, has been performed by Dr. Raut from NIBSC. He presented the study at the SSC meeting in Oslo in 2006. However, the report has not submitted to the 2006 SSC Business Meeting because of major concerns expressed in the Subcommittee. These mainly related to the overall poor quality of the data that the 22 participants had generated, both inter- and intra-laboratory variability being undesirable high (CVs up to 36%). Moreover, recalculating the results relative to any of the 5 candidate preparations only slightly improved the inter-laboratory CVs, suggesting that the new standard would have been of limited benefit. Other concerns were the viral status of candidate standard 05/206 (HCV and HIV positive), and the uncertainty with regard to statistical validity of the inhibitor assays. In 2007, a Working Party was established (Chair: K. Mertens) with the aim to assess the feasibility a ‘post-hoc’ statistical validity check in order to identify reliable inhibitor assays. At the 2008 SSC meeting, Dr. Mertens discussed several options to proceed, including a new, smaller study under more controlled conditions. However, this would create the need for additional candidate materials from human origin. These now have become available as recombinant antibodies cloned from the immune repertoire from haemophilia patients. The next step will be a limited feasibility study, that is intended to be reported at the 2011 SSC meeting. The audience was encouraged to send any additional suggestions to Dr. Mertens (k.mertens@sanquin.nl) for further discussion in the FVIII inhibitor Standardisation Working Party.

FVIII field study – Comparison of a BDD product with a full length product. D. Lillicrap (Canada).

This study has evaluated the reliability and reproducibility of routine FVIII assays used to quantify FVIII levels with two FVIII concentrates, one, a new B domain truncated product, N8, and the second, a full-length product, Advate. Four spiked hemophilic plasmas were used as the test materials with levels of FVIII of 0.03, 0.20, 0.60 and 0.90 U/mL. Routine FVIII assays were used in 33 laboratories around the world, in the majority of cases one stage assays, but in 5 laboratories, chromogenic assays. The results of this field study show that the two products show very similar results with the two FVIII assays. With the one stage assay, both products show overestimates of FVIII at the low concentrations and slight underestimates of FVIII at the highest level tested (0.90 U/mL). With the chromogenic assay, all results were slight overestimates for both products. The CV for both assays and both products was very similar.

Closing remarks – A Srivastava (India)
In his closing remarks, the chair thanked all the co-chairs, speakers and attendees for their participation and closed the meeting at 1:00pm on the 25th of May, 2010.
Factor XIII and Fibrinogen

25 May 2010
Cairo, Egypt

Chairman:  Hans Kohler (CH)
Co-Chairmen:  Moniek de Maat (NL), Aida Inbal (IL), Jaap Koopman (NL), Muriel Maurer (US), Leonid Medved (US), Marguerite Neerman-Arbez (CH), John Weisel (US)

Part 1: Fibrinogen

Dr Sanj Raut of the National Institute of Biological Standards and Controls (NIBSC) already informed during the Boston Meeting 2009 that in 2011 the stocks of the 2nd International Standard for Fibrinogen Plasma (98/612) and the 1st International Standard of Fibrinogen Concentrate (98/614) will be exhausted. Material has been sourced to replace these reference materials. The plan is to start collecting data on the material in June 2010. An update was given by Dr Colin Longstaff on both replacements as well as on the concomitant calibration of Lot 4 of the SSC/ISTH Secondary Coagulation Plasma Standard. Definitive candidate fills for all three standard materials are completed. The samples for the International Collaborative Studies will be sent out in July 2010. Analysis of returned data will be performed in October 2010. Stability studies will be completed in November 2010. Reports to participants and to the WHO will be made in 2011. The final report will also be presented at the next SSC meeting in Kyoto, Japan, 2011.

Dr Nicola Mutch gave an interesting overview about the role of polyphosphate in modifying structure and function aspects of the fibrin network. Polyphosphates are negatively charged polymers that are released from dense granules upon platelet activation. Dr Mutch showed that polyphosphate binds to fibrinogen and soluble fibrin, induces formation of denser fibrin clots and downregulates fibrinolysis, when present during fibrin formation, by attenuating binding of t-PA and plasminogen to fibrin.

Dr Marlien Pieters gave an update on standardisation of fibrin network characterisation methodologies. At the last SSC meeting in Boston, 2009, Dr Pieters showed an inventory of the methods and levels measured in healthy controls for permeability of a fibrin clot in order to decide whether standardisation was required. A wide range in mean permeability (> 4-fold) was reported for different studies. The question was brought up whether standardisation is needed for this variable since it is not used in clinical diagnosis. It was decided then that some standardisation would be welcome to enable comparisons between different studies.

At today’s meeting, Dr Pieters proposed standard criteria and conditions for the measurement of fibrin clot permeability in regard to plasma preparation, permeation buffer, thrombin and CaCl₂ concentrations in the activation mix, incubation times, clot and buffer containers, and permeate collection. The proposed method has been sent out for comments and an inter-laboratory variability study will be planned.
Dr Leonid Medved described molecular mechanisms of fibrin-VE-cadherin interaction and anti-inflammatory properties of fibrin-derived peptides. Interactions of fibrin with endothelial cells via VE-cadherin are important for angiogenesis and wound healing. Earlier, the interaction of the β15-42 sequence of fibrinogen with VE-cadherin was shown to be important for capillary formation. Dr Medved showed results from mapping studies confirming the binding sites in fibrin and VE-cadherin. Furthermore, fibrin-derived peptides β15-42 and β15-66, and particularly the dimeric form, showed anti-inflammatory effects by inhibiting leukocyte migration and exhibited a cardioprotective effect by reducing reperfusion injury after myocardial infarction.

Part 2: Factor XIII

Unfortunately the presentation by Dr Muszbek, entitled “Challenges of assigning a FXIII-B value to the 1st International FXIII standard plasma; scientific and methodological considerations” had to be cancelled.

As discussed earlier there is a need for determining the total FXIIIB in the FXIII WHO reference material since FXIIIA half-life depends on the amount of available FXIIIB. To address this issue Dr Akitada Ichinose proposed a project of a pilot study to establish the normal value of FXIIIB concentration using the international standard plasma. Dr Ichinose first gave an overview over the heterogeneity, function, and possible role of FXIIIB in various diseases incl. congenital and acquired FXIII deficiency, emphasizing the need for a FXIIIB standard as well as standardisation of FXIIIB measurement. In regard to a FXIIIB standard material, Dr Colin Longstaff from NIBSC explained, that in order to add a value (in this case FXIIIB value) to an existing International Standard (in this case the International FXIII Plasma Standard), approval must be seeked from WHO and a significant global need (in respect to numbers of people affected, costs, resources etc.) must be justified. In regard to methods for FXIIIB measurement, it was agreed that antigen assays are easier to establish than functional assays, but specific antibodies are needed to distinguish between tetrameric and free FXIIIB. Dr Diane Nugent recommended to approach Novo Nordisk in regard to an antibody specific for free FXIIIB (formerly from Zymogenetics).

Little data is available on acquired FXIII deficiency states. However, acquired FXIII deficiency can lead to clinically relevant bleeding complications. Dr Akitada Ichinose presented the methodology and results from a national survey he carried out in Japan in 2009, together with plans for a second study in Japan later this year. From these results it was estimated that there could be 1 case per million. Dr Ichinose proposed an international collaborative study to collect more data on this topic. He suggested to establish a working party since consensus on many issues such as definitions and diagnosis of acquired FXIII deficiency, laboratory tests, preparation of questionnaires, data collection and analysis must be reached first.

The source of plasma FXIIIA is still under debate. Furthermore, FXIIIA lacks a signal peptide and cannot be secreted via the classical ER-Golgi pathway. Earlier results by Dr Paul Cordell suggested that platelets are unlikely to be the main source. In his current
work Dr Cordell investigated the distribution of FXIIIA in monocyte-macrophages (primary cells and THP-1 cell line) and he showed that FXIIIA is enriched in podosome-like structures and is associated with Golgi proteins. Dr Cordell explained that some Golgi proteins are involved in non-classical secretion. He concluded that the association of FXIIIA with Golgi proteins may suggest a mechanism of non-classical secretion and the podosome-like structures may represent machinery for secretion.

Dr Verena Schroeder presented data on a recently discovered link between the complement system and coagulation involving fibrinogen and FXIII. MBL-associated serine protease 1 (MASP-1), a serine protease of the complement lectin pathway, was shown to be able to activate FXIII and fibrinogen, however, this was investigated only in purified systems so far. Dr Schroeder showed that MASP-1 activates FXIII in plasma in a dose and time dependent manner. Furthermore, FXIII activation by MASP-1 is also dependent on FXIII Val34Leu genotype. Using a turbidimetric assay, it was shown that MASP-1 also induces fibrin clot formation in plasma and has an additive effect even in the presence of thrombin. MASP-1 may therefore be relevant in physiological and/or pathophysiological clot formation.

Recombinant FXIIIA will soon be available for treatment of FXIII deficiency. Dr Diane Nugent showed data on pharmacokinetics of recombinant FXIIIA2 in patients with congenital FXIII deficiency and reported that rFXIII was well tolerated (no serious adverse events, no inhibitor formation) and efficient in restoring normal clot solubility. Intriguingly, after administration of rFXIII, free B-subunit levels were reduced and back to normal levels only after two weeks which raises interesting questions about normal association/dissociation rates of the tetramer or regulation of FXIIIB. In addition, B-subunit polymorphisms such as His95Arg may also influence association/dissociation of the subunits and hence the pharmacokinetics. Taken together, these studies again raise important issues in regard to the specific measurement of both FXIII subunits in plasma and emphasises the need for standard materials of recombinant FXIIIA2 and, as mentioned above, FXIIIB since FXIIIA half-life depends on the amount of available FXIIIB as shown by Dr Nugent.
Chairman: Colin Longstaff (UK)
Co-chairmen: Carl-Erik Dempfle (DE), Ann Gils (BE), Dirk Hendriks (BE), Osamu Matsuo (JP), Michael Nesheim (CA), Tetsumei Urano (JP)

The WHO 2nd International Standard for Streptodornase

Calibration of tPA antigen in the SSC/ISTH secondary plasma standard lot 3 and lot 4?

Update on standardising PAI-1 antigen measurements in plasma
Colin Longstaff, UK

Dr Longstaff opened the meeting with an update on previous WHO International Standards (IS) that had been through the Fibrinolysis Subcommittee. The WHO 2nd IS for Streptodornase, approved by the SSC in 2009 was subsequently endorsed by the ECBS of WHO in October 2009. The earlier WHO 1st IS for tPA antigen in plasma, approved in 2007 was the subject of an Official SSC Communication, currently in press in J Throm Haemost. tPA antigen was approved for inclusion on the list of analytes for the SSC/ISTH plasma coagulation secondary standard lot 3 in 2007 with a value of 3 ng/vial. Currently calibration of the next batch, lot 4, is underway and participants in the earlier study were contacted to assess the interest in including tPA antigen as an analyte in lot 4, however, there was not a good response and currently there is not enough interest among manufacturers to conduct a calibration study. Dr Longstaff then presented an update on the determination of PAI-1 antigen in plasma. Previously a collaborative study was conducted in 2007 and results were extremely scattered indicating that the ng/ml assigned to the calibrators in different methods and kits are not consistent. The spread of results was significantly improved using a harmonisation process but this does not enable assignment of a value to a standard in real gravimetric units. A proteomics approach was proposed and outlined as a possible new approach to establish the actual PAI-1 concentration in plasma, which could lead to the establishment of an IS for PAI-1 antigen.

Program to replace the WHO International Standard for Urokinase and other serine proteases
Craig Thelwell, UK

Dr Thelwell discussed the current WHO 1st International Standard for HMW-Urokinase (87/594) which is becoming depleted, with only 300 ampoules remaining and an average usage of around 80 per year. The program to replace this standard is expected to be approved by WHO this year, and will take around 2 years to complete in association with the SSC Fibrinolysis subcommittee. The replacement IS will be used
by manufacturers to assign potencies to urokinase used clinically as a thrombolytic. The source material should be a purified preparation from human urine, however the manufacturer has yet to be identified. Urokinase has been largely superseded by alternative thrombolytics although it has retained a niche market. With few manufacturers and laboratories performing urokinase assays, recruitment to a collaborative study may be difficult and the study size may be small. It is expected that the replacement will be calibrated in IU relative to the 1st IS (87/594), using fibrin clot lysis methods. It is also proposed to include the molar concentration of the replacement standard, as a dual label alongside the IU. This could be achieved using active-site titration, and the methodology is being optimised at NIBSC using stopped flow kinetics. Data were presented on possible methods and potential sources of error for active site titration of urokinase. Associated reference materials to calibrate the titrations are also being trialled in order to improve results between laboratories. It is hoped to identify other laboratories with the capability to perform this method to take part in the study. Laboratories interested in participating in any part of the study are encouraged to contact Craig Thelwell or Colin Longstaff.

**PAI-1 and TAFI measurements on samples from inflammatory bowel disease patients**
Ann Gils, Belgium

Dr Gils presented work on changes in PAI-1 and TAFI levels in subjects with the most prevalent inflammatory bowel diseases, (IBD): Crohn’s disease (CD) and ulcerative colitis(UC). Although the pathogenesis of IBD is not well understood, there is strong evidence that coagulation and the immune system could play a role. A number of studies have reported a higher incidence of thromboembolic events in IBD patients. Current treatment is the administration of either calcium heparin or low molecular weight heparin. An alternative approach to prevent thromboembolic complications in IBD patients is to increase the fibrinolytic capacity. PAI-1 and TAFI levels were investigated in IBD patients to investigate whether targeting PAI-1 and/or TAFI would be a valuable strategy in the prevention of TE events. Data from a number of different ELISA assays were presented revealing decreased intact TAFI antigen levels and a tendency to increased TAFI activation peptide levels in IBD patients versus healthy controls.

**Applying functional TAFIa (CPU) assays to samples from hemophiliacs**
Mike Nesheim, Canada

A further update was presented regarding a sensitive assay for functional TAFIa (CPU) in plasma. Assays for the active enzyme TAFIa, are challenging due to the vast excess (~5000-fold) of zymogen, and the presence in plasma of constitutively active CPN. The assay described is based on the ability of TAFIa to release bound fluorescent plasminogen from soluble high molecular weight fibrin degradation products that have covalently attached QSY moieties which quench the fluorescence of the bound plasminogen. When the plasminogen is released the fluorescence intensity increases and the rate of this can be used to measure the level of TAFIa in the sample. Details of a refinement were presented which greatly improves the yield of QSY-labelled FDPs. The basal level of TAFIa in a small group of normals was shown to be 20 pM. Previous data had suggested a hypothesis that bleeding in hemophilia is due not only to impaired
coagulation but also to impaired down regulation of fibrinolysis and the role of TAFIa generation in haemophiliacs was investigated in collaboration with Dr. Brummel–Ziedens and Dr. Rivard who provided whole blood samples from controls and haemophiliacs using the sensitive assay. The results showed impaired TAFI activation in the hemophiliac samples and the magnitude of the defect tracked with the severity of the disease as defined by the FVIII level at the time of the blood draw. In addition, a significant correlation was found between the TAFIa potential and the average five year bleeding score. These data support the further collaboration with Dr Dirk Hendriks and coworkers to continue to compare results obtained with their assay and this method. Wider application of the assays should yield new insights into the physiology and pathophysiology of the hemostatic and fibrinolytic systems.

Comparative study on proCPU/TAFI assays
Evelien Heylen, Belgium

Evelien Heylen from Dirk Hendriks’ laboratory presented initial results from a study comparing the most widely used commercially available proCPU (TAFI) assays, including antigen kits as well as activity based assays. For many years, the lack of thorough validation of proCPU assays and the desirability of an internationally recognized standard have been subjects discussed at the Fibrinolysis Subcommittee of the SSC. Problems with standardisation mean results from numerous published studies describing the biological variation in proCPU plasma concentration and possible correlations with thromboembolic events must be interpreted with caution. In the present study, a thorough validation of these assays was performed, using genotyped plasma samples of healthy individuals (n = 130), aiming to identify reliable proCPU assays. A reference method was selected and used to compare other commercially available kits. Good agreement was seen with some assays but others showed discrepancies due to sensitivities to known variants at Thr/Ile 325 leading to underestimation of proCPU concentrations.

Global fibrinolysis assays
Tetsumei Urano, Japan

Dr Urano discussed the utility of Euglobulin Clot Lysis Time (ECLT) to measure the whole process of fibrinolysis and the efficacy of thrombolytic drugs. Data were presented on an investigation of ECLT, tPA-supplemented plasma clot lysis time (tPA-PCLT) and Spontaneous Clot Lysis Time (SCLT). These studies are needed to investigate the effect of drugs such as those that promote formation of latent PAI-1. ECLT appeared to well represent the capacity of plasma to express tPA activity which is essentially determined by the balance between tPA and PAI, though ECLT did not detect TAFIa and FXIIIa dependent inhibition of fibrinolysis. tPA-PCLT appeared to well detect TAFIa dependent inhibition of fibrinolysis, though it did not detect the capacity of plasma to express tPA activity. SCLT appeared to mainly represent TAFIa and FXIIIa-dependent inhibition of fibrinolysis. Each so-called global fibrinolytic assay has both advantage and disadvantage and appeared not to equally describe the whole process of fibrinolysis. There is a need to establish a new method which covers whole process of fibrinolysis. At this moment, it seems important to understand these limitations of existing global fibrinolytic assay.
Comparison of methods used to study fibrinolysis
Nicola Mutch, UK

Dr Mutch presented an overview of commonly used methods to measure fibrinolysis potential in plasma, highlighting their various advantages and disadvantages. Methods which rely on monitoring clot turbidity changes during lysis are simple and adaptable and provide the opportunity for manipulation of clot composition. However, these systems are static. Flow is investigated in the Chandler Loop system and this system also retains a high degree of flexibility in terms of clot composition, so haemostatic proteins, antibodies and cells can be investigated easily. However, this system is not suited to high throughput screening. A system that does include the possibility of studying the effect of the endothelial cell surface is currently lacking but is desirable.

TEG and ROTEM results from fibrinolysis studies were also briefly reviewed. These methods suffer from poor reproducibility and have limited capacities for handling multiple samples. Some other methods may be particularly suited to specific studies and should not be overlooked, such as in situ zymography and confocal microscopy.

No method can be applied to all studies but the choice of method needs to be tailored to the questions being asked.

Dr C-E Dempfle chaired to final part of the program on D-dimer.

Update on the NEQAS EQA for D-dimer
Ian Jennings, UK

Dr Jennings presented the latest findings from UK NEQAS studies on D-dimer determination in clinical laboratories. Several problems have been identified which contribute to the poor comparability of data across laboratories and between methods. Significant sources of problems and errors include the large range of different reporting units in use and standardisation of D-Dimer units is required to improve this situation. Conversion of units to/from DDU and FEU – against proposed guidelines from the CLSI, is a source of concern, whilst guidelines for cut-off values for the exclusion of VTE are also not applied consistently. Consequently, laboratories use inappropriate cut-off values or fail to interpret results correctly. A clear source of within-centre variation is, in some cases, instrument-related. This may be influenced by lyophilised plasma, which in turn may have implications for future reference material preparation. Over many years of exercises it has been observed that between-centre variation is improving, but is still sub-optimal. Availability of a reference plasma for D-Dimer measurement would likely improve variability and is recommended.

Production and analysis of fibrin degradation products
Mike Nesheim, Canada

Many calibrators in commercial D-dimer kits are made from fibrin clots digested with plasmin and Dr Nesheim summarised a series of studies on the nature of the soluble products released from crosslinked fibrin. These in vitro studies were performed by perfusing a clot with plasmin, passing the perfusate through a MALLS light scattering device coupled in tandem with a UV detector, and collecting fractions for subsequent analysis by SDS-PAGE. The light scattering and UV measurements allow
determination of the weight-average molecular weight of the soluble FDPs in the
perfusate and the SDS-PAGE provided an assessment of the covalently bound
components and the extents of alpha, beta and gamma chain cleavage by plasmin.
The results showed that soluble products with an average molecular weight of
6,000,000 are released from the clot. These were subjected to gel filtration
chromatography. A broad distribution of products was found with molecular weights
ranging from 250,000 to 10,000,000. About 50 percent cleavage of alpha, beta and
gamma chains was observed. Twenty percent cleavage through all three chains at D-E
junctions was found. These studies provide the identity and concentrations of soluble
FDPs and allow for the preparation of well characterized isolated FDPs with a wide
range of molecular weights to be used as standards or tools to better characterize D
dimer assays.

**Plans for a collaborative study to prepare a reference preparation for D-dimer**
Steve Kitchen, UK

Dr Kitchen outlined proposals for a study to develop an IS for D-dimer and highlighted
the need for such a standard based on results from numerous studies where wide
variations in reported values of D-dimer were observed in laboratories measuring
common samples. It has been demonstrated that the spread of data can be improved
by using a common calibrator. The nature of the optimum common calibrator was
discussed and it was suggested that a pool consisting of a large number of patients’
plasma samples with high D-dimer levels may be the most appropriate material. Such a
pool has been collected and will be filled and lyophilised at NIBSC by Dr C Longstaff to
produce around 500-600 ampoules, sufficient for trial studies. Initially a collaborative
study will be organised which may include other samples, for example fibrin degradation
products prepared from plasmin digestion of a fibrin clot. Details of the project,
samples, methods, calibration etc, will be discussed by a proposed Working Party,
which was announced by Dr Kitchen.

There was no further business and the meeting was adjourned.
Hemostasis and Malignancy

25 May 2010
Cairo, Egypt

Chairman: Agnes YY Lee (CA)
Co-chairmen: Giancarlo Agnelli (IT), Dominique Farge (FR), Charles Francis (US), Alok Khorana (US), Marina Marchetti (IT), Martin Prins (NL), Wolfram Ruf (US)

Attendance: A Lee, D Farge, W Ruf, M Marchetti, M Prins
Invited Speakers: M Carrier
Regrets: A Khorana, C Francis, G Agnelli, J Zwicker, H Liebman

There were 7 presentations updating subcommittee activity and discussing new proposals.

Dr. Wolfram Ruf presented data on tissue factor (TF) and intracellular signalling in cancer growth and metastasis. The data for TF's proangiogenic, prometastatic and prothrombic activities were presented. Tumor cell PAR2 signaling promotes the angiogenic switch in cooperation with the TF cytoplasmic domain. Selective inhibition of TF signaling is sufficient to attenuate tumor growth and angiogenesis and has potential synergistic benefit with anti-VEGF therapy. TF phosphorylation may represent a biomarker for ongoing TF signaling and identify those who may benefit from anti-TF therapy. The utility of TF+ microparticle (MP) as a marker for TF-dependent tumor progression and cancer-associated thrombosis requires further investigation.

Dr. Marina Marchetti presented an update on the development of a reference material for measuring tumour tissue factor. TF standard preparations are not suitable for measurement of TF in cancer cell cultures. A tumour cell line with very high expression of TF has been identified as a possible source of standard material for performing a pilot study and developing the standard materials. The importance of the influence of culture environment and other experimental conditions were discussed. It was also brought up that the heterogeneity of TF expressed by different tumour types could make standardization even more challenging.

Dr. Martin Prins presented expanded analysis of the IMPACT study, which evaluated the effect of nadroparin on survival in patients with lung, pancreatic or prostate cancer. The overall results showed no effect with nadroparin on survival, although the regimen was well-tolerated. Adjusting for potential imbalance between the groups (e.g. contamination) and for prognostic factors (cancer type, country, metastasis, Karnoskfy performance, and creatinine clearance) did not show any benefit. It was discussed whether this negative result may indicate a dose issue (i.e. under dosing) rather than a true lack of antitumour effect. This may be answered pending the results of the CONKO and FRAGEM studies (see below).
Dr. Khorana’s presentation on “Prophylaxis in outpatients – time for action?” was given by Dr. Lee. The review highlighted the current evidence for outpatient prophylaxis. Although previous studies have shown negative results with LMWH prophylaxis in a variety of patients with solid tumours receiving outpatient chemotherapy, three major trials recently found significant reductions in venous thrombosis with LMWH. The PROTECHT study showed standard prophylaxis dose of nadroparin reduced the combined endpoint of arterial and venous thrombosis in a mixed population of patients with solid tumours, while the CONKO 004 study and the FRAGEM study found significant reductions in symptomatic thrombosis in patients with advanced pancreatic cancer who received full or half-therapeutic doses of a LMWH. It was discussed whether these results will lead to a change in practice or indeed, a change in current guidelines to recommend prophylaxis in these patients.

Dr. Carrier presented the results of a meta-analysis determining the incidence of venous thromboembolism in patients receiving either thalidomide- or lenalidomide-based chemotherapy for multiple myeloma. He emphasized that the events are largely captured as adverse events rather than predefined study outcomes, that the reporting is inconsistent and that prophylaxis with ASA, warfarin or LMWH are often used in an uncontrolled fashion. Furthermore, there are no randomized controlled trials or adequately performed cohort studies to establish the efficacy and safety of any antithrombotic agent for primary prophylaxis in this setting. Hence it was concluded that the available data used to estimate thrombotic event rates are unreliable and also call into question the regimens used for prophylaxis in these patients. It was discussed whether the subcommittee should produce a position paper to encourage the introduction of venous thromboembolism as a standard safety outcome in oncology trials, especially in phase III protocols evaluating new agents or regimens.

Dr. Liebman’s presentation on “Unexpected pulmonary embolism in patients with cancer – is it relevant?” was given by Dr. A. Lee. Studies have reported that up to 3.4% of CT scans done for routine re-staging reports the presence of unexpected or incidental pulmonary embolism. Based on data from his institution (Casey et al J Clin Oncol 2006;24:4928), many cases involve proximal pulmonary vasculature and symptoms of pulmonary embolism (PE) (e.g. fatigue, shortness of breath) are often present. Patients with proximal PE also share the same prognosis as symptomatic patients with PE. Clinical factors associated with incidental PE include previous history of thrombosis and recent surgery. Discussion revealed that there is consensus that patients with incidental proximal PE should be treated the same way as those with symptomatic disease, while those with subsegmental PE should be confirmed with further imaging, such as leg ultrasonography. Another issue raised was whether these events should be included as outcomes in clinical trials.

Based on the above presentations, Dr. Lee proposed new projects for the subcommittee focusing on: standardizing venous thromboembolism outcomes in oncology trials and creating clinical guidance in evidence-poor areas. There was support that the subcommittee considers tackling the following issues/initiatives:
1. Establishing diagnostic criteria for incidental venous thromboembolism. This is important to improve the accuracy and consistency of reporting the incidence of this outcome in clinical studies.
2. Encouraging the inclusion of venous thromboembolism as a standard safety outcome in oncology trials of new agents.

Dr. Farge updated the activities of her proposal to establish ISTH VTE guidelines in cancer-associated thrombosis. Much work has already been done with support from the French INCa group with respect to infrastructure and methodology support. Dr. Lee stated that there are ongoing discussions amongst the ISTH leadership regarding the participation of ISTH in developing guidelines and that the current proposal is not an ISTH SSC activity.

Dr. Lee thanked the audience for their participation. The meeting was adjourned at 13:00.
Lupus Anticoagulant/Phospholipid-Dependent Antibodies

24 May 2010
Cairo, Egypt

Chairman:  *Thomas L Ortel (US)*
Co-Chairmen:  *Ph de Groot (NL), Bas de Laat (NL), Vittorio Pengo (IT), Jacob Rand (US), Guido Reber (CH), Armando Tripodi (IT)*

The subcommittee meeting was opened with a brief overview of the broad topics to be covered, including approaches to optimize existing assays, the optimal use of currently available assays in the diagnosis of antiphospholipid syndrome, the development of new diagnostic tests that potentially report on disease mechanisms, and the role of antiphospholipid antibodies in special populations.

First, Silvia Pierangeli provided a summary of some of the activities from the 13th International Congress on Antiphospholipid Antibodies which was held in Galveston, TX, in April 2010. First, she presented preliminary results from the wet workshop, which compared different methods for the detection of antiphospholipid antibodies. She then discussed information relevant to the taskforce on anticardiolipin antibodies, including the recommendation for guidelines on anticardiolipin and anti-β-2-glycoprotein I antibody testing.

Vittorio Pengo then discussed the laboratory diagnostic criteria for antiphospholipid syndrome, emphasizing the importance of ‘triple positivity’ in anticardiolipin, anti-β-2-glycoprotein I, and lupus anticoagulant testing. This led to a discussion of a proposed consensus document for the diagnosis of APS based on laboratory criteria. While there was general agreement on most points, it was felt that the identification of a lupus anticoagulant alone, in the absence of anticardiolipin and/or anti-β-2-glycoprotein I antibodies, was sufficient for the diagnosis of APS in certain patients. There was also some discussion concerning laboratory requirements for APS manifested by obstetrical complications should be the same as for APS manifested by thrombotic complications.

This was followed by a presentation by Philip de Groot on the importance of structural conformation of β-2-glycoprotein I in the circulation, and how this might impact on reactivity with antibodies and pathophysiology of the syndrome. Importantly, conformation of purified β-2-glycoprotein I would also have an impact on the sensitivity of anti-β-2-glycoprotein I antibody ELISA’s.

Armando Tripodi then presented a strategy for attempting to standardize reporting of data from lupus anticoagulant testing. The strategy was based on concepts drawn from development of the INR to standardize PT results obtained from patients on chronic warfarin therapy. Initial results, particularly from testing done with a DRVVT-based approach, were very promising.
Bas de Laat then presented information on ongoing efforts to develop anti-β₂glycoprotein I domain 1-specific monoclonal antibodies that could be used as potential tools for standardizing anti-β₂glycoprotein I ELISA results. Importantly, the issue of β₂glycoprotein I conformation also impacted on the results from these efforts.

Thomas Ortel then gave a presentation for Jacob Rand on a proposal to conduct a multicenter, multi-national study investigating the utility of an annexin A5 resistance assay in the diagnosis of patients with APS. This generated some discussion concerning whether this study could be run concomitantly with an effort by Dr. de Laat looking at the role of endogenous thrombin potential in patients with APS, and De. Pengo stressed that it would be important to critically correlate the results with the diagnostic clinical testing performed on these patients (anticardiolipin, anti-β₂glycoprotein I, and lupus anticoagulants).

The final two presentations represented special patient populations with APS. Ulrike Nowak-Göttl presented information about pediatric patients with APS, and the impact of the diagnosis of antiphospholipid antibodies on treatment strategies for pediatric patients with thromboembolic complications. Andra James then presented data on obstetrical complications in patients with antiphospholipid antibodies. The general consensus among the members of the subcommittee was that the laboratory testing strategy for APS would be the same for these patient populations as for adult patients with thrombotic manifestations.
The SSC has decided to have two new major aims over the next two years. First to develop standard protocols that will enable uniform approaches to clinical trials of anticoagulants in children, as well as defining areas that needed further research in this field. The aim is to develop a number of position papers, each driven by a co chair and assisted by a working party, which will be ready for finalisation by the meeting in Kyoto.

The second aim is to provide guidance for the interpretation of laboratory assays in children, as this is not provided by any other guidelines or authoritative body worldwide. Once again a co chair will take responsibility for driving each project.

There are a number of ongoing projects that require completion. In terms of bleeding there are two ongoing projects. The development of bleeding scores for VWD is a joint project with the VWD SSC, and the Seven Bleep registry is collecting data on non haemophiliac use of recombinant factor VIIa in children.

Thus the following presentations were made at the meeting

**From adults to neonates: Proposing a standard approach to introducing new anticoagulant drugs in children.**

**Responsible Co-chair Guy Young, Assisted by: Christoph Male**

New anticoagulants will likely begin replacing the standard agents in the prevention and treatment of thromboembolic disease over the next decade. The licensing of these agents will be based on studies performed in adults. There are numerous differences in adults and children when it comes to the etiology, diagnosis and management of thrombosis. Therefore, prior to the application of these agents to children, studies will need to be performed to assess the safety, pharmacology, and efficacy. The recently published and current studies of novel anticoagulants in children vary widely in their patient population and design. Going forward, a more uniform approach to assessing these new drugs in children is warranted. Thus, a set of principles for anticoagulant drug development in children will be proposed to the SSC with the goal of publishing a manuscript outlining these principles which future drug development programs should be held to.
Safety and efficacy endpoints in paediatric anticoagulant trials: Developing an ISTH position paper

Responsible co chair Christoph Male, Assisted by: Lesley Mitchell, Neil Goldenberg, Ulrike Nowak Gottl

A key element to a standardised approach to clinical trials of anticoagulation in children, is uniform agreement of safety and efficacy endpoints. Without standard endpoints, comparison of results of different trials will be very difficult to achieve. Further the lifespan of children, their underlying physiology/anatomy and their normal growth and development, mandate consideration of some specific endpoints in children that are not usually of major importance in adults. This working group will develop a position statement which recommends a uniform approach to determination of study outcomes for paediatric anticoagulant trials.

PTS as an outcome measure of paediatric thrombosis therapy: can we develop standardised criteria

Responsible Co chair Anthony Chan  Assisted by Neil Goldenberg, Shoshana Revel Vilk, Leo Brandou, Janna Journeycake, Anjali Sharathkumar

Post-thrombotic syndrome (PTS) is a known complication of deep vein thrombosis. Reported incidences of PTS in children are quite variable depending on the studied populations, site of thrombosis, type of intervention and the measurement being used. PTS was first discussed at the SSC 2001. Peters reported PTS using an adult PTS scale [1,2]. In the same meeting, a modified pediatric PTS score was also reported and subsequently published and used [3,4,5]. The PTS was not discussed again until 2008 and the importance of studying PTS were highlighted. Another modified adult PTS score has been developed over the years but complete validation has not been published [6,7].

Current scales may be too sensitive and thus diagnosed too many patients with clinical insignificant PTS. Furthermore, a separate PTS score for the upper and lower venous system may be necessary.

The working party recommends

1. Unifying the two current pediatric PTS scores and have formal validation.
2. Investigate the need to develop separate score for upper and lower venous system.
Quality of life as outcome measure for anticoagulant trials in children: Developing a standardised tool

Responsible Chair: Paul Monagle Assisted by Patti Massicotte, Aisha Bruce, Sophie Jones

Advances in health care in primary illnesses in children eg cancer, heart disease and organ transplantation have been curative. As a consequence of these medical and surgical successes, complications have resulted with thromboembolism being one of the most common. Although mortality due to TE occurs infrequently, morbidity is common and can be lifelong. Treatment of TE, carried out in many children has associated adverse effects (bleeding, clot recurrence/extension, rare events) but also results in a burden for the child negatively affecting how they feel and potentially resulting in non adherence. New therapeutic agents that become available for treatment of TE require evaluation in children in clinical trials. The incorporation of quality of life as an outcome measure in these clinical trials is mandatory as other traditional outcomes measured in TE trials (mortality, safety, efficacy) may change minimally. Large studies which are not feasible would be required to study these differences. In this way, more informed choices re TE therapy can be made, the child can be empowered by playing a role in their disease therapy and adherence will be maximized.

Developing a measurement tool has challenges especially in children including parent child diad, medical team child relationship, developmental changes and life trajectory. The following must be considered when designing the tool: purpose, focus, origins of items, opportunity for self reporting, threat of negative wording to self esteem, number of items & time to complete, proxy reporting, adequate psychometric properties and culture & language of population to be tested. The disease specific tool is superior to the general overall tool having increased sensitivity to detect small clinically important changes in QOL thus are ideal for clinical trials designed to evaluate new therapies.

Laboratory monitoring of anticoagulants in children: developing a uniform approach

Responsible Chair Paul Monagle Assisted by: Anthony Chan, Fiona Newall, Vera Ignjatovic

Monitoring of all anticoagulant drugs in children is required for a variety of reasons.

There remains uncertainty as to the optimal tests, therapeutic ranges, frequency of monitoring and clinical outcomes related to monitoring results in children. Current clinical strategies are extrapolated from adult guidelines despite the increasing body of evidence that the currently utilised monitoring strategies are suboptimal in children because of developmental haemostasis, changes in drug binding and even mechanism of activity. This working party will define the specific issues related to monitoring anticoagulation in children, identify the current shortcomings and make
recommendations for immediate research priorities. A suggested standardised strategy will be put forward for laboratory monitoring of currently used anticoagulants in children.

**Diagnostic criteria for thrombosis in children: Which Radiological techniques are validated?**

**Responsible chair : Janna Journeycake  Assisted by: Leo Brandau**

Once considered rare in children, thrombotic events (TE) represent a new “epidemic” condition within this population. Objective testing is a crucial aspect in the management of these patients: clinical assessment alone is unreliable in the recognition of TE; undiagnosed events may be accompanied by significant morbidity and mortality; and because anticoagulant treatment is effective. However, therapy can be costly and associated to complications, and its inappropriate use should be avoided. In adults, the use of non-invasive imaging modalities in comparison to their respective gold standard radiological methods enabled a critical assessment of their clinical role. Conversely in children, there is limited literature (i.e. upper extremity deep vein thrombosis and cerebral venous sinus thrombosis) available regarding the role of diagnostic imaging in pediatric patients TE (arterial and/or venous). Currently, many pediatric patients are submitted to several diagnostic studies at diagnosis, leading to a potential unnecessary increase of costs, delay in commencing therapy, and lack of an evidence-based standardized diagnostic imaging approach. Similarly, the lack of studies precludes the comparison of current modalities with alternative non-invasive methods, delaying their application in clinical practice. We suggest the identification of knowledge gaps to establish a strategy for planning the future actions of the Paediatric SSC on this subject.

**Developmental Haemostasis : Recommendations for laboratories reporting paediatric samples**

**Responsible co chair Gili Kennet   Assisted by : Paul Monagle, Vera Ignjatovic**

Developmental haemostasis is now a well accepted phenomenon and relates to the measurable changes in the haemostatic system with age. This concept has important biological implications, but more importantly has significant clinical implications. Reference ranges for most haemostatic parameters are analyser and reagent dependent, and this has critical implications for the definition of normal children, diagnosis of disease states and monitoring of anticoagulant therapy. The working party will develop a position paper outlining the expectations for diagnostic laboratories processing paediatric samples, how reference ranges should be developed for children, and how results can or cannot be compared across laboratories.

**Role of thrombophilia testing in children: development of standard indications for testing, and assay methodologies**

**Responsible Co chair Ulrike Nowak Gottl**
Thrombophilia testing is very frequently performed in clinical practice. Data in adult studies over recent years has dramatically changed the way we view the role of this testing. The working group will review the evidence for thrombophilia testing in children and provide a position statement about the current role of thrombophilia testing in clinical practice and incorporation of thrombophilia testing in outcome based clinical trials in children.

Laboratory assessment of bleeding disorders in children: Standardisation protocols

Responsible Co Chair Wolfgang Muntean Assisted by Nicole Schlegal, Margaret Rand

A number of acquired bleeding disorders and most inherited bleeding disorders, at least the most severe, present manifestations during childhood. Variations of hemostasis components during development, small blood volume and difficult blood drawing explain pitfalls and dilemmas of diagnosing bleeding disorders and/or evaluating bleeding risk during the first years of life. Taking into account the existing procedures and recommendations of other SSC for testing hemostatic capacity, specific coagulation components, fibrinolysis factors, and platelet number and functions, we intend to propose specific pediatric protocols for clinical application. They include:

- Blood collection procedures according to age, blood volume, body weight and venous access
- Algorithms respecting both blood saving and coagulation testing priorities, especially for neonates, babies with very small body weight and infants or children with poor venous access. These algorithms should make a selection among usual and new tests.
- Interpretation of results of hemostasis tests according to age-related reference ranges, established with the same analytical system (analyzer and reagents).

These protocols should also take in account ethical guidelines for clinical diagnosis and clinical research trials in pediatrics and should be clinically validated.

Seven bleep Registry update

Responsible Chair Paul Monagle Assisted by Jan Blatny, Prasad Matthew

SeveN Bleep (Seven A in Nonhemophilia bleeding in Pediatrics) is a web-based Clinical registry for collecting data on the use of rFVIIa in the treatment of severe and/or life threatening bleeding in children without haemophilia, endorsed by the Paediatric/Perinatal SSC of ISTH. So far 191 cases have been recorded into it, of which 164 (86%) records fulfilled the validation criteria and were eligible for further analyses. In 27 (16%) cases, rFVIIa was used to prevent severe bleeding, and 137 (84%) records were related to administration of rFVIIa for the treatment of bleeding. There were 42 (30%) neonates and infants, and 95 (70%) older children.
Survival rate in older children was 76.1% and neither thrombembolic event nor death related to rFVIIa treatment were recorded. In neonates and infants, the survival rate was 50%, with no deaths attributed to use of rFVIIa, but with one TE (2.4%) related to rFVIIa treatment.

The registry should now conclude as a formal registry of the subcommittee, and proceed to present its findings.

Summary

In summary, the Paediatric/perinatal subcommittee is embarking on an ambitious program focusing on developing a framework for clinical trials of anticoagulants in children. This involves the framework of the trials, the definition of outcome measures, clarifying the radiological methods and anticoagulant monitoring strategies validated in children and specifically including PTS and quality of life. Working groups have been constituted with the aim of producing position papers and recommendations by the next meeting in Kyoto. In addition separate working groups will make recommendations around laboratory haemostatic testing in children.

Each co chair will take responsibility for a working group and will progress the work through conference calls and email contact.
Plasma Coagulation Inhibitors

23 May 2010
Cairo, Egypt

Chairman: Herbert C Whinna (US)
Co-chairmen: Francesco Bernardi (IT), Elaine Gray (UK), Tilman Hackeng (NL), Steven Kitchen (UK), Richard Marlar (US), P Meijer (NL), Laurent Mosnier (US)

Inconsistencies in protein C assay sensitivity to protein C defects
Peter Cooper, CSci, FIBMS

Peter Cooper reported on the discrepancies of the use of chromogenic-based protein C activity assays compared to clotting-based protein C assays. The majority of UK clinical laboratories use the chromogenic protein C activity assay for their routine protein C test. The chromogenic-based protein C activity assay may be more specific since it appears to be insensitive to Lupus Anticoagulant, elevated Factor VIII levels and Factor VLeiden. However the chromogenic-based protein C activity assay will miss some abnormal protein C molecules (Type IIb). He presented data on several families in which the chromogenic protein C activity assays correlated to antigen levels but the clotting-based protein C activity assays were significantly decreased into the abnormal range consistent with the picture presentation. The Type IIb defects that are not detected by the chromogenic-based protein C activity assay are in the phospholipid binding regions and the protein S binding domains of the protein C molecule.

Based on the reported data, the chromogenic-based protein C activity assay is more advantageous for the routine coagulation laboratory in that it is more accurate, precise and has better linearity and less interference. However if a phospholipid binding or protein S binding domain mutation in the protein C molecule is suspected, then a clotting-based protein C activity assay must be performed.

Variation in protein S activity measurement in clinical samples between different methods
Ian Jennings, PhD

Ian Jennings reported that based on UK External Quality Assessment results for protein S activity assays, there appears to be significant variation in reported values of protein S activity assays. Upon investigation, part of the issue for this discrepancy is due to variability in the method (manufacturer's kits). However that does not explain all of the variation. He undertook a methodology questionnaire to the participant's to attempt to determine other contributing factors. Of the three available kits, all averaged significantly different mean values over multiple years however within the last two years two of the commercial kits results are slowly reporting similar results. These differences have been noted in other international EQA programs. In his studies, he has found that it is not due to lyophilization or any other discernable cause.
Ian Jennings has started developing a protocol with Piet Meijer and Richard Marlar to attempt to determine the cause of the variation of the protein S activity assays. This proposal is being developed and will be discussed at the next SSC meeting (2011).

At this time the recommendation for performing clinical protein S assays is to initially perform a free protein S antigen assay and if abnormal then perform the protein S activity assay and the total protein S antigen.

Collaborative study on the establishment of the 3rd International Standard for Antithrombin, Plasma
Elaine Gray, PhD

Elaine Gray summarized the report on the 3rd International Standard for Antithrombin, Plasma. Summary of report:

Twenty-four laboratories from 13 countries participated in a collaborative study to establish a replacement for the 2nd International Standard for Antithrombin, Plasma (93/578) and to calibrate antithrombin functional and antigenic potency estimates for the ISTH/SSC secondary coagulation standard Lot#4. Locally collected normal pooled plasmas were also included in the study to assess the relationship between the International Unit and the normal plasma unit. The laboratories were able to perform the functional and antigenic assays with high precision; when assayed against the 2nd International Standard (IS), the geometric coefficient of variation (GCV) ranged from 0.6 to 9.7% and 1.0 – 13.9% for the functional and antigenic assays respectively. For functional assays, excellent agreement was observed between laboratories. This was evidenced by low inter-laboratory % GCV for the candidate material, the ISTH/SSC Lot 3 and proposed Lot 4 (2.5%, 2.0% and 2.5% respectively). For the antigen measurement, the inter-laboratory variation was slightly higher, at 4.4%, 4.3% and 4.4% for the candidate material, the ISTH/SSC Lot 3 and proposed Lot 4, respectively. Although there were significant differences between the functional potency estimates obtained against the 2nd IS and the local normal pooled plasmas for all the samples, the differences were all below 2% and there was no significant difference between the antigenic values. It is recommended that the candidate material, sample B (NIBSC code, 08/258) be considered as the 3rd International Standard for Antithrombin, Plasma, with assigned potencies for function: 0.95 IU/ampoule and antigen: 0.96 IU/ampoule. For the ISTH/SSC Lot# 4 Secondary Plasma standard, it is proposed that the following potency values should be assigned: function - 0.92 IU/vial, antigen - 0.93 IU/vial.

The report has been approved by the Working Party and subcommittee. The report will be submitted to the SSC Committee for approval. The approved report will be then submitted to the WHO for their approval as the International Standard for Antithrombin, Plasma at their October, 2010 meeting.
Availability of reference plasmas for thrombin generation tests
Elaine Gray, PhD

Elaine Gray announced the availability of 2 reference plasmas for thrombin generation tests. One of the reference plasma is aimed for fluorogenic assays while the other reference plasma is for chromogenic assays. Both plasmas have been evaluated in a multi-centre collaborative study involving 118 laboratories and will be available in August, 2010 from the National Institute for Biological Standards and Control (NIBSC) who is the custodian for these plasmas donated by industry. The plasmas are available free to laboratories or commercial companies; however the requestor will have to pay for shipping. The only requirement is that the laboratory must provide written feedback to Elaine Gray on the performance of the plasmas.

Report from the Working Party on Thrombin Generation Tests
Elaine Gray, PhD

Elaine Gray briefly described the protocol for the investigation of Thrombin Generation Test for monitoring Factor VIII Inhibitor Bypassing Agent (FEIBA) in factor VIII inhibitor plasma samples. The samples were sent out to and evaluated by 20 laboratories (5 laboratories each for the 4 different Thrombin Generation Time assay methods). Data has been returned and the Working Party is evaluating the data. The Working Party will discuss the data and conclusions before reporting the results to the subcommittee (2011).

Elaine Gray also discussed the dissolution of the current Working Party on Thrombin Generation Time assays (which is too board and has not specify an specific application). She suggested creating a new Working Party on Thrombin Generation Test assays concentrating on Thrombophilia.
Plasma Kallikrein-Kinin System

25 May 2010
Cairo, Egypt

Chairman: Thomas Renne (SE)
Co-chairmen: David Gailani (US), Keith McCrae (US)

Factor XI and the plasma contact system Program and speakers for the SSC meeting in Cairo, Tuesday May 25. 9:00-13:00, about 30-40 participants in average followed the session and we had lively and comprehensive discussions following every presentation.

Welcome note: by Thomas Renné. Based on the results of the election at the Vienna SSC meeting the name of the subcommittee was changed to “Factor XI and the contact system”. Suggestions for new co-chairmen to substitute for Keith McCrea are asked for.

Paola van der Meijden, Maastricht, NL: Role of factor XII in thrombus formation on collagen.

Dr. van der Meijden gave a brief overview about the critical role of FXII for arterial thrombus formation. FXII is activated by contact to polyanions and the aim of her study was to analyze activation of FXII by collagens under flow and implications of this pathway for thrombus formation. Using mice with FXII and factor XI deficiency, human plasma and specific inhibitors, Dr. van der Meijden showed that collagen drives thrombin generation in the absence of tissue factor in an FXII-dependent manner. Type I collagen fibers binds directly to FXII in the presence and absence of cofactors HMWK and PPK. In plasma free systems, in PPP, and PPR collagens activate FXII. Both inhibition of FXII and GPVI interfered with collagen driven thrombus formation and PS exposure in flow chambers. Effects of FXII/GPVI-inhibitors were synergistic. Mice with deficiency in collagen-GPVI signaling, LAT and PLC\(_2\) suggested a dual role of collagen in thrombus formation. Collagen activates platelets via GPVI signaling resulting in PS exposure and additionally activates FXII directly.

Volker Pönitz, Stavanger, NO: Activated factor XII as a risk predictor for cardiovascular disease?

Dr. Pönitz gives a brief overview about the biological roles of the contact system driving the intrinsic pathway of coagulation, the fibrinolytic system, the complement, and the kallikrein-kinin system. The aim of Dr. Pönitz study is to analyze the role of activated FXII (FXIIa) for arterial heart disease in humans. So far there are contradictory data regarding the role of FXIIa levels as predictor for thrombotic disease in patients. In the
RACS study Dr. Pönitz analyzed the FXIIa form FXIIaA as predictor for mortality, association of FXIIa with established cardiovascular risk factors, and analyzed of FXIIa-inhibitor complexes as marker for thromboembolic disease. More than 800 ACS patients form the Stavanger Hospital were included in the RAC study. FXIIa is not associated with elevated plasma lipid levels. Especially in patients with low TnT levels FXIIa is an independent risk factor for all cause mortality at 24 months follow up. FXIIa-inhibitor complexes were not independent risk factors for mortality at 24 months. Dr. Pönitz also initiated the RIST study that showed the role of FXIIa as novel marker for stent rethrombosis. In summary, the data support a role of the contact system for coronary heart disease.

Yi Wu, Philadelphia, USA: The plasma kallikrein-kinin system and regulation of endothelial progenitor cell function.

Dr. Wu introduced historical and current models of the plasma kallikrein kinin system and the role of the system for disease states. The KKS has proinflammatory, proangiogenic and profibrinolytic activities. Dr. Wu analyzed the role of kinin-free HMWH (HKa) for inhibition of mature endothelial cells. He focuses on the role of HKa for endothelial progenitor cell (EPC) functions and the role of the KKS for EPC dysfunction and vascular pathology. Using isolated human EPC Dr. Wu showed that HKa inhibited large colony and vessel formation by EPC. HKa accelerates onset of EPC senescence involving reactive oxygen species signaling. The proscenescence molecule p16INK4a was identified as a key player in HKa signaling. Dr. Wu confirmed the role of HKa for vascular reendothelization in vivo using rat models. He analyzed the role of HKa-EPC for arthritis in a proteoglycan-induced arthritis model in rats. Inhibition of bradykinin reduced synovial recruitment of EPCs. Bradykinin stimulated transendothelial migration of EPC involves homing receptors CXCR4. The KKS may have dual roles for EPC dysfunction in rheumatoid arthritis.

Marco Chicardi, Milano, IT: Diagnosis and treatment of hereditary angioedema — in 2010.

Dr. Chicardi introduces the audience into pathology, clinics, diagnostics, and treatment of hereditary angioedema (HAE). There are several classes of HAE involving C1 esterase deficiency of defects, FXII mutations and yet unknown causes. Leakage in angioedema involves endothelial junction reorganization driven by histamine or bradykinin. Defects in bradykinin degradation may also lead to edema formation. Complement activation seems to be of minor experiments in HAE. Bradykinin plasma levels are largely elevated in acute HAE associated swelling attacks. Bradykinin formation is a locally controlled process and new types of B2 receptor antagonists (icatibant) interfere with bradykinin signaling. Dr. Chicardi comments on long-term prophylaxis (low frequency of swellings) with attenuated androgens (100 mg/day) or tranexamic acid. He introduces the new drugs that have appeared within the last years including recombinant C1INH from transgenic rabbits, DX88 and icatibant. In contrast to C1INH DX88 and icatibant can be applied subcutaneously. Dr. Chicardi gives an
overview about licenced drugs for HAE treatment indicating that HAE therapy is still a puzzling situation with different approved drugs in the US and in Europe.

**Creig Thelwell, South Mimms, UK:** Presentation of the C1 inhibitor standard.

There is currently no standard for C1 Inhibitor. Sanquin initiated establishing two C1INH standards for diagnosis and treatment, respectively. A plasma and a concentrate-derived standard were presented and compared. Stability of both standards (08/262 and 08/256) is excellent and has been reviewed by expert in the filed. For more information we would like to refer to the ISTH/SSC homepage where detailed information about the new standards is presented and soon will be ready for download.

**Klaus T. Preissner, Giessen, DE:** Role of Factor XII in lung fibrosis.

Dr. Preissner’s very interesting presentation focused on the role of FXII for lung fibrosis. FXII has procoagulant and promitogenic activity in the lung. Dr. Preissner started with an overview of lung diseases and highlighted the role of coagulation factors for fibrotic lung disease. Dysbalance in the alveolar space is the key event for lung fibrosis and also drives inflammatory processes. Dr. Preissner showed close homology of bleomycin-induced fibrosis in mouse models and idiopathic pulmonary fibrosis (IPF) in humans. In both disease states tissue factor and FVII expression is strongly upregulated. In various disease states extracellular RNA can be detected in the alveolar space and promotes autoactivation of FXII and stabilizes PAI-1. As fibrinogen and PAR1 deficient mice are protected from bleomycin-induced lung fibrosis Dr. Preissner analyzed role of FXII for the pathology of the disease. He showed that FXII expression is upregulated in IPF lungs and that FXII activity is high in bronchoalveolar lavage from patients. Expression of FXII is TGF-1B dependent in fibroblasts, which were identified as the major source of FXII in diseased lungs. TGF-1B signals via Smad3 in lung fibrosis. In mice infusion of CTI protects mice from bleomycin-induced lung fibrosis. Similarly, genetic ablation of FXII is protective against lung fibrosis in the bleomycin model. Dr. Preissner concluded his presentation with a model of FXII-induced proliferation of lung fibroblasts that is of importance for lung fibrosis.

**Henri Spronk, Maastricht, NL:** Factor XI activation in vivo.

Dr. Spronk showed recent data that address the relative importance of FXI feedback activation versus FXIIa-mediated FXI activation. He introduced his talk with a comprehensive overview about six previous studies that have addressed FXI activation in vitro. In summary the results from these studies seem contradictory and it is still not clear, whether FXI-feedback activation by thrombin occurs in vivo. Differences in methods and concentrations may account for the discrepancy in the previous studies. FXI can be either activated by thrombin resulting from tissue factor-driven coagulation (feedback activation) or FXIIa. Dr. Spronk used thrombin bound to microspheres to analyze the feedback activation loop in real time thrombin generation (TG) assays. These studies indicated a critical role of the feedback loop for TG in plasma that was independent of FXII. Inhibition or deficiency in FXI largely abolished thrombin driven TG
supporting the critical role of the feedback loop in this system. In animals, crossing mice with deficiency in intrinsic and extrinsic pathways indicated that FXI has a critical role during development, that the feedback loop is existing in vivo, and that this pathway is of critical importance for hemostasis under special conditions.

**Jonas Emsley, Nottingham, UK:** Plasmakallikrein/factor XI structure function relations.

Dr. Emsley gives an introduction into structural aspects of PK and FXI binding to HMWK. He summarized previous studies that have addressed interaction of FXI with proteins such as thrombin and HMWK. HMWK binding to FXI is mediated by a minimal D6 domain sequence that is also capable to bind PK. Dr. Emsley introduced scanning for FXI binding peptides that identified a specific motif (DFPD) that accounts for binding to the zymogen. Dr. Emsley presented an update on co-crystallisations of FXI and HMWK and the first preliminary structure. Data suggest HMWK binding to FXI apple domain. Dr. Emsley showed high-resolution analysis of the FXI-HMWK interaction. Based on the structural data FXI missense-mutants confirmed the critical role of Apple 2 and identified residues for FXI binding to HMWK. Dr. Emsley showed structural consequences of FXI point-mutations using the previously published FXI structure. Based on the homology of FXI and PK he analyzed HMWK binding to PK. The PK structure is modeled on the FXI crystal structure. Dr. Emsley concluded his presentation showing the importance of HMWK-dependent platelet binding and its role for FXI activation. On platelet surfaces FXI seems to be activated by ½ FXIa.

In summary, Dr. Emsley has identified the minimal HMWK binding site in Apple domain 2 of XI. This motive is essential for the interaction and missense mutation interfere with the binding.

**Nicola Mutch, Leeds, UK:** Polyphosphates, novel procoagulant activators.

Dr. Mutch addresses physiological polyanionic activators for FXII. Polyphosphates are released from platelets and have a length of 60-100 phosphate units. Polyphosphates initiate FXII-mediated contact activation in plasma. Polyphosphates directly bind to contact system proteins and promote autoactivation of FXII in the absence and presence of PK. The aim of their presentation is on mechanistic insights into polyphosphates-mediated procoagulant reactions. Polyphosphate-mediated activation of FXII is reversible and results in a single-chain form. Polyphosphate-driven FXII activation is concentration dependent and bell- shaped. Polyphosphates weakly stimulate FXIIa activation of PK and HMWK does not stimulate autoactivation of PK by polyphosphates. Polyphosphates do not protect FXIIa from inhibition by C1 esterase inhibitor. Dr. Mutch explains structural differences in polyphosphate and dextran sulfate-mediated FXII activation.
Chairman: Andreas Greinacher (DE)
Co-Chairmen: Donald Arnold (CA), Beng Chong (AU), Yves Gruel (FR), Hartmut Kroll (DE)

**Autoimmune thrombocytopenia**
*Chairs: A. Greinacher (Germany)*

Platelet antibody testing in ITP: The need for standardization (*D. Arnold, Canada*)

Dr. Arnold discussed the requirements for implementation of platelet autoantibody testing in ITP clinical trials. It is only with large scale studies and clinically well-characterized patients that the value, limitations and application of platelet antibody testing in ITP can be realized.

The Canadian R-ITP showed that it is feasible to systematically include platelet autoantibody testing at regular points in time. Experience gained from this trial were the basis for the discussion on methods of test implementation in large multicenter trials in ITP.

*Round table discussion on platelet antibody standardization workshop, focus on application of platelet antibody tests to clinical studies.*

Key issues of the implementation of platelet antibody testing on a large scale were discussed:

<table>
<thead>
<tr>
<th>Issue</th>
<th>Discussion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Need for a central lab?</td>
<td>Only small number of laboratories well trained for platelet autoantibody testing. Clinical trial REQUIRES a central lab. Select labs according to their strength for different assay</td>
</tr>
<tr>
<td>Define the purpose of testing</td>
<td>Treatment outcome? Prognosis and risk for bleeding?</td>
</tr>
<tr>
<td>Which test method?</td>
<td>Glykoprotein specific assay, uncertainty whether other targets are important. Unresolved whether there are other antigens. Whole platelets are easier to standardize than lysed platelets</td>
</tr>
</tbody>
</table>
Use a method which allows later testing
MACE easier to apply than MAIPA

<table>
<thead>
<tr>
<th>Direct vs indirect?</th>
<th>Direct abs more sensitive than indirect testing. For clinical studies use both methods.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Which Monoclonal?</td>
<td>MoAbs to IIb or IIIa subunit not IIbIIIa complex abs</td>
</tr>
<tr>
<td>Which anticoagulant?</td>
<td>ACDA superior to EDTA if blood is sent to central lab</td>
</tr>
<tr>
<td>What volume of collection?</td>
<td>??? As much as ethically possible. Min 10ccs (children different than adult), 30 mL in adults</td>
</tr>
<tr>
<td>On site processing?</td>
<td>Central if preparation can be made within 3 days. Ideally central lab per larger region (continent). Important when different countries are involved. Define a network for potential central labs.</td>
</tr>
<tr>
<td>Shipping method?</td>
<td>Courier</td>
</tr>
<tr>
<td>How to batch/store samples?</td>
<td></td>
</tr>
</tbody>
</table>

**Alloimmune thrombocytopenia**

Chairs: D. Arnold (Canada)

**Update on platelet alloantigen systems: New approaches in antigen/antibody detection** (S. Santoso, Germany)

Dr Santoso updated the list of current human platelet alloantigens (HPAs) with the new published rare platelet alloantigens. Six HPA biallelic systems (HPA-1, -2, -3, -4, -5 and -15) and 10 rare HPAs (HPA-6bw until -14bw and -16bw) are approved by the Platelet Nomenclature Committee (PNC) of the ISBT Working Party on Platelet Immunology and ISTH SSC Platelet Immunology (see minutes of 54th SSC Meeting 2008 in Vienna, Austria). Five published new rare alloantigens (Va, Cab, Sta, Kno and Nos) are now included in the list of HPA systems (HPA-17bw until -21bw). The official information is available at the IPD–HPA Database of EBI (http://www.ebi.ac.uk/ipd/hpa).
The new rare platelet alloantigen, Hit, associated with a case of neonatal alloimmune thrombocytopenia represents the third allelic form of HPA-7bw, which forms by a point mutation 407Pro > Ser in GPIIIa (Koh et al, 2010). Single point mutation responsible for the third allelic form of HPA-1 (33Leu > Val) has been described (Santoso et al, 2006). In contrast to Hit alloantigen, no platelet reactive alloantibodies against GPIIIa Val33 isoform were identified so far. All these alloforms may hamper the genotyping of HPA and platelet antibody detection, and should be therefore taken into account. The nomenclature of the third alleles and their inclusion into the HPA nomenclature should be discussed. According to the current policy of the Platelet Nomenclature Committee only immunogenic isoforms can be included into the HPA nomenclature.

Several methods for the detection of platelet alloantibodies have been developed in the last decades. Antigen capture assay using monoclonal antibodies such as MAIPA assay represents currently the gold standard of platelet antibody testing. In EDTA whole blood GPIIb will be degraded by a plasma enzyme. This does not happen with washed platelets. Accumulated evidence, however, indicated that this method may overlook low-avidity platelet alloantibodies (Socher et al, 2009) and may cause false negative result due to competitive inhibition by monoclonal antibody (Peterson et al, 2009). The surface plasmon resonance (SPR) technology allows the measurement of biomolecular interaction in real-time with a degree of sensitivity without the need of label, washing and monoclonal antibody. Recently, XPR technology has been introduced to overcome the limitations of conventional SPR technique. The use of this method for the detection of platelet antibodies and its advantages and disadvantages was presented.

**Low affinity platelet alloantibodies, detection and its relevance** (S. Santoso, Germany)

In a retrospective study of HPA-1bb-mothers of thrombocytopenic HPA-1ab newborns a significant portion of anti-HPA-1a antibodies could only be detected by the use of surface plasmon resonance technology, but not by our standard serological methods (e.g flow cytometry, antigen capture assay). Low-avidity antibodies are characterized by low-titer and high “off-rate”. The clinical relevance of such antibodies was demonstrated by the use of NOD/SCID mouse model. The implementation of SPR technology for the improvement of NAIT diagnosis was discussed.

The method depends on purified GPIIbIIIa and will be restricted to reference laboratories. The platelet immunology SSC discussed whether an SSC statement should be published in JTH alerting clinicians about these low affinity antibodies.

**Discussion:** The antigens used in the XPR assay are purified from human platelets. Background of low avidity autoantibodies has not been assessed systematically yet.

**Prediction of NAIT** (B. Skogen, Norway)

Until recently there was no predictor for severity of the thrombocytopenia in the fetuses of HPA 1a negative mothers available. Clinically the severity of thrombocytopenia in a
previous child has been used as a risk factor for the next pregnancy. The positive predictive value of this clinical approach is ~ 50% and is not applicable in first pregnancies of mothers known to be HPA-1a negative.

More recently, the maternal anti-HPA 1a antibody level during the pregnancy has been claimed to be a better predictive factor. The association between antibody level and severity of thrombocytopenia was first reported by Williamson, who found correlation between antibody titer in the last trimester and severity of thrombocytopenia. This observation has been further substantiated by antibody measurements performed with the MAIPA technique. Jaegtvig et al reported in 2000 on MAIPA recorded antibody levels in the mothers plasma at delivery which correlated with neonatal thrombocytopenia. Other more recent studies using the same technique has revealed similar results. Thus, Killie and coworkers found that both mean antibody level at delivery (23.7 IU/ml) and weighted mean of antibody level (22.0 IU/ml) was significantly higher in the plasma of women having babies with severe thrombocytopenia than those having babies with only mild to moderate thrombocytopenia (4.2 IU/ml and 5.3 IU/ml respectively). By setting a cut off level of the antibody concentration to 3 IU/ml, to identify cases at risk of NAIT, the clinical sensitivity was 93% and specificity 63%, compared to 13% and 92% for clinical history of a previous thrombocytopenic child as risk factor. The obstetric history, however, had about the same positive predictive value as antibody quantitation.


Discussion: The issue is to standardize the units in the HPA-1a assay. This can be achieved by using the international HPA-1a standard.

Genetic pitfalls in platelet genotyping and new platelet antigen(s). (G. Bertrand G., V. Jallu, C. Kaplan)

The first part of the presentation showed genetic mutations that have consequences on platelet genotyping. Two mutations were described on the gene coding for GPIIb and induce a new digestion pattern by PCR-RFLP for HPA-3 (2614C>A) or HPA-9 (2645C>T). Three mutations were reported on the gene coding for GPIIIa. They all interfere with the HPA-1 genotyping by PCR-SSP or by a high throughput method (262T>C; c.281+28G>A; c.166+40C>T (dbSNP n°988684)). The second part of the
presentation was dedicated to new platelet antigens. Cab\textsuperscript{a} is located on the GPIa (Gln716His) and Cab2\textsuperscript{a} on the GPIIb (Ser472Asn). Both platelet antigens were discovered in a context of severe neonatal thrombocytopenia. These antigens have no consequence on the function of the glycoproteins. The Cab\textsuperscript{a} antigen is officially included in the HPA nomenclature (HPA-18bw).

\textbf{Antenatal management of 81 high risk pregnancies: maternal immunization and consideration for a less invasive strategy.} (G. Bertand G. and Kaplan C. for the French working group)

In a retrospective study including 75 HPA-1bb women with feto-maternal incompatibility (239 pregnancies, 159 newborns) half of the cases of fetal/neonatal alloimmune thrombocytopenia (FNAIT) were diagnosed during/at delivery of the first pregnancy. The severity of the disease was associated with the gynecologic history of the women (78\% of the cases of ICH in multigravida), but not with the maternal HLA DRB3 allele or the blood group. Subsequent pregnancies were managed with corticoids, IvIG, or both IvIG and corticosteroids: newborns of women who received the combination therapy of IvIGG and corticosteroids had the lowest incidence of severe thrombocytopenia (25\%). The maternal anti HPA-1a antibody concentration measured before any treatment was predictive of the fetal status. If the antibody titer was > 28 IU/mL (using the international standards) all fetuses were severely thrombocytopenic.

\textbf{Discussion:} FcRn should be also considered as a target for treatment, anti-HPA-1a IgG isotypes should be tested, as they may influence the transplacental transport.

\textbf{Discussion: Which of the new aspects should be considered for diagnostic approaches and treatment in neonatal alloimmune-thrombocytopenia}

The SSC discussed that a working party should be formed to summarize the information of pitfalls in genotyping of HPA-1a with the data on low affinity antibodies and antibody titer determination in an SSC statement. Responsible Dr Kroll/Dr Skogen/Dr Santoso/Dr Bertrand/. Delivery to the SSC by December 2010.

\textbf{Heparin-induced thrombocytopenia}
\textit{Chairs: Y. Gruel (France)}

\textit{Thrombin generation potential in HIT. Does it correlate with clinical manifestation?} (B. Tardy)

HIT is often complicated by thrombosis. IgG antibodies targeting the platelet factor 4 – heparin complex activate platelets and generate microparticles with procoagulant activity. Recently it was shown that the thrombin generation assay is capable of detecting procoagulant activity induced by patient platelet poor plasma (PPP) with HIT IgG antibodies in donor platelet rich plasma (PRP) in presence of a low heparin concentration. Figure 1: The “HIT thrombogram profile”
Dr Tardy and coworkers assessed the HIT thrombogram profile in 34 patients with a clinical diagnosis of HIT, a positive platelet aggregation assay, a positive H-PF4 ELISA. Donor PRP and patient PPP (1:1) were incubated either with unfractionated heparin (0.2 U/ml) or with saline. Thrombin generation was assessed by calibrated thrombinography. TF concentration was 6 pM. All the parameters of the basal thrombogram (without heparin) and the ratios wH/woH of all the thrombogram parameters were compared between the patients with and without thrombosis. The HIT thrombogram profile with a high TF concentration (6pM) did not correlate with clinical manifestation in HIT patients. With low TF concentration (1pM) the basal peak of thrombin correlated with thrombosis.

Discussion: Test depends on the quality of the platelets. Interdonor variability remains unclear. PPP thrombin generation potential was not different between the groups. But in the PPP of patients often contains heparin. Same observation has been made by other groups. Pattern of HIT antibody induced thrombin generation is similar to the pattern seen, when platelets with phosphatidylserin exposure are used in the experiment.

Towards standardization of anti-PF4/heparin antigen assays (A. Greinacher, Germany)

Dr Greinacher reported a study performed between the Greifswald laboratory and the McMaster laboratory (Dr Warkentin) on the correlation of PF4/heparin EIA results (expressed in OD units) and the prevalence of platelet-activating antibodies. In patients with suspected HIT, EIA-IgG OD reactivities (Greifswald laboratory; n=2,821) were compared with the heparin-induced platelet activation assay (HIPA). In addition correlation of reactivities of another EIA-IgG (McMaster laboratory; n=1,956) were compared with the serotonin-release assay (SRA). The percentage of sera testing positive in the functional assays strongly correlated with PF4/heparin-IgG EIA OD reactivities in both laboratories with very similar results (correlation coefficient >0.95) when normalized OD ranges (maximum OD divided by 10) were used instead of absolute OD values. Results of PF4/heparin-IgG EIA should not be reported as only
positive or negative but in ranges which allows for risk-stratified prediction for presence of platelet-activating antibodies. As ODs are expressed in arbitrary units, use of normalized OD ranges permits a standardized approach for inter-laboratory comparisons.

**Discussion:** functional assays not needed anymore in future? Yes it is stil required as the standard curve gives an estimate only. The commercial assays should be assessed by this approach to confirm that the correlation curve can also be applied to these assays.

**PF4/polyanion complexes are there other implications?** (K. Krauel, Germany)

The early onset (4-6 days) of anti-PF4/heparin IgG after first heparin contact and the presence of these antibodies in the normal population with a very low likelihood of previous heparin treatment may result from previous infections. This is supported by the finding that PF4 binds to different bacterial species leading to the exposition of the HIT antigen. In addition anti-PF4/heparin IgG enhanced phagocytosis of PF4-coated bacteria and mice developed anti-PF4/heparin antibodies after polymicrobial sepsis. Heparin-induced thrombocytopenia appears to be a misdirected secondary immune response, wherein PF4/heparin-coated platelets mimic previously-encountered PF4/bacteria complexes. This may explain the high prevalence of PF4/heparin antibodies in certain patient populations. It remains to be clarified, whether there is a difference in antibody binding characteristics of “natural” PF4/heparin antibodies and heparin treatment induced antibodies.

**Microparticle Generation Assay: a new tool to diagnose and study the physiopathology of type-II HIT** (F. Mullier et al. UCL Mont-Godinne, Yvoir, University of Namur, Namur, Belgium)

Dr Mullier presented the Platelet Microparticles Generation Assay (PMPGA), a whole blood assay for diagnosis of HIT. In this assay platelet-poor patient plasma is first incubated 20 minutes at 37°C with diluted citrated 109 mM whole blood from a healthy donor containing 0, 1 or 500 IU heparin/ml. In the second step, Platelet microparticles positive or negative for annexin-V FITC (phosphatidylserine) and anti-CD62P-PE (P-selectin) are quantified on a FACS Aria®. The PMPGA assay was compared with the 4T score, PF4/ELISA assay, aggregometry, and 14C-serotonin release assay and correlated well, although the numbers are still too low to draw definite conclusions.

**Discussion:** Concerns were raised that plasma constituents usually inhibit the activation of platelets by PF4/heparin antibodies and this problem is not related to the readout system. Therefore the PMPGA needs to be compared prospectively with a larger number of sera.
In the first part of the sessions, 3 lectures overviewed the current status of diagnosis of congenital platelet function disorders, setting the scene for the presentation of a Working Party on Diagnosis of Congenital Platelet Function Disorders, and discussion of its aims and organization.

Dr Chris Van Geet presented an overview of the genetically unraveled inherited platelet function disorders and proposed a classification that classifies these disorders according to their mode of inheritance and to the different stages in pathophysiology: defective platelet-vessel wall interaction, defects in the cytoskeleton, defective platelet-platelet interaction, defects in signaling, secretion defects, megakaryocyte maturation defects and defective procoagulant activity. As many of the involved genes are expressed also in other tissues or even ubiquitously, the clinical phenotype is often much broader than the platelet phenotype. The following, less known and/or more recently discovered platelet function disorders were discussed in more detail: the collagen receptor GPVI defect, the Duchenne Muscular Dystrophy, the Kindlin3 defect, the P2Y12 defect, the thromboxane synthase defect and the Gs hypo- and hyperfunction disorders. Moreover, the heterogeneity of the secretion defects was emphasized. These naturally occurring mutations disclose the physiological relevance of these molecules in platelet function. Many more inherited thrombopathies are still waiting to be discovered.

Dr Andrew Mumford presented a diagnostic strategy of congenital PFD, which is based on the evaluation of the bleeding history of patients with suspected PFD, measurement of platelet aggregation and ATP secretion (lumiaggregometry), sequencing of candidate genes, which are then further studied in stably transfected CHO-K1 cells. The cases of patients with defects of P2Y12 (in some cases, associated with type-1 VWD) or abnormalities in the arachidonic acid/thromboxane A2 pathway were described.

Dr Alberto Tosetto reviewed the current status of application of Bleeding Assessment Tools (BAT) in patients with platelet function disorders (PFD). BAT are semiquantitative indexes of bleeding severity that have been primarily devised for use in patients with type-1 von Willebrand disease, a model of mild bleeding disorder. Their use in other bleeding disorders has not been extensive. Preliminary data of studies in PFD patients were reviewed, which suggest that BATs may have a positive impact in the diagnosis of these disorders. A consensus "new" BAT has been designed and approved by an ad hoc WP jointly formed by members of the VWF/FVIII, Pediatric Haemostasis and
Womens’ Health Issues SSC, and it will undergo further validation studies in different patient populations, possibly including PFD patients.

Finally, the panel of Co-chairs of the Subcommittee on Platelet Physiology presented the new WP on *Diagnosis of Congenital Platelet Function Disorders*, whose aims can be summarized as follows:

1. What patients should be screened for platelet function disorders?
   - Type of bleeding manifestations
   - Usefulness of bleeding scores?
   - Criteria orienting towards an inherited defect
   - Presence of associated alterations in other cells/organs
   - Any role for global tests of primary hemostasis?
   - Do we need to rule out other bleeding disorders (e.g., VWD) before studying platelet function?
   - Drug history

2. What first-line screening tests should we use?
   - Should platelet secretion be measured in parallel with platelet aggregation in all patients?

3. What second-line, confirmatory tests should we use to test the diagnostic hypothesis that was raised based on the results of the first-line screening tests?

4. Proposal of a diagnostic algorithm

In the second part of the session, Dr Robert Storey reviewed the factors that contribute to the high inter-individual variability in response to the antiplatelet agent clopidogrel, a pro-drug, whose active metabolite targets the platelet P2Y12 receptor for ADP. These factors include: age, body weight, lifestyle (e.g., smoking), abnormalities of ABCB1 (affecting the absorption of the drug), loss-of-function and gain-of-function mutations of isofroms of cytochrome C450 (which plays a major role in the biotransformation of the pro-drug into its active metabolite), diabetes mellitus, renal function, interaction with other drugs. The roles of genotyping and laboratory monitoring of platelet function of patients on clopidogrel treatment, aiming at optimizing its antiplatelet and antithrombotic effects, have also been addressed during Dr Storey’s presentation and its discussion by the audience.

Finally, Dr Marco Cattaneo presented the final report of the WP on Standardization of Light Transmission Aggregometry (LTA), which were slightly modified in the last year, since their presentation at the 2009 SSC Meeting in Boston, based on the results of a thorough literature search. As the working party concluded that LTA is clinically useful ONLY for studying platelet function disorders, the following recommendations apply to the study of patients with suspected platelet function disorders only:
• Blood samples for LTA should be collected from subjects who:
  - refrain from smoking for at least 30 minutes
  - abstain from caffeine for at least 2 hours
  - rest for a short period
• A record of all drugs that the subject has taken in the week prior to testing should be collected
• Treatment with drugs known to reversibly inhibit platelet function (e.g. NSAIDs) should be stopped at least 3 days before sampling
• Treatment with drugs known to irreversibly inhibit platelet function (e.g. aspirin, thienopyridines) should be stopped at least 10 days before sampling
• When treatment with drugs that inhibit platelet function cannot be stopped before sampling, drug-induced effects on platelet function should be considered when interpreting the LTA results
• It is uncertain whether blood samples for LTA should be collected from fasting patients, and whether treatment with any drug should be stopped before sampling
• Blood samples for LTA should be drawn:
  - with minimal or no venostasis
  - using a needle of at least 21 gauge
  - into plastic (polypropylene) or siliconized glass tubes
  - into 109 or 129 mM sodium citrate, buffered anticoagulant
• The first 3-4 ml of blood drawn should be discarded or used for tests other than LTA
• When difficulties are encountered in obtaining sufficient blood for LTA, underfilled tubes may only be used to exclude severe platelet function disorders, such as Glanzmann Thrombasthenia or Bernard-Soulier Syndrome
• Blood samples should be allowed to “rest” at room temperature for 15 min before centrifugation
• Preparation of PRP for LTA:
  - should be prepared by centrifuging blood samples at 200 x g for 10 min, at ambient temperature (approximately 21°C), without using a brake
  - should be prepared by blood sedimentation for samples with very large platelets (it is uncertain whether it is advisable to keep the tubes at 45°)
• Preparation of PPP for LTA: PPP should be prepared by centrifuging whole blood, or the tubes of blood from which PRP was removed, at ambient temperature, at 1500 x g for 15 min
• Grossly hemolyzed samples should be discarded
• If the sample tested is lipemic, the final report should indicate this
• It is necessary to check the platelet count of the PRP sample tested
• The results of LTA studies could be inaccurate when the platelet count in the PRP samples is lower than 150 x 10⁹/L, therefore, caution should be taken when interpreting abnormal results in samples with low platelet counts
• PRP with low platelet counts may be tested to exclude severe platelet function disorders (BSS, type 2B and platelet type von Willebrand disease)
- Platelet count of PRP samples should NOT be adjusted to a standardized value with autologous PPP (uncertain for PRP samples with platelet counts > 600 x 10⁹/L)
- LTA studies must include a known normal subject, run in parallel with the subject(s) under study
- After centrifugation, PRP samples should be allowed to sit at room temperature for 15 min before testing
- PRP should be used to set 0% light transmission in the aggregometer
- Autologous PPP should be used to set 100% light transmission in the aggregometer
- LTA studies should be performed at 37°C
- During LTA testing, PRP samples should be constantly stirred at 1,000 rpm using a disposable stirrer, unless otherwise specified by the manufacturer of the aggregometer
- Before adding an agonist, baseline tracings for LTA should be observed for oscillations and stability for at least 1 minute
- The volume of agonist added for LTA should be consistent, and never more than 10% of the total sample volume
- Platelet aggregation should be monitored for:
  - a minimum of 3 minutes after adding an agonist
  - a minimum of 5 minutes after adding an agonist that does not cause maximal aggregation by 3 minutes with most control samples
  - a minimum of 10 minutes after adding an agonist that does not cause maximal aggregation by 5 minutes with most control samples
- LTA studies should be completed within a maximum of 4 hours after blood sampling

The following platelet agonist should be used for diagnostic LTA studies:

- **ADP**: 2 µM (higher concentrations if abnormal results with 2 µM)
- **Epinephrine**: 5 µM (higher concentrations if abnormal results with 5 µM)
- **Collagen**: 2 µg/mL (Horm collagen) (higher concentrations if abnormal results with 2 µg/mL)
- **Thrombin Receptor Activating Peptide (TRAP)**: 10 µM (higher concentrations if abnormal results with 10 µM)
- The **thromboxane A2 mimetic U46619**: 1 µM (higher concentrations if abnormal results with 1 µM)
- **Arachidonic acid**: 1 mM (higher concentrations if abnormal results with 1 mM)
- **Ristocetin**: 1.2 mg/mL
  - In case platelet agglutination induced by Ristocetin 1.2 mg/mL is normal, testing should be repeated using Ristocetin 0.5-0.7 mg/mL
  - In case platelet agglutination induced by Ristocetin 1.2 mg/mL is absent, testing should be repeated using Ristocetin 2 mg/mL.
- The platelet aggregation tracing should be evaluated based on:
- presence of shape change
- length of the lag phase
- slope of aggregation
- maximal amplitude or % aggregation
- amplitude or % aggregation at the end of the observation
- disaggregation
- visual examination of the aggregation tracings

- The presence of a "secondary wave" induced by epinephrine should be evaluated
- Studies completed more than 4 hours after blood collection should be reported with a comment of this
- Clinical laboratories must establish an appropriate reference interval and validate test performance with each lot of reagents

Members of the WP on Standardization of Light Transmission Aggregometry:

1. M. Cattaneo, Milano, Italy (CHAIR)
2. A.D. Michelson, Worcester, MA, USA (Co-CHAIR)
3. C. Cerletti, Campobasso, Italy
4. P. Harrison, Oxford, UK
5. C.P.M. Hayward, Hamilton, Ont., Canada
6. D. Kenny, Dublin, Ireland
7. D. Nugent, Orange, CA, USA
8. P. Nurden, Pessac, France
9. A.K. Rao, Philadelphia, PA, USA
10. A.H. Schmaier, Cleveland, OH, USA
11. S. Watson, Birmingham, UK
Predictive Variables in Cardiovascular Disease

May 24, 2010
Cairo, Egypt

Chairman: James D. Douketis, Canada
Co-chair: Alberto Tosetto, Italy

Predictive Variables in Cardiovascular Disease Subcommittee (PVCDS)

Business Meeting

- Factor IX Malmö and recurrent VTE: Dr. Willem Lijfering (Netherlands) provided updated findings on effect of factor IX, patient sex and recurrent VTE as an ongoing PVCDS endorsed activity, initially presented at the SSC Meeting in Vienna. The presentation questioned the purported association between factor IX and thrombosis.

- microRNA and venous thrombosis: Dr. Carla Vossen (Netherlands) presented data from her research group assessing various single nucleotide polymorphisms (SNPs) are their relationship to the development of cardiovascular disease (coronary artery disease, stroke), outlining strengths and limitations of associations.

- D-dimer and Recurrent VTE Collaborative Working Party: Dr. Campbell Tait (UK) provided an update of research by an SSC Working Party assessing D-dimer and other predictors of recurrent VTE, including patient sex, timing and type of D-dimer measured post-anticoagulation. This group was developed at the SSC meeting in Vienna and met again at the SSC meeting in Boston. The ultimate aim is to develop a clinical prediction guide to help clinicians decide about the duration of anticoagulation after unprovoked VTE. The ongoing activities of this Working Party are described below.

- New molecular markers of CV disease: Drs. Shu He (Sweden), on behalf of Dr. Margareta Blomback presented work from her research group assessing new molecular markers of cardiovascular disease.

- Global tests to predict VTE: Dr. Astrid van Hylckama Vlieg (Netherlands) considered emerging molecular markers, related to thrombin generation, aimed at identifying patients at risk for a first episode of VTE.

- Residual vein thrombosis and recurrent thrombosis: Dr. Walter Ageno (Italy) presented preliminary findings of an SSC Working Party aimed at undertaking a patient-level meta-analysis to assess the effect of residual thrombosis as a predictor of recurrent VTE. Study plans and methodology were discussed.
Educational Session

Dr. Mary Cushman (US) provided a comprehensive and practically-oriented review of the importance of conventional risk factors for cardiovascular disease. This was followed by a lecture by Dr. Moniek de Maat (Netherlands) describing new and emerging molecular risk markers of cardiovascular disease and where they may be utilized in clinical practice. These presentations were followed by a debate entitled: “C-reactive protein should be used in everyday practice to risk-stratify patients without established cardiovascular disease”, with Dr. Cushman taking the “yes” position and Dr. de Maat taking the “no” position.

Overall, the business meeting presentations were well-received, with multiple questions from the audience. The highlight of the session was the informative and provocative presentations and subsequent debate by the two excellent speakers.

PCVDS Working Party Meeting

A meeting was convened that involved a working party which is undertaking collaborative research using pooled data from several studies assessing patients with VTE and risk for recurrence. During this meeting, ongoing and future collaborative initiatives relating to determinants of recurrent VTE were discussed. Plans were made for future research collaborations. The attendees of this meeting included: T. Baglin (UK), M. Cushman (US), F. Dentali (It), J. Douketis (Can), J. Geersing (Neth), S. Eichinger (Aut), A. Iorio (It), G. Le Gal (Fr), M. Marcucci (It), C. Moons (Neth), G. Palareti (It), D. Poli (It), M. Reghini (Ch), C. Tait (UK), A. Tosetto (It).
As for the previous two SSC meetings, the session was divided into three parts, addressing key topics in vascular biology: Topic1 "Shed proteins/receptors", in the educational part of the session, Topic2 "Detection and characterization of circulating endothelial cells and their progenitors", and Topic 3 "Determination and characterization of (circulating) microparticles", the last two topics constituting the business part of the session.

The first topic, chaired by M. Berndt, covered the mechanism of Shed proteins/receptors release, their relevance as clinical biomarkers of vascular disease and their biological function.

Robert Andrews (Australia) presented the mechanisms involved in GP VI shedding and the factors regulating GPVI release. Mechanisms involved for inducing GPVI shedding in vitro involves activation-dependent and - independent processes with a role of calmodulin. Using ELISA assays and western blot analysis of cleaved fragments, R Andrews showed that GPVI shedding is 1/ADAMS 10-mediated 2/shear stress-dependent and 3/induced by coagulation activation. This last mechanism involves factor Xa related pathways whereas thrombin was poorly involved. The next steps are now to elucidate the biological function of the shed form of GPVI.

Elisabeth Gardiner (Australia) presented the clinical relevance of soluble GPVI (sGPVI). GPVI is shed as a 55kDa soluble fragment from the platelet membrane after having undergone an activation cycle initiated upon ligand binding or engagement of FCyRIIa, signalling, calmodulin dissociation and cleavage. Soluble GPVI can be measured by ELISA, in citrated plasma. In healthy subjects, its circulating levels are not affected by age, gender and smoking. In pathological situations, sGPVI levels are significantly increased in patients with ischemic stroke and correlated with soluble PECAM-1. The interest of sGPVI in patients with antibody mediated thrombocytopenia (Heparin Induced Thrombocytopenia, Idiopathic Thrombocytopenia) was also discussed.

Nathalie Bardin (France) provided a brief historical overview of CD146, a transmembrane glycoprotein of 120 kDa belonging to the immunoglobulin superfamily and involved in cell permeability, monocyte transmigration and angiogenesis. CD146 is also detectable as a soluble form in human plasma. Although sCD146 alterations have been reported in different pathological settings, significance of sCD146 variations remains unknown. The role of sCD146 in vascular biology was investigated with a specific focus on angiogenesis. In vivo, sCD146 displays chemotactic activities on
several types of vascular cells including mature and progenitor endothelial cells (EPC), and smooth muscle cells. In vitro, sCD146 increases EPC migration, proliferation and vascular tube formation. In a mouse model of hind limb ischemia sCD146 was shown to promote angiogenesis and tissue reperfusion. These data identify sCD146 not only as a biomarker, but also as a potential effector of vascular regeneration.

The second topic, chaired by F Dignat-George, was dedicated to the analysis of circulating endothelial cells (CEC) and their progenitor (EPC). Technical challenges of standardization rely both on their scarcity and the overlapping phenotype between progenitors (EPC) and mature endothelial cells (CEC). CEC and EPC have emerged as potential biomarkers and therapeutic targets in cardiovascular disorders and cancer angiogenesis. However, lack of consensus in nomenclature and identification markers hampered the full development of EPC and CEC in clinical application.

Jamie Case (USA) described improvement in FCM methods allowing accurate detection of rare events and distinction between EPC and CEC. He presented validation of an FCM assay that identified EPC as viable cells expressing CD34, CD31 and CD146 while negative for CD45 and CD133. Interestingly, the clonogenic potential of this sorted population was attested together with their capacity to proliferate as endothelial colony forming cells expressing vWF.

Francoise Farace (France) reported on the development and validation of a multi-parameter flow cytometry assay to enumerate CEC in whole blood, with specific recommendations for rare event detection. CEC are defined as C31+, CD45-, CD146+ viable events. With such an assay, normal values were in the same order of magnitude compared to Immunomagnetic separation assay used as the consensus method. Higher levels were observed in patients with metastatic cancer. Data from a cohort of 99 patients with colorectal cancer treated with chemotherapy and bevacizumab indicated that CEC values at baseline or at the end of the first cycle may predict patient response to treatment. Advantages and limitations of the FCM method were discussed.

Patrizia Mancuso (Italy) provided data on a flow cytometry assay for CEC enumeration based on the following criteria: CD45- nucleated cells, CD31+, CD146+, CD133-. This assay allowed distinction of viable versus necrotic cells. P. Mancuso showed that after sorting, such events exhibit Webel-Palade bodies and express VE-Cadherin m-RNA, consistent with their endothelial origin. Definition of analytical performance for CEC counting indicated an inter-operator variability of 17% and a reproducibility of 26%. In healthy subjects, CEC mean levels were about 150 cells/ml. The interest of this assay for monitoring anti-angiogenic therapy in cancer patients was discussed.

The last topic, chaired by Nigel Key, was dedicated to the analysis of cell-derived microparticles. New directions related to the impact of pre-analytic steps, the development of new generations of flow cytometers or alternative technologies allowing enumeration and characterization of particles of smaller sizes, were presented.

Romaric Lacroix (France) emphasized the importance of pre-analytics in microparticle (MP) analysis, showing the impact of different parameters on healthy subjects. Data showed that 1/Time delay before first centrifugation influences PMP counts and activity.
as soon as 1h. 2/Agitation during transportation is a critical pre-analytical altering PMP analysis. This effect can be prevented with a more controlled transportation system. 3/A centrifugation protocol (2x2,500g 15min) generates less artefactual PMP, indicating that a routine adapted centrifugation protocol could be appropriate for MP determination. 4/One freezing/snap thawing cycle at -80°C does not significantly affect PMP analysis up to 6 months. These preliminary data indicated that there is a need for standardization of pre-analytical procedures. Based on these data, a pre-analytical protocol for MP analysis was proposed. A new collaborative workshop to validate this proposal will be set up.

Nigel Key reinforced the importance of pre-analytics focusing on the pre-analytical variables for plasma MP tissue factor activity determination. TF activity in plasma was detected both by immobilization methods and in the pellet of high speed centrifugation. In all cases, centrifugation speed was showed to be a critical variable. Furthermore, addition of exogenous phospholipids increased TF independent activity but not the TF dependent counterpart. Finally, platelet free and/or poor plasma TF activity was not affected by a freeze/thaw cycle.

Chris Gardiner (UK) presented the last version of a laser tracking instrument (Nanosight) based on the measurement of Brownian motions. This technology measures particles with a size between 50 nm and 1 μm. Analysis of preparations of particles from several cell types suggests that the concentration of particles is 1000 fold greater than estimates by conventional flow cytometry. However, the specificity of this technique, related to interference of lipidic vesicles, remains to be improved. The fact that particles different from real MP or exosomes may interfere, advocates for specific labeling. Since standard fluorochromes are not appropriate due to photobleaching, other reagents, such as antibody-coated Q-dots, are under investigation for specific staining of MP subsets.

Bernard Chatelain (BE) reviewed technological factors influencing size-measurement using either forward or size scatters. He compared the resolution capability of different types of new generation flow cytometers (including optional version for some instruments) using size-calibrated beads. He proposed simple means and a formula to calculate resolution index and provided a table of values illustrating the great differences in resolution among instruments, even of the latest generation. From this overview, it appears that new FCM instruments now have improved sizing performance that allow resolution of smaller sized MP (0.3 μm and even lower), with significant impact on the total amount of microparticles detected by flow cytometry. Alternative threshold strategies have been proposed based either on side scatter or fluorescence. These approaches need to be challenged on various instruments and models.

Technologies such as flow cytometry (FCM) and capture assays (CA), provide complementary information related to the enumeration and procoagulant activity of microparticles and have been proved useful in clinical studies. Francoise Dignat George and Jean Marie Freyssinet (France) presented the results of a multicenter French study aimed to investigate whether the variations reported by the two methods are correlated. Circulating levels of Platelet MP and annexin V^-MP, measured by flow
cytometry and capture assays were highly correlated ($r= 0.86$, $p < 0.001$ for annexin V$^+$-MP ($n=229$) and $r= 0.85$, $p < 0.001$ for PMP ($n=291$), respectively.

Multivariate analysis also showed that sedimentation rate and fibrinogen were positively correlated to PMP and annexin V$^+$-MP, whatever the methods used for their determination. These results are mutually supportive to qualify both flow cytometry and capture assays for MP determination. Although both techniques may detect different fractions of circulating MP, they point towards similar trends for variations linked to inflammation.

Finally, Francoise Dignat George presented an update and perspectives of the ISTH Vascular Biology Working Group related to the Measurement of microparticles by flow cytometry. During the ISTH SSC that took place in Vienna, in 2008, a collaborative workshop on the standardization of microparticle counts was set up to define the inter-laboratory reproducibility of PMP counts using flow cytometry. To avoid any pre-analytical or reagent-linked variation, all participating laboratories analyzed aliquots of PFP samples prepared by F Dignat George laboratory, using common reagents and the same flow cytometry protocol that was calibrated using Megamix beads. The conclusions of this workshop, presented in ISTH Boston in 2009 indicated that 1/ It is possible to define flow cytometry performances in terms of background and resolution using Megamix™ beads, regardless of the type of instrument 2/ these calibrated beads represent a useful tool to standardize MP enumeration in a reproducible manner on Beckman Coulter flow cytometers with inter assay coefficients of variation of about 15 % whereas other strategies may need to be validated for Bekton Dickinson instruments.

Taken as a whole, it was concluded that standardization is possible but is dependent on intrinsic characteristics of both the flow cytometers and the calibration strategy. Results of this first ISTH SSC collaborative workshop" has been submitted to publication to the Journal of Thrombosis and Haemostasis and F Dignat George thank all the people who kindly helped to set up this workshop and those who actively participated.

During the Boston meeting, important questions were raised, in particular related to 1/ the representativeness of the measurable part of MP (the “visible part of the iceberg”) of the clinically relevant biomarkers we are seeking, 2/ the need for new generations or types of flow cytometer (or alternative technologies with similar immunological capabilities) that would allow enumeration and characterization of particles of smaller sizes 3/ the impact of pre-analytic parameters on MP determination and 4/ the potential correlations between flow cytometry and functional tests available for MP determinations. Some of these questions have been partially addressed in 2010, during the Cairo meeting. In particular, recent technological improvements of new generation flow cytometers, in term of size resolution and background noise, maintain flow cytometry as a highly competitive analytical method to measure microparticles. Challenging these evolutions in pathological situations is a mandatory step to validate their real impact in clinical practice. There is also a need to use more “objective” methods (AFM, DLS, …) to qualify homogeneous preparations of MP suspensions to be circulated as standards and used within the community.
questions open directions for future collaborative working parties in our VB SSC. Because standardization of the pre-analytical step was identified as a prerequisite and a limiting/critical step for future studies, the SSC ISTH Cairo was the opportunity to propose a new collaborative workshop on the impact of pre-analytic variables on MP determination. To that aim, participant laboratories will have to prepare Plasma free platelets (PFP) from healthy patients using predetermined conditions, and the core laboratory will analyse these PFP, using flow cytometry and functional assays. Instructions and documents to download will be available from the ISTH SSC website very soon.
von Willebrand Factor

23 May 2010
Cairo, Egypt

Chairman: Jeroen Eikenboom (NL)
Co-chairmen: Thomas Abshire (US), Imre Bodo (HU), Giancarlo Castaman (IT), Jorge DiPaola (US), Emmanuel J Favaloro (AU), Anne Goodeve (UK), Bernhard Lämmle (CH), David Lillicrap (CA), Reinhard Schneppenheim (DE)

Audience: approximately 100-120 on Sunday, 70 on Monday

Summary of VWF Subcommittee Approvals and Working Parties

- WHO 2nd International standard VWF, concentrate (NIBSC code 09/182) advised to be accepted
- WP on Bleeding Assessment Tool ended, manuscript submitted as SSC Official Communication
- WP on VWF assays in VWD diagnosis has ended, closure of website (www.vwfassays-in-vwd.com), manuscript submitted as SSC Official Communication
- Set up a steering committee for the VWF mutation database registry to review submissions (www.vwf.group.shef.ac.uk)
- WP on DDAVP in the management of VWD will be finished this year, closure of website (www.ddavp-in-vwd.com)
- WP on standardization of VWFpp will probably despatch samples for testing in January 2011, and report to the SSC VWF Subcommittee July 2011
- Registry on platelet-type VWD (www.pt-vwd.org) ongoing
- Registry on Acquired Von Willebrand Syndrome (www.intreavws.com) ongoing
- A document by the Medical Standards Committee Haemostaseology of the German Institute for Standardization (Normenausschuss Medizin – DIN, Deutsches Institut für Normung e. V.) describing a framework for a reference method for the assay of VWF multimers (DIN 58988) will be circulated among the VWF Subcommittee co-chairs for evaluation and possible endorsement.
- A proposal by the Biorheology Subcommittee for a joint WP on flow based assays for the diagnosis of VWD will be considered, but needs further elaboration and discussion before a decision can be made.

Standardized bleeding scores:
Session chairs Thomas Abshire (USA)/David Lillicrap (CA)

A joint working party of the VWF and Perinatal/Pediatric Hemostasis subcommittees has been working on a standardized scoring system for mild bleeding to improve diagnosis, treatment and communication about bleeding and to develop guidelines for a quantitative bleeding score. During this VWF subcommittee meeting a joint session was organized with other subcommittees interested in this subject (Perinatal/Pediatric
Hemostasis, Factor VIII & Factor IX, Women’s Health Issues in Thrombosis and Haemostasis, and Platelet Physiology) to report on the results of the WP.

Alberto Tosetto (IT) reported on bleeding scores in adults and on the final report of the WP on standardized bleeding scores that has been submitted as an SSC Official Communication (Bleeding Assessment Tool: A Standardized Questionnaire and a Proposal for a New Bleeding Score for Inherited Bleeding Disorders) to JTH. He gave an introduction on the basic principles of such questionnaires: it starts first with collection of clinical data (paper or web-based), then interpretation of data through a bleeding scale, and in the end a bleeding score will be assigned depending on the scale you use. Validity of a score depends on its use. For diagnostic purposes (PPV, NPV) the scores perform well. For prognostic purposes there is no gold standard for severity (what is the relation with factor level?). Scores perform well in mild bleeding disorders, but saturate in severe bleeding.

Margaret Rand (CA) discussed that the accurate assessment of mucocutaneous bleeding symptoms can be particularly difficult in children. A quantitative Pediatric Bleeding Questionnaire (PBQ), based on the MCMDM-1VWD Bleeding Questionnaire, was developed that includes pediatric-specific bleeding symptoms, eg post-circumcision bleeding, cephalohematoma, macroscopic hematuria and bleeding from the umbilical stump. A score of ≥2 is predictive of a diagnosis of VWD. Scores are higher in more severe forms of VWD and correlate positively with age in types 1 and 3 VWD. The PBQ is also useful in assessing bleeding severity in children with inherited platelet function disorders.

Flora Peyvandi (IT) reported on bleeding scores in rare bleeding disorders. Clinical severity is very variable even in severe FV, FVII and FXI deficiency. We need a quantitative approach to symptoms and we should use a similar definition. It is necessary to be able to distinguish normal and affected subjects and to quantify severity among affected subjects. FXI deficiency shows no association between FXI levels and bleeding score. For the other rare deficiencies there is a clear association between bleeding score and the laboratory severity of the deficiencies.

Claire Philipp (USA) reported on menorrhagia and postpartum hemorrhage as bleeding symptom. She focused on the impact of menorrhagia on invasive procedures, transfusion, iron deficiency anemia, work/school loss, impaired quality of life. Bleeding score was used as a screening tool for gynecologists to identify women with menorrhagia to be screened for an underlying bleeding disorder.

**VWF assays for VWD diagnosis**
Session chair Anne Goodeve (UK)

- **Alternative assays for GPIb binding**

Hans Deckmyn (BE) presented data on an alternative assay for VWF:RCo that they have developed. It was developed as an ELISA method: coating with mAb a-rGPlbα to
which rGPIbα binds, then plasma plus ristocetin is added, GPIbα-bound VWF is detected by a conjugated mAb a-VWF. The assay has been modified for automation by a turbidimetric method. Nanoparticles coated with rGPIbα fragment agglutinate by binding VWF in presence of ristocetin. Assay discriminates between normal, type 1 and type 2 VWD. Assay performs at least equally well with regular platelet agglutination assay.

Bob Montgomery (USA) presented data on their ELISA based GPIbα binding assay that does not require ristocetin. The mutant rGPIbα that is used spontaneously binds to VWF without the need for ristocetin. The D1472H polymorphism present in 67% of African Americans causes falsely reduced VWF:RCo results due to interference with ristocetin binding. This effect of the polymorphism is overcome with the new assay.

Juergen Patzke (GE) reported a similar assay in which a recombinant GPIbα fragment containing two gain of function mutations was used to establish a VWF:GPIb binding assay that does not require ristocetin. A particle enhanced agglutination assay format allowed the application on automated instruments. The measuring range covered 3 to 150% VWF. An excellent precision and a good correlation to the VWF:RCo assay was observed. As expected from an activity assay, the VWF:GPIb binding/VWF:Ag ratio was lower in type 2A, M and B than in type 1. The new assay has the potential to replace the VWF:RCo assay.

A future activity of the subcommittee could be focused on the comparison/evaluation all these and other alternatives to the regular VWF:RCo assay.

- **Standardization of multimer analysis**

Peter Turecek (AT) reported on a document (DIN 58988) by the Medical Standards Committee Haemostaseology of the German Institute for Standardization (Normenausschuss Medizin – DIN, Deutsches Institut für Normung e. V.). This document describes the framework for a reference method for the assay of VWF multimers (the number and size distribution and the substructures of the multimers in citrated plasma or factor concentrates). It focuses on collection and processing of blood, transport and storage conditions of samples, test and evaluation methods, and quality control, quality assurance and validation considerations. It is proposed that VWF-SSC should consider DIN 58988 as a guidance for more in depth standardization of methods to analyze multimers. The document will be circulated among co-chairs for evaluation and possible endorsement by the VWF Subcommittee.

- **Flow based assays**

Johan van Heemskerk (NL) reported that flow based assays have high potential in detecting functionality of VWF, however applicability of these assays is still a problem because the assays are also sensitive to other variables like platelet count and hemoglobin levels. Furthermore, the assays are not standardized. He suggested to form a joint WP between Biorheology and VWF Subcommittee. It was decided that in the
next few months we will further elaborate on this to work out the best way to proceed with this. No formal WP was formed yet.

Keith Neeves (USA) reported that blood flow assays are a promising approach for measuring VWD phenotypes because they capture the shear stress dependent functions of VWF. To date, flow assays have been primarily used as a research tool for measuring receptor-ligand dynamics at physiologic shear stresses. A goal of his lab is to translate flow assays from a research tool to a clinical assay. He presented a technology platform for processing small volumes (<1 mL) of whole blood over a range of shear stresses (100-2600 1/s) using micropatterning and microfluidic techniques. He showed some of the technical challenges they have encountered and preliminary data using VWD whole blood in their microfluidic flow assays.

**VWF propeptide studies**
Session chair Anne Goodeve (UK)

Tony Hubbard (UK) reported on the plans of the Working party on standardization of VWFpp assays. Although there is an agreed international unitage (IU) for VWF:antigen, there is no agreed IU for VWFpp and laboratories rely on local reference preparations which may be a cause of considerable inter-laboratory variability as well as leading to problems of long-term continuity. The SSC/ISTH Subcommittee on VWF has established a Working Party on Standardization of VWFpp Assays with the objectives of assessing the inter-laboratory variability of VWFpp (and VWF:antigen) estimates and the calibration of a reference plasma with an agreed unitage for VWFpp. The WP will organise a multi-centre study which will involve the testing of “common” plasma samples relative to local reference preparations for VWFpp and VWF:antigen. This comparison will allow the assessment of inter-laboratory variability of both analytes. Inclusion of the WHO 6th IS FVIII/VWF Plasma and the SSC/ISTH Secondary Coagulation Standard (Lot #3) as the “common” plasma samples may also allow the assignment of a value for VWFpp to these reference preparations, however, this will depend on the variability and quantity of the data in the study.

**VWF plasma and concentrate standards**
Session chair Anne Goodeve (UK)

Tony Hubbard (UK) reported that declining stocks of the WHO 1st IS VWF Concentrate (00/514) has made it necessary to prepare a replacement. Value assignment of the proposed WHO 2nd IS was undertaken in an international multi-centre study involving 45 laboratories. Three candidate VWF concentrates were assayed for VWF:Ag, VWF:RCo and VWF:CB relative to the WHO 1st IS VWF Concentrate (00/514) and the WHO 6th IS Factor VIII/VWF Plasma (07/316). For all candidates and all analytes the inter-laboratory variability was reduced when estimates were calculated relative to the WHO 1st IS Concentrate as compared to the WHO 6th IS Plasma; this finding supports the use of the WHO IS Concentrate for the assay of therapeutic concentrates. Candidate D was proposed as the WHO 2nd IS Concentrate since it was associated with the lowest inter-laboratory variability for all 3 analytes and resembled the WHO 1st IS most closely in
terms of multimer profile and Ag/RCo ratio. Proposed assigned values for VWF:Ag and VWF:RCo were derived from assays relative to the WHO 1st IS VWF Concentrate to ensure best continuity of the International Unit (IU). The proposed assigned value for VWF:CB was calculated from assays relative to the WHO 6th IS Plasma since the WHO 1st IS Concentrate does not have an assigned value. No significant difference was found between VWF:CB estimates obtained using type 3 and type 1/3 collagen reagents and the overall inter-laboratory variability (GCV) of 18.7% was considered acceptable for the calculation of a consensus mean. **It is proposed that candidate D (NIBSC code 09/182) be accepted as the WHO 2nd IS VWF Concentrate with the following assigned values: VWF:Ag (10.7 IU/ampoule); VWF:RCo (9.2 IU/ampoule); VWF:CB (10.3 IU/ampoule).** All VWF Subcommittee co-chairs have approved when the document was circulated.

**ADAMTS13**

Session chair: Thomas Abshire (USA)

Koichi Kokame (JP) reported that at present, we have no absolute standard for ADAMTS13 measurement, and therefore, pooled normal plasma is commonly used as a standard. How many plasma samples should be combined to make a standard? The number of male and female samples should be the same, because the ADAMTS13 activities are significantly different between males and females. Their data suggested that more than forty plasma samples are required for rigorous analysis of the ADAMTS13 activity, and twenty samples may be sufficient for diagnostic analysis.

Yoshihiro Fujimura (JP) reported on ADAMTS13 inhibitor levels in patients with acquired TTP. Measurement of ADAMTS13 antibodies titer may be necessary for optimal treatment choice. In the discussion it was mentioned that this may not be the case considering the high variability of antibody titers among patients.

Hans Deckmyn (BE) reported on an animal model of acquired TTP in baboons. Murine anti-hADAMTS13 mAbs were developed (anti-metalloprotease domain and anti-TSR2). Injection of antibodies in baboons induced trombocytopenia, schistocytes, hemolysis, decreased hemoglobin levels, some mucous bleeding, and microvascular thrombosis. No deaths or organ failure were observed, recovery after cessation of administration of antibodies. This could serve as a animal for acquired TTP.

**VWF and VWD registries**

Session chair: Imre Bodo (HU)

Maha Othman (CA) reported on the status of the ongoing platelet-type VWD registry (www.pt-vwd.org). 106 cases suspected for PT-VWD were submitted for analysis (67 cases from Canada and 38 from outside Canada). GPIba mutations were identified in 17/106 cases: mutation G233S in 18%, G233V in 82%. In 44/106 cases type 2B VWF mutations were identified. PT-VWD is really rare. Website will be maintained, it should be indicated on the website that this registry is endorsed by the ISTH/SSC. Registry should be linked to both VWF and Platelet Physiology Subcommittees.
Dan Hampshire (UK) reported on the VWF mutation database registry (www.vwf.group.shef.ac.uk). In light of recent research developments within the field of VWF and VWD, the information contained in VWFdb has been re-evaluated. There are currently 541 mutations and 218 polymorphisms listed, of which 382 and 167 respectively are unique entries. Thirty (18%) of the unique polymorphisms represent missense alterations. He proposed some changes to VWFdb, including formation of a steering group to evaluate mutation and polymorphism submissions to the database and to recommend further areas for development. It was decided to form a steering committee. In the next few months an SSC Official Communication will be submitted.

**Maintenance of Websites of different Working Parties**
Session chair: Imre Bodo (HU)

Augusto Federici (IT) discussed whether websites of different WP should be maintained or ended.

**www.ddavp-in-vwd.com:** WP on desmopressin in the management of VWD will be finished in 2010; data to be analysed. The VWF Subcommittee voted in favor of closure of the website.

**www.intreavws.com:** Although submissions to the international registry on acquired VWS were limited, the registry will be continued. Additional funding will be obtained to keep the website open for at least 2010-2015. The VWF Subcommittee voted in favor of continuing the website. A steering committee will be formed for evaluation of submissions.

**www.vwfassays-in-vwd.com:** WP on VWF assays in VWD diagnosis has finished and a manuscript has been submitted as SSC Official Communication. The VWF Subcommittee voted in favor of closure of the website.

**Ongoing and starting multicenter studies on VWD**
Session chair: Imre Bodo (HU)/Jeroen Eikenboom (NL)

In this session several multicenter VWD studies were discussed. The VWF Subcommittee is becoming more and more a fruitful platform for discussing items related to initiating and maintaining (inter)national collaborations. Many of those collaborative studies will be independent and no formal WP of the Subcommittee, however they may be endorsed by the Subcommittee.

- **Working party on desmopressin in the management of VWD**

Augusto Fedrici (IT) reported that this study is closed. Results so far: 268 entries into the study, 263 eligible patients, 225 evaluable patients, 222 made correct evaluations for biological response. Patients were prospectively followed for 24 months. 83 patients underwent DDAVP treatment (35 for bleeding, 48 for minor & major surgeries) and can
be analyzed for clinical efficacy. Formal statistical analyses to be performed. Manuscript will be prepared and submitted to BLOOD.

- **Working party on VWF assays in VWD diagnosis**

  Augusto Federici (IT) explained that the work for this WP had already finished in 2008. Initially a full manuscript was written, which was agreed among by Subcommittee members. The manuscript was, however, not accepted by JTH as a full report. Now it has been submitted as a short SSC Official Communication to JTH, review underway. There are still lyophilized plasma samples left from the study, stored at NIBSC (Tony Hubbard). In the next months we will discuss among co-chairs about possible studies to be performed with these samples.

- **European Project on type 3 VWD**

  Augusto Federici (IT) presented the European Project on type 3 VWD, which is an independent study, but greatly supported and officially recognized by the VWF Subcommittee. Also, recognized by EAHAD and received travel grant from EAHAD. Four year international (Europe and Iran) study on type 3 VWD. Aims: Role of bleeding severity score and levels of VWF and FVIII as predictors of bleeding tendency in type 3 VWD; response to VWF/FVIII concentrates; risk of anti-VWF inhibitors. Network: European and Iranian Centers. Data to be obtained: bleeding score, plasma/DNA analyses of VWF, methods for anti-VWF antibodies. Two year prospective evaluation of bleedings, doses of concentrate and exposure days. Funding is being discussed with pharmaceutical companies.

- **EUVWD Cooperative Group**

  Ian Peake (UK) explained that a new group (EUVWD) was formed as continuation of the internal and external MCMDM-1VWD collaborations. Several projects are still being published as outcome of the MCMDM-1VWD project. A number of other projects are running, also in collaboration with the NIH Zimmerman project.

- **Zimmerman Project (ZPMCB-VWD)**

  Bob Montgomery (USA) summarized some results from the Zimmerman Project. Recruited: 456 VWD index cases, 1336 affected and non-affected family members, 71 patients with menorrhagia pilot study, controls 246, menorrhagisa controls 65; total recruited 2179. Several patients have higher VWF levels than expected, but many of those still have VWF gene mutations identified. No mutations in 36/152 type 1 patients. Those basic data are very similar to the previous MCMDM-1VWD and the Canadian type 1 studies.

- **VWD International Prophylaxis (VIP) Study**
Tom Abshire (USA) presented the VWD International Prophylaxis (VIP) Study, which is an independent study, but greatly supported and officially recognized by the VWF Subcommittee. It studies the effect of prophylaxis in VWD (both retrospective and prospective study). Prospective part: follow-up for 1 year.

- **WiN (Willebrand in Netherlands)**

Jeroen Eikenboom (NL) reported on the Willebrand in the Netherlands study (WiN) that has included 806 patients (participation rate 76%) with moderate-severe VWD in the Netherlands that are all known in hemophilia treatment centers. Inclusion criteria were VWF:Ag and/or VWF:RCo and/or VWF:CB <30% and/or FVIII:C <40%. Bleeding score questionnaires, health-related quality if life (HR-QoL) questionnaires, historic laboratory data and laboratory data at recruitment have been obtained.

*Submitted by Jeroen Eikenboom (NL)*
Women’s Health Issues in Thrombosis

24 May 2010
Cairo, Egypt

Chairman: Sabine Eichinger (AT)
Co-chairmen: Margareta Blomback (SE), Benjamin Brenner (IL), Jacqueline Conard (FR), Andra James (US), Barbara Konkle (US), Peter Kouides (US), Claire McLintock (NZ), Claire Philipp (US)

Start: 3:00 pm

Attendants: ~ 80

Welcome and Introduction of Co-chairmen (S. Eichinger)
Sabine Eichinger welcomed all participants also on behalf of the co-chairmen. She provided an overview of the program and reasons for changes in the program (Benjamin Brenner and his coworker Anahat Aharon could not attend the meeting because of difficulties to obtain visa for Israeli visitors to Egypt). Menno Huisman was excused for personal reasons.

She then provided an overview of the aims of the SSC Subcommittee on Women’s Health Issues on Thrombosis and Haemostasis (WHITH) and past activities and achievements.

Overview of Educational Activities (S. Eichinger)

This year’s Educational Session of the Subcommittee on WHITH was organized by Flora Peyvandi and Andra James. There were three speakers on the following topics:

- The thrombotic risk associated with oral contraceptive use - A. van Hylckama-Vlieg, The Netherlands
- The risk of TTP in congenital and acquired AD13 deficient women - F. Peyvandi, Italy
- Management of pregnancy in women affected with coagulation disorders – R. Kadir, UK

The session was very well attended (~150), and it was well appreciated that about 1/3 came “from the region” (Africa, Middle East).

On behalf of Benjamin Brenner, Sabine Eichinger presented the current state of activities related to the 4th International Symposium on Women’s Health Issues in Thrombosis and Haemostasis, which is organized by Benjamin Brenner and Ian Greer, and will be held from February 4-6, 2011, in Berlin.

Update on Registries
Andra James provided an overview on the current status of registries. There are four registries, two have ended (Registry on Thrombosis and Thrombotic Risk in Women Receiving Ovarian Stimulation for Pregnancy; LMWH in patients with artificial heart valves), one is running (Registry for Levonorgestrel Intrauterine System In Women with Inherited Bleeding Disorders), and one is in the process of being posted on the ISTH website (Corpus luteum bleeding in women with bleeding disorders registry). None of these registries recruited either no or just a few patients. Dr. James explained reasons for lack of participation. The major problem is funding, e.g. for IRB approval, promotion, incentives for participation.

Sabine Eichinger provided information that funding would be available from the SSC/ISTH. The amounts are limited and are mainly intended to facilitate communication between members of working parties/groups or registries, e.g. to organize telephone or video conferences. The ISTH website will be cleared from all non-active registries. Future registries should only be proposed if the person responsible for conduct, the aim, an estimated time frame, the location for hosting, and the form of funding are ascertained though the chairman and the co-chairmen of our Subcommittee.

**SSC initiated projects**

Barbara Konkle gave an update on her work on outcomes of pregnancy in patients with type 1 vWD.

On behalf of Menno Huisman Sabine Eichinger proposed a new initiative of our Subcommittee, Diagnosis of deep-vein thrombosis and pulmonary embolism in pregnancy, of our SSC. This issue is of great importance as clinical prediction rules are not validated in pregnancy and standards for D-Dimer in the pregnant state are not available. There is also a lot of controversy with regard to extent and hazards of fetal and maternal irradiation during CT and lung scanning. The activity received much support and was well accepted. Collaboration with the SSC on Fibrinolysis particularly for standardization of D-Dimer will be sought. The format of this new initiative (working party, working group, Subcommittee driven activity) needs to be clarified with the chairman of the SSC.

Jacqueline Conard provided an overview on the risk of venous thromboembolism during hormonal contraception, with a particular focus on hormonal patches, rings and hormone-releasing IUDs.

**Thrombophilia and pregnancy outcomes**

This topic has been extensively discussed in the light of many controversies during several meetings of our Subcommittee. Meanwhile, results from several large studies are available and the data set became more robust. Importantly, interventional studies with a clinical outcome are available. The whole spectrum of thrombophilia and pregnancy outcomes was highlighted during our session by Claire McLintock, followed
by Saskia Middeldorp, who presented data on the ALIFE Study, a randomized controlled trial which compared placebo, aspirin and aspirin combined with LMWH in women with unexplained recurrent miscarriage. She also presented data from another recently published interventional trial (SPIN study).

It is aimed to summarize recommendations on thrombophilia screening and management of women with pregnancy complications in a SSC document.

Activities related to the Menorrhagia Working Party

The menorrhagia working party has very successfully been led by Claire Philipps and consists of another 4 members (Peter Kouides, Rochelle Winikoff, Rezan Kadir, Christine Lee). One of the major achievements was the contribution of the group to developing a "Bleeding Assessment Tool" in a joint project with the Subcommittee on vWF and Perinatal/Pediatric Thrombosis and Hemostasis. Dr. Philipps presented on standardization processes for assessing bleeding in women with menorrhagia.

It has been agreed that the working party will continue to be active in the validation study for the "Bleeding Assessment Tool".

Bleeding disorders are a major problem for women in underprivileged countries. To highlight this topic, Flora Peyvandi gave a presentation on the risk of miscarriage in women with rare bleeding disorders, who are also more prevalent in underprivileged countries.

Magdy El-Ekiaby explained the problem of bleeding disorders in women under the background of cultural and social differences that make fertility is essential. He provided information on a program that will help to improve the management of these women in Egypt.

It has been agreed that activities of our Subcommittee should expand on the issue of bleeding disorders in women in underprivileged countries. To fulfil this task the Working Party on Menorrhagia will be modified and restructured.

The presentation of two new projects (Travel and thrombosis in women; Assay of TF and TFPI on microparticles in pregnancy complications) by Benjamin Brenner and Anahat Aharon had to be cancelled.

Closing Remarks (Sabine Eichinger)

The next meeting of the Subcommittee on WHITH will take place during the 57th Annual SSC Meeting on the occasion of the XXIII ISTH Congress in Kyoto, Japan, from July 23-28, 2011.

It is planned to have an Educational Session of the Subcommittee on WHITH during this meeting.

End: 6:35 pm
Review of Lot #3 (A Hubbard)

Lot #3 is now in the fourth year of use and this presentation gave an update on the stability testing and the despatch to customers.

Stability testing. In February 2010 the final stability testing was undertaken by the Royal Hallamshire Hospital (Sheffield, UK) and NIBSC. This included the testing of samples stored at elevated temperatures for over 6 years (accelerated degradation study) and also the comparison of samples stored at -70° C with ampoules stored at the bulk storage temperature of -20° C (real-time study). The estimates of residual activity for four factors (V, VII, VIII, XI) after storage of vials at elevated temperatures fitted the Arrhenius model extremely well and gave robust predictions of loss evident from the small 95% upper confidence limits of loss. The combined data indicated that FVIII was the least stable of the four factors with a mean predicted loss of 0.098% per year at -20° C, whereas FXI was the most stable with a mean predicted loss of 0.004% per year. The upper 95% confidence limit of loss for FVIII supported a shelf-life of 25 years. Predictions of loss for storage at higher temperatures supported the shipment of Lot #3 at ambient temperature for short periods. Comparison of the measured loss for the four factors after 6 years with the calculated loss based on the predictions showed extremely good agreement. The mean potency of vials stored at -70° C for 6 years differed by less than 5% from vials stored at -20° C confirming the extremely good stability of Lot #3 at -20° C. It was suggested that this stability study was of sufficient general interest to justify publication.

Despatch to customers. Between the beginning of July 2009 and the end of April 2010 a total of 5,830 vials were despatched. This included the use of 1,230 vials in the calibration exercise for Lot #4. In total 38,288 vials have been despatched from the initial stock of 54,800 vials. The remaining stock of 16,512 vials is expected to be exhausted in 2012.

Use of Lot #3 by EQA schemes

UK NEQAS Troubleshooting: Dr Kitchen summarised the conditions of use of Lot #3 for troubleshooting purposes. Over the last year there has only been one issue of the standard for this purpose. This related to a problem in the estimation of Antithrombin function in a single laboratory. This laboratory used a system which incorporated instrumentation and reference plasmas from different manufacturers and hence help from either manufacturer was limited. The laboratory reported results approximately 12% higher than the median on 2 separate surveys. The SSC Standard Lot #3 has
been supplied to help identify the source of variation. Results in subsequent surveys will indicate whether this has been successful.

**College of American Pathologists: Dr Teruya** discussed the inclusion of Lot #3 in two surveys (VWF and thrombophilia) during 2010. For most of the analytes the deviation of the measured mean potency from the assigned value was less than 10% which was considered acceptable. However, for two factor VIII:C methods the bias was greater than 10% and for one Protein C clotting method the bias was close to 20% which agreed with the finding for this method in 2009. One method for Protein S activity was also associated with a bias around 20%. Highest inter-laboratory variability was found for one VWF:ristocetin cofactor method and two Protein S activity methods. These results emphasise the need to include as many different methods in the calibration exercises in order to avoid any bias in the assigned values.

**Progress towards Lot #4**

**Procurement of Lot #4 (A Hubbard)**

The purchase order for Lot #4 was submitted to the manufacturer with the successful bid in June 2009. Lot #4 was manufactured from a pool of plasma from 186 US normal donors collected by apheresis. The pool was filled into 100,000 screw-capped vials (1ml plasma per vial) and lyophilised. The CV of the fill was 2.8% and the residual moisture was 0.1%. NIBSC took delivery of the 100,000 vials on 21 October 2009 and the stock was placed into -20°C storage. Vials were placed into storage at elevated temperatures (+4, +20, +37, +45 °C) in December 2009 for subsequent testing in the accelerated degradation study.

**Calibration strategy and update (E Gray)**

The calibration of Lot #4 for the 19 analytes commenced in January 2010. The value assignment will involve multi-centre studies which will include Lot #4, Lot #3 and the relevant WHO International Standard (WHO IS). Where applicable the value assignment will be combined with studies to replace the current WHO IS, otherwise there will be studies dedicated only to the calibration of Lot #4. It is planned to complete the calibration exercises in time to submit the results for endorsement at the SSC Business meeting in July 2011.

There were 32 attendees.