



**55th Annual  
Scientific and Standardization  
Committee Meeting**

**Boston, USA**

**July 11-12, 2009**

# SCIENTIFIC SUBCOMMITTEE MINUTES

11-12 July 2009, Boston, MA, USA

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# Animal, Cellular, and Molecular Models of Thrombosis and Haemostasis

11 July 2009  
Boston, MA, USA

Chairman: *Timothy Charles Nichols, USA*

Co-chairs: *Edward M Conway, Belgium; Shaun Coughlin, USA; Jay L. Degen, USA; Nigel Mackman, USA; Eva-Maria Muchitsch, Austria; Susan Smyth, USA; Hugo Ten Cate, The Netherlands; Hartmut Weiler, USA*

The focus of this Animal Models Subcommittee meeting was to discuss specific strengths and limitations of animal models in hemostasis and thrombosis research. The goal was to identify relevant topics that could be developed into reports for potential publication in JTH. There was a consensus that an effort should be made to describe the variables that should be considered standardizing methods of thrombosis and hemostasis testing such as the ferric chloride arterial thrombosis model and the hemophilia mouse tail bleeding time.

## BRIEF TALKS

**1. Hartmut Weiler - Human gamma fibrinogen in mouse coagulation.** Dr. Weiler described the role of specific fibrinogen domains in hemostasis studies that result from differential RNA processing, the differences of these domains across species, their impact on thrombosis and hemostasis experiments, and their potential role in venous thromboembolism.

**2. Hugo ten Cate - Coagulation protease-inhibitor effects in models of cardiovascular disease.** Dr. ten Cate described the differences in coagulation factor content between early and late atherosclerotic plaques, the potential clinical importance of these changes over time, and mouse modeling of these issues.

**3. Paul Monahan – Intra-articular factor IX protects hemophilia B mice from developing hemophilic synovitis.** Dr. Monahan the role of intra-articular coagulation factor function in the development and prevention of hemophilic arthropathy. Both F.IX protein and AAV-gene therapy expressed F.IX prevented hemophilic synovitis in hemophilia B mice.

**4. Nigel Mackman – Tissue factor and tissue-specific hemostasis.** Dr. Mackman described the scientific basis for tissue-factor mediated tissue-specific hemostasis. He provided data on tissue factor levels in a panel of tissues, suggested how these differences might alter bleeding phenotypes and emphasized how experiment must account for the potential impact of these differences on experimental results.

**5. Caterina Gallippi – Novel ultrasound methods to monitor hemostasis.** Dr. Gallippi described novel ultrasound methods that her laboratory is developing to detect bleeding and hemostasis.

**6. Graca Almeida-Porada – Hemophilia A in sheep.** Dr. Porada's laboratory has rederived sheep with hemophilia A. She described the phenotype and genotype and her laboratory's plan for future experiments.

**7. Timothy C. Nichols – Canine hemophilia A and B.** Dr. Nichols described studies that document reduction in spontaneous bleeding in canine hemophilia following chronic (years) replacement therapy or gene transfer of canine factor VIII, IX, and VIIa.

#### **EDUCATIONAL LECTURES.**

**1. Thomas G Diacovo - Modifying murine von Willebrand factor A1 domain for in vivo assessment of human platelet therapies.** The approach to cross species interactions of murine VWF and human platelets was discussed by Dr. Thomas G. Diacovo.

**2. Eva-Maria Muchitsch - Key questions for animal models of hemostasis in developing new drugs and obtaining approval.** Issues to be considered in the utilization of animal models in developing new hemostatic agents and obtaining approval for use was discussed by Dr. Eva-Maria Muchitsch.

**3. Mortimer Poncz – Stressing the limits of mice models of hemophilia: When mice are not small people.** Dr. Mortimer Poncz discussed key differences between murine and human hemophilia with emphasis on how it related to the presence or absence of F.VIII in platelets.

## Biorheology

11 July 2009  
Boston, MA, USA

Chairman: *J.W.M. Heemskerk, The Netherlands*

Co-chairmen: *Thomas Diacovo, USA; Marc Hoylaerts, Belgium; Michael King, USA; Gerard B Nash, UK; J J Zwaginga, The Netherlands*

The Biorheology meeting session started with an educational part which was followed by section on the translational use of thrombosis models. This part attracted an audience of about 500 people. After the break, about 180 people participated in the second section on molecular interactions in flow, and the third section on standardization of thrombus formation under flow.

The educational program aimed to demonstrate and explain the importance of flow in thrombosis and hemostasis (chaired by dr. J. Heemskerk and dr. G. Nash). Dr. Nash (Birmingham, UK) discussed the basic rheological principles and critical parameters implicated in the adhesion of platelets and leukocytes to the vessel wall. The size and possibilities of deformation of these cells, as well as of erythrocytes, in part explains their different behavior in adhesion. Dr. Heemskerk (Maastricht, the Netherlands) gave a basic overview of the different ways to measure thrombus formation *in vivo* and *in vitro*. The importance of interactions between platelet and coagulation activation was stressed in thrombus formation *in vivo*, as well as the need to introduce coagulation in measurements of thrombus formation with flow devices *in vitro*.

Section 1 contained 4 lectures on flow-dependent experimental thrombosis models and translation (chaired by dr. M. Hoylaerts and dr. J. Heemskerk). Dr. S. Jackson (Melbourne, Australia) spoke on novel molecular interactions in thrombus formation. His talk focused on the partly reversible formation of platelet tethers and the capturing into a thrombus of non-stimulated platelets. Dr. P. Mangin (Strasbourg, France) discussed similarities and differences in measurements of *in vivo* and *in vitro* thrombus formation in mice. He stressed the importance of using more clinically relevant thrombosis models, for instance by measuring thrombus formation on (ruptured) atherosclerotic plaques. Dr. L. Brass (Philadelphia, PA, USA) a number of key examples of how to use flow-dependent models *in vitro* and *in vivo* to explore new ideas about platelet activation. He showed that the use of different (*in vivo*) models can lead to different conclusions regarding the involvement of platelet signaling proteins in thrombus formation. Models to use should be well-chosen and well standardized. The last speaker in this section, Dr. M. Hoylaerts, (Leuven, Belgium) discussed the limitations of animal thrombosis models. Pointing to the need for alternatives, he showed the possibilities of a hypoxia mouse model, which mimicked a prothrombotic state.

After the break, section 2 started on the determination of flow-dependent molecular interactions (chaired by dr. M. King and dr. M. Hoylaerts). Dr. A. Reininger (Munich, Germany) presented new data showing how platelet-fibrin-thrombus formation in arterial flow required on high and low shear regions. Therefore, he used a newly developed flow device designed to mimic the flow conditions in stenotic coronary arteries.

Dr. B. Fuchs, (Berlin, Germany) discussed measurements of flow-based VWF binding to collagen. She measured the flow-dependent binding characteristics of VWF to collagen types I and III. Subsequently, dr. K. Vanhoorelbeke (Kortrijk, Belgium) demonstrated a new principle of gene therapy, resulting in increased VWF expression in murine liver, in which the flow-dependent function of VWF was restored.

Section 3 focused on the standardization of thrombus formation under flow, with particular attention to the use of flow devices (chairs: dr. G. Nash and dr. J. Heemskerk). The second chairman on behalf of dr. J. Cosemans (Maastricht, the Netherlands) presented a new way of measurement of thrombus formation on (ruptured) atherosclerotic plaques. Data were provided that the binding of factor XII to (plaque-derived) collagen contributed to the formation of thrombin-clot clots under flow. The parallel-plate flow device used in this study was discussed. Also the two subsequent speakers showed their modified versions of a parallel-plate flow chamber. Dr. M. Roest (Utrecht, the Netherlands) presented a first study of the use of flow assays to characterize patients with unknown bleeding disorders. The methods employed for quantitative analysis of thrombus formation were explained as well. Dr. R. Farndale (Cambridge, UK) discussed the analysis of shear-dependent thrombus deposition on synthetic collagen surfaces. Both the advantages and disadvantages of the use of immobilized collagen peptides were indicated.

As a final remark, the subcommittee chairman, on behalf of the co-chairmen, concluded that this afternoon significant steps were made towards standardization of the measurements of flow-dependent thrombus formation in vivo and in vitro.

## Control of anticoagulation

12 July 2009  
Boston, MA, USA

Chairperson: Trevor Baglin (UK)

Co-Chairpersons: Walter Ageno (IT), Job Harenberg (DE), Clive Kearon (CA), Aharon Lubetsky (IL), John Olson (US), Gualtiero Palareti (IT), Sam Schulman (CA), AMHP van den Besselaar (NL)

### Introduction and update on activities

Trevor Baglin summarised the activities of the Working Parties:

**Definition of major bleeding in surgical studies** (Sam Schulman): input from a variety of invited surgeons had been received and a final draft communication for circulation to the SSC subcommittee and publication in the journal has been prepared by Sam Schulman.

**Standardisation of classification for post-thrombotic syndrome** (Susan Kahn) This Working Party had completed a recommendation published in the journal (JTH 2009;7:879-83).

**Recommendation for requirements on biosimilar anticoagulants** (Job Harenberg) This Working Party had completed a recommendation published in the journal (JTH 2009;7:1222-5)

**Standardisation of measurement of platelet-dependent thrombin generation** (Trevor Baglin) A final draft is in preparation for circulation to the SSC subcommittee and submission for publication in the journal.

A report on screening **computer dosage programs for warfarin and other vitamin K antagonists for their safety and effectiveness** had been prepared by Leon Poller and submitted to JTH as an SSC communication with a copy of the full report to be available on the ISTH website.

The activities of other Working Parties and an updated on registries was presented by SCC Co-Chairs.

### Registry Report - Splanchnic vein thrombosis

WALTER AGENO

The registry had planned to enrol 500 patients with 10 patients from each of 40 international centres. 208 patients had been enrolled from centres in 26 countries after 1 year and so some centres had enrolled more than 10 patients. Dr Ageno indicated that involvement from centres in more countries would be favourable. It was concluded that enrolment may not be limited to 500 and progress over the next year would determine the future of the registry. It was envisaged that patients would be followed for up to 2 years.

ACTION: registry to continue.

## **Discussion / Potential registry - Cerebral Vein Thrombosis –**

WALTER AGENO

Dr Ageno reviewed the characteristics of the condition emphasising that recurrence rates are probably lower than previously appreciated. A registry similar to the Splanchnic vein thrombosis registry was discussed and this possibility will be considered subsequent to the meeting.

ACTION: Registry to be further considered.

## **Working Party Report - Recommendation for requirements on biosimilar anticoagulants**

JOB HARENBERG

Dr Harenberg indicated that the report was finished and published in the Journal. Based on the heterogeneity of LMWHs biosimilar preparations should be demonstrated to be non-inferior in pre-clinical and clinical investigations. Simplified pharmacological, pharmacokinetic and clinical studies may be required for biosimilar LMWHs whose compositional profiles and physicochemical properties are similar to those of the originator.

ACTION: Completed.

## **Working Party Report - Standardization of methods to determine direct factor Xa inhibitors**

JOB HARENBERG

In some circumstances there is a need to measure plasma levels and hence a requirement for standardisation. A protocol of work was presented and an invitation to interested participants to contact Dr Harenberg directly.

ACTION: Dr Harenberg to formalise protocol and await expressions of interest.

## **Working Party Report - Results of international collaborative study for calibration of two candidates for International Standard for thromboplastin, human, plain**

TON VAN DEN BESSELAAR

The completed study was presented. 21 laboratories participated and measured PTs by tilt tube on 10 different days from 6 patients with INRs between 1.5 and 4.5. Standard rTF/95 and RBT/05 were used with 2 candidates 07/314 and 08/144 according to predefined performance criteria. Candidate 08/144 met all three predefined criteria and performed better than 07/314. The report had previously been circulated to experts who all chose candidate 08/144.

ACTION: Report completed and additional decisions from experts on choice of candidate to be completed in next few weeks.

## **Working Party Report - Variability of INR in stabilized patients and requirements for INR analytical precision**

TON VAN DEN BESSELAAR

Work in progress was presented. Biological variation was calculated from INR results from 150 patients with 75 in each of two target ranges taking either phenprocoumon or acenocoumarol. Patients had had no change in drug dose with at least 6 consecutive INRs in the therapeutic range and an interval of at least 2 weeks between INR measurements. Required analytical precision was determined after determination of biological precision. Imprecision goals for the two target ranges were 2.4% and 4.9% for phenprocoumon and 2.7% and 5.3% for acenocoumarol. The process would be particularly relevant to point of care instruments.

ACTION: Work to continue by Dr van den Besselaar.

### **Reporting INRs in liver disease**

ARMANDO TRIPODI explained the use of the INR in the MELD score for prioritising patients with end stage liver disease for liver transplantation. He presented data illustrating the discrepancy between ISI (VKA) and ISI (LIVER) and showed how thromboplastins can be calibrated for ISI (LIVER). Options would include ISI assignment by the manufacturer or locally or centralisation of plasmas from patients. Consideration will be given to producing guidance after consideration of the logistics of different options.

ACTION: SSC formal communication to be considered.

An introductory session on new oral anticoagulants was presented with a view to preparing practical guidance on patient management in the future. The subject was introduced by Trevor Baglin. An overview of drug development programmes was presented by Harry Buller, considerations for monitoring, bleeding and reversal by Jeff Weitz, an overview of potential advantages and disadvantage by Jack Ansell and an overview of what might be considered for phase IV studies with collaboration between companies and anticoagulation clinics by Gulatiero Palareti.

ACTION: SSC formal communication to be considered.

The formal business meeting was followed by an educational session covering antithrombotic therapy for patients with atrial fibrillation, the need for bridging therapy and the potential role of pharmacogenetic testing in oral VKA naïve patients.

# Disseminated Intravascular Coagulation (DIC)

12 July 2009  
Boston, MA, USA

Chairman: *Cheng-Hock Toh, UK*

Co-chairs: *Nigel S. Key, USA; Hyun Kyung Kim, Rep of Korea; Jorn Dalsgaard Nielsen, Denmark; Hideo Wada, Japan*

## Educational Session

Theme: Disease-specific molecular mechanisms in DIC

Drawing upon the validation of the ISTH overt DIC score in diagnosis, the next translational step towards patient benefit is in working towards therapeutic strategies. Whilst studies have shown that the DIC score is clinically useful in prognostication, irrespective of its aetiology, the underlying molecular mechanisms are different for sepsis as opposed to trauma. This session drew upon the experience of 3 experts in the fields of sepsis, trauma and acute promyelocytic leukaemia (APL), respectively.

- Dr Peter Ward from University of Michigan, Ann Arbor described the importance of IL-17 in experimental sepsis. Its neutralisation could rescue mortality in a cecal ligation model, even when administered later in the sepsis course. This may hold promise in future human studies and discussion was had re-best ways of stratification patients in trials of sepsis.
- Prof Karim Brohi from Barts and the London Medical School discussed acute trauma-induced coagulopathy (ATC). The accompaniment of shock to tissue injury was particularly potent at inducing ATC and this was associated with significant activation of protein C. Discussions followed on whether this was an anti-inflammatory response to the pro-inflammatory insult of tissue injury and acidosis.
- Dr Katherine Hajjar from Cornell, New York discussed the relevance of annexin A2 in the pathogenesis of APL. The overexpression of this receptor is attenuated by all trans retinoic acid treatment and appears to enhance fibrinolytic activity following thrombin stimulation and in concert with uPAR.

## Business Session

Standardisation issues

- Prof Cheng Hock Toh from Liverpool, UK presented an overview of work accomplished in the last 4 years of his chairmanship of the sub-committee. Further to the publication of recommendations on diagnostic scores for overt DIC in JTH 2007; 5: 604-6, another communication has been accepted for publication in JTH. This is the work led by co-chair Dr Kim on application of the INR instead of the PT in the DIC scoring system.
- Dr Hideo Wada from Mie University, Japan discussed the non-overt DIC scoring system. 2 Japanese studies had shown that this could identify pre-overt DIC whilst a Liverpool study had

shown that this was prognostically independent of the development of overt DIC. Differences in design and choice of cut-off levels might be explanatory, especially in relation to D-dimer kits available in Japan versus those in Europe.

- Dr Gary Kinasewitz from Oklahoma Cardiovascular Research Foundation presented his experience with an evolving scoring system of coagulopathy that relied on PT and platelet parameters only. In extending upon previous presentation of data in patients with sepsis, his recent experience shows the value of this evolving scoring system that does not include a fibrin-related marker in patients with severe trauma as well as sickle cell disease. Trend analysis based on a second sample at 12 hrs was equally informative as 24hrs.
- Dr Robinder Khemani from the paediatric ICU (PICU) in Los Angeles confirmed that the overt DIC scoring system was also applicable in the paediatric setting. Furthermore, it had independent value to severity scoring systems used on PICU. His impression was that an evolving scoring system would also be informative in that setting.
- Dr Hyun Kyung Kim examined the impact of platelet count methodology (impedance, optical, immunological, flow cytometry) in DIC. 8 methods were assessed using samples from patients with DIC with varying severity scores. She found that all methods were broadly comparable with deviation mainly in severe coagulopathy. The degree of platelet activation could also induce inter-method variations.

#### Collaborative studies

- Dr Tina Dutt from the UK reported on the ongoing collaboration with SSC in Plasma Coagulation Inhibitors and UK NEQAS in Coagulation on the standardised determination of acquired protein C and antithrombin levels. Samples had now been prepared and awaiting viral studies before sending to identified participating laboratories. Several more volunteer laboratories were signed-up today.
- Dr Nigel Key presented the findings from the collaboration with SSC in Vascular Biology on microparticle enumeration. This has been completed and overall, two-thirds of flow cytometers demonstrated good resolution and acceptable background interference. The performance of individual brand cytometers was discussed.

#### **Summation**

- Prof Toh stated that this was his last year as chair of this SSC and suggested a steer for the work ahead. He stressed the importance of combining work on understanding pathway-specific mechanisms linking on to haemostatic dysfunction in conjunction with clinical/laboratory refinement work on identifying DIC as well as strong collaborations with relevant SSCs.

## Registry of Exogenous Hemostatic Factors

12 July 2009  
Boston, MA, USA

Chairman: *Mary Ann McLane, USA*

Co-Chairmen: *Kenneth J Clemetson, Switzerland; Manjunatha R Kini, Singapore; Francis S Markland Jr, USA; Takashi Morita, Japan*

In attendance were Chair McLane, Co-Chairs Kini, Markland, Clemetson, Morita and 55 guests.

1. Meeting was brought to order by Chairman Mary Ann McLane.
2. The minutes of the last meeting (Vienna 3 July 2008) were read and there were no questions or corrections.
3. Dr. McLane reported on the preparation for the 4<sup>th</sup> International Conference on Exogenous Factors Affecting Thrombosis and Haemostasis (EFATH09), taking place 17-19 July 2009 at the Campus Center of the University of Massachusetts-Boston. The meeting has been planned since 2007, with a website established to facilitate registration, speaker invitations and accommodations at the Doubletree Bayside Hotel. A reception on Friday evening July 17 will open the meeting, followed by two full days of talks (29 speakers), including seven (7) oral poster presentations. Registration was planned for 100-125, with 45 registrations currently received. The economy has definitely affected attendance, with two invited speakers pulling out due to finances. Additionally, the IST meeting in Recife, Brazil, in the spring may have pulled attendees. Registration fees will cover the costs for the meeting rooms but not for the food to be used at the reception, breaks and lunches. An NIH-R13 application by McLane and her contacts at the University of Massachusetts was not funded. We were successful, thanks to Dr. Kini's efforts, in getting sponsorship from Venom Supplies in South Australia. Our Registry leadership will have to realistically project the possibility of having EFATH#5 in Amsterdam in 2013. Dr. Jan Rosing has agreed to facilitate this planning.
4. Dr. Clemetson gave a final report on the 2009 manuscript publication, in JTH and Toxicon, for the classification and nomenclature of C-type lectins. He also noted that the Toxicon notice did not include the link to JTH despite that notice being in the proof. Dr. Clemetson will (1) request an erratum be published in Toxicon, especially since that link was the main reason for requesting the publication in Toxicon in the first place, and (2) write an article for the IST newsletter to publicize more widely the JTH article and the correct URL link. For the JTH article, some author information is incorrect; Dr. Clemetson will contact the ISTH office to get this corrected. *NOTE: After the SSC meeting, Dr. McLane met Cary Clark in the ISTH meeting office, and he made the needed changes.*
5. Dr. McLane presented "Interaction of Disintegrins with Natural Killer Cells". Discussion which followed centered on assays for 2D versus 3D analysis, and the role of exogenous factor proteins, in general, on innate immunity.
6. Dr. McLane reported that the next Registry meeting will be in Cairo Egypt, 22-25 May 2010. The next EFATH meeting will be in the Netherlands, with the ISTH meeting happening 29 June – 4 July 2013. See item #3.
7. Future projects: Dr. Kini, on behalf of Drs. Fox and da Silva, provided an outline of the nomenclature and classification schemes currently in place for the exogenous factor metalloproteases. The International Society on Toxinology has recently appointed a

nomenclature committee, and Dr. Kini has been nominated to it. He described that while classifications of these proteases is clear, there is confusion in their nomenclature, especially from a structural and functional basis. Dr. Kini will be discussing the possibilities of coordinating the efforts of the IST with this Registry, with the understanding that any project from this Registry would be published in JTH.

8. Other matters:

- a. Dr. McLane reported on the SSC chairs' dinner meeting from 11 July 2009: there was an emphasis again on the link between projects of the SSCs and their publishability in JTH; a new working party has been formed, involving multiple SSCs, on the definition of "bleeding" to be used both clinically and as an endpoint in research. Currently, members of the vWF, Platelet and Women's Health SSCs have already begun meetings, and there will be a significant presentation in Cairo in 2010. Since a major result of envenomation is bleeding, it was suggested that members of our Registry could provide valuable insights into the efforts of this working party. McLane will discuss this with Dr. Gerhard Johnson at her meeting with him on Monday 13 July 2009.
- b. Dr. Morita is on the planning committee for ISTH 2011 in Kyoto, and will work toward getting targeted sessions/symposium dealing with exogenous factor-related topics within the program.
- c. The business meeting recessed until 10:30.

9. Two speakers spoke for the Educational Program:

- a. Ivo Francischetti, National Institutes of Health, on Transcriptomics and Functional Genomics of Salivary Glands from Hematophagous Arthropods
- b. Frank Markland, University of Southern California: Fibrolase and Its Evolution to Clinical Trials: A Long and Winding Road

10. There being no additional business, the meeting was adjourned at 12:00 noon.

## Factor VIII and IX

11 July, 2009.  
Boston, USA

Chair: A. Srivastava (India)

Co-Chairs: C.A. Lee (UK), C. Negrier (France), F. Peyvandi (Italy), J-M. Saint-Remy (Belgium), C. Hay (UK), J. Oldenburg (Germany), E. Tuddenham (UK).

The meeting started at 8:00 am with the chairman's welcome to over 500 participants. He also explained that while the overall layout of the program remained the same, there were new topics related to standardization of issues in the clinical management of hemophilia such as protocols for factor replacement as well as assessment of outcome. It was also mentioned that there was a special educational program this year in the afternoon.

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

The data from the registry of inhibitors to factor IX had been recently analyzed and published in the journal *Haemophilia - Inhibitors in factor IX deficiency a report of the ISTH-SSC international FIX inhibitor registry (1997–2006)*; (<http://www3.interscience.wiley.com/cgi-bin/fulltext/122454238/PDFSTART>).

The process for publication of such data as recommended by the SSC was discussed and should be followed in all cases.

### **Session -1: Rare Bleeding Disorders (RBDs)**

*Chairpersons: Flora Peyvandi (Italy) and Christine A Lee (UK)*

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

The 'European Network of Rare Bleeding Disorders: (EN-RBD)' project is supported by the European community through the Executive Agency for Health and Consumers. The aim of EN-RBD was to develop a homogeneous on-line database to collect and manage data on clinical manifestation, phenotype, genotype, treatment and treatment-related complications of patients affected by Rare Bleeding Disorders (RBDs). 10 European countries have been involved to date and 521 patients entered. The distribution of RBDs and their phenotype severity (severe deficiency was defined for factor levels <5% and fibrinogen level <10mg/dl, moderate 5-10% and hypofibrinogenemia <50mg/dl and mild >10% or dysfibrinogenemia >50mg/dl) were calculated on the entire group of patients, showing that FVII and FXI deficiencies (30%) were the most prevalent RBDs., FV+FVIII and FII deficiencies (2%) were found to be the rarest disorders. The second part of analysis was based on the prevalence and severity of bleeding manifestations in 221 patients with complete data required by EN-RBD questionnaire. 260 cases from French registry contained only demographic information and annual amount of treatment with no detailed clinical information; in addition 40 records have been removed after the first quality control. Further analyses were performed to compare clinical severity of patients

and their lab phenotypes. These results showed that severe bleeding episodes such as haematoma, haemarthrosis, umbilical cord, GI and CNS, were significantly more frequent in patients with severe phenotype (coagulant activity less than 5% and afibrinogenemia) ( $p < 0.0001$ ). Mucocutaneous type of bleedings such as epistaxis, cutaneous and oral cavity, were also present in patients affected with moderate or mild deficiency (more than 5% of coagulant activity and hypo- and dys-fibrinogenemia) ( $p < 0.05$ ). Menorrhagia and haematuria were present in all three groups of patients with different type of phenotype severities. A good correlation between lab phenotype results and clinical severity was observed for patients affected with afibrinogenemia and severe FXIII and FX deficiencies since most of these patients (>75%) have had at least one severe bleeding. There was no report on patients with afibrinogenemia, severe FXIII and FX deficiencies who remain asymptomatic in their entire life. One third of patients with severe FXI deficiency remain asymptomatic confirming a large heterogeneity in clinical manifestations of patients affected with RBDs. Additional data and improvements are required to address the following: spontaneous onset of bleeding symptoms vs. triggered, bleeding during surgery, the efficacy of prophylaxis during surgery. These modifications have led to developing the second version of data collection tool (EN-RBD 2.0) with new functions and page layout, scheduled to be ready in July 2009.

#### **North American Registry of Rare Bleeding Disorders – Amy Shapiro (USA)**

In the US there had been concern about product availability for RBDs and in June 2005 there was an FDA workshop. MASAC had driven the agenda. The CDC under the umbrella of the Rare Protein Deficiency Group had overseen data collection for RBDs. There was an electronic system overseen by the American Thrombosis and haemostasis Network (ATHN) which had developed a master template for data collection. ATHN was funded by Baxter. ATHN was formed in July 2006, held a summit in July 2009 and contributed papers on RBDs to a special issue of *Haemophilia* in November 2008. In the US rFVIIa, Protein C, Fibrinogen and ATIII were licensed. FXIII was under IND. FX and FXI were sourced from outside the US.

#### **Standardisation of assays: FVII and FXI – Ian Jennings (UK)**

Data from approximately 300 UK NEQAS laboratories registered for FVII:C and FXI:C assays can help indicate areas where there is scope for improvement in assay standardisation. Due to variable reagent sensitivity, mild deficiencies of these factors may be missed with screening tests (PT and APTT), and it is important that this is recognised when investigating a bleeding disorder. Differences of up to 30% in FVII:C assay levels have been observed in UK NEQAS exercises; analysis of data indicates this may relate to source of thromboplastin. Rabbit extract reagents tended to produce higher FVII results. FXI:C assay standardisation has been aided by introduction of an WHO international standard in 2005; however, not all manufacturers have commenced calibration of their commercial reference plasmas against this standard, and between-method discrepancies remain. Further areas requiring standardisation are approaches to reference ranges and assay design. It was of some concern that in 25% laboratories only a single dilution was made for the FIX assay. It was emphasised that the reagent, the reference plasma and the assay design were all critical components to achieve a good result.

#### **Thrombin Generation in RBDs – Waander van Heerde (Netherlands)**

There were two processes involved in haemostasis – coagulation and fibrinolysis. Global tests of haemostasis could measure both. The NHA measured both thrombin and plasmin generation using two fluorogenic agents. Fibrin was important for plasmin generation. In FV deficiency there was no fibrinogen generation and no plasmin generation. Consideration has to be made as to using platelet poor or platelet rich plasma. As yet there is no clinical application of this methodology.

#### **A new FX concentrate: Clinical issues – Joanne Lloyd (UK)**

BPL (UK) has developed a high purity factor X concentrate for the treatment of hereditary factor X deficiency. The concentrate is characterised by a low reconstitution volume, high final potency, high purity and low thrombogenicity. BPL has been granted orphan drug designation for the concentrate in the US and Europe. The clinical programme for factor X has been developed after receiving regulatory advice, through parallel scientific advice and detailed discussion with clinicians from around the world. The programme has been designed to obtain good quality data from very few patients while meeting both US and EU regulatory requirements. Three studies are planned: a pharmacokinetics, safety and efficacy study in adults with severe and moderate factor X deficiency; a paediatric study on children aged less than 12 years with severe or moderate factor X deficiency; and a study on prophylaxis in surgery in adults with severe to mild factor X deficiency. The three trials planned would recruit as follows: Ten 01, PK study for adults >12 years; Ten 02, a paediatric study for severe and moderate disease in adults > 12 years; and Ten 03 prophylaxis in surgery. There is a variable half life in the literature up to 144 hours. The trial would exclude patients on prophylaxis. The numbers to be included would be 12-16 in Ten 01 and 02 and up to 10 procedures in five patients in the surgical study. For this study, severe deficiency is defined as those with <1% factor level and moderate as up to 5% factor level. Patient recruitment to the pharmacokinetic, safety and efficacy studies is now commencing in US and Europe

#### **Recombinant FXIII: Early data from clinical studies – Kim Jacobsen (Denmark)**

Novo Nordisk has developed a recombinant FXIII concentrate. Recombinant FXIII (rFXIII) is manufactured as a soluble protein in a yeast (*Saccharomyces cerevisiae*) production strain. The purified rFXIII [A<sub>2</sub>] homodimer protein is formulated, standardized and freeze dried into vials containing 15.0 mg (equivalent 2505 IU). There are two clinical trials on going with this product at present - in FXIII deficiency and cardiac surgery. The product is FXIII-A2 homodimer. The vial contains 2505 IU in 3ml after reconstitution (835 IU/ml). The dose is 35 IU/kg per month. The duration of these trials will be Aug 2008 to April 2010. After a four week run-in there is a year of treatment with monthly dosing and a final month of follow up. Participants will be provided with free drug thereafter until a license is obtained. At present there were 41 participants who had received > three doses and in total > 225 doses. The cardiac surgery trial is for acquired FXIII deficiency on by pass. FXIII treatment would result in transfusion avoidance. The total numbers were 408 in three groups, placebo, 17.5 IU/kg and 35 IU/kg. There are no major adverse effects so far. Efficacy data is to be analyzed.

#### **Session 2. Clinical issues- I (Assay / EQA / Phenotype)**

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

**Systematic approach to the assay discrepancies between one and two-stage assays –  
*J. Oldenburg, H. Brondke, A. Pavlova, (Germany) J. Ingerslev (Denmark), M. Makris (UK)***

Causative missense mutations in the coding region of the F8 gene are predominantly resulting in mild or moderate haemophilia. Such patients can generally be equally well diagnosed with either the one-stage or two-stage assay systems. However a significant subset of mutations that may represent about 30% of patients with non-severe haemophilia A have been described, in which the two systems yield discrepant activities. These discrepancies can be that strong that patients show normal FVIII:C in one assay while the other clearly indicates the presence of a mild haemophilia A. . The currently identified discrepant mutations can affect both test systems, however the majority of these mutations have been reported to give higher FVIII:C in the one-stage clotting assay. Mutations which are characterized by a higher FVIII:C activity in the one-stage assay have been shown to result in enhanced instability of the A2 domain in the activated FVIII protein. This instability facilitates A2 subunit dissociation and leads to premature loss of cofactor activity. The chromogenic assay is more sensitive to this dissociation, thus showing lower FVIII:C.

Mutations which are resulting in higher or even normal two-stage FVIII:C while the one-stage assay indicates low FVIII:C affect the cleavage kinetics of FVIII by thrombin. As these FVIII variants can still, albeit slowly, be activated by the serin protease thrombin. In the two-stage assay the impaired thrombin cleavage kinetics are compensated by high thrombin concentrations, diluted FVIII-protein and the long incubation times employed. The present study aims to establish an international registry of such mutations with discrepant FVIII:C in different assays. The role of this interesting phenotype will be further addressed by exchanging plasma samples between experienced laboratories and by expression studies of recombinant FVIII variants. We expect that this initiative will enlight the underlying pathomechanisms and clinical implications of this interesting phenomenon.

**The clinical application of a sensitive assay system measuring FVIII antigen – *Ed Gomperts, Saulius Butenas, Ken Mann (USA)***

A highly sensitive Fluorescence Luminex Immunoassay for FVIII antigen has been developed utilizing a murine  $\alpha$ -FVIII mAb ( light chain specific ) as capture antibody coupled to microspheres. In addition, a biotinylated murine  $\alpha$ -FVIII mAb (heavy chain specific) coupled with R-phycoerythrin-streptavidin is employed as the probe and the measurement of the antigen presence is via fluoroluminescence using a Luminex system. The detection limit for FVIII antigen is 3 pM. The assay has special features including the use of FVIII-depleted plasma as the diluent. In addition, non-specific isotype matched mAb-coupled microspheres are used both in the calibrator and all test samples to eliminate the non-specific signal. Finally, it is calibrated with a well-characterized recombinant FVIII standard. This assay was applied to FVIII measurement under pharmacokinetic (PK) circumstances in 7 patients with severe hemophilia A without an inhibitor, with comparison assays using the APTT system: Four individuals received 5 different commercial clotting factor concentrates and 3 study subjects received 3 different lots of one commercial product. These studies generated 174 samples with the one stage APTT and the immunoassay results revealing a correlation between the 2 assays of  $r = 0.8468$ . The immunoassay demonstrated small amounts of FVIII antigen in 6 of the 7 study subject pre-infusion samples. The FVIII activity and antigen PK recovery results correlated closely within each concentrate and within each individual. FVIII APTT and antigen T<sub>1/2</sub> differences were

apparent showing associations with mean pre-infusion antigen levels. In conclusion, this research documented the applicability of a highly sensitive FVIII antigen assay to the PK analyses of FVIII infusions and also the concentrates employed. Further evaluation is warranted.

### **Assays for global haemostasis in haemophilia : Correlation with adjuvant therapeutic options – *Benny Sorensen (UK)***

Benny Sorensen reviewed a series of studies that have suggested recording thrombin generation and/or whole blood clot formation for global assessment of substitution therapy with FVIII or by-passing agents. However, more than lack of propagation of thrombin characterizes the biochemical pathology of haemophilia. Studies point toward abnormal clot stability, increased susceptibility toward fibrinolysis, reduced clot weight, as well as delayed and reduced activation of FXIII resulting in discordant fibrin lateralization. Whole blood thromboelastography / thromboelastometry can be used to evaluate physiological clot formation by tissue factor activation of corn trypsin inhibitor stabilized whole blood. In addition, new assays are developed based on simultaneous activation with tissue factor and tissue plasminogen activator in order to evaluate resistance toward accelerated fibrinolysis and clot stability. Recent data has shown that infusion of FVIII (50%) following by concomitant adjuvant treatment with tranexamic acid 10mg/kg is associated with up to 5-fold increased clot stability. Pilot studies with combination of rFVIIa and plasma derived FXIII shown a comparable pattern on clot stability. In conclusion, accelerating thrombin generation and clot formation may not be the only goal to reach in correcting the haemostatic deficits of haemophilia. Adjuvant therapy with e.g. tranexamic acid may be a highly effective intervention. Thromboelastography / thromboelastometry may prove useful tools in exploring new adjuvant treatment regimens.

### **Tests of global hemostasis (TEG / TGT / Wave form analysis) in clinical samples of hemophilia A – *Sukesh C. Nair, Alok Srivastava (India)***

The tests that reflect global haemostasis namely the TGT, TEG and Clot WA has shown good linearity of their parameters with FVIII levels especially on spiked samples. But there is limited data on such responses in these tests in clinical samples of patients with Haemophilia A (HA). Moreover data on Global Haemostasis Tests (GHT) performed on clinical samples of patients with severe HA (SHA) have been limited either by smaller numbers or the type/types of tests. This study was done to document the pattern of Global haemostasis (GHT) namely TEG (Haemoscope, 1/36000 Recombiplastin), TGT (Calibrated Automated Thrombography, 1pM rTF) and WA (Clot Curve analysis –CCA, ACL 10000) in a large number of patients with HA (70 Severe HA, 18 Moderate HA, 8 Mild HA and 20 healthy controls). For standardization TEG, TGT and CCA was done on carefully selected FVIII deficient clinical sample spiked with rFVIII concentrate to obtain random concentrations ranging from 30 to 0.1 iu/dL. Measured FVIII levels on the spiked samples correlated well with the expected FVIII levels on replicate (10) measurements with good reproducibility by one stage FVIII assay on the ACL 10000 (detection limit up to 0.2iu/dL). There was very good correlation between the GHT parameters and FVIII:C and good inter test correlation ( $r > 0.9$ ). Median GHT parameters on patient samples [(R time (TEG), peak thrombin (TGT) and Max2 (CCA)) could discriminate well between normal, SHA, moderate HA. Comparing the GHT parameters in the 70 SHA samples with the FVIII levels obtained on ACL 10000 showed wide distributions of the GHT parameters and poor correlation with FVIII:c levels ( $r < 0.3$ ) unlike spiked samples even when FVIII levels were same implying that FVIII is not the only determinant for the GHT parameters in clinical samples. Inter test correlation was also very poor ( $r < 0.3$ ). This is largest cohort of clinical samples from patients with SHA to be analyzed by all three GHT. Though a wide range was noted in the parameters,

they need to be correlated with the clinical profile to assess the usefulness of these tests in this regard.

**Global hemostasis tests in hemophilia : Early data and a systematic study plan. – C. Negrier (France)**

The potential use of global assays for monitoring coagulation in the bleeding disorder area was addressed in the second session. An introductory presentation was done in order to remind the principles, the pros and cons of the 2 technologies that have been mostly investigated, i.e., rotational thromboelastography, thrombin generation and wave form analysis of clot formation. These assays have mainly been used for discriminating patients with various severities of bleeding disorders (mainly haemophilia) but a proposal has been made that given the current standardization, one should enter the clinical application of these technologies. Multiple variables have to be taken into consideration to minimize the variations, and a proposal was made to share with the community a detailed video presenting all the technical details that are associated with a proper measurement of thrombin generation. It is the intention of the FVIII and FIX SSC to provide the video on the ISTH website in order to facilitate setting up of this technology and accumulate comparable data. A validation process has however to be done. Further refinements of the current assays can be envisaged and the monitoring of clot lysis could also be a useful addition to the clot formation monitoring.

***A proposal to initiate three working parties within the FVIII / IX subcommittee to suggest ways for standardization of the three tests is to be put up soon. Once standardized, applications in different fields may be evaluated by all interested using those methods.***

**Agenda of the FVIII / IX subcommittee of the SSC – A Srivastava (India)**

Alok Srivastava explained the process of formulation of the agenda of the subcommittee. He explained that in accordance with the mandate and mission of the SSC itself, issues related to standardization of assays, concentrates and preparation of standards need to be discussed related to hemophilia and rare bleeding disorders. In addition, scientific issues related to inhibitors, phenotypic heterogeneity, genotype-phenotype correlations, tests of global hemostasis and their applications to these disorders and clinical issues such as surgery in hemophilia have been included in the past. This year, apart from continuing these subjects, the science of standardization of clinical issues such as the use of factor concentrates (protocols for clinical management) and standardization of the assessment of outcome were included as these issues are also very significant considering the fact that >90% of the high cost of the management of hemophilia is related to factor concentrates.

The process involves discussion between the subcommittee chair and co-chairs regarding potential topics that are current and relevant for inclusion in the agenda for that year and also consideration of direct requests from any scientist regarding the potential inclusion of their work if it fell within the broad mandate of the subcommittee.

**Session 3. Clinical issues- II (Inhibitors / Prophylaxis / Novel Therapies)**

*Chairs: C. Hay (UK) and J-M. Saint-Remy (Belgium)*

**International ITI Study : Update – D. DiMichele (USA)**

An update on the International ITI Study was presented by Donna DiMichele on behalf of co-PI, Charles Hay, and the I-ITI study group. The study is a prospective randomized multi-center trial of the safety and efficacy of high (200 u/kg/d) vs. low (50 u/kg thrice weekly) dose ITI in a good risk cohort of high titer hemophilia A inhibitor patients. The data for the cohort as a whole was presented; investigators remain blinded to interim arm-specific results. To date, 133 of the targeted 150 subjects have been enrolled from 17 participating countries. As of 7/09, 110 have been randomised at a median age of 23 months) and a median pre-ITI titer of 5.1 BU (0.6 - 9.8), achieved after a median 5.0 (0 – 23) months from inhibitor diagnosis. This cohort has been on ITI for a median 13.0 (0.8 – 33.0) months. To date, 55/110 have achieved a negative Bethesda titre; 46 have achieved a normal FVIII recovery; and 43/110 have so far become tolerant after a median of 15 (3.0– 30.0) months from ITI initiation. Fifty-four subjects have reached a study endpoint; a total of 12 subjects have failed ITI, based on one of two failure criteria. Two interim data safety analyses are mandated by the protocol when 50 and 100 subjects completed the study. In May 2008, the DSMB conducted its first *in camera* interim comparative analysis of study treatment arms with respect to safety and efficacy, and concluded that it was safe to continue the trial. Another DSMB review of the data is in process. Independent investigators are conducting 3 satellite studies in conjunction with the trial. The PIs continue to welcome any additional participating centers until target enrolment is achieved.

**International recommendations on the diagnosis and treatment of patients with acquired haemophilia A – Angela Huth-Kühne (Germany)**

Acquired haemophilia A (AHA) is a rare bleeding disorder characterised by autoantibodies directed against circulating coagulation factor (F) VIII. Typically, patients with no prior history of a bleeding disorder present with spontaneous bleeding and an isolated prolonged aPTT. AHA may, however, present without any bleeding symptoms, therefore an isolated prolonged aPTT should always be investigated irrespective of the clinical findings. Control of acute bleeding is the first priority, and we recommend first-line therapy with bypassing agents such as recombinant activated FVII or activated prothrombin complex concentrate. Once the diagnosis has been achieved, immediate autoantibody eradication to reduce subsequent bleeding risk should be performed. We recommend initial treatment with corticosteroids or combination therapy with corticosteroids and cyclophosphamide and suggest second-line therapy with rituximab if first-line therapy fails or is contraindicated. In contrast to congenital haemophilia, no comparative studies exist to support treatment recommendations for patients with AHA, therefore treatment guidance must rely on the expertise and clinical experience of specialists in the field. A set of recently published international practice guidelines based on the collective clinical experience of the authors in treating patients with AHA aim to contribute to improved care for this patient group. <http://www.haematologica.org/cgi/content/full/94/4/566>

**Prophylactic factor replacement in hemophilia – What studies do we need to standardize practice – Victor Blanchette(Canada)**

Prophylaxis is defined as the regular infusion of factor concentrates in anticipation of, and in order to prevent, bleeding. If started before joint damage, prophylaxis is termed primary. A recent prospective, randomized controlled trial (RCT) has demonstrated that primary full-dose prophylaxis is superior to episodic ("on-demand") therapy in young boys with severe hemophilia A with respect to frequency of joint hemorrhages and the development of early osteochondral

changes in index joints (ankles, knees and elbows) detected by MRI examination at age 6 years (Manco-Johnson MJ et al N Engl J Med 2007;357:535-544). Questions arising out of this study include the following : 1) What is the long term clinical (including functional) significance of the very early osteochondral changes detected by MRI in boys with severe hemophilia A? 2) Can the results from the USA Joint Outcome Study be extrapolated to young boys with severe hemophilia B? and 3) Is the very high cost of life-long full dose primary prophylaxis justifiable? Future prospective clinical trials are needed to address these and other questions, including: 1) What is the role of tailored ("patient driven") lower dose primary prophylaxis regimens; 2) When and how to adjust or even stop prophylaxis in adolescents and young adults; and 3) What is the role of prophylaxis in inhibitor positive patients who are started on programs of immune tolerance induction ( ITI ) regimens, or who have failed such regimens. The challenge is that the long-term clinical studies needed to address these questions are very expensive and difficult to complete successfully. We need, therefore, to consider : 1) The value of carefully designed, short-term studies that use surrogate but valid end-points eg joint damage detected by ultrasound / MRI examinations of index joints; and 2) Longer-term observational studies that incorporate a broad range of clinically relevant outcome measures eg number and type of hemorrhages with a priori definitions of the types of hemorrhage (trauma-related vs spontaneous, significant soft tissue, re-bleed); joint scores determined by physical examination eg the Hemophilia Joint Health Score (the HJHS); functional scores eg the functional independence score (FISH); imaging studies (with definition of the relative roles of X-rays , ultrasound and MRI examination); activity profiles eg the Hemophilia Activities List (HAL), and quality of life measures (the Haemo-QoL and the CHO-KLAT). Finally when classifying a given prophylaxis regimen as successful or not, it is important to consider possible causes of failure such as : 1) Patient / family non - compliance with the prescribed prophylaxis regimen; 2) Individual patient pharmacokinetic ( PK ) profiles; and 3) Development of target joints.

**A simple, safe strategy for hemophilia gene therapy with plasmid DNA. – Michele Calos, USA**

Michele Calos is collaborating with Pain Therapeutics, Inc. to develop a plasmid gene therapy for the treatment of hemophilia B, caused by Factor IX (FIX) deficiency. The concept is to insert the human FIX gene, expressed under a liver-specific promoter, into the genome of host hepatocytes with the help of a sequence-specific phage integrase. The result is permanent integration of the FIX gene into a limited number of sites in the liver genome and robust, long-term FIX expression. She presented data describing the proof-of-principle experiments in murine models in which FIX and integrase plasmids were co-delivered using high pressure tail vein injection. Robust expression of human FIX was detected within hours, and stable therapeutic levels were seen for the life of the mouse. Hepatocyte FIX expression was confirmed by immunohistochemistry showing sustained expression in about 20% of hepatocytes, and FIX plasmid integration was monitored by PCR. PTI is developing clinically acceptable delivery methods for plasmid DNA in liver. They have completed large animal studies in the pig and are currently assessing safety and efficacy in a non-human primate, the baboon.

## **Session 4. Standardization issues**

*Chairs: E. Tuddenham (UK) and J. Oldenburg (Germany)*

### **An International Collaborative Study to Assign Value to the WHO 8<sup>th</sup> International Standard for Blood Coagulation Factor VIII Concentrate – *Sanj Raut, Sarah Daniels and \*Alan Heath (UK)***

Stocks of the current WHO 7<sup>th</sup> International Standard (IS) are expected to be exhausted by 2010 and as such four candidate materials, 2 plasma-derived (samples A and C) and 2 recombinant (samples B and D), have been evaluated as potential replacements by assays relative to the current WHO 7<sup>th</sup> IS and the secondary standard BRP 3/Mega 2 US (sample E), in an international collaborative study involving 38 laboratories. All laboratories were instructed to use their routine validated methods and to follow the ISTH/SSC recommendations, including pre-dilution of concentrate samples in FVIII deficient plasma. Laboratories used either one-stage (22 data sets), or chromogenic methods (30 data sets). Several laboratories performed both assays (12 data sets). Intra-laboratory geometric coefficient of variation (GCV) ranged from 0.3-36.3% with GCVs from the majority of the labs below 10%. There was good agreement between laboratories using each of the two methods with the lowest inter-laboratory variability for candidate A (GCVs < 5%) and an identical mean potency of 9.44 IU/ml by one-stage (n=22) and chromogenic (n=30) methods, when assayed relative to the 7<sup>th</sup> IS and with a combined mean value of 9.4 IU/ml (n=52; GCV=5.0%). Relative to the BRP 3/Mega 2 US standard, one-stage and chromogenic potencies for sample A differed by < 5% with a combined mean value of 9.32 IU/ml (n=52; GCV=8.4%). For sample C, there was a significant difference in potencies between one-stage and chromogenic assays by 7.3% and 11.7% relative to WHO 7<sup>th</sup> IS and BRP 3/Mega 2 US, respectively. The chromogenic potencies however were in very good agreement relative to the two standards, being 10.4 IU/ml (n=30) when assayed against both standards. The inter-laboratory GCVs are 4.8% and 7.1% relative to WHO 7<sup>th</sup> IS and BRP 3 (sample E), respectively. For the 2 recombinant candidates (samples B and D), there was larger inter-laboratory variability with GCVs ranging from 14.5%-19.7%, nevertheless the difference in potencies between one-stage and chromogenic assays was less than 5%.

Overall, sample A is the favoured candidate due to its complete agreement in mean values obtained by the one-stage and chromogenic methods, and the lowest overall inter-laboratory variability for combined estimates (GCV=5%) from both methods. The excellent parallelism of assays, relative to all standards included in the study, and the results from the initial stability studies, validates its suitability to serve as the replacement standard for measurement of both plasma-derived and full length recombinant FVIII concentrates. In the interests of continuity, commutability and following total agreement by all participants of the study, it is proposed that candidate A (NIBSC code 07/350) be accepted as the WHO 8<sup>th</sup> International Standard for FVIII concentrate with an assigned potency of 9.4 IU/ampoule.

### **Value assignment of the proposed WHO 6<sup>th</sup> IS FVIII / VWF plasma (07/316) – *A R Hubbard (UK)***

The current WHO 5<sup>th</sup> International Standard (WHO 5<sup>th</sup> IS) Factor VIII/von Willebrand factor, plasma (02/150) was established in 2003 and is the primary standard for the estimation of 5 analytes in plasma (Factor VIII:coagulant activity; Factor VIII:antigen, VWF:antigen, VWF:ristocetin cofactor, VWF:collagen binding). Since stocks of the WHO 5<sup>th</sup> IS are extremely low a replacement preparation has undergone value assignment in an international collaborative study involving 44 laboratories in 14 different countries. The collaborative study required each laboratory to assay the proposed WHO 6<sup>th</sup> IS relative to the WHO 5<sup>th</sup> IS and locally collected normal plasma pools. Estimates for all analytes calculated relative to the WHO 5<sup>th</sup> IS were associated with lower inter-laboratory variability compared to estimates relative to the local normal pools. There were significant differences for estimates of FVIII:C, FVIII:Ag and VWF:Ag calculated relative to the WHO 5<sup>th</sup> IS and the local normal pools. In consideration of the known stability of the WHO 5<sup>th</sup> IS, the variability of estimates relative to the fresh normal pools, and in order to maintain continuity between the WHO 5<sup>th</sup> and 6<sup>th</sup> IS it is proposed that the WHO 6<sup>th</sup> IS be assigned the mean values obtained relative to the WHO 5<sup>th</sup> IS. All 44 participants in the collaborative study have accepted the proposal that the preparation coded 07/316 be accepted as the WHO 6<sup>th</sup> International Standard Factor VIII/von Willebrand Factor, Plasma with the following assigned values:

Factor VIII:C	0.68 IU per ampoule
Factor VIII:Antigen	1.04 IU per ampoule
VWF:Antigen	1.00 IU per ampoule
VWF:Ristocetin cofactor	0.87 IU per ampoule
VWF:Collagen binding	1.03 IU per ampoule

**Factor VIII and von Willebrand Factor – the confusion of the 70’s persists to today. – Robert Montgomery (USA)**

In the 1970’s Factor VIII (FVIII) and von Willebrand factor (VWF) were demonstrated to be two immunologically distinct proteins that associated in plasma. Hemostasis scientists and members of the VWF and FVIII SSC Subcommittees clearly recognize the differences between these proteins and their immunological identification. For the most part, studies of FVIII and VWF are correctly interpreted when well characterized antisera and monoclonal antibodies are used. Some textbooks, however, still discuss endothelial cells as being FVIII positive resulting in the incorrect identification of FVIII antigen (FVIII:Ag) and FVIII related antigen (FVIII:RAg) as the same protein. This has led to some commercial antibodies and endothelial cells being incorrectly identified. FVIII:RAg has been supplanted for years by the correct designation of VWF:Ag. Multiple commercial antibody suppliers state that monoclonal antibodies to FVIII:Ag react with FVIII and are negative against VWF, but even these may not be correctly identified. Polyclonal antibodies, monoclonal antibodies and endothelial cells are commercially available with the incorrect designation of protein reactivity and have been used by the scientific literature to identify proteins incorrectly. Cell-synthesized or soluble recombinant VWF, plasma VWF, plasma FVIII, and recombinant FVIII were used to characterize protein targets and probed with poly- and monoclonal antibodies to FVIII and VWF. While local antibodies correctly identified FVIII and VWF, some commercial antibodies unambiguously demonstrated incorrect protein identification. These commercial antibodies have unfortunately been used in published journal articles and have resulted in incorrect published conclusions. Careful standardization of these antibodies is needed so that these proteins are correctly identified. Measuring FVIII clotting activity will identify the clotting factor, but the cellular and extracellular association may still lead to ambiguities.

*He therefore proposed that FVIII / IX subcommittee would recommend to manufacturers to verify antibody reactivity with rVWF and rFVIII by some defined method. If neither was positive for some reason such as being an anti-peptide, then it should be listed as undetermined.*

**Standardizing assessment of musculoskeletal outcome in hemophilia. – P. Poonnoose, A. Srivastava (India)**

In the management of haemophilia, assessment of long term outcome is important, not only to document the impact of the condition on the individual, but also to compare different treatment strategies. Till recently, most long term musculoskeletal outcome studies have used bleeding frequency, in addition to clinical (WFH Physical Examination) and radiological (Pettersson) scores, to determine the efficacy of different treatment regimes. With the advent of early prophylaxis, the incidence of joint arthropathy has significantly reduced and it became necessary to improve these tools, and develop new disease specific assessment tools, to make them more sensitive to early changes. Under the International Prophylaxis Study Group (IPSG), the Haemophilia Joint Health score (HJHS) was developed to improve on the assessment of the joint structure and function, and the Compatible MRI score (combining the Denver and the European Pednet scores) was developed to quantify early joint damage. The use of ultrasonography to document early soft tissue changes is also being evaluated. The implications of small differences in these clinical/radiologic scores in terms of overall musculoskeletal function and patients' quality of life is still not clear. The development of functional scores like the Functional Independence Score for Haemophilia (FISH), and the Haemophilia Activities List (HAL), and other quality of life (QoL) measurement tools have helped in providing a more comprehensive assessment of health. An improved understanding of musculoskeletal outcome, using such a core group of clinimetric tools, should assist with the development of contextually relevant guidelines for the management of hemophilia.

**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 6:00pm.

# Fibrinogen and Factor XIII

11 July 2009  
Boston, MA, USA

Chairman: *Moniek P. M. de Maat, The Netherlands*

Co-Chairs: *Robert A S Ariens, UK; Aida Inbal, Israel; Hans P Kohler, Switzerland; Jaap Koopman, The Netherlands; Muriel Maurer, USA; Leonid Medved, USA; Marguerite Neerman-Arbez, Switzerland; Rainer Seitz, Germany; John W Weisel, USA*

## Educational

In the education session, first John Weisel told us about Fibrin gels and their use in bioengineering and clinical applications. Since fibrin gels have unique structural and rheological properties, they are well suited for numerous biomaterials applications in medicine and engineering. Fibrin is highly extensible with non-linear elasticity, and polymerization can be modulated to produce a variety of soft substrates. Fibrin is commonly used as a sealant to stem bleeding and promote wound healing. Newer applications include its use as a matrix for drug delivery, cell migration and differentiation, tissue engineering, and patterning.

Next, Aida Inbal discussed the non-coagulation activities of Factor XIII (FXIII). FXIII is especially known as a plasma transglutaminase that catalyzes the cross link formation between fibrin chains and thereby stabilizes the clot. Beside this function in haemostasis, FXIII has a role in wound healing and embryo implantation processes that involve angiogenesis. In this review the role of FXIII in angiogenesis and wound repair as well as the molecular mechanisms underlying these effects were discussed. Finally, a novel activity of FXIII-protein disulfide isomerase (PDI) was described.

## Factor XIII

Drs. Kohler and Muszbek presented their concept position paper on the diagnosis of factor XIII deficiency. They showed that the correlation between results of various FXIII tests is acceptable for levels over 20%, but poor for levels under 20%. A flow-chart is suggested for the analysis of FXIII deficiency:

- Screening using quantitative functional tests
- Classification of type of deficiency, using specific ELISA's and measurements in plasma and platelet lysates
- Detection of auto-antibodies, both neutralizing (mixing experiments) and non-neutralizing (binding study) antibodies
- Cross linking studies
- Genetic investigation

After this presentation there was discussion on whether the names of the companies should be mentioned when describing the assays. The proposal was to mention the names on the supplemental information on the website, but not in the JTH manuscript.

The second presentation was by dr. Ivaskevicius, who reported that mutations in FXIII could be detected in a proportion of the patients with mild FXIII deficiency: 17 mutations in F13A and 14 in F13B in a total of 134 patients. In patients with a low F13B, also a decreased F13A was present, even though there is a 2 times excess of F13B.

For the measurement of the F13 activation peptide (AP) an ELISA exists that uses a combination of monoclonal and polyclonal antibodies. It would be ideal to use two monoclonal antibodies and therefore dr. Schroeder developed a second MoAb with a high Kd, no crossreactivity with FXIII zymogen or other plasma proteins and that measures independent of the Val34 and Leu34 variants. After complete activation of FXIII, this assay measured 38.6% of expected AP when 2 AP are released. This is more than with the old assay, but still recovery is incomplete. Measuring the AP, using this new assay, in patients with acute stroke showed that the AP is increased on day 1 after severe stroke and also in patients where the NIHSS score did not improve.

When FXIIIA is infused, its half life depends on the amount of FXIIIB that is available. If FXIIIA cannot directly bind to FXIIIB in the circulation, it is rapidly cleared. Dr. Ariens discussed the need for determining the total FXIIIB in the FXIII WHO reference material. By subtracting this from the FXIII A2B2 levels, the amount of free FXIII can then be calculated.

### **Fibrinogen**

In diagnostic routine laboratories both the Clauss assay and the PT-derived method are used to determine the fibrinogen concentration. Dr. Miesbach showed that the PT-derived method correlates well with the protein concentration, but misses to detect dysfibrinogenaemia's. In a selection of dysfibrinogenaemia patients normal levels for PT-derived fibrinogen were measured, while the Clauss method gave strongly decreased fibrinogen levels. This should be realised when samples are analysed. A number of dysfibrinogenaemia patients, but not all, could be identified by a prolonged PT.

Dr. Neerman-Arbez presented a concept guideline for the assays that should be performed when a mutant fibrinogen is identified. The manuscript will be distributed.

Dr. Pieters made an inventory of the methods and levels measured in healthy controls for permeability of a fibrin clot in order to decide whether standardization is required. Differences in permeability were given by the type of plasma and the thrombin concentration, while dialysis had no effect. A wide range in mean permeability (> 4-fold) was reported for different studies. The question was brought up whether standardization is needed for this variable since it is not used in clinical diagnosis: it was decided that some standardization would be welcome to make it possible to compare different studies. An attempt will be made to incorporate this method in the above-mentioned guideline.

Finally, dr. Raut of the NIBSC announced that in 2011 the stocks of the 2nd IS Fibrinogen Plasma (98/612) and the 1st IS Fibrinogen Concentrate (98/614) will be exhausted. Currently material is being sourced to replace these reference materials. The plan is to start collecting data on the material in June 2010 and report the results in 2011.

## Fibrinolysis

12 July 2009  
Boston, MA, USA

Chairman: *Colin Longstaff, UK*

Co-chairs: *Carl-Erik H. Dempfle, Germany; Ann Gils, Belgium; D Hendriks, Belgium; Osamu Matsuo, Japan; Michael E. Nesheim, Canada; Tetsumei Urano, Japan*

Report

Fibrinolysis SSC -2009

### **WHO International Standards**

#### Report on a new International Standard for Streptodornase

Colin Longstaff

Osamu Matuso introduced Colin Longstaff who presented a brief summary of the recent collaborative study to calibrate the proposed WHO 2<sup>nd</sup> International Standard (IS) for Streptodornase, as a replacement for the first IS 62/007. Stocks of the first IS are very low and there is a need for a replacement to allow manufacturers of streptokinase to measure and quantify contaminating streptodornase (a DNase enzyme) in their products. The candidate IS, coded 08/230 consisting of 10 000 ampoules of a partially purified streptokinase preparation, kindly provided by a manufacturer was found to contain 3200 IU/ampoule from the results of an international collaborative study including 7 laboratories from 5 countries. The calibration exercise was generally successful with the typical variation seen within and between laboratories in such exercises. Dr Longstaff reported a number of potential issues with the study, including the age of the existing IS and potential for some loss of activity, the small number of laboratories taking part and the unfamiliarity of the assay format to participants who may not routinely perform the type of dose response methodology needed to calibrate the new preparation of streptodornase. It was also reported that it was not yet possible to make any predictions about the long term stability of 08/230 as no degradation of samples or loss of activity could be seen after 6 months storage at 37, 45 and 56 deg C. Stability studies will be continued.

The proposal was made at the meeting, as in the report, that preparation 08/230 should be recommended for establishment as the WHO 2<sup>nd</sup> IS for Streptodornase with a potency of 3200 IU/ampoule and there were no objections voiced.

#### Update on standardisation of PAI-1 antigen determinations in plasma

Dr Longstaff also provided an update on a collaborative study performed in 2007 on the measurement of PAI-1 antigen in plasma. The original study highlighted the wide variation between methods in apparent ng/ml PAI-1 antigen observed for 5 different plasma pools provided to 12 laboratories. In that study a harmonisation procedure was demonstrated that was able to significantly reduce the variability

between methods and enable recalculation of PAI-1 antigen values for the various methods on a common scale. However, the harmonisation process results only in consensus values for PAI-1 antigen in the 5 different plasma pool samples, but this may not be the same as the real, gravimetric concentration of PAI-1. Dr Longstaff reported his attempts, using surface plasmon resonance, to measure the concentration of PAI-1 in 2 of the plasma pools used in the study. Results looked promising and more work will be performed to attempt to determine the concentration of PAI-1 in plasma, with the longer term view of establishing an IS for PAI-1 antigen in plasma calibrated in ng/ml.

#### Approaches to dual labelling of International Standards for enzymes and inhibitors

Craig Thelwell

Dr Thelwell presented a proposal to calibrate some of the WHO IS used in the fibrinolysis area with additional units. WHO International Units (IU) are arbitrary and defined by the ampouled reference material, independent of any one method. This is in contrast to the ISO system where traceability to SI units is essential through associated reference methods. ISTH and IFCC initiatives to promote standardisation have highlighted the need to develop both reference methods and reference preparations, and it was recommended that for future standardisation projects enzymes should be calibrated in SI units where possible. Active-site titration is a method that could be used to calibrate some enzymes and inhibitors with an active molar concentration. Active-site titrated trypsin was used to calibrate the 1<sup>st</sup> IS for alpha-1 antitrypsin, which has a potency assigned in mg and moles of active inhibitor. Active-site titrations are quantified relative to a standard curve of the released product: 4-nitrophenol for NPGB titrations and 4-methylumbelliferone for MUGB titrations. Reference preparations of these chemicals could be used to standardise active-site titrations and reduce inter-laboratory variation. Trial fills of 4-nitrophenol and 4-methylumbelliferone have been prepared at NIBSC as liquid standards in both sealed ampoules and screw capped vials. They are concentrated solutions in weak storage buffer to allow dilution into the assay buffer. The 4-nitrophenol standard was validated in a collaborative study, and the 4-methylumbelliferone was validated against the 4-nitrophenol standard by comparing active-site titration results for a trypsin solution. MUGB was then used to measure the active molar concentration of some of the current IS quantified by the 4-methylumbelliferone standard. Initial estimates of these IS were made based on assays at NIBSC as follows: 3<sup>rd</sup> IS for Plasmin (97/536) = 1.5 μM; 1<sup>st</sup> IS for HMW uPA (87/594) = 629 nM; 2<sup>nd</sup> IS for Thrombin (01/580) = 1.2 μM and the 1<sup>st</sup> IS for FIXa = 238 nM. The next fibrinolysis IS due to be replaced is the 1<sup>st</sup> IS for HMW uPA. The 2<sup>nd</sup> IS could be calibrated in multiple units: IU for continuity of the current unit (using the traditional collaborative study approach involving biological plasminogen activation assays in the presence of fibrin); Katal, the derived SI unit for enzyme activity (the 4-nitroaniline standard prepared at NIBSC could be included in the study) and in moles of active enzyme by active-site titration (either using NPGB or MUGB relative to 4-nitrophenol or 4-methylumbelliferone standards). Ideally this should be done using a reference method performed by an accredited laboratory. If this approach of dual labelling is agreed upon NIBSC could develop accredited reference methods and chemical standards and serve as an official reference method laboratory for enzyme active-site titrations.

## TAFI/CPU

### Update on the functional assay for plasma TAFIa

Michael Nesheim

A further update was presented regarding a sensitive assay for functional TAFIa (CPU) in plasma. The assay 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. The update comprises a change in procedure for producing the QSY-labelled FDPs. In the past, selective reduction of disulfide bonds in fibrinogen was performed and then QSY was coupled to the new SH groups. The fibrinogen was then clotted with thrombin and the fibrin was lysed with plasmin to produce the FDPs. Although this procedure provided QSY-labelled FDPs suitable for the assay, the labeled fibrinogen often had a tendency to precipitate to various extents when it was dialyzed to remove excess reagent. This, in turn, reduced yields and added to the expense of preparing the reagent. Thus, the procedure was modified so that the reduced, but unlabelled, fibrinogen was clotted and then lysed to yield unlabelled FDPs. These were then labeled with QSY. Very little, if any precipitation occurred when excess reagent then was removed by dialysis. The extent of labeling was the same with both methods (about 10 QSY/ fragment X), as was the  $K_d$  and extent of quenching upon plasminogen binding to the labeled FDPs. In addition, the labeled FDPs prepared by the two methods functioned identically in the TAFIa assay. The new method, however, required only one fifth as much QSY reagent and greatly improved the yield of QSY-labelled FDPs. This makes preparation of the reagent easier and cheaper, and thereby makes the assay more accessible to those who might wish to use the method. The basal level of TAFIa in a small group of normals was shown to be 20 pM. In whole blood *in vitro* with clotting induced with tissue factor in the presence of and intrinsic pathway inhibitor, TAFIa was found to increase from about 20 pM to levels of the order of 2500 pM after clotting, with a time course that tracked very closely to the thrombin-antithrombin complex time course. Collaboration with Dirk Hendriks and coworkers was continued to compare results obtained with our assay and theirs. Wider application of the assays should yield new insights into the physiology and pathophysiology of the hemostatic and fibrinolytic systems.

### Determination of CPU/TAFIa in plasma samples

Evelien Heylen and Dirk Hendriks

Dr Heylen presented recent work on attempts to measure active CPU in plasma which is made technically difficult due by low levels of active enzyme in the presence of high levels of proCPU and another carboxypeptidase, CPN, with constitutive activity. The proenzyme, proCPU also has intrinsic activity against small peptide substrates. A method was described that enabled determination of active CPU down to a levels of 20 pM and below in plasma, employing inhibitors of CPN and a substrate optimised for specificity for CPU. Results were shown using this method on a number of samples from patients treated for stroke with thrombolytic therapy and normal plasma. There was good agreement

using this method and the method described by Dr Nesheim, but there was a discrepancy in the basal level of active CPU/TAFIa determined by the 2 methods, being zero in this method, but above zero, around 20 pM, in the Nesheim method. Possible reasons for the discrepancy were discussed and the work will be continued.

## **D-Dimer**

### Update on D-Dimers – UK NEQAS data.

Ian Jennings

Previously reported data from UK NEQAS (Blood Coagulation) exercises has shown poor between-centre precision for D-dimer assays, which can be improved by harmonisation/calibration with standard plasmas. Recent data shows that variation in results is a consequence not only of kit used, but also instrumentation, with significant differences in results obtained with the same commercial kit on different instruments, underlining the need for improved standardisation. Furthermore, there is a lack of consensus on cut-off values used for exclusion of VTE, even amongst centres using the same kit and instrument, and further guidance on the derivation of cut-off values is required.

The business session was followed by an educational session on D-dimer.

### **Educational Session**

The meeting concluded with an educational session of 90minutes, organized by Dr C-E Dempfle, and featured 3 presentations reviewing the application of D-dimer testing in different clinical situations. The session focused on the primary clinical applications of D-dimer and related assay systems from a practical perspective. D-dimer currently is the most prevalent marker for detection of coagulation activation. Various assay systems are available, including automated assays for high throughput laboratory instrumentation, as well as point of care or near patient assays. D-dimer may discriminate conditions associated with intravascular fibrin formation from conditions without intravascular fibrin formation, and most commonly D-dimer assays are used to for the exclusion of venous thrombosis or pulmonary embolism. However, other applications are possible, including the differential diagnosis of acute chest pain. Furthermore, in the perioperative setting, D-dimer and other markers of fibrin generation may identify patients with high clinical risk, including patients prone to thromboembolic complications. Finally, D-dimer measurement has emerged as a prognostic tool in secondary prevention of venous thromboembolism, for monitoring of pregnancy complications and in cancer. The session was very well attended and received.

### D-dimer in the emergency room (venous thromboembolism exclusion, aortic dissection).

Carl-Erik Dempfle

### Fibrin generation markers in the perioperative setting.

Wolfgang Korte

D-dimer as prognostic parameter in venous thromboembolism, pregnancy and cancer.

Saskia Middeldorp



## Haemostasis and Malignancy

11 July 2009  
Boston, MA, USA

Chairman: *Martin H Prins, The Netherlands*

Co-chairs: *Giancarlo Agnelli, Italy; Dominique Claire Farge, France; Charles W Francis, USA; Alok A. Khorana, USA; Agness Y Y Lee, Canada*

There was an educational session, as specified in the ISTH 2009 program book, at the beginning of the session. This program was well attended, with many attendees standing at the back wall, as chairs were in shortage.

Hereafter the business meeting started, which was probably not a well chosen name, since it suggested privacy rather than an open meeting.

The proposals for new studies presented in the business meeting were closely matched with the deficiencies in study data identified in the educational session.

A. Khorana presented a proposal for a registry of incidental PE and thrombosis encountered on (staging) CT-scan for patients who underwent a CT-scan for other reasons than suspected PE. Discussion focused on the appropriate control group: Similar cancer without incidental PE? - Symptomatic PE with similar thrombus load? There was also discussion about the ability of such a registry to adjust/correct for, or to detect, treatment effects, since many patients with incidental but important PE receive anticoagulant treatment.

PW Kamphuisen presented a proposal for 3 clinical studies. The first one considered primary prophylaxis in cancer patients with high levels of procoagulant microparticles. This study is planned to be double blind, placebo controlled. The discussion focused mainly on two issues: best test for procoagulant microparticles and choice of strategy of defining ambulant patients with cancer at high risk for thrombosis. The test Dr Kamphuisen is proposing is reported to be reproducible, although research is ongoing. Regarding the strategy, 3 options seem available – focus on cancer type/stage only, risk profiles or biomarkers. To choose between these studies on all approaches seem necessary. The second study proposal concerned the optimal treatment (VKA or LMWH) after 6 months of LMWH in patients with cancer and thrombosis. The design was to be open label, randomized. There was no discussion on the need for this study, but the feasibility of the sample size (1000 patients) was challenged. The final study concerned the diagnostic algorithm for the diagnosis of arm vein thrombosis, a common problem in cancer patients. The purpose is to demonstrate the safety of withholding treatment based on a decision rule and a normal D-dimer. This proposal was not meeting opposition. For all three studies an eCRF will be made available, to ease participation

Potential participating centers can apply via a dedicated website:

[www.examine thrombosis.com](http://www.examine thrombosis.com).

D. Farge presented a joint effort of a France/European group to join forces with an Oncology guideline group, to come to evidence based guidelines (European) for the approach to prevention and treatment of VTE in cancer patients. This proposal fits nice with the ISTH initiative for being more proactively involved in guideline development.

Finally, the format of the SCC-subcommittee meeting was discussed. In general, there was a consensus that educational activities in combination with a focus on new initiatives would be a good combination for future meetings.



## Lupus Anticoagulant /Phospholipid-Dependent Antibodies

11 July 2009  
Boston, MA, USA

Chairman: *Vittorio Pengo, Italy*

Co-chairs: *Ph G De Groot, The Netherlands; Thomas Ortel, USA; Jacob H Rand, USA; Guido Reber, Switzerland; Armando Tripodi, Italy*

**Round Table (chair: A Tripodi): 'Standard reference material for detecting antiphospholipid antibodies: problems and possible solutions'. Devreese K, Favaloro E, Gray E, Pierangeli S, Reber G.**

**(Pierangeli)** An important problem in aPL tests is the use of properly validated calibrators and reference material. There is no doubt that utilization of validated ELISA assays, whether commercial or in-house, with well-tested calibrators may enable more reproducible measurements. Our laboratory, the Antiphospholipid Standardization Laboratory (APLS/LAPL) has been involved in the preparation, evaluation and distribution of aCL standards since 1980's. Because of the large demand, three generations of secondary calibrators have been prepared and distributed since then. Each generation of calibrators used the first group of calibrators for comparison and was carefully prepared and exhaustively tested in our own and other well-recognized laboratories before distribution. As another means of demonstrating the performance of these calibrators, results of six samples (from the second standardization workshop, the "KAPS" standards) were compared using each generation of calibrators. Our laboratory also prepares reference material used in national and international survey programs. However, it is our opinion that reference material with well-defined positive values for IgG, IgM and IgA aCL and anti- $\beta_2$ GPI antibodies should be made widely available to clinical laboratories, research centers and diagnostic companies.

Proposal to produce aPL (aCL and anti- $\beta_2$ GPI) Positive Reference Material:

1. Purpose of the reference material: to be used by requestors to validate their own assays, or as an “external” positive control or to prepare their own “in-house” calibrators or to QC aPL assays.
2. Preparation of large quantities of reference material (serum) with blendings of sera of well-characterized patients with APS. Testing for infectious agents to make sure the material is negative for: HIV, HBsAg, HCV, CMV, etc.
3. Thorough evaluation of the reference material and validation in various assays at the manufacturing center and also in multiple recognized centers and diagnostics companies before processing in aliquots and distribution. Cross-validation against the monoclonal aPL antibodies (originally produced by Koike *et al*).
4. Definition of the positive range. Units of measurement: GPL, MPL, APL for aCL and SGU, SMU and SAU for anti- $\beta_2$ GPI. Target positive range: medium positive values.
5. Stabilization, preservation, storage conditions, real time stability studies.
6. Lyophilization. Pre and post-QC tests.
7. Distribution (COA, MSDS, insert provided)
8. Issues related to custody and distribution of the material to be discussed and resolved.
9. Financing of the project to be discussed and resolved.

**(Devreese)** Diagnosis of the Antiphospholipid syndrome (APS) is complicated by lack of a golden standard. Lupus anticoagulant (LAC) coagulation assays as well as enzyme-linked immunosorbent assays (ELISA) for anticardiolipin (aCL) and beta2-glycoprotein I (b2GPI)

antibodies have methodological shortcomings. The lack of standardization and the misinterpretation of results remain major problems often leading to overdiagnosis.

Use of an international standard for antiphospholipid (aPL) antibody detection can help solve these problems.

aPL antibodies are polyclonal and their heterogeneity will be difficult to reflect in an international standard. Mixtures of, preferable human, monoclonal antibodies against the most frequent antigens of the aPL antibodies can approximate the complex spectrum of natural occurring aPL antibodies in patients.

This standard material should be used in coagulation assays as well as in immunoassays as calibration material, in order to express results quantitatively.

In parallel with the aCL and the  $\beta$ 2GPI antibodies, quantification of LAC may help to stratify patients into risk groups. The quantitative expression of the aPL antibody titer measured with coagulation assays as well as immunoassays, may further evaluate the relationship between the clinical symptoms and the presence of aPL antibodies, as well as their potency and fluctuation over time.

**However, preparation, characteristics and intended use of such a standard material should result out of a consensus meeting of experts in the field.**

**(Reber)** I propose as reference material mixture of mAb directed to different domains/epitopes of  $\beta$ 2GPI and prothrombin and a procedure to validate it. The custodian laboratory should be NIBSC and the intended use of this reference material should be method validation and expression of results.

**Laboratory diagnosis: coagulation assays (new guidelines). A Tripodi**

Dr Tripodi described point by point the new guidelines on LA testing with particular reference to major changes introduced with respect to previous 1995 guidelines. These guidelines are being published in JTH.

### **Detection of antibodies against domain I of beta2-glycoprotein I improves the diagnosis of APS: an international multicenter study. B de Laat**

Diagnosis of the antiphospholipid syndrome (APS) is difficult due to limited specificity of existing assays for detecting clinical relevant antiphospholipid antibodies. Anti-beta2-glycoprotein I (beta2GPI) antibodies play a central role in the disease process of APS. We have investigated the relation between antiphospholipid antibodies with specificity for domain I of beta2GPI and thrombosis/pregnancy morbidity in an international multicenter set-up. 539 patients derived from ten different centres met the inclusion criterion of having anti-beta2GPI antibodies in their plasma/serum. Clinical data and results of tests for lupus anticoagulant, anti-cardiolipin antibodies and anti-beta2GPI antibodies were established at the different centres of inclusion. After being re-tested for presence of IgG and/or IgM anti-beta2GPI antibodies, the samples were tested for presence of IgG directed against domain I of beta2GPI and results were correlated to the thrombotic and obstetric history. Re-testing for the presence of anti-beta2GPI antibodies resulted in inclusion of 511/539 patients. IgG class anti-domain I antibodies were present in plasma of 275/511 patients (54%). 227/275 (83%) had a history of thrombosis. This resulted in an odds ratio of 3.8 (2.5-5.7, 95% confidence interval) for thrombosis. Anti-domain I IgG antibodies were also significantly correlated with obstetric complications (odds ratio: 2.4 (1.4-4.3, 95% confidence interval)). Conclusion: In this multicenter study the detection of IgG antibodies that are directed against domain I of beta2GPI proved to be stronger associated with thrombosis and obstetric complications than the classic anti-beta2GPI antibody assay.

Predictive value of the Antiphospholipid Score. T Atzumi

To define the Antiphospholipid Score (aPL-S) by testing multiple antiphospholipid antibodies, and to evaluate its efficacy for the diagnosis of antiphospholipid syndrome (APS). Method: From our database of autoimmune disease clinic, the results of antiphospholipid antibodies in 233 patients were used to define aPL-S. Five clotting assays (the mixing studies: activated partial thromboplastin time (APTT), kaolin clotting time, the dilute Russel's viper venom test (dRVVT), and the confirmatory tests: APTT and dRVVT) and 6 ELISAs (IgG/M anticardiolipin antibodies, IgG/M anti-beta2-glycoprotein I antibodies and IgG/M phosphatidylserine dependent antiprothrombin antibodies). The aPL-S was calculated according to the number of positive test and their titers (range 0-83), and compared with the history of thrombosis/pregnancy morbidity. Results: The aPL-S was higher in patients with such manifestations (n=46) than those without (22.3±26.3 vs. 4.13±10.8, p=0.00001). For the diagnosis of APS, the receiver operating characteristics (ROC) curve of aPL-S showed hyperbolic, and the area under the ROC curve (ROC AUC) are 0.752 and 0.686 for aPL-S and for the Sydney revised Sapporo criteria, respectively. Conclusion: The aPL-S is a useful quantitative index for diagnosing APS, and a potential predictive marker of thrombosis in autoimmune diseases.

Educational Session (T. Ortel, J Rand, PG de Groot, V Pengo)

The four speakers covered all the relevant aspects of the antiphospholipid syndrome. An Historical background was initially be given by T Ortel starting from the discover of antiphospholipid antibodies at the beginning of past century. To follow dr Rand described the current hypotheses on the pathogenesis of the syndrome. Dr de Groot then discussed the very important issue on the laboratory diagnosis of the syndrome and finally Dr Pengo described the clinical features of the disease as well the infrequent associated clinical manifestations.

## Perinatal/Pediatric Hemostasis

11 July 2009  
Boston, MA, USA

Chairman: *Gili Kenet, Israel*

Co-chairs: *Janna M Journeycake, USA; Prasad Mathew, USA; Paul Monagle, Australia; Wolfgang Muntean, Austria; Ulrike Nowak-Gottl, Germany; Nicole Schlegel, France*

### Anticoagulant trials:

Chair: P Massicote, Ca

#### **Development of a Pediatric Quality of Life Inventory For Children Requiring Long-term Warfarin-** Aisha AK Bruce, Canada, and Fiona Newall, Australia

Health related quality of life (HQOL) measurement is paramount to ensure both patient satisfaction with medical care and adherence to treatment recommendations, and serves as the “gold-standard” measurement for patient relevant outcomes as a basis for clinical research. Since treatment with long-term warfarin affects the child and families’ HQOL independently of child’s pre-morbid condition, a validated pediatric anticoagulation HQOL (already applied in Canada and Australia) helps identify barriers in management and to evaluate therapeutic options. Extended use of HQOL was suggested as an assessment tool for new anticoagulant trials in pediatric patients.

#### **Trial design and outcomes- for evaluation of anticoagulant drugs for use in children-** Christoph Male, Austria

About 50-90% of the drugs used for pediatric patients are not authorized. New regulatory pediatric initiatives are currently taken in order to generate valid data on use of drugs in children (special formula, variable dosage, safety and efficacy measures), not only via extrapolation from adult data. New safety and PK studies should always be performed in children, with adjustment to various age- groups. The EMEA currently requires presentation of pediatric investigational plan (PIP), for each new drug or new pediatric indication. SSC was encouraged to take a stand defining the needs, priorities and feasibilities required for this process.

#### **Limitations and definitions in pediatric thrombosis studies-** L Mitchell, Ca

Since the different background co-morbidities, acute trigger, location of thrombosis as well as vessel wall properties and developmental hemostatic changes affect thrombosis epidemiology in children (and in neonates as compared to teenagers) as compared to adults, unique study design is required for pediatric patients. Specific end point definitions for prophylaxis and treatment studies in children including thrombus burden and the significance of asymptomatic DVT were addressed.

Pediatric Thrombosis and stroke – suggestions, definitions and trials:

Chairs: J Journeycake, USA and G Young, USA

**PTS in Children: A Review of the Evidence, Instrument Validation, and Call-to-Action- NA Goldenberg and MJ Manco-Johnson**

Post-thrombotic syndrome (PTS) is a syndrome of chronic venous insufficiency following DVT. Several scales for PTS assessment have been developed in adults. In 2002, Manco-Johnson reported the development of a pediatric PTS outcome instrument that combined the adult CEAP with the Wong-Baker pain scale previously validated for pediatric use. The training module for this scale, that was prospectively applied and further validated (Goldenberg et al, 2004, Manco-Johnson, 2007), is now available online via the Kids-DOTT trial webcenter, [www.kids-dott.net](http://www.kids-dott.net).

Standardized evaluation of PTS should serve as a co-primary endpoint (along with recurrent VTE) in future trial of pediatric VTE.

The Kids-DOTT trial is a multicenter investigator-initiated RCT of the duration of therapy for venous thrombosis in children, funded via NIH and industry, using this end point. Investigators are encouraged to contact Neil Goldenberg for details.

**International Pediatric VTE Collaboration—Multicenter Cohort Study on Residual Occlusive Thrombosis as a Prognostic Factor for Recurrence- N Goldenberg- USA, AK Chan, Canada, U Nowak- Gottl- Germany**

A multi center international study evaluating residual occlusive thrombosis as a putative prognostic factor for recurrence was suggested. Occlusive thrombosis has been defined as a prognostic factor for PTS in children (Revel-Vilk et al), but an association between acute or persistent veno-occlusion and recurrence risk has been insufficiently examined to date. Investigators, who have local institutional-based cohort studies in place for collection of clinical, laboratory, and radiological data in pediatric VTE, and who are interested to participate- may contact Neil Goldenberg, Ulrike Nowak-Gottl, and Anthony Chan for further details.

**Genetic association studies for juvenile thrombosis and stroke- Ulrike Nowak-Göttl and Monika Stoll, Germany**

The influence of genetic factors underlying pediatric venous thrombosis and stroke, and their interaction as well as environmental factors, was discussed. Data covered a broad range of genetic-epidemiological studies including the estimation of heritability, examples from candidate-based association studies as well as the prospects of whole genome association studies in understanding the complex basis of these diseases. International multi center collaboration was encouraged in order to continue such studies.

Neonatal Thrombosis:

Chairs: AK Chan, Canada and M Bonduel, Argentina

**Neonatal renal vein thrombosis- K Lau, Ca**

Renal vein thrombosis is a complication that occurs in neonates with various underlying risk factors. It carries a grave prognosis for affected kidneys. Prospective controlled trials are still lacking and no evidence-based guideline is available to date. Different therapies, including anticoagulation and fibrinolysis have been promoted in the past with variable success. Data extracted from current literature suggest that most of the involved kidneys became atrophic, and current treatment strategies are not necessarily offering better prognosis.

Cooperative prospective studies that involve multiple centers are needed to elucidate the optimal treatment modality for neonatal renal vein thrombosis.

#### **Neonatal Portal Vein Thrombosis- S Williams , Ca**

Neonatal portal vein thrombosis is a common thrombotic event associated with the use of umbilical venous catheter placement for supportive therapy. There is spectrum of outcomes, with a proportion of neonates having spontaneous resolution. However, portal hypertension, a chronic condition with significant morbidity, is a recognized complication following portal vein thrombosis, which may occur years after the thrombotic event. Interventions with the potential to alter outcomes, or decrease the risk of portal hypertension are not established. As a result, there is no standard approach to therapy, which ranges from observation, umbilical catheter removal, anticoagulation to thrombolysis.

There is a need for development of a standardized approach to the care of neonates with portal vein thrombosis.

#### **Neonatal Cerebral Vein Thrombosis (NCSVT)- M Moharir , Ca**

NCSVT is still under-diagnosed despite improving awareness and better neuroimaging techniques. The outcome from NCSVT appears to be adverse in the majority. Use of anti-coagulant therapy (ACT) in NCSVT is highly variable; but most centres in US do not seem to be offering treatment due to lack of established safety and efficacy. There are emerging data to suggest the safety of ACT and the potentially serious risks of no treatment in NCSVT. The feasibility of multicentric pooling of data and clinical trials was discussed.

# Plasma Coagulation Inhibitors

11 July 2009  
Boston, MA, USA

Chairman: *Herbert C Whinna, USA*

Co-chairmen: *Francesco Bernardi, Italy; Elaine Gray, UK; Tilman M Hackeng, The Netherlands; Steven Kitchen, UK; Richard Marlar, USA; Piet Meijer, The Netherlands; Laurent O Mosnier, USA*

-Differences in protein S activity assay outcomes in the external quality assessment programme of the ECAT Foundation (2:00 - 2:20 PM)

Piet Meijer, PhD

In the surveys of the external quality assessment programme of the ECAT Foundation significant differences in the average test outcome between different protein S activity assays were observed. This is in line with data presented last year by the UKNEQAS. So far the reason for these differences is unknown. It was suggested to install a project group including representatives from EQA programmes and diagnostic companies to further investigate this phenomenon.

-Proposal for a scoring system of the analytical performance of coagulation inhibitor testing (2:20 - 2:45 PM)

Piet Meijer, PhD

In the past the ECAT Foundation introduced a model for the evaluation of the long-term analytical performance of a laboratory test by a particular laboratory-based on the results of external quality assessment surveys. This model allows us to assess the long-term within-laboratory analytical coefficient of variation (LCVa), which represents imprecision, as well as the bias. Briefly, for each participant, linear regression is applied using the consensus values of each survey as the denominator (independent variable) and the corresponding laboratory values as the numerator (dependent variable). The slope and the variability of the regression line, the mean and the standard error of the consensus values as well as the mean value of the laboratory results are calculated. The formulae for the calculations are given below.

Bias:

$$B = \frac{\sqrt{\frac{n-1}{n} \cdot (b-1)^2 \cdot s_x^2 + (\bar{Y} - \bar{X})^2}}{\bar{X}} \cdot 100\%$$

Imprecision:

$$LCV_a = \frac{(s_{y|x} / b)}{\bar{X}} \cdot 100\%$$

(X) is the consensus value and ( $\bar{X}$ ) is the mean value for X. ( $s_x$ ) is the standard error of (X). (Y) is the laboratory value and ( $\bar{Y}$ ) is the mean value for Y. (b) is the slope and ( $s_{y|x}$ ) is the variability of the regression line, which is calculated on the basis of the least-square method. The number of laboratory results included is expressed by (n).

In the presentation given the focus was on the LCVa as a measure of a stable test performance over time. Data were shown for Antithrombin, Protein C and Protein S. As an objective criteria for the

evaluation of the laboratory performance the concepts of the biological variation as a basis for analytical quality specifications were discussed. Based on the evaluation model for the long-term performance (LCVa) and the concept of biological variation as a basis for analytical quality specifications a scoring system was introduced for minimum, desirable end optimum performance. On the basis of these criteria laboratories can be graded. For instance, Grade A means an LCVa fulfilling the criteria for optimum performance. With this approach the long-term analytical performance of a laboratory can be assessed and compared to objective criteria for analytical performance. This comparison shows the laboratory whether further improvement in the analytical quality is necessary. This model can also be used for the evaluation of test performance.

-Thrombin generation testing: Sample collection and processing (2:45 - 3:05 PM)  
Peter Cooper, CSci FIBMS

Pre-analytical variables can greatly affect assay results. Looked at following pre-analytical variables:

- Samples taken using 21G 'Butterfly'
- Immediate vs delayed sample processing
- Transport by vacuum tube
- Effect of corn trypsin inhibitor

These were studied on following instrument platforms:

- 1) Thrombinoscope assay  
Fluoroskan Ascent fluorometer  
5pM rTF and 4µmol PL
- 2) Technoclone Technothrombin TGA assay (manual)  
Fluoroskan Ascent fluorometer  
Reagent RC Low (~ 0.5pM rTF and low concentration PL)
- 3) Technoclone Technothrombin TGA assay (automated)  
Ceveron analyser  
Reagent RC Low (low concentrations rTF and PL)

The following conclusions were reached:

- Avoid sample transport by vacuum tube (TGA assay)
- Slightly underfilled samples may have a reduction in thrombin generation
- 21G Butterfly (with minimum stasis) can be used to obtain samples for Thrombinoscope assay with 5pM TF but 21G Butterfly is less reliable for Technothrombin TGA assay with RC Low
- CTI reduces thrombin generation with TGA assay
- Ceveron & manual TGA assays require different reference ranges
- Added lipid (1:500 and 1:250 dilutions of 20% ClinOleic) had minimal effect on assays
- Sample pre-analytical must be taken into account

- Thrombin generation in mouse plasma (3:05 - 3:30 PM)  
S. N. Tchaikovski, PhD

Mouse models become increasingly important in thrombosis research. However, only a limited number of assays are available for assessment of the coagulation system in mouse plasma.

Calibrated automated thrombography was applied to quantify tissue factor-initiated thrombin generation in murine platelet-rich and platelet-free plasma and to develop a test for measurement of resistance to activated protein C (APC) in mouse plasma.

To overcome the higher activity of coagulation inhibitors in mouse plasma as compared to human plasma, the reaction temperature was lowered to 33°C and the assay was carried out at a 2-fold higher final plasma dilution (1:3) than commonly used for CAT in human plasma. This increased the ETP 4- to 5-fold and enabled reliable measurement of thrombin generation in both platelet-free and platelet-rich mouse plasma. For the APC resistance measurement the reaction conditions were further optimised with respect to tissue factor, phospholipid, APC and CaCl<sub>2</sub> concentrations. The test was validated using plasma of mice with different genetic background with respect to the factor V<sub>Leiden</sub> mutation (FV<sub>Leiden</sub>). APC resistance increased in the order: wild-type mice < heterozygous FV<sub>Leiden</sub> mice < homozygous FV<sub>Leiden</sub> mice.

Similar to what is observed in humans, protein S deficiency and elevated plasma levels of prothrombin in mice also caused APC resistance. In contrast, pregnancy induced a decrease in APC resistance in wild type and in factor V<sub>Leiden</sub> mice, whereas opposite changes are observed in pregnant women. The opposite changes in APC resistance in mice and in humans during pregnancy could be explained by opposite changes in TFPI activity and plasma levels of protein S.

Our data demonstrate species specificity in the regulation of the protein C and TFPI systems and suggest that protein S and TFPI play an important role in the development of pregnancy-induced APC resistance in mice and in humans.

-Report from the Working Party on Thrombin Generation Tests (3:30 - 4:30 PM)

Pre-analytical variables for Thrombin Generation Tests (3:30 - 3:45)

Barry Woodhams, PhD

Barry Woodhams presented preanalytical variables that should be considered when carrying out thrombin generation tests. These variables include blood collection, processing of plasma samples and sample storage. He concluded that there are many pre-analytical variables that can influence the results of thrombin generation tests. Not all of these parameters have been fully investigated and there is conflicting information from publications. Formal studies are needed to evaluate the influence of these variables.

Collaborative study to investigate the behaviour of FEIBA in FVIII inhibitor plasma by thrombin generation tests(3:45 - 4:00)

Katalin Varadi, PhD

Katalin Varadi described the protocol for investigating the behaviour of Factor VIII Inhibitor Bypassing Agent (FEIBA) in FVIII inhibitor plasma samples. The study is expected to be completed by the end of 2009 and the study results will be presented at the next SSC meeting in 2010.

Thrombin Generation Tests - overview of ISTH abstracts (4:00-4:15)

Peter Giesen, PhD

Peter Geisen carried out a survey of on the ISTH abstracts related to thrombin generation tests. Two hundred and sixty eight abstracts were found referencing thrombin generation tests. The majority of

the abstracts present data on understanding the mechanism of haemostasis, thrombosis and risk factors, haemophilia and diagnosis and treatment of diseases.

Availability of reference plasmas for thrombin generation tests (4:15 -4:30)

Elaine Gray, PhD

Elaine Gray announced the availability of 2 reference plasmas for thrombin generation tests. One of the reference plasmas 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 September 2009 from the National Institute for Biological Standards and Control (NIBSC) who is the custodian for these plasmas donated by Industry.



## Plasma Kallikrein-Kinin System

July 12, 2009  
Boston, MA, USA

Chairman: *David Gailani, USA*

Co-chairs: *Keith R McCrae, USA; Thomas Renné, Sweden; Alvin Schmaier, USA*

The meeting of the Plasma Kallikrein-Kinin subcommittee SSC was held from 8 AM to Noon on July 12, 2009 in Room 156ABC of the Boston Convention Center. The session included an **Educational Session** from 11 AM to noon. We were very impressed (astounded, frankly) at the attendance for this session, which was easily 150-200 people for the first two hours, increasing to the room's capacity (probably 300+ people ) for the Educational Session. I cannot recall a group this large for a session devoted to this topic since I began attending ISTH meetings in 1991.

The session started by addressing an issue raised at the 2008 session in Vienna regarding the subcommittee name. The current name was adopted six years ago, because of concerns that the old name, "contact activation", described an in vitro phenomenon. However, the current name is often used to describe roles for factor XII, prekallikrein and kininogen that are unrelated to hemostasis or thrombosis. With recent observations indicating that factor XII contributes to thrombosis through a mechanism similar or related to contact activation, it was felt that the current name was not adequate to describe the areas of interest of the subcommittee. Dr. Alvin Schmaier presented an eloquent argument for keeping the current name, but when the issue was put to a vote, there was near unanimous consensus that the name should be changed. "The Plasma Contact System" was proposed as a new name, and it was agreed that the chair of the subcommittee (Dr. Gailani) would carry this forward to Drs. Johnson and Falanga.

**The Scientific Presentations** portion of the meeting followed, and are described here:

**Dr. Alvin Davis** of Harvard University presented an update on the biochemistry and physiology of C1-inhibitor. In addition to briefly covering the known role of C1-INH as a protease inhibitor, Dr. Davis discussed contributions of this protein in regulating inflammation, complement activation, and contact activation that appear to be unrelated to its serpin function. This includes a role in regulating the alternative complement pathway, and inhibition of the activities of gram negative endotoxin.

**Dr. Andras Gruber** of the Oregon Health and Sciences University presented a review of work in animal models showing an antithrombotic effect of therapeutic factor XI inhibition, with an emphasis on his work in primates. The anti-human factor XI monoclonal antibody designated 1A6 prevents three-dimensional growth of a platelet-rich thrombus in an arterio-venous shunt thrombus model in baboons. The antibody appears to be mediating its effect through blockade of thrombin production. Also covered was work in factor XI deficient mice showing that these animals have a significant survival advantage over wild type mouse in a model of polymicrobial peritonitis/sepsis.

**Dr. Owen McCarty** of the Oregon Health and Sciences University is an engineer who has become interested in the biomechanics of platelet activation. He presented new work showing that factor XI binds to platelets through a previously unrecognized mechanism involving the receptor ApoER2. Many years ago, it was established that factor XI bound to platelets through glycoprotein 1b. However, the number of binding sites for factor XI (~1500 per platelet) was far lower than the number of GP1b receptors per platelet (~25,000). There are about 2000 ApoER2 receptors per platelet, and they form a complex with GP1b. A new model is being developed in which both ApoER2 and GP1b are required for factor XI binding. Dr. McCarty also presented interesting flow based studies showing the importance of factor XI to fibrin formation in flow, and the importance of platelets for factor XI activity to become manifest under flow.

**Dr. José Govers-Riemslog** from the Cardiovascular Research Institute in Maastricht gave us an update on her work looking for evidence of activated contact proteases in patients with coronary artery disease and other acute vascular syndromes. The work is based on antibody capture assays that look for factor XIIa, XIa, and kallikrein in complex with serpin inhibitors. While increased protease activation can be identified in certain clinical situations, it is clear that there is wide variation in activation of the contact system during acute arterial events, and that patterns of activation vary between individuals, possibly reflecting differences in underlying pathologies.

**Dr. Cornelius Kluft** from the Gaubius Laboratory TNO-PG Leiden finished the scientific program by discussing assays for detecting oversulfated chondroitin sulfates in plasma. These compounds were used to adulterate crude preparations of heparin in 2008, resulting in the deaths of a number of patients from an anaphylactic process. It is clear from Dr. Kluft's work that variations in the way these assays are set up affect both sensitivity and specificity for various glycosaminoglycans. While we do not feel that there is sufficient information at this point for the Subcommittee to come up with a formal position on this topic, it was suggested that Dr. Kluft post recommendations for the assays on the ISTH website, and solicit feedback on steps to be taken to standardize the assays. Based on the feedback, it may be possible for the Subcommittee to formalize recommendations at a later time.

An **Educational Session** was presented for the first time in our subcommittee meeting. We chose the topic "Physiologic and Pathologic Activation of the Plasma Contact System" based on the renewed interest in contact activation as a trigger for pathologic coagulation.

**Dr. Martijn Gebbink** of the University Medical Center in Utrecht presented work showing that misfolded proteins were capable of triggering activation of the contact system without enhancing thrombin generation. The session started with a very instructive discussion of how common protein mis-folding is, and the variety of disease processes it contributes to. This was followed by a discussion of how tissue plasminogen activator and factor XII bind to a variety of misfolded proteins, enhancing fibrinolysis and non-coagulant activities of the contact system.

**Dr. Thomas Renné**, the subcommittee Co-Chair, from the Karolinska Institute in Stockholm finished the session with an excellent overview of work over the past five years demonstrating that factor XII can contribute to coagulation in vivo, and may play a central role in certain types of pathologic coagulation.

He also, briefly discussed elegant work, subsequently presented in detail by Dr. Henri Spronk in the Plenary Session, confirming that factors XI and IX, but not factor XII are required for hemostasis *in vivo*. Specifically, mice expressing with low expression of tissue factor need factor XI and factor IX for viability, but do not require factor XII.

Overall, we were very pleased with this years SSC subcommittee meeting in terms of the quality of presentations, audience participation, and particularly the large turnout. It is clear that general interest in the topic of contact activation is increasing, largely based on the possibility that targeting the contact proteases with therapeutic inhibitors may be effective anti-thrombotic strategy that would be associated with a relatively low risk of bleeding.



## Platelet Immunology

11 July 2009  
Boston, MA, USA

Chairman: *Andreas Greinacher, Germany*

Co-chairmen: *Beng H Chong, Australia; Yves Gruel, France; Volker Kiefel, Germany; Hartmut Kroll, Germany; Theodore Warkentin, Canada*

Part 1. 08:00 - 10:30 Autoimmune thrombocytopenia

*Chairs: B. Chong (Australia), H. Kroll (Germany)*

08:05 - 08:20 Standardization of terminology and definitions in ITP: on which topics has a consensus reached and what are the open issues *F. Rodeghiero (Italy)*,  
([Rodeghiero@hemato.ven.it](mailto:Rodeghiero@hemato.ven.it))

Dr Rodeghiero provided an update on the work of the committee for standardization in ITP: A group of worldwide recognized clinical experts in immune thrombocytopenia (ITP), endorsed by the Scientific Working Group on Thrombocytopenias of the European Hematology Association ([www.tcpeha.org](http://www.tcpeha.org)), reached a consensus on definitions and terminology in ITP. The European funding group was enlarged to include experts from Canada, USA and Australia. Presentations on the progress of the project were made at the meetings of the SSC/ISTH Subcommittee on Platelet Immunology each year from 2006 to 2008.

The final document was published in *Blood* (Rodeghiero F et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood* 2009; 113:2386-93).

Agreed on definitions encompass: minimal criteria ( $< 100.000/\mu\text{L}$ ) to define thrombocytopenia, the distinction between primary and secondary ITP, definition of newly diagnosed (0-3 months), persistent (4-12 months), and chronic ( $> 12$  months) ITP, criteria for response and complete response and some criteria for inclusion and response evaluation for investigational trials.

The definition of refractory ITP requires that patients fail splenectomy and also need active treatment for bleeding or excessive risk of bleeding. This definition was not agreed on for children. The group identified a need for further investigation into development of reliable scales, based on clinical criteria, for measuring the risk of bleeding.

08:20 - 08:30 Discussion: does the Platelet Immunology SSC agree with the consensus?

Discussion ensued regarding what should be considered to be “refractory” ITP. The definition of “refractory” ITP should depend on treatments failed, then platelet count, then the presence of bleeding. Perhaps a definition contingent on failing splenectomy is insufficient

for many patients who should not be considered refractory if they do not bleed, despite persistently low counts.

Concerns were raised that the consensus statement was not clear regarding what is “secondary” ITP: e.g., should hepatitis B and C be routinely tested for, and is HBV- or HCV-associated ITP considered “secondary”? Screening for serologic markers such as ANA was not addressed by the Standardization committee. There is a need for further studies to characterize these subgroups of patients and determine the course of their disease with clinical outcomes. Platelet antibody testing also needs to be studied to identify patient subgroups.

The lack of standardization of bleeding definitions is clearly a gap in ITP clinical practice and needs to be addressed in future studies. Current difficulties in implementing criteria to differentiate mild vs severe bleeding were acknowledged.

There was some discussion about whether the standardization criteria should be used to evaluate new papers for publication. This may be a future goal, but concern was raised that it may be premature to accept these criteria as standards for reporting until their validity and acceptability was assessed.

The platelet immunology SSC keeps in close contact with the ITP nomenclature working group and supports the inter-society approach of standardization.

08:30 - 08:45 Update: PARC study new lessons learned, new questions arising from systematically collecting and following ITP adult and pediatric ITP patients. *P. Imbach/T. Kühne (Switzerland)*, ([paul.imbach@unibas.ch](mailto:paul.imbach@unibas.ch))

Dr Imbach presented an update on the registries on ITP: In 1997 the motivation for ICIS was the many unresolved issues of ITP. Within 12 years ICIS developed 4 registries with a total of more than 6,000 patients with newly diagnosed ITP from over 70 centers worldwide. The experience with these registries over 12 years shows the high demand of good quality assurance; especially critical issues are: excellence of software, clear formulation of question sheets, verification of data. Data analyses are most critical and time consuming.

The pediatric and adult registry of chronic PARC ITP registry has now 1860 patients including 399 adults. The follow-up numbers of patients at 6-, 12-, 24-months are 1419, 1079, and 677, respectively. After 9 months of data verifications and statistical analyses the evaluation and reporting of results can be started.

New development: an Amendment concerning thrombopoietin agonists and comorbidities/adverse effects has been accepted by the local ethical committee and is now available for local centers.

ICIS have published 6 main articles and 2 supplements of 86 and 98 pages, respectively, from their ITP Expert meetings in Switzerland, all in peer reviewed journals providing new and

important information on the natural history and management of ITP. More adult ITP patients should be included into the registry!

08:45 - 08:55 Discussion. Are there specific questions of the Platelet Immunology SSC which could be answered by the PARC study.

The discussion was focused around the findings of the registry data and less on the development of implementation of the registry itself. The investigators are hoping to recruit more adults to the registry targeting n=1000 (currently at about 400 adults).

One notable finding from the registry data was that there was a decreasing trend for splenectomy and that there was not much difference between children and adults with respect to certain clinical outcomes (?).

08:55 - 09:10 (incl 5 min discussion) [Platelet antibodies in well-defined populations of patients with ITP \(D. Arnold, Hamilton Canada\)](#)

Dr Arnold, McMaster University, Hamilton, Canada reported on the prevalence of anti-platelet antibodies in patients with clinically well characterized ITP. They determined the frequency of GPIIb/IIIa and GPIb/IX autoantibodies in non-splenectomized patients with acute ITP (n=36) and patients with chronic refractory ITP (n=25) using a glycoprotein specific assay (antigen capture). They found detectable anti-IIb/IIIa or Ib/IX antibodies in 18 of 36 (50%) of patients with acute ITP and in 20 of 25 (80%) patients with chronic ITP. Treatment with either intensive immunosuppression or TPO agonists had little effect on platelet antibodies in chronic ITP. This indicates that the sensitivity of anti-platelet antibody testing may be higher in patients with chronic compared with acute ITP, and that platelet autoantibodies do not explain the mechanism of thrombocytopenia in all patients.

Discussion:

Open issues

1. Does the presence of platelet-reactive Abs has any prediction for the clinical course of the patient or the likelihood of response to treatment?
2. Do the OD levels reactivity relate to the platelet count?

Although direct assays (antibodies on the platelets) have the highest yield and should be the focus of continued investigations, investigators should continue to investigate patient plasma.

Although direct assays (antibodies on the platelets) have the highest yield and should be the focus of continued investigations, investigators should continue to investigate patient plasma.

Other aspects of platelet antibodies should be examined also including antibody subclass, variability in Fc portions, and other platelet antibodies including IALLA.

09:10 - 09:25 (incl 5 min discussion) are GPIIb/IIIa and GPIb/IX glykoproteins sufficient for platelet autoantibody testing? (Tamal Bakchoul, Giessen, Germany)

Dr Bakchoul reported a retrospective study on referred sera of patients with suspected ITP. They found that 21% had IIb/IIIa antibodies; 11% had Ib/IX antibodies, and 10% had GPV antibodies. In 10% of positive samples, GPV was the only detectable antibody.

Discussion:

GPV may not be stable in stored samples as it has been shown to be cleaved by enzymes; thus storage time and conditions of the sample before testing may influence the results of anti-GPV testing. The correlation between anti-GPV antibodies and severity of disease has not been explored. Testing methods for anti-GPV must take into account the similarities with GPIb; inhibitions assays with GPIb (in addition to GPV as shown) should be considered.

09:25 - 09:35 BREAK

09:35 - 10:05 Discussion for a Statement of the Platelet Immunology SSC on the role of platelet antibody testing in the time of new therapeutic options for ITP:

Statement for the SSC chair: V. Kiefel (5 min)

On behalf of Volker Kiefel, Andreas Greinacher presented a discussion basis for the platelet immunology SSC on the role of platelet antibody testing in ITP. The current assays for platelet antibody testing either measure platelet associated IgG (PAIgG) or glycoprotein specific antibodies. The PAIgG assays are non-informative assays with low specificity and low sensitivity. One possibility to improve specificity is to prepare an eluate of a patient's platelets by a low pH buffer and to test the eluate in a whole platelet assay. Otherwise platelet glycoprotein specific assays should be applied.

- ❖ Probability of autoantibody detection:  
acute AITP < chronic AITP < Evan's syndrome
- ❖ Probability of positive result in autoantibody analysis in patients with chronic AITP
  - 50-70 % "GP-PAIgG"
  - 10-30 % "free autoantibodies (GPs IIb/IIIa, Ib/IX, V, no alloab-pattern)"
- ❖ Autoantibody testing usually not required
  - patients with acute, probable "post-infectious" AITP, esp. in childhood
  - patients with uncomplicated AITP responsive to corticosteroids, IVIG, ...
- ❖ Autoantibody testing useful or mandatory
  - patients with comorbidities which can cause TP, e.g. hematologic malignancies
  - patients with refractory TP especially before institution of invasive (splenectomy), expensive/experimental therapy

- ❖ Recommendation
  - always try testing of autologous platelets in addition to serum or plasma at least in adult patients
  - provide testing report with appropriate comment/interpretation in case of negative/positive result
- ❖ Important differential diagnoses
  - drug-induced TP (DITP)
  - post transfusion purpura (PTP)
  - thrombotic thrombocytopenic purpura (TTP)
  - hereditary TPs

General discussion: Is there a role for platelet autoantibody testing in patients with ITP?

There is renewed interest in platelet antibody testing in light of TPO agonists to understand mechanisms in ITP and also to ensure that treatment with TPO agonists (and other therapies) could be dangerous in patients who are misdiagnosed as ITP (ie hereditary TTP).

A general recommendation of antibody testing for diagnosis of ITP was considered problematic by the clinical hematologists in the discussion, as the diagnosis can not be based on antibody tests only given the low sensitivity. There was general agreement that platelet antibody testing has a role in patients with comorbidities which can also cause thrombocytopenia.

Differential diagnosis of TCP is so wide, that we need clear definitions to deal with the heterogeneity of the disease: First, we need reliable clinical criteria to define subsets of patients with ITP (primary vs secondary); and second, we need accurate diagnostic testing (platelet antibody) to define patient subgroups to predict response to treatment. There is also a need to define Ab+ and Ab- groups of ITP patients and compare their clinical courses.

Given that the frequency of antibodies in patients with acute disease is low, patients with a typical response to IVIg do not need platelet antibody testing done. However there is a need for (informative) platelet antibody testing when the diagnosis of ITP is uncertain.

There was agreement that prospective data are needed, whether the clinical outcomes and the outcomes after intervention in ITP patients differ according to the antibody status or antibody specificities.

A study was proposed in which all patients with clinical ITP should undergo lab testing for platelet antibodies. "Autoantibody-positive" and "autoantibody-negative" ITP should be defined just like there is "primary" and "secondary" ITP. These subpopulations should be assessed for and their clinical features and the response to treatment

Cecile Kaplan alerts the Platelet Immunology SSC that there is a workshop of the ISBT looking for standardization of lab test approaches for platelet antibody detection.

The Platelet immunology SSC will set up a working party to further specify how current assays for determination of platelet autoantibodies could be adopted to be applied in a multicenter observational study. Those who are interested to participate should contact the chair of the PI SSC ([greinach@uni-greifswald.de](mailto:greinach@uni-greifswald.de)).

PART 2. 10:05 - 10.45 Heparin-induced thrombocytopenia  
 Chairs: Y. Gruel (France), T. Warkentin (Canada)

How to improve the clinical use of antigen assays in HIT

10:05 - 10:20 Combining quantitative interpretation of PF4-dependent EIA's and high heparin  
 K. Althaus (Greifswald, Germany)

Two approaches to improve specificity of assays detecting PF4/heparin antibodies are currently discussed. One is to use a higher OD cut off, the other to use a confirmatory step with high heparin. The Greifswald laboratory systematically assessed 1,000 consecutive sera of patients with clinical suspicion of HIT in a PF4/heparin EIA and a washed platelet functional assay, the heparin-induced platelet activation test (HIPA).

Results

-heparin/-PF4-EIA	OD <0.5 (negative) n=662	OD 0.5-1.0 (weak positive) n=218		OD >1.0 (strong positive) n=120	
	Inhibition of OD with 100 IU/ml heparin				
	not done	<40% n=157	>40% n=61	<40% n=27	>40% n=93
HIPA	0.15% (positive) n=1	1% (positive) n=7	16% (positive) n=11	40% (positive) n=11	62% (positive) n=58

Althaus K et al. Presented at the ISTH Platelet Immunology SSC July 11, 2009

Results

	classified positive	sensitivity for platelet activating antibodies	specificity for platelet activating antibodies	PPV for platelet activating antibodies
n=1000	IgG ELISA >0.5	98%	72%	24%
	IgG ELISA > 1.0	84% ↓	94% ↑↑	58% ↑↑
	IgG ELISA > 0.5 and inhibited >40% by high heparin	84% ↓	91% ↑	45% ↑
	ELISA OD 0.5-1.0 and inhibited >40% by high heparin + all OD >1.0	98% ↔	89% ↑	44% ↑

Althaus K et al. Presented at the ISTH Platelet Immunology SSC July 11, 2009

- 10:20 - 10:35 Towards genetics in HIT Yves Gruel (Tours, France)

Yves Gruel presented data on SNPs on genes coding for proteins that have been previously identified to be involved in auto-immune diseases and in platelet activation. Three groups of patients were studied, one with subjects treated by heparin and without antibodies to PF4 (Ab<sup>neg</sup>), one including patients with antibodies to PF4 but without HIT (Ab<sup>pos</sup>), and the third group with definite HIT patients.

Immune genes: No significant difference was found for CTLA-4 -318 C/T, + 49 A/G and PTPN22 1858 C/T. The TNFA A allele was less frequent in HIT patients (7%) compared to other groups of patients (15.8 and 16.7%, p=0.007).

Effector protein genes: P2Y1 and P2Y12 SNPs: no differences. G-protein  $\alpha$  subunit *GNB3* (825 C/T) T allele was found to be associated with higher risk of HIT.

10:35 - 10:45 Last Minute Contributions

Andreas Greinacher and Ted Warkentin presented preliminary data showing that a major issue for standardization of antibody testing are the different values obtained by different photometers. It might be very problematic to use general cut offs for EIAs who are performed in different laboratories. This situation reflects somewhat the problem of standardization of the prothrombin time.

PART 3. Educational 11:00 - 12.00 *Chairs: Y. Gruel (France), T. Warkentin (Canada)*

11:00 - 11:30 Laboratory diagnosis of antibodies in Heparin-induced thrombocytopenia *T. Warkentin (Canada)*

11:30 - 12:00 Drug dependent thrombocytopenias *R. Aster (USA)*



## Platelet Physiology

12 July 2009  
Boston, MA, USA

Chairman: *Marco Cattaneo, Italy*

Co-chairs: *Joel S Bennett, USA; Paul Harrison, UK; Catherine P M Hayward, Canada; Dermot Kenny, Rep of Ireland; Alan D Michelson, USA; Diane Nugent, USA; Steve Watson, UK*

The first part of the session was dedicated to the discussion of the Experts' Consensus of the Working Party on Light Transmission Aggregometry.

Due to the lack of clear scientific, published evidence on how Light Transmission Aggregometry should be performed, the consensus among experts was reached using the RAND method.

The RAND method – developed in the '80s – is intended to obtain a formal consensus among expert groups about the appropriateness of health care interventions, particularly when scientific evidence is absent, scarce and/or heterogeneous (*Brook RH, Chassin MR, Fink A, et al. A method for the detailed assessment of the appropriateness of medical technologies. Int J Technol Assess Health Care 1986;2:53-63*). Technically, for each clinical scenario (usually defined as patient population, treatment, comparison, one or more relevant outcome) a form is prepared, where each member of a Working Group scores appropriateness from 1 (completely inappropriate) to 9 (fully appropriate). Ballots are blinded to the other members. The two extreme scores (highest and lowest of the experts) are discarded, and the area containing the majority of the ballots defines the classification of the intervention (inappropriate, uncertain, appropriate).

Eleven experts accepted to participate in the Consensus, chosen among the current co-chairs of the SSC subcommittee on Platelet Physiology, the co-chairs of the SSC subcommittee on Platelet Physiology in the years 2005-2007 (when the the decision was taken to form a Working Party on Light Transmission Aggregometry), and scientists with experience on LTA standardization in other organizations.

The first run of RAND methodology was organized in February 2008. The second and third runs, in which forms modified according to the results/comments of previous runs were used, were organized in April-June 2008. The preliminary results of the Consensus were presented and discussed during the meeting of SSC Subcommittee on Platelet Physiology in Vienna, July 2008. The Consensus was refined, based on the comments and suggestions of the audience, and a final, fourth run of RAND methodology was organized in February 2009.

### **Statements of the Consensus have been grouped in 8 categories:**

- Indications for LTA
- Pre-analytical variables (subjects)
- Pre-analytical variables (blood collection)
- Pre-analytical variables (preparation of PRP and PPP)
- Assessing the quality of the PRP sample
- Methodology
- Platelet agonists for LTA
- Evaluating and reporting LTA results

The recommendations of the panel of experts for each category were:

- Indications for LTA
  - LTA is clinically useful for the study of subjects with bleeding disorders
  - LTA should NOT be used for the identification of subjects at risk for thrombosis
  - LTA should NOT be used to monitor subjects on anti-platelet therapy
- Pre-analytical variables (subjects)
  - 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
- Pre-analytical variables (blood collection)
  - 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
- Pre-analytical variables (preparation of PRP and PPP)
  - Blood samples should be allowed to “rest” at room temperature for 15 min before centrifugation
  - Platelet-rich-plasma 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 patients with very large platelets (it is uncertain whether it is advisable to keep the tubes at 45°)
  - Platelet-poor-plasma for LTA 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
- Assessing the quality of the PRP sample
  - Grossly hemolyzed samples should be discarded
  - If the blood 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  $150 \times 10^9/L$ , 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  $> 600 \times 10^9/L$ )
- Methodology
  - 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
- Platelet agonists for LTA. The following platelet agonists should be :
  - **ADP:** 2  $\mu M$  (higher concentrations if abnormal results with 2  $\mu M$ )
  - **Epinephrine:** 5  $\mu M$  (higher concentrations if abnormal results with 5  $\mu M$ )
  - **Collagen:** 2  $\mu g/mL$  (Horm collagen) (higher concentrations if abnormal results with 2  $\mu g/mL$ )
  - **Thrombin Receptor Activating Peptide (TRAP):** 10  $\mu M$  (higher concentrations if abnormal results with 10  $\mu M$ )
  - **The thromboxane A2 mimetic U46619:** 1  $\mu M$  (higher concentrations if abnormal results with 1  $\mu M$ )
  - **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**.
- Evaluating and reporting LTA results
  - 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.

In the second part of the session, the formation of a Working Party on the Diagnosis of Inherited Platelet Function Disorders was proposed. The Working Party will address the following issues:

1. What patients should be screened for platelet function disorders?
  - Type of bleeding manifestations
  - 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 be used?
  - 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

Guidelines for platelet function testing are being prepared also by the British Committee for Standards in Haematology: Paul Harrison (Oxford, UK) gave a progress report on this.

Finally, during the Educational Session, Alan D. Michelson (Boston, MA) reviewed platelet function tests as a guide to thrombotic risk, and Catherine P.M. Hayward (Hamilton, ONT) reported on recent advances in the understanding and diagnosis of inherited platelet function disorders.

## Predictive Variables in Cardiovascular Disease

11 July 2009  
Boston, MA, USA

Chairman: *Gordon D.O. Lowe, UK*

Co-chairs: *James Douketis, Canada; Veikko Salomaa, Finland; Alberto Tosetto, Italy*

Educational Session – Predictive hemostatic variables: a user's guide to applying clinical/laboratory predictors of venous and arterial thromboembolism in everyday clinical practice

M. Woodward (US) reviewed the stages in assessment of new predictors of arterial thrombotic events (coronary heart disease and stroke). Using a recent study of plasma fibrinogen in a large population cohort in Scotland as an example, he explored its associations with thrombotic events and its ability to add to currently-used risk factor scores such as the Framingham score. M. Rodger (CA) reviewed the levels of evidence for decision rules; and the development of clinical-laboratory decision rules for recurrent venous thromboembolism. He described development of a rule including gender, post-thrombotic leg signs, oestrogen therapy and plasma D-dimer level; which was currently being assessed in a large validation study. H. Bounameaux (CH) reviewed published clinical-laboratory prediction rules for confirmation/exclusion of clinically-suspected acute venous thromboembolism, including results from a recent systematic review.

Business Session

Predictors of recurrent venous thromboembolism

T. Baglin (UK) presented a preliminary analysis of an ongoing collaborative study of recurrent venous thromboembolism in persons with severe thrombophilias (deficiencies of antithrombin, protein C or protein S; homozygous Factor V Leiden; or the combination of Factor V Leiden and the prothrombin G20120A mutation). The highest risk of recurrence was observed in antithrombin deficiency; however final results of this ongoing study will be presented at the 2010 subcommittee meeting. F. Rosendaal (NL) reviewed clinical risk factors for recurrence, including current studies exploring possible explanations for the lower risk of recurrence in women: this did not appear to be explained by the low recurrence rate in women with previous events while on estrogen therapies. He presented a preliminary analysis of an ongoing collaborative study of the association of Factor IX Malmo with recurrence: the final results will be presented at the 2010 subcommittee meeting. A. Tosetto (IT) presented results of a collaborative individual patient – based meta-analysis of the association of plasma D-dimer with recurrence; which suggested that D-dimer merited consideration in prediction of recurrence, together with clinical risk factors. J. Douketis (CA) briefly reviewed ongoing subcommittee collaborations on clinical risk predictors for recurrence, some of which would be presented as communications during the ISTH 2009 Annual Meeting.

Predictors of arterial thromboembolism

G. Lowe (UK) informed the meeting that the subcommittee report on associations of hemostatic variables with risk of first arterial thrombotic events had been submitted to SSC following a second round of expert reviews; following review by SSC a final report was in preparation for the SSC website as

well as submission for publication. He asked the meeting for expressions of interest in developing a further report on hemostatic variables in prediction of recurrent arterial thrombotic events.



## Vascular Biology

11 July 2009  
Boston, MA, USA

Chairman: *Jean-Marie Freyssinet, France*

Co-chairs: *Michael C Berndt, Rep of Ireland; Françoise Dignat-George, France; John H Griffin, USA; Peter J. Newman, USA*

As for the previous two SSC meetings, the session was divided into three parts, addressing key issues in vascular biology and related (athero)-thrombotic disorders. Again, none of the three topics is specific of the scope of ISTH, which emphasizes the efforts to be made by the SSC to maintain ISTH leadership in the field.

**Shed proteins/receptors as clinical biomarkers of vascular disease** was the first topic, presented as an **Educational Session** moderated by P. Newman. There is growing realization that certain cell surface receptors are shed from the surface of blood and vascular cells as a result of their activation - perhaps as a mechanism to dampen the adhesive properties of the cell. A second consequence of platelet activation is the secretion of the contents of specialized granules, some of which have the potential to support endothelial cell growth, stem cell differentiation, and tumorigenesis. This 90 minutes session has highlighted recent progress in the identification and characterization of the so-called platelet sheddome and platelet secretome, with an emphasis on how such fluid-phase proteins can both affect vascular physiology and report vascular disease.

The platelet sheddome can be explored using a proteomics shotgun approach identifying ~740 proteins including 120 membrane proteins, all involved in receptor/ligand interactions, or being secreted or proteolytically cleaved proteins. One obvious question is why platelets shed proteins? The answer is not unique as they can do it for several reasons, modulation of platelet-platelet interactions with end of "discussion" upon shedding, modulation of interactions with the vessel wall, production of bioactive fragments, or for no coherent reason, or for all of the above ones. Four examples generated by proteomics were developed, Semad4D, Semad7A, ESAM and JAM-A, showing that further functional studies can nominate them candidate markers of disease to be confirmed by ELISA determinations (L. Brass).

GPVI is shed as a 55kDa soluble fragment from the platelet membrane after having undergone an activation cycle initiated upon ligand binding, signalling, calmodulin dissociation and cleavage. Significant elevation of circulating soluble GPVI levels has been shown in autoimmune disorders such as idiopathic thrombotic thrombocytopenic purpura or heparin-induced thrombocytopenia, or cardiovascular pathologies such as acute coronary syndromes or

ischemic stroke. Since platelet activation occurs in all of these situations, soluble GPVI could therefore be considered a disease marker (M. Berndt).

Ectodomain shedding is also a feature of platelet ageing. In nucleated cells, ageing can lead to apoptosis for “clean” elimination of senescent cells by phagocytosis. Because platelets are non-nucleated cells, the issue is whether ageing results in activation or whether apoptosis can take place. Platelets possess apoptotic machinery, and platelet storage lesion is reminiscent of apoptosis. Platelet apoptosis promotes phosphatidylserine exposure and subsequent development of procoagulant activity, and also accounts for GPIIb/IIIa and GPVI release, through mechanisms different from activation by conventional agonists. Hence, the noxious procoagulant function could be counterbalanced by decreased adhesion and aggregation ability (S. Schoenwaelder).

**Circulating endothelial progenitor cells (EPC)** are viewed with regenerative potential, at least in cardiovascular disorders knowing they can also be mobilized in tumor development. There is however a lack of consensus on their definition.

Identification of EPC relies on two types of complementary approaches: culture assays detecting the ability of peripheral blood mononuclear cells to yield colonies with endothelial characteristics, and on flow cytometry methods. Culture assays distinguish CFU-EC and circulating angiogenic cells as “early appearing” cell subsets of myeloid origin, and “late endothelial colony forming cells” displaying functional properties of progenitor cells with *de novo* blood vessel formation capacity. Regarding flow cytometry, main issues are related to the scarcity of EPC and the absence of specific markers. General recommendations for rare event detection are proposed including, the use of fluorescence minus one controls, exclusion of monocytes and non-viable cells and the use of bi-exponential scales to help visualize events with negative features. Antigen combinations are proposed based on optimized cell sorting experiments: ECFC precursors are found among CD31(+), CD34(+), CD45(-), CD133(-)/CD146(+), CD105(+) populations whereas CD34(+)/CD133(+)/CD31(+) cells are enriched in circulating angiogenic cells of hematopoietic origin. Better understanding of EPC heterogeneity opens new insights into consensual identification criteria for clinical applications in cardiovascular disorders and cancer (M. Yoder).

Current definition for optimal protocol allowing specific isolation of ECFC from adult peripheral blood are still a matter of debate regarding parameters such as blood collection, matrix, or cellular seeding density. Commercially available cell culture device also provides opportunities for cell expansion in a clinical grade-approved culture condition (D. Smadja).

Regarding **microparticles** (MPs), new features were presented. Megakaryocyte MPs can be directly visualized by video microscopy and discriminated from platelet MPs according to specific antigen expression. Circulating MPs isolated from mice were CD62(-) and LAMP-1(-) and expressed full-length filamin A, indicating a megakaryocytic origin. In healthy volunteers, MPs are also CD62(-) and contained full-length filamin A. Cultured human megakaryocytes shed microparticles that are CD41(+), CD42b(+), and express surface phosphatidylserine. This indicates that direct production by megakaryocytes represents a physiologic means to generate circulating platelet MPs (J. Italiano). MPs generated from cultured human microvascular endothelial cell lines have been shown able to promote plasmin generation. This process involved expression of urokinase-type plasminogen activator (uPA) and its receptor (uPAR) at the surface of endothelial MPs and was further increased by their ability to bind exogenous uPA on uPAR. Hence, endothelial MPs act as vectors supporting efficient plasmin generation and dissemination, a new pathway in the regulation of endothelial proteolytic activities with potential involvement in inflammation, angiogenesis, and atherosclerosis (R. Lacroix).

Physical techniques can help to enumerate MPs and assess their size distribution. Dynamic light scattering (DLS) has been used to measure either purified MPs or diluted normal platelet free plasma (PFP) showing polydisperse MP distribution (up to 1000nm) but with a predominant population from < 50 nm to above 300 nm. Analysis of diluted PFP in PBS (1:40-1:160) suggests that the concentration of particles is  $\sim 200\text{-}260 \times 10^9/\text{L}$ , which is 1,000 fold greater than estimates by flow cytometry. However, the specificity of this technique, related to possible interference of non-membraneous lipid particles remains to be improved, especially when analyzing samples from hyperlipemic subjects. Procoagulant red cell-derived MPs (50 to >1000nm) within fresh frozen plasma (characterised by flow cytometry, ELISA and procoagulant assays) can also be detected and all signals > 200 nm are removed after filtration. DLS technology may therefore prove to be useful for rapidly measuring the entire size distributions and concentration of MPs within biological fluids (P. Harrison). Atomic force microscopy (AFM) offers another approach as shown by the detection of platelet-derived MPs and the definition of their size distribution. MPs from healthy individuals are immobilized on a modified mica surface coated with an antibody to CD41 prior to AFM imaging. AFM detected CD41(+)MPs ranging from 10-475 nm (mean  $67.5 \pm 26.5$  nm). Again, numbers of CD41(+) MPs were 1,000-fold higher than those measured by flow cytometry ( $32\text{-}702 \times 10^9/\text{L}$  plasma respectively  $11\text{-}291 \times 10^6/\text{L}$  plasma). Because these methodologies yield more objective values than flow cytometry, they could be precisely used to confirm whether MP variations reported by other methods (flow cytometry, functional capture assay, ELISA) appear relevant through appropriate correlations. In any case, it has to be emphasized that exosomes should not be considered and should therefore be eliminated from MP preparations (see the 2007 report of this Subcommittee).

A complete description of an experimental protocol is essential to allow interpretation and reproduction of results. Such a description includes information about sample origins and preparation, details about pre-analytical and analytical procedures, and data processing and analysis. To improve the reporting and presentation of flow cytometry methods and results, the International Society for Advancement of Cytometry (ISAC) has developed guidelines that were recently summarized as MIFlowCyt: Minimum Information About a Flow Cytometry Experiment (Cytometry 73A: 926-930, 2008). These guidelines apply to all types of flow cytometry measurements, but there are subsets that are especially critical for the analysis of cell-derived MPs and deserve particular attention (J. Nolan).

The confusing situation in the enumeration of MPs by flow cytometry (FCM) has led Françoise Dignat-George (France) and Nigel Key (USA) to propose a workshop on standardization by using calibrant microbeads that have been provided free of charge by the manufacturer, in agreement with the conflict-of-interest guidelines indicated by the ISTH Executive Director and SSC Chair. Calibrated microbeads have enabled to standardize instrument settings in the 40 participating laboratories, including those recruited in DIC and Malignancy Subcommittees, from 14 countries, representing 57 instruments! Overall, 66% of the cytometers were validated (stage A). At stage B, biological samples were distributed by the core laboratory (F. Dignat-George), after having solved shipment issues with respect to specific national security regulations. The first conclusions are, (i) it is possible to define FCM performance in terms of background and resolution using calibrated beads, regardless of the type of instrument (stage A), (ii) these calibrated beads represent a useful tool to standardize MP enumeration in a reproducible manner on flow cytometers from one manufacturer with a CV < 20% (stage B), (iii) other strategies may need to be validated for instruments from a second manufacturer. The next questions to be addressed are, (i) is it correct to focus on the top of the MP iceberg, (ii) how representative is this visible part of the MP iceberg of the clinically relevant biomarkers we are seeking? (iii) can new generation or types of flow cytometers be developed (or alternative technologies with similar immunological capabilities) that would allow enumeration and characterization of particles of smaller sizes? This workshop should lead to a SSC publication in a rather close future.

The **slides** detailing the main features of this workshop should be available soon for download from the ISTH website.

(<http://www.isth.org/LinkClick.aspx?fileticket=6afs2tNl0wo%3d&tabid=60>)

In summary, the Vascular Biology group has been in existence for 8 years now, first as a working party for 5 years, and as a full SSC for the past 3 years. Our attendance has never been better, and there is clearly a growing interest amongst our membership in the themes and programs that the Scientific Subcommittee on Vascular Biology undertakes.

## von Willebrand Factor

12 July 2009  
Boston, MA, USA

Chairman: *David Lillicrap, Canada*

Co-chairmen: *Thomas Abshire, USA; Imre Bodo, Hungary; Giancarlo Castaman, Italy; Jorge DiPaola, USA; Jeroen C.J. Eikenboom, The Netherlands; Emmanuel J Favalaro, Australia; Anne Goodeve, UK; Bernhard Lämmle, Switzerland; Reinhard Schneppenheim, Germany*

### **VWF Assays for VWD Diagnosis:**

Standardization of ristocetin-based VWF assays: Augusto Federici

- Still central assays for VWD diagnosis
- We need better functional VWF assays
- Critical role of VWF A1 domain-GPIb interaction
- Both platelet aggregometry and ELISA assays available – both have high CVs
- Can either use a fixed concentration of ristocetin (0.5-1.5 mg/ml) or calculate the ristocetin threshold that produces 30% platelet aggregation
- RIPA is still a key test for diagnosis of type 2B VWD

Automated VWF:RCo : Andreas Hillarp

- Discussion of automated VWF:RCo test protocol
- Minor modifications made to manufacturer's protocol (including VWF-free plasma as diluent) + establishment of low and high calibration curves
- These modifications improve the manual/automated derived results – very good accuracy and reproducibility
- Can be used for both plasma-based and concentrate VWF:RCo assays

Alternative functional assays – GPIb binding assays: Hans Deckmyn

- Using WT rGPIb, coat microtiter plate with mAb to GPIb alpha. Add VWF +ristocetin. Detect with labeled anti-VWF Ab.
- Now test is commercialized
- Chemoluminescent readout available
- Turbidometric method also developed – nanoparticles coated with rGPIb alpha and binding VWF in presence of ristocetin. This is the assay that has been further developed
- Turbidometric assay is linear and detection limit 4 u/dl. Within device CV ~6%
- Good results with VWD clinical samples (123 samples tested – mix of type 1 and type 2 variants). Very comparable to platelet VWF:RCo assay data

VWF-FVIII antibody specificities: Bob Montgomery

- Still issues of Ab specificity with some anti-FVIII Abs – some detecting VWF (old term VWF:RAG)

- Need some process to ensure antigen specificity
- All anti-FVIII Abs should be carefully evaluated for their specificity before use

## **VWF Propeptide Studies**

### Standardization and Clinical Utility

Sandra Haberichter

- Both VWF and VWFpp circulate in plasma. Circulation time variable in populations
- Used in various situations – endothelial perturbation, acquired VWD and subtypes of VWD
- Large normal population studies - >200 subjects
- Higher levels of plasma VWF:Ag and VWF:pp in African Americans
- Formal standardization of VWFpp concentration to an international standard is necessary

Bas De Laat

- Comparative studies of the Sanquin and GTI assays. Very comparable on normal plasma samples
- Need to pursue international standardization
- Multi-center participation – ISTH #3 standard as the test material – aiming for 15 labs
- Need to assign value to WHO 6<sup>th</sup> plasma standard
- Timeline to present data at the Cairo 2010
- Nomenclature - assignment of VWFpp as official propeptide title

## **Standardization of Bleeding Scores (joint session with Pediatric SSC)**

Adult scores – Francesco Rodeghiero

- Definition of the aims of a bleeding score tool
- Should the tool be disease-specific. Also issue of age-influences
- Development of validated tools during the past several years
- Attempt to have a unified tool for adults and children
- Should the survey be self-reported or should there be a formal interview by a health care professional?

Pediatric scores – Paula James

- Differences for children. Starting point for development - the original Vicenza survey.
- Pediatric scoring system - about 20 mins to complete. Questionnaire very similar the Vicenza document. Scores for each symptom -1 to +4

- Evaluation of new Pediatric Scoring Questionnaire (PBQ) in a) normal children (142 children – normal range: 1.5 to +2.5 thus normal <+2) . Most frequent symptoms - minor wounds, nose bleeds b) VWD diagnostic analysis (151 children) c) Known VWD or platelet function defect cases (123 children)
- Very good negative predictive value and receiver operator curve
- All symptoms (apart from seasonal nose bleeds) were discriminating
- The PBQ is the first validated pediatric bleeding tool

### **VWF Plasma Standard**

Tony Hubbard

- WHO 5<sup>th</sup> IS plasma standard established in 2003. Will run out shortly
- Urgent requirement for new 6<sup>th</sup> WHO plasma standard
- >50,000 vials available of new 6<sup>th</sup> standard
- 44 labs participated in standardization – 4 sets of testing performed
- VWF:Ag, VWF:RCo, FVIII:C, VWF:CB assayed – against 5<sup>th</sup> WHO standard and local plasma pools
- Assignment of VWF:Ag value against 5<sup>th</sup> WHO standard of 1 IU/mL
- VWF:RCo 0.87 IU/mL against 5<sup>th</sup> WHO standard. Major variation using local pools
- Collagen binding against 5<sup>th</sup> WHO – 1.03 IU/mL/ampoule
- Establishment of 6<sup>th</sup> VWF plasma standard by October 2009
- 2<sup>nd</sup> IS VWF concentrate standard – aiming for fall 2010

### **ADAMTS13**

ADAMTS13 assays: Koichi Kokame

- Historical review of ADAMTS13 assays
- Full-length and small molecule substrates. Antigen and functional assays
- International review of ADAMTS13 assays by Tripodi et al (JTH 2008) - compared to prior study published in 2004. All methods were much improved compared to 2004
- Proposal for a standard ADAMTS13 material
- Discussion of mechanistic aspects of ADAMTS13 cleavage of VWF

Fast screening method for ADAMTS13: Bas De Laat

- Time for current assays – 2 to 4 hrs. Done in central reference laboratories
- More convenient system required. ? possibility of a fast screening method
- Development of an ADAMTS13 antigen dip stick
- Development of an ADAMTS13 Ab dip stick
- Testing of normal population and TTP samples for sensitivity and specificity
- Potential for using this assay in the non-academic settings

## VWF Registry and Nomenclature

VWF Registry and Nomenclature: Anne Goodeve

- VWF Mutation and polymorphism database. Hosted at University of Sheffield
- Website updated recently – June 2009
- 523 VWF mutations
- 216 VWF polymorphisms (assignment of rs #s)
- Sequence variations identified by difference to VWF RefSeq
- Manuscript describing VWFdb in preparation
- Nomenclature – change to HGVS system
- Nomenclature manuscript will be submitted to JTH shortly

Type 2B/PT-VWD: Maha Othman

- Initiated in 2007 – web-based database – on ISTH website
- 18 families - 44 cases of GPIIb alpha mutations: 3 missense changes and 1 deletion
- Prospective international study of potential PT-VWD cases – 86 cases referred
- Results of study presented - some cases have neither VWF A1 domain nor GPIIb alpha mutations
- Presentation of results of a recent international survey on PT-VWD diagnosis

## Women's Health in Thrombosis & Haemostasis

11 July 2009  
Boston, MA, USA

Chairman: *Andra H James, USA*

Co-chairs: *Margareta Blomback, Sweden; Benjamin Brenner, Israel; Jacqueline Conard, France; Sabine Eichinger, Austria; Barbara A Konkle, USA; Claire McLintock, McLintock; Claire S Philipp, USA*

### **Minutes: Women's Health in Thrombosis & Haemostasis Scientific Subcommittee (SSC)**

**Chair** – Andra H. James, MD

**Co-Chairs:**

- Magareta Blomback - present
- Benjamin Brenner - present
- Jacqueline Conard - present
- Sabine Eichinger - absent
- Barbara Konkle - present
- Claire Phillip - present
- Claire McLintock - absent

**Attendance** – approximately 200

Dr. James gave an introduction and overview

**Education:**

Dr. Brenner reported on the 3<sup>rd</sup> successful Women's Health Issues in Thrombosis and Haemostasis symposium held February 6-8, 2009, in Prague and announced the next symposium will be held in Berlin February 2011.

**Publications:**

Dr. James reported on the publication of a subcommittee inspired consensus report on, "VWD and Other Bleeding Disorders in Women," which was published this month in the *American Journal of Obstetrics and Gynecology*.

**Registries:**

- Dr. James reported on the Pregnancy outcome in women with mechanical heart valves registry. No new patients have been added this year.
- Dr. Kadir reported on the Mirena IUD for menorrhagia in women with bleeding disorders registry.
- Dr. Hoffman reported on the Corpus luteum bleeding in women with bleeding disorders registry. Necessary forms will be added to the website.

### **Other New and Proposed Projects:**

- Dr. McLeod – Toronto - presented the protocol for the HEPRIN trial, a randomized trial of anticoagulation (low-dose heparin) in women with poor placentation. Members were invited to participate.
- Ms. Lester – Ottawa – presented the protocol for the TIPPS trial, a randomized trial of low-molecular-weight heparin for women with thrombophilia and a history of poor pregnancy outcome. Members were invited to participate.
- Dr. Blomback presented preliminary results from a study of laboratory analyses in habitual abortion.

### **Educational Program:**

#### **Attendance: Between 400 and 500**

Our educational program consisted of a presentation by Dr. Phillip based on her work on identifying women with menorrhagia for hemostatic evaluation, followed by a debate between Dr. Brenner and Dr Marc Rodger on anticoagulation during pregnancy to prevent pregnancy complications for inherited thrombophilia.

## Working Group on Coagulation Standards

12 July 2009  
Boston, MA

Chairman: *Anthony Hubbard, NIBSC, Potters Bar UK.*

Review of Lot #3 (A Hubbard)

### *Decisions 2008/2009*

The intended uses, conditions of despatch and Working Group decisions over 2008/9 for Lot #3 were summarised: - the assignment of an additional analyte to Lot #3 (tPA antigen 3.0 ng/ml) was approved by the Executive Board in September 2008 and this will be presented for formal endorsement at the SSC Business meeting; - the use of Lot #3 for “trouble-shooting” was reviewed after 1 year and approved for continued use by the SSC Executive in September 2008; - the handling charge for Lot #3 was increased from £2.80 (GBP) per vial to £3.60 per vial from February 2009 in response to significant changes in the dollar/sterling exchange rate.

### *Stability of Lot #3*

The results of the accelerated degradation study for Lot #3 were reviewed. The four analytes tested were found to be extremely stable with predicted losses below 0.1 % per year at -20 °C. The results support a shelf life in excess of the expiry date of 2014. Final stability testing will be carried out in 2010 together with real-time comparisons of vials stored at -20, -70 and -150 °C.

### *Despatch of Lot #3*

Between July 2008 - June 2009 a total of 9,170 vials were despatched in 30 orders to 13 manufacturers. The total despatch for Lot #3 over the last 3 years is 30,400 leaving a remaining stock of 21,500. Based on the average despatch the stocks of Lot #3 will be exhausted in 2011. Action point: review list of orders against EQA schemes list of calbrant plasma manufacturers to check for uptake of Lot #3.

### *Towards Lot #4*

Based on the stability of previous Lots and the predicted despatch rate for Lot #3 it was agreed that the optimum size for Lot #4 would be 100,000 vials – this would also reduce the frequency of replacement and provide longer term continuity for users. The tendering process for Lot #4 has been completed. Invitations to tender were despatched to 15 manufacturers and two bids were received. The Executive Board agreed on the choice of manufacturer and the purchase order was submitted in June 2009 with delivery by September/October 2009. Calibration of Lot #4 will be coordinated through NIBSC and will be performed either by inclusion in collaborative studies related to replacement WHO International Standards or in dedicated studies involving Lot #3, Lot #4 and the relevant WHO International Standards. It is expected that calibration of Lot #4 will be completed in time for endorsement at the SSC Business Meeting in July 2011. A handling charge of \$5.00 per vial has been proposed for Lot #4.

### *Experience of EQA schemes – UK NEQAS (Ian Jennings)*

Between July 2008 and July 2009 UK NEQAS supplied Lot #3 to 6 centres for trouble-shooting purposes related to the assay of Protein C activity (2), Free Protein S antigen, VWF:antigen, Factor VIII:C and Fibrinogen. All of the centres successfully resolved their local problems as indicated by accurate results in the following NEQAS surveys. In one centre the discrepant results for VWF:Ag were traced to mis-

calibration of a particular lot of commercial reference. The manufacturers have since withdrawn this product lot. The inclusion of Lot #3 in EQA surveys and in the resolution of assay discrepancies provides an additional route to influence the harmonisation of commercial reference plasmas.

Experience of EQA schemes – College of American Pathologists (Jun Teruya)

Lot #3 has been included in two surveys during 2009 – the VWF survey (CGS3-A) and the Thrombophilia survey (CGS2-A). Data were analysed by “method specific” statistics. Most results were consistent with a bias away from the assigned value of less than 10% consistent with good harmonisation between manufacturers, however, some discrepancies were identified where the bias exceeded 10%, eg. 11% for Factor VIII:C using one instrument/reagent combination, 20% for Protein C activity by one clotting assay, 12% by two methods for Protein S activity and 15% by one method for free Protein S antigen.

