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Animal Models

Chair: H. Weiler (USA)
Co-Chairs: Shaun Coughlin (USA), Jay L. Degen (USA), Cornelis Kluft (Netherlands), Nigel Mackman (USA), Tim Nichols (USA), Susan Smyth (USA)

The focus of the session was to give attendants a review of emerging technology and animal models relevant to thrombosis and hemostasis. The session addressed three themes:

Session 1. Genetic manipulation of hemostasis in mice

**Dr. Degen** reviewed available mouse strains with altered function of key hemostatic factors, thrombin and fibrinogen. In addition to loss-of-function models knockouts for prothrombin and fibrinogen, several mutations have been introduced into the fibrinogen gene cluster to abolish specific interactions of fibrinogen with integrins that are relevant in inflammation and infection. Novel models included mice with selective expression of Aalpha-chain isoforms (“long” and “short”), and a “non-clottable” form of fibrinogen that lacks the thrombin cleavage sites for fibrinopeptide removal. This model will be instrumental in dissociating functions of fibrinogen, as compared to fibrin. A novel approach was presented to delete the prothrombin gene in a temporally and spatially regulated manner. This model allows an investigator to temporally induce a state of almost complete prothrombin deficiency in adult mice.

**Dr. Conway** provided an excellent overview of approaches to alter gene functions in a cell type-restricted manner in endothelial cells. He introduced experimental concepts that will allow not only endothelial cell-specific manipulation of gene expression, but in addition target the endothelium of specific organs, in particular the brain. An important aspect of the presentation was to emphasize that several approaches to achieve endothelial cell-selective gene expression/inactivation also affect bone marrow-derived cells.

**Dr. Coughlin** focused on the role of protease activated receptors in platelet function, i.e. Par4, and gave a comprehensive summary of available data validating Par4 function in platelet-driven hemostasis and thrombosis in mice, as compared to humans.

**Dr. Mackman** reviewed existing data from the analysis of mice expressing lower or higher amounts of TF in different organs. Findings emphasize the concept of organ-selective functions of tissue factor, and of an organ-selective balance of hemostasis.

**Dr. Isermann** addressed experimental approaches and use of animal models to study the role of altered hemostasis in chronic, as opposed to acute models of disease. This concept was illustrated in the paradigms of atherosclerosis and of the role of the protein C pathway in diabetes. This presentation stressed the value of animal models that introduce—as compared knockout models—more subtle alterations in the hemostatic balance that more closely mimic the situation in human populations.

Session 2. Coagulation – inflammation axis
Dr. Ploplis reviewed published data from a set of transgenic mice expressing various levels of protein C. These animals, as opposed to complete knockouts for protein C, are viable but exhibit numerous derangements causing spontaneous thrombosis and inflammation, and cause pregnancy failure.

Dr. Lupu gave an overview over models of septic inflammation and DIC in Baboons. These models have been exploited to examine the function of the protein C pathway in inflammation. An important outcome of these studies is the recognition that inflammatory disorders such as sepsis with or without DIC comprise several different pathogenic mechanism in different stages of disease. In contrast to mouse models, Baboons can be analyzed with reagents/methods developed for, and applicable to humans, because of evolutionary conservation of proteins and pathways.

Dr. Nichols gave an overview over the use of several pig breeds in research relevant to atherosclerosis, and an update on technological aspects of large vessel function analysis in these animal models.

Session 3. Analytical tools in rodents

Dr. Ruf presented data from experiments in mice using pharmacologic reagents to manipulate coagulation in settings of inflammation (sepsis/LPS); and dissect the role of coagulation and coagulation receptors in hemostasis as compared to cell signaling. An important outcome of these studies in mice was that coagulation activation appears to make only a minor contribution to the inflammatory derangements in mouse models of inflammation triggered by LPS.

Dr. Poncz reviewed strategies to manipulate and target gene expression in murine platelets. Using the paradigm of fVIII gene delivery to platelets and correct hemophilia, analytical methods were discussed to monitor functional outcomes of gene manipulation, such as in vivo imaging of platelet thrombus formation and measurement of bleeding times.

Dr. Smyth could not attend. The theme of her presentation was scheduled to be the use of genetic analysis to delineate novel traits relevant to thrombosis and hemostasis. This topic will be the lead theme for the coming sessions of the Animal model SSC.

The session was extremely well attended. The Chairs / Cochair came to the consensus that similar sessions with an educational focus on technology will be valuable in future sessions during regular ISTH meeting years.
Biorheology

Chair: J.W.M. Heemskerk (The Netherlands)
Co-Chairs: T. Diacovo (USA), E. Grabowski (USA), M. Hoylaerts (Belgium), M. King (USA), G. Nash (UK), J. Zwaginga (The Netherlands)

The Biorheology session was an intensive meeting with 13 presentations divided into 4 parts, corresponding to two working parties and two special projects. The session was well attended by about 190 participants throughout.

Introduction
Chairman Dr. J. Heemskerk presented a short overview of the activities of the Subcommittee on Biorheology in the past year. He continued with an overview of the working parties and special projects of the subcommittee, as also reflected in the program of this year’s session.

WP1: Practical biophysics of cellular bond characteristics mediated by flow (chaired by T. Diacovo and M. King)
Dr. T. Diacovo reiterated the mathematical basis to describe bond characteristics: from on-off rates and affinity state description, equilibrium kinetics to modern (Bell) modeling of dynamic disruption of chemical bonds by mechanical (shear) force. By the use of a glycoprotein Ib (GPIb)-coated microspheres in interaction with wildtype and von Willebrand disease (VWD) type IIb peptides of VWF A1 domain, Dr. Diacovo presented experimental data that validated the new mathematical modeling system. Mutant VWF A1 domains were engineered on the basis of expected changes in their binding to GPIb. Adhesion changes could be shown in the microsphere system, and be quantified as specific changes in bond life time and Kd using this modeling approach. The in vivo relevance of the modified A1 domains was furthermore shown in a mouse knock-in models. Dr. M. King subsequently showed advances in mathematical modeling of the platelet tethering to VWF and the platelet aggregate formation. Using the Bell model, determinants like shear, platelet and glycocalyx dimensions and GPIb density, he presented algorithms that describe dynamic interactions in 3D of multiple platelets with the vessel wall and with each other. These algorithms are experimentally validated for platelets at lower shear rates, but in the future additional complexity will be added, e.g. shear induced platelet activation, signal transduction, and affinity changes of surface receptors.

WP2: Flow-determined modulation of molecular processes in thrombosis and haemostasis (chaired by J. Zwaginga and K. Sakariassen)
Dr. A. Bonnefoy demonstrated novel results and techniques that in addition of the A3 domain also the A1 domain of VWF is involved in the binding of VWF to collagen. This binding is inhibited by heparin, suggesting that anticoagulation with heparin can also interfere in platelet-VWF-collagen interaction. This recognition is of immediate importance for the attempt in WP2 to further characterize patients with aberrant VWF, being recognized as VWD patients, using flow-mediated assays. The penetrance of flow-incorporating assays in the clinical routine, however, is limited. Dr. J. Eikenboom gave an overview of the current way of classification of VWD patient, such as recently agreed by the SSC on VWF. In most types of VWD (type 1, types 2ABNM, and type 3), patients display a variable extent of bleeding. In addition to the antigen level of VWF, also qualitative abnormalities of VWF are instrumental to the diagnosis. Dr.
Eikenboom concluded that assays which incorporate flow conditions might well increase diagnostic sensitivity in regard of clinical symptoms. Dr. J. Zwaginga followed up on this subject and reported on a currently reviewed position paper of the Subcommittee on Biorheology, stating which and how flow assays could best contribute to further explaining remarkable phenotypes of VWD patients. Although cone-and-plate analyzers are commercially available, perfusion chamber-based assays because of their extensive validation in VWD variants, are regarded as most promising in this respect. Several modifications in these assays, however, should be dealt with before clinical validation and prospective multi centre trials can be set up. For the latter, collaboration with the SSC Subcommittee on VWF is indispensable and will be investigated in the coming year.

**SP1: Standardization thrombogenic surfaces in flow: potential of synthetic surfaces (chaired by E. Grabowski and J. Heemskerk)**

On behalf of Dr. R. Farndale, Dr. N. Pugh presented the methodologies – type of flow chamber, staining procedure and confocal microscopic imaging – used to measure thrombus formation in vitro under flow conditions. Novel triple helical collagen peptides, designed for binding to glycoprotein VI (GPVI), integrin alpha2beta1 and VWF, are synthesized by this group and used as an adhesive surface for aggregating platelets. Dr. Pugh showed that the presence of all three motifs is required for optimal thrombus formation at high shear rates. He discussed the various ways of quantification of this process, and the importance of a standardized coating procedure for reproducible results. Dr. K. Sakariassen gave an overview of results from ex vivo perfusion studies, where non-anticoagulated human blood is flowed over human type III collagen. He stressed the importance of quality control of the collagen preparation and also of the way of application of collagen on the surface (e.g., spraying). He underlined the importance of thrombus volume as an endpoint parameter, and he pointed to the phenomenon of axial dependence of thrombus formation. The reproducibility of thrombus formation over a 10 year period appeared acceptable, particularly in studies where only non-smoking healthy individuals were included as blood donors. As the last speaker in this session, Dr. J. Cosemans compared the process of flow-induced thrombus formation on various types of adhesive surfaces. While the thrombotic process on plaques was found to be variable from one plaque to another, it was more standard on purified collagen type I preparations. She presented evidence that the degree of processing of plaque-derived collagen by matrix metalloproteinases contributes to the variable results obtained with plaques. On the other hand, synthetic triple-helical peptides with collagen-based motifs for adhesion to alpha2beta1 or GPVI, alone or in combination with VWF gave highly promising results in achieving reproducible thrombus formation under flow. These results are promising for the generation of artificial adhesive surfaces to check for the activity of specific adhesive platelet receptors.

**SP2: Models of thrombus formation in vitro and in vivo (chaired by M. Hoylaerts and G. Nash)**

Dr. S. Jackson gave an overview and showed examples of the advantages and pitfalls of in vitro and in vivo models to study thrombus formation. He demonstrated the great potency of microcapillary flow chambers in combination with high-resolution confocal microscopy and other recently developed multi-imaging systems. He showed how total internal reflection fluorescence microscopy can be used to get detailed insight into the adhesive properties of single platelets subjected to flow. Dr. Jackson then focused on the additive contributions of
immobilized collagen and VWF in platelet adhesion and activation. Comparable in vivo studies, however, seem to indicate that platelets adhering to the vessel wall are in a lesser activated state than is anticipated from the in vitro flow studies. Various factors appear to mediate thrombus instability in FeCl3 in vivo models (which are partially different from the in vitro situation), i.e. the vascular substrate, the local rheology, the activation state of the endothelium and the contribution of thrombin. Dr. L. Brass presented evidence for novel mechanisms that regulate thrombus growth and stability. He discussed current insight into the factors determining platelet-platelet interaction in a laser-induced vascular damaging model. Particularly studies with genetically modified mice have given support to the idea that the thrombotic process consists of three phases: a phase of rapid platelet accumulation, a slower growth and plateau (RGD insensitive) phase, and a gradual declining phase. Mice with mutated G12, lacking semaphorin 4D, or lacking ESAM show different profiles of thrombus formation, in which one or two of these phases are altered. Such modeling is likely to help understanding the multi-molecular interactions that are involved in the thrombotic process.

Dr. G. Nash gave an overview of the blood rheological and haemodynamic factors influencing platelet deposition in various flow models. He stressed the importance of margination of cells to the outer part of the vessel wall (determined by haematocrit and red cell aggregation). This results in blunting of the velocity flow profile, which for example is different for horizontal and vertical vessels, and for thin and thick vessels. The last speaker, Dr. E. Grabowski, informed on the factors determining the adhesion of platelets to cultured endothelial cells in flow. Treatment of the cells with alpha-shiga toxin and factor VIIa or TNF-alpha caused massive adhesion of strings of platelets, as a consequence of the activation of endothelial cells. This adhesion was tissue factor-dependent. Dr. Grabowski informed on the measurement method, the adhesive receptors involved and on the pathological conditions at which this platelet adhesion is relevant.
Control of Anticoagulation

Chair: S. Schulman (Canada)
Co-Chairs: W. Ageno (Italy), T. Baglin (UK), J. Harenberg (Germany), C Kearon (Canada), A. Lubetsky (Israel), J. Olson (USA), G. Palareti (Italy), A.M.H.P. van den Besselaar (The Netherlands)

Chairmen: S. Schulman (Canada) and A.M.H.P. van den Besselaar (The Netherlands)

S. Schulman opened the meeting of the committee and briefed on the activities over the past year. He also instructed on the importance of publishing work from within the SSC as official SSC publications.


Jörgen Jespersen (Denmark) and Leon Poller (UK) presented first the design of this multicentre study.

Previous studies claiming benefit for computer-dosage have depended solely on laboratory results and have not been sufficiently large to determine whether observed improvement in international normalised ratio (INR) control resulted in clinical benefit or whether computer-dosage was as safe clinically as that by experienced medical staff.

The aim of this study was to compare dose control with computer-assistance versus dosage by experienced medical staff at establishments with considerable experience of administration of oral anticoagulants. Forty centres were invited to provide data from 400 patient-years thus to achieve a 16,000 patient-years’ target. Patients at participant centres were to be randomised to manual (medical staff) dosage or to one of two commercial computer programs, either a new version of the PARMA program [PARMA 5], or to the established DAWN AC program. The use of two different commercial computer programs aimed to preserve the study’s independence from industry.

New definitions of major and minor bleeding have been introduced in a standardised Clinical Events Assessment Form designed by the Project Adjudication Committee. Clinical Events forms were returned as the events occurred to the EAA Central Facility by participant centres and were assessed “blind” by the Project Adjudication Committee. The study was monitored by the Project Steering Group and by the Project Safety Committee.

A continuous programme of international sensitivity index (ISI) calibration and external quality control of INR testing was conducted at participant centres.

The first 6-12 months were devoted to recruitment and training of staff from centres, to familiarisation with the computer programme and to enlisting patients.
EAA: Cost-Effectiveness of Computer-Assisted Anticoagulant Dosage: Adjudication of clinical events

Gordon Lowe (UK), also on behalf of M. Moia (Italy), A. Turpie (Canada) described the process of the EAA Adjudication Committee, then the definitions and total numbers of clinical events, followed by a brief comparison with the literature.

EAA: Cost-Effectiveness of Computer-Assisted Anticoagulant Dosage: Clinical study results.

Jörgen Jespersen (Denmark) and Leon Poller (UK) continued by reporting clinical results from the randomized study by the European Action on Anticoagulation (EAA). 32 centres with a special interest in oral anticoagulation participated and incorporated 13,219 patients, which provided 18,617 patient-years. One computer program was used much more than the other program in this study. INR tests numbered 193,890 with manual dosage and 193,424 with computer-assistance giving “time-in-range” (Rosendaal) of 64.7% and 65.9% respectively. The overall number of confirmed adjudicated clinical events although 7.6% lower in all clinical groups with computer-dosage was not significantly different but the number of clinical events in the 3208 patients with deep vein thrombosis was significantly lower (115) with computer-assisted dosage compared to 152 in the medically dosed arm of the study (p<0.01).

The study confirms the clinical safety and effectiveness of computer-assisted dosage using two different programs (PARMA 5 and DAWN AC) compared to the standards of the 32 participant centres with a special interest in oral anticoagulation.

Anticoagulant dosing using computer dosing systems.: Results of a UK NEQAS survey on current practice

R. Maclean on behalf also of I. Jennings, S. Kitchen and I. Walker (all from UK) reported on a survey on computer-assisted dosing resulted in particular cases. They examined 3 hypothetical scenarios, provided clinical details, past INRs and warfarin dosages. 955 UK NEQAS participants completed the exercise. They had to recommend a continued dose and the time to next test. The participants were subgrouped as 250 used CDSS, 129 dosed manually, Blood coagulation programme 71 vs 210. Dawn most common followed by RAID. Huge variability in recommended recall times was observed. For patients with a recent historic change in INR there was also a huge variability in recommended new dose. Clinicians tend to override computer doses in 30%.

EAA: INR correction by local ISI calibration or “Direct INR”. An international study

Michelle Keown (UK) presented data from an international collaborative study at 77 centres which compared local INR correction using the two alternative methods recommended in the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis guidelines: Local ISI calibration and “Direct INR”.
Success of INR correction by local ISI calibration and with Direct INR was assessed with a set of 27 certified plasmas (20 from warfarin patients and 7 from normals).

At 49 centres using human thromboplastins, 3.0% initial mean local INR deviation from certified INR was reduced by local ISI calibration to 0.7% and at 25 centres using rabbit reagents, from 15.9% to 7.5%. In contrast with “Direct INR” mean deviation using human thromboplastins increased from 3.0% to 6.6% but there was some reduction with rabbit reagents from 15.9% to 10%.

Local ISI calibration gave INR correction for the majority of PT systems but failed at the small number using combined rabbit reagents suggesting a need for a combined reference thromboplastin. Direct INR gave correction overall with rabbit but not with human reagents. INR correction was better than local ISI calibration with combined rabbit reagents.

**Outlier detection and exclusion: effects on ISI calibration in multicentre studies.**

A.M.H.P. van den Besselaar presented this project also on behalf of V. Chantarangkul, A. Tripodi.

ISI calibration is performed with fresh samples from many individuals, both normals and patients treated with oral anticoagulants. According to 1999 WHO guidelines, samples outside the 1.5-4.5 INR range should be excluded followed by exclusion of data points with greater distance than 3 residual standard deviations about the regression line. Dr van den Besselaar et al analyzed three multicentre ISI calibration studies of international thromboplastin standards (performed in 1990, 1995, and 2005), to determine the effects of INR and outlier exclusion. Obviously, the within-laboratory variation was reduced by outlier exclusion, but there was also a tendency to reduced between-laboratory variation. The effect on the mean ISI was only minor: in most cases <1% and in all cases <3%. It is recommended to follow the 1999 WHO guidelines for outlier exclusion.

**The in-built QC of the CoaguChek XS strip, technical and electrochemical principle, and validation data**

Winfried Plesch, Roche Diagnostics, Germany, presented data on the quality control of the CoaguChek XS strip. The CoaguChek® XS system is based on the amperometric measurement of the thrombin activity initiated by starting the coagulation cascade using a human recombinant thromboplastin. The concept of the CoaguChek XS test strip integrated QC function is to check the performance of the system at the spot of the coagulation testing. This could be done by adding the blue indicator Resazurin and a mixture of redox partners for this molecule to the PT test formulation. This means that the integrated control testing is performed exactly within the physical and chemical environment of the thromboplastin, which activates the clotting cascade in the blood sample. Under conditions of mishandling of the test strip, such as exposure to high humidity or longer exposure to daylight, the Resazurin is transformed to the purple by-product Resorufin. After blood application both molecules are quantified electrochemically using the same electrodes and the same meter functions as used for the clot detection. The reliability of detecting mishandling of test strips was validated under conditions reflecting the different climate zones in the world. Even under extreme conditions the integrated QC performed reliably.
In conclusion, incorrect handling of test strips, e.g. exposure to high humidity, will lead to deterioration of the thromboplastin in the reagent mixture, which in turn would result in unreliable INR values. Such mishandling is reliably detected by the integrated QC of the CoaguChek XS system, which ensures that only correct INR results are displayed to the user. Therefore, liquid quality control samples are no longer needed to check the CoaguChek XS system performance.

External Quality assessment of NPT/POC devices, Coaguchek XS and XS Plus

Steve Kitchen also on behalf of D. Kitchen, I. Jennings, T. Woods, I. Walker (all from UK) reported on this quality assessment. UK NEQAS Blood Coagulation has successfully established an External Quality Assessment (EQA) programme for INR testing using 2 new Point of Care devices - the CoaguChek XS (CUC XS) and the CoaguChek XS Plus (CUC XS Plus) during 2006. Their end point detection is based on the amperometric measurement of the thrombin activity initiated using a human recombinant thromboplastin, and a new formulation of EQA material was required. 2 lyophilised plasmas and diluents for reconstitution and recalcification were developed and tested by centres routinely using these devices. Results from pilot exercises (n=23 participants) showed good precision: sample 1 CUC XS median INR = 3.0, CV 8%, CUC XS Plus median INR =3.2, CV 6%; sample 2 CUC XS median INR = 3.45, CV 4%, CUC XS Plus median INR = 3.4, CV 6%, but included a number of test error messages from devices. Sample modifications were made and after further pilots a full programme has been launched in which the reproducibility of results has been better than that seen with other NPT devices in UK NEQAS programmes, and with very few testing errors. For the CUC S programme analysis of EQA data from lyophilised plasma samples has on rare occasions identified test strip lot numbers giving discrepant INRs (compared to other lot numbers), but these same lot numbers have had smaller or absent differences when native whole blood is tested. This means that findings from plasma testing can only safely be considered genuine if there are supporting data based on whole blood analysis since the devices are calibrated only for whole blood testing.

PART 2.

Chairs: S. Schulman (Canada) and J. Harenberg (Germany)

Prothrombin time (PT) expression and standardization in liver disease

Valerie Eschwège on behalf of also L Bellest, R Poupon, O Chazouillères and A Robert from Unité d’hémostase et service d’hépatologie, Hôpital Saint-Antoine, Paris, France discussed the use of INR in liver disease. International Normalized Ratio (INR)/International Sensitivity Index (ISI) system developed to standardize PT reporting during oral anticoagulation (OA) has been extended in liver disease (LD) and included in prognostic models such as the Model for End Stage Liver Disease (MELD) prioritizing liver transplant. Dr. Eschwège and colleagues have previously reported that, in LD, INR fails to yield a PT reporting independent of the thromboplastin used. A new standardization of PT in liver disease is evaluated using the thromboplastin calibration model proposed by the World Health Organization (WHO) using plasmas from LD instead of OA patients and leading to a new INR LD /ISI LD system specific for LD.
1) The ISI LD of 5 thromboplastins (#1, 2, 3: rabbit, 4: human, 5: human recombinant) were
determined by calibration, following WHO guidelines, against the reference preparation rTF/95
using 60 plasmas of patients covering the whole range of LD severity. 2) In 34 other patients, the
differences between mean PT reported as seconds, ratio, INR and INR LD across the 5
thromboplastins were analysed by ANOVA and multiple comparison test.

The ISI LD/ISI were 0.98/1.67, 0.94/1.54, 0.70/1.05, 0.84/1.03 0.85/0.83 for thromboplastins 1
to 5 respectively, demonstrating the difference in reagents sensitivities to defects induced by LD
and by OA. For the 2 most different reagents (1 and 5) and for the 5 patients with INR>3, the
discrepancy between INR was >60% of the mean INR and consequently the difference between
MELD scores (scale from 0 to 40) calculated with these INR was ³ 7. Mean PT differed
significantly for most pairs of reagents in all reporting modes except INR LD that eliminated all
results variability.

Adoption of INR LD instead of INR as an international scale of PT reporting appears as an
important goal in hepatology.

Use of the PT and the INR scale as a measure of prognosis in liver disease

Armando Tripodi discussed the use of prothrombin time (PT) for staging severity of liver
disease. The model for end-stage-liver-disease (MELD) is a mathematical score used to prioritize
patients for liver transplantation and includes results for creatinine, bilirubin and PT expressed as
international normalized ratio (INR). The rational of using the MELD rests on the assumption
that the score would be the same across the country if the methods used to measure the variables
yield the same numerical results regardless of the testing laboratory. Evidence was provided that
specific methodologies may influence the MELD and the PT-INR was identified as the most
important. This study was designed to provide information on the between-thromboplastin
variability and to explore alternatives to obviate such variability. Fifty-seven cirrhotics were
selected and their PTs were measured with 7 thromboplastins. The thromboplastins were
previously calibrated by testing plasmas from patients on vitamin-K-antagonists (VKA) and
healthy subjects to assign the international sensitivity index (ISI vka) needed to convert PT into
INR. Each of the thromboplastins was also assigned an ISI liver by substituting in the calibration
the plasmas from VKA-patients with plasmas from cirrhotics. INR and MELD values for
individual patients were calculated by using the ISI vka or the ISI liver. The mean INR vka
obtained with the 7 thromboplastins were significantly different (p<0.001). Conversely, the mean
INR liver were not. Similarly, the mean MELD vka were significantly different (p<0.001), but
those differences were abrogated for the MELD liver. In conclusion, the alternative
thromboplastin calibration using plasmas from cirrhotics instead of from VKA-patients is
feasible and may resolve the variability of the MELD to prioritize patients for transplantation.

- Discussion: Several commentators applauded the studies by Drs. Eschwège and Tripodi.
It was suggested to form a working party, preliminary chaired by Dr. Tripodi, and with
Dr. Eschwège, Dr. Schulman and others involved. There will be a need to include
hepatologists. The aim will be to create a guideline for the use of INR in patients with
liver disease or alternatively to change the formula for MELD to include another
parameter than INR.
Development of generic low-molecular-weight heparins. What are the guidelines?

Jawed Fareed (USA) discussed the issue that several commercially available low molecular weight heparins (LMWHs) are now widely used in the management of thrombotic and cardiovascular disorders. Although derived from porcine mucosal heparin, these drugs are manufactured by distinct chemical and enzymatic methods with products, which can be differentiated in chemical and biologic assays. The preclinical pharmacological profile of these products is also distinct and profoundly impact on their therapeutic actions. More recently several generic versions of enoxaparin and dalteparin have been introduced in some Asian and South American countries. In addition, numerous generic suppliers have applied for the approval to sell the generic versions of enoxaparin and dalteparin in the US and European union. Neither the US FDA nor EMEA have any guidelines for the generic interchange of branded products at this time. The current pharmacopeial guidelines are inadequate to accept the generic version of the branded LMWHs since these apply the older guidelines. The LMWHs represent a hybrid of the biologic and chemical manufacturing processes. The European Pharmacopeial description of each of the individual LMWHs is incomplete and US Pharmacopeia is working towards developing monographs for such drugs as enoxaparin and dalteparin. Considering the complexities related to chemical and biologic profiles of LMWHs, additional guidelines for the therapeutic and generic interchangeability are warranted. Product characterization and structural equivalence are not adequate to validate the generic versions of branded LMWHs. Data obtained on the currently available generic versions of LMWHs show that while these products exhibit similar molecular and pharmacopeial profile, marked differences in their in vivo pharmacology are noted. Thus, animal studies and qualified clinical trials may be needed as acceptance criterion for the generic LMWHs. Moreover, process controls including starting material and pharmacodynamic characterization in valid models may be needed. The ISTH/SSC has played a role in the biologic standardization and characterization of LMWHs, it is proposed that ISTH/SSC on the Control on Anticoagulation, consider the development of specific guidelines for the requirements to accept a generic version of the branded product.

• Discussion : Dr Trevor Barrowcliffe commented that there is already some development towards guidelines within EMEA. Dr Harenberg responded that he, Drs A Kakkar, Samama and Casu and others met very recently and were encouraged to give input from coagulation expertise.

• It was therefore decided to form a working party for development of guidelines on comparisons of generic LMWHs with the original brand. This should take into account the lot-to-lot variations seen in the brand product and should concentrate on pharmacokinetic and hemostatic properties, whereas cell interactions are so far beyond the scope of characterization of any LMWH on the indications presently approved. Dr Harenberg agreed to chair this working party and it will mainly communicate by e-mail and have the goal to finish the guideline recommendation before next SSC.

Problems with anti-Xa measurement during treatment with LMWH in subjects with antithrombin deficiency

Gualtiero Palaret and colleagues studied a pregnant woman with antithrombin and protein S deficiencies who had had two previous DVT episodes. The latter episode occurred at the
beginning of a previous pregnancy that ended in intra-uterine death, even though the patient was receiving antithrombin concentrate together with therapeutic LMWH doses. Reporting to their clinic years later on occasion of a new pregnancy, the warfarin therapy was stopped and treatment started with antithrombin concentrate (twice a week) and therapeutic LMWH doses b.i.d. When anti-Xa measurement was performed with different methods the levels varied according to the presence or absence of antithrombin supplementation in the assay reagents. Anti-Xa levels were within the therapeutic range using reagents that included antithrombin, but were clearly below the therapeutic levels after exclusion of antithrombin from the reagent, except when the antithrombin level in the patient was normal thanks to supplementation. This prompted an increase of antithrombin concentrate supplementation and LMWH doses in order to maintain adequate anti-Xa levels (excluding antithrombin from the reagent) throughout the pregnancy. The pregnancy ended well, with no complications for either mother or foetus. Dr Palareti is following two similar cases in collaboration with colleagues from another Institution.

He would like to warn against the use of anti-Xa assays containing antithrombin in the reagents to regulate a LMWH anticoagulation in subjects with antithrombin deficiency/reduction.

**Registry on recurrent venous thromboembolism on anticoagulation in patients with cancer – update**

Sam Schulman reported that the recruitment to this registry has been very slow in spite of announcements at several conferences, mass distribution e-mail via the ISTH roster and promotion by the sponsor of the unrestricted grant, Leo. Efforts will continue to encourage reporting.

**New registry on visceral vein thrombosis**

Francesco Dentali and Walter Ageno from Italy presented a suggestion for a new registry. Splanchnic vein thrombosis is an uncommon, but potentially life-threatening disease. Symptoms are non-specific, and clinical presentations are variable. Advances in imaging techniques have facilitated its early diagnosis, but information on how these techniques are applied in clinical practice is scant. Several etiologic factors have been reported, but their true prevalence is uncertain.

The treatment of splanchnic vein thrombosis may involve anticoagulation alone or in combination with surgery. The timing and intensity of antithrombotic treatment in this setting are unknown, as well as the natural history of the disease. Because information on risk factors, clinical features, diagnosis, treatment and outcome of splanchnic vein thrombosis is mainly based on small, uncontrolled retrospective series of patients, the aim of the proposed prospective registry is to improve the knowledge on this important disorder. Information on most common clinical presentations, most used diagnostic approaches, most common risk factors, and on the natural history of splanchnic vein thrombosis in a large prospective cohort of patients will be collected. Use of anticoagulation, duration of anticoagulation, thromboembolic and major bleeding complications during anticoagulant therapy and when anticoagulant therapy is stopped will also be registered.
Disseminated Intravascular Coagulation (DIC)

Chair: C.-H. Toh (UK)
Co-Chairs: N. Key (USA), J.D. Nielsen (Denmark), K. Okajima (Japan), H. Wada (Japan)

Theme: From Scoring System to Patient Care

Chairman’s report

CH Toh presented an overview of work from this subcommittee and referred to the Communication from 2001 (Thromb Haemost 2001; 86: 1327) as a key reference point with development of the diagnostic criteria. The first phase of this work culminated with this year’s SSC Communication (J Thromb Haemost 2007; 5: 604). As the past 5 years’ work has primarily concentrated on the DIC of sepsis, the next phase of activities will include further diagnostic refinement especially into the DIC of trauma and obstetric disorders as these are likely to have pathogenic differences to sepsis. Emphasis would continue to be about Prognostication and Pathogenesis.

DIC in obstetric disorders

J Thachil highlighted the unique properties of the uteroplacental system whose procoagulant potential is set at a higher level than in other vascular systems and therefore prone to perturbation. The main causes of DIC are pre-eclampsia and post partum haemorrhage. The former has an inflammatory component albeit at significantly lower levels than those seen in sepsis. Evidence was also presented that the DIC of obstetrics mainly represents overspill of uteroplacental site coagulation activation rather than a systemic state of events. As such, delivery of the placenta usually leads to resolution of the DIC.

D de Prost from hôpital Louis Mourier, Colombes, France evaluated the usefulness of fibrinogen level in the management of PPH. She presented data showing that a low level of fibrinogen during labour can predict the later occurrence of PPH; moreover, in declared PPH, a low level of fibrinogen is an independent predictor of the severity of haemorrhage. These results suggest that the fibrinogen level can be used to guide the management of PPH and monitor coagulopathy. The interest of using a point of care device to shorten the delay for getting the laboratory result should be evaluated.

DIC in severe trauma

Uri Martinowitz considered coagulopathy in trauma as a distinct entity whose pathogenesis was about localised endothelial damage and clot formation rather than the diffuse process in sepsis, albeit fairly similar coagulation abnormalities. Concern was expressed that as DIC-sepsis management is increasingly about the value of anticoagulant treatment that haematologists might mistake this to be treatment in DIC trauma.
Bertil Bouillon with his experience as a trauma surgeon, presented coagulopathy as a clinical problem in the trauma population. The current concept is of primary and secondary injury with coagulopathy contributing to the latter. Data was presented of how coagulopathy contributes to an adverse outcome. A new classification concept of bleeding and coagulopathy in severe trauma was proposed as was a concept for diagnostics and treatment.

Markus Huber-Lang presented an educational lecture on the interaction between coagulation and complement pathways. This adds to our knowledge of thrombin ubiquity and may well have potential in early recognition of maladaptive host responses to injury.

**DIC in sepsis**

Marcel Levi highlighted the value of the ISTH DIC score as the strongest predictor of mortality in multivariate analysis of sepsis trials. In addition, present evidence indicates that administration of recombinant human activated protein C should be considered in the DIC of sepsis but it increases the risk of major bleeding. Interestingly, analysis of the Kybersept trial of high dose antithrombin concentrate in severe sepsis showed the subgroup of patients that had DIC and that did not receive heparin showed a remarkable survival benefit, but this finding requires prospective validation. As regards heparin, a recent large trial in patients with severe sepsis where in press has shown a non-significant benefit of low molecular weight heparin on 28 day mortality and underscores the importance of not stopping heparin in patients with DIC and abnormal coagulation parameters.

Hideo Wada found the level of thrombin-antithrombin complexes to be similar in DIC subgroups but other parameters such as protein C and antithrombin levels were not always the same suggesting that disease specificity affected the functional consequence of thrombin generation. He also presented data that antithrombin levels below 70% had value when incorporated into the DIC scoring system.

B. Jilma informed us that only limited methodologies exist that can be used in human volunteers to mimic the physiologic alterations observed in critically ill patients. The coagulation response to experimental endotoxemia is self-limited, and the consumption of coagulation factors and natural anticoagulant is relatively modest. Similarly, the use of endotoxin is not necessarily reflecting the pathophysiology of microorganisms other than Gram negative bacteria. Thus, while experimental endotoxemia presents a well standardized translational tool for the development of novel anticoagulants, precautions are necessary in the extrapolation of data to septic patients with DIC.

Hidehiko Saito concluded the session with promising study results of using recombinant soluble thrombomodulin ART-123 in DIC from infection and malignancies. The molecule has a long half-life of 20 hours but does not appear to have bleeding complications. Current results show no effect on mortality but quicker resolution of DIC.

**Closing remarks**
CH Toh concluded this well-attended session whose numbers were consistently above 300. There was broad agreement without dissenting comments on adopting the presented plan of activities for the future. Laboratory standardisation will continue on various fronts as will continued refinement to the DIC score for disease-specific relevance. Collaboration will continue with SSCs of Fibrinolysis and Haemostasis in Malignancy respectively on the issue of D-dimer; with SSC on Vascular Biology over measuring microparticles and SSC on Control of Anticoagulation over the issue of PT/INR.
Registry of Exogenous Hemostatic Factors

Chair: N. Marsh (Australia)
Co-Chairs: K. Clemetson (Switzerland), M.R. Kini (Singapore), F. Markland Jr (USA) M. McLane (USA), T. Morita (Japan)

Nine members of the Registry including Co-Chairs (Kini, Markland, Clemetson, McLane and Morita) were in attendance plus some 25 guests. Chair Marsh were absent with apologies.

Welcome: R. Manjunatha Kini, Co-Chair (Chaired the meeting)

1. Meeting was brought to order by the Chairman Dr. Manjunatha Kini.
2. The minutes of the last meeting were read and there were no questions.
3. Classification and nomenclature of disintegrins (M. A. McLane) A report on the classification and nomenclature of disintegrins from snake venoms was prepared by with inputs from Dr. Markland and was presented to the committee by Dr. McLane. This new nomenclature system was sent as an official communication of the Registry to SSC. It was published as a short notice in the the Journal of Thrombosis and Haemostasis. Dr. McLane updated the list of disintegrins. The subcommittee agreed unanimously with the new nomenclature and classification. As disintegrins are part of larger precursors containing metalloprotease domains, there was a question how these have to classified. Dr. Kini informed the subcommittee that the classification of metalloproteases will be looked into in the near future by Drs. Ana Moura De Silva and Jay Fox.
4. Classification and nomenclature of C-type lectin related proteins (K. Clemetson) A report on the classification and nomenclature of C-type lectin related proteins from snake venoms was prepared by Dr. Clemetson and Dr. Morita was presented. It is a difficult task as the new proteins are being identified through proteomics and transcriptomics. The new classification and nomenclature was agreed upon by the sunbcommittee. Some minor suggestions were made to fine tune and improve the nomenclature and classification system. Dr. Clemetson agreed to complete the manuscript to describe the details of the new classification and nomeclature soon. This report will be circulated among the members of subcommittee and after approval will be sent to SSC for final approval and publication.
5. Two new studies on procoagulant proteins from Lonomia obliqua was presented by Dr. Ana Marisa Chudzinski-Tavassi (Brazil) and on new three-finger toxins with anticoagulant activities was presented by Dr. Kini. This was part of our strategy to update some of the new developments in the field.
6. Organization of the International Conference – We are planning to organize the Fourth International Conference on the Exogenous Factors Affecting Thrombosis and Hemostasis as a satellite meeting to the XXII World Congress to be held in Boston, USA. Dr. McLane, Chairman of the Organizing Committee presented the details of the organization. This conference for three days is planned to commemorate the 25 th anniversary of the first meeting on Exogenous factors in San Diego. The subcommittee agreed with the plan. There were suggestions to include poster sessions and some oral presentations to encourage the participation of graduate students, postdoctoral fellows
and young scientists. The subcommittee also unanimously agreed to publish the proceedings in the form of a book (about 500 pages).

7. Other activities: Dr. Kini asked the subcommittee members for suggestions/ concerns in any other areas of exogenous factors. We agreed to look into metalloproteases and their classification.

8. The next meeting of the Registry will be held in Vienna (2-5 July, 2008).

9. Any other matters: As there were no other matters, the meeting was adjourned.
Factor VIII and IX

Chair: A. Srivastava (India)
Co-Chairs: C. Hay (UK), C. Lee (UK), K. Mertens (The Netherlands), C. Negrier (France), F. Peyvandi (Italy), J. Saint-Remy (Belgium), E. Tuddenham (UK), H. van Den Berg (The Netherlands)

The Chair opened the meeting at 15:45 and welcomed the audience of about 200 attendants. He confirmed that there were no modifications in the agenda and mentioned that all topics of interest could not be included this year due to lack of time.

Completed/Submitted reports and recommendations

In the last year, SSC activities resulted in the following publication in the Journal of Thrombosis and Haemostasis:


Section 1. SSC Working group on Rare Bleeding Disorders. Chair – F. Peyvandi Co-Chair – C. Negrier.

Since its inception in 2004 within the FVIII/IX subcommittee, this SSC working group on "Rare Bleeding Disorders" (RBDs) has attempted to improve our understanding of prevalence, diagnosis and treatments of these diseases by developing the Rare Bleeding Disorders database (www.rbdd.org). Some international data is also available from an annual survey conducted by the World Federation of Hemophilia (WFH) (www.wfh.org). Various national registries have also begun collecting epidemiological data on these conditions (Swiss, North American, United Kingdom, French, Egyptian and other EMBRO countries, Iranian and Indian).

RBD in North America – M. Soucie / A. Shapiro

Dr. Soucie provided information about rare bleeding disorders in the U.S. This data is collected using a public health surveillance system called the Universal Data Collection (UDC) system. The UDC has a national, IRB-approved protocol and collects standardized clinical data and a blood specimen that is centrally tested for transfusion transmitted viruses. Patients in any of 135 federally funded comprehensive care centers with a factor deficiency (<50% of normal) or VWD are included, with informed consent. Data collected include demographic, clinical and treatment information, and a self-assessed quality of life tool. Since May, 1998, over 20,000 patients have been enrolled. The distribution of disorders among enrolled patients is 59% hemophilia A, 16% hemophilia B, 22% VWD, and 3% other factor deficiencies. Dr. Shapiro described the efforts that are also underway to establish a Rare Coagulation Disorders Resource Room, a project of the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation (NHF). This web-based Resource Room will be devoted to a variety of rare blood disorders, each
having a separate manuscript covering clinical, laboratory and genetic aspects authored by an expert.

**RBD in South America – E. D’Amico:**

Dr. D’Amico presented the South American data. Comprising of 12 countries (total population ~390,000,000) ranging from 458,000 to 191,790,900 inhabitants / country, there is very little published data on RBDs from these countries. Dr. D’Amico had conducted a survey among some of the leading centers in these countries and was able to get data from Brazil, Columbia, Panama, Peru (regional) and Venezuela (national). The reported numbers show wide variability in prevalence of these conditions as documented so far. In all this data, shows the presence of 59 fibrinogen, 62 prothrombin, 41 FV, 132 FVII, 78 FX, 164 FXI, 20 FXIII and 25 combined FV/VIII deficiency patients. The most frequent RBDs are FVII and FXI deficiency. There is limited access to laboratory diagnosis of these conditions. A wide range of products ranging from fresh frozen plasma to factor concentrates are used for treatment, though access is variable depending on the country.

**RBD - The WFH survey 2006 – P. Bolton-Maggs**

Dr. Bolton-Maggs mentioned that the World Federation of Hemophilia (WFH) had been collecting epidemiological data on hemophilia and other bleeding disorders since 1998 from its national member organizations, which now are over 100 and represent >85% of the world’s population. 56 out of the 101 countries that provide data have some form registry. From 2004, countries have been asked to provide information about the rare bleeding disorders in addition to hemophilia and von Willebrand disease. The number of patients with RBDs reported increases every year and currently runs at more than 17,000. Till 2005, the following numbers of patients with RBD had been reported: fibrinogen-599; prothrombin–167; FV-769; FV/VIII-188; FVII-1689; FX-597; FXI-2446; FXIII-435; platelet disorders-2648. There are many unclassified patients as well. The quality of the data is variable since health care standards vary and the registries may be managed by medical or lay people. This data is accessible on www.wfh.org.

**SSC Working Group on inherited RBD- Proposed plan of Action: F. Peyvandi**

Dr. Peyvandi described the efforts of above mentioned registries and surveys providing data on RBDs (www.wfh.org/2/7/7_0_Link7_GlobalSurvey2005.htm and www.rbdd.org). The former has information on 6,934 RBD patients in 98 participating countries and the latter has data on 3,017 RBD patients in 64 participating countries. The prevalence of RBDs are similar in the two data sets. However, in both these, only very basic information is available on each patient. About 50% of this data refers to patients in Europe. As a consequence, a network of 10 European treatment centres has been formed to develop a new and homogeneous communication tool for reporting, managing, editing and viewing information on RBD patients (www.rbdd.eu)
To help focus on the development of the science around each RBD, it has been decided to establish small expert groups to prepare a roadmap for the development of the science of that condition.

Section II. F VIII / IX: Clinical issues – I (Assay/EQA/Phenotype) Co-chairs: C.A. Lee and H.M. van den Berg

Optimizing the 1-stage assay – J. Polgar

Dr. Polgar stated that current factor assays do not optimally cover the clinically relevant range particularly at the lowest levels by a reliable single calibration curve. Further improvement of the one-stage assays would be possible through introduction of a ‘zero calibration point’ and the establishment of calibration curves with an optimization approach. In the optimization approach: A) More than ten mathematical transformations can be used both for time/activity. B) Two curves can create the calibration, by selecting two groups of calibration points. C) Curve fittings can be linear, 2nd or 3rd order polynomials. D) A cut-off point defines the end of one, and the beginning of the next curve on the combined calibration curve with a smooth transition. Factor assays on analyzers, which use calibrations established by an optimization approach, have a wide range for accurate assays (0%-150% activity) with excellent linearity in the calibrated region and increased tolerance for reagent variations. Widespread use of ‘zero calibration points’ and establishing calibration curves by the ‘optimization approach’ could lessen variability in factors results among clinical laboratories.

EQA for tests of global haemostasis : ROTEM and TEG – S Kitchen, DP Kitchen, I Walker

Dr. Kitchen presented data from the UK National External Quality Assessment Scheme (UK NEQAS) for Blood Coagulation that has assessed the use of lyophilised plasma samples for thromboelastography (TEG) and Rotational thromboelastometry (ROTEM). Lyophilised plasmas from a series of normal subjects and patients with deficiency of FVII, IX or XI were analysed in a single centre. These studies confirmed that some parameters were similar to those obtained for whole blood which is the sample material usually analysed for patient study. After this preliminary study, 2 pilot exercises were performed in which 7-10 ROTEM users and 13-14 TEG users received samples from normal subjects and a patient with severe FXI deficiency. The participant group included expert haemostasis centres and anaesthetists in operating theatres. The Coefficient of Variation for clotting times was between 10 and 121% and some clearly outlying results were observed. The clot firmness measurements showed CVs of 8 -33% between centres and were lower for plasma samples with higher fibrinogen concentrations. Overall our data show that lyophilised plasma samples can be used for EQA of TEG and ROTEM, and the degree of variability observed in 2 exercises suggests that such EQA could be of benefit in identifying outlying results. Further exercises are planned.

EQA for genetic testing of haemophilia – D. Perry

Dr. Perry presented the evolution of the EQAS for genetic testing of hemophilia in the UK. A pilot scheme was set up by NEQAS in 1998-2000 using whole blood for the intron 22 inversion.
At that time many laboratories were using southern blotting and only a few long range PCR. In 2003, an advisory group was set up to provide a robust scheme for genetics. It was planned to provide samples, set up immortalised cell lines and set up a scoring scheme which would also depend on the family history. There are now two exercises each year which provide a clinical history and whole blood or DNA samples for analysis. A collaboration has been set up with NIBSC to set up immortalised cell lines. The lyophilised DNA has provided conflicting results but the liquid DNA has performed well. There has been encouragement to laboratories to use standard gene monocenclature – the CMGS (Clinical Molecular Genetics Society) system. Participants are required to 1) identify the individual 2) identify the mutation 3) answer the question and 4) be precise and this is the basis of the scoring system. At present the exercises have been based on FVIII but there are plans to move onto FIX and VWF genetic analysis.

**Phenotypic heterogeneity of severe hemophilia – Newer players. A. Srivastava**

Dr. Srivastava mentioned that clinical observation of minimally treated patients with severe hemophilia suggested that the phenotypic heterogeneity among them exists not only in terms of the frequency of bleeding but also at the level of the inflammatory response in the joint. It was therefore hypothesized that polymorphisms in the genes of hemostatic factors and inflammatory cytokines could both affect clinical phenotype. Based on this hypothesis, patients with severe hemophilia were classified as mild (<5 bleeds/year, <10 WFH clinical and <10 Pettersson radiological score) or severe phenotypes (all others). Of the 114 patients evaluated, 14 were classified mild by these criteria. Among these patients, ‘severe’ mutations (inversions / deletions) (RR: 4.8), FVII 353 polymorphisms (arg/gln-gln/gln; lower levels) (RR: 5.9), protein C 1476 AT/TT (higher levels) (RR: 4.0) and TNF a 308 GA/AA (higher levels) (RR: 3.9) were found to be associated with clinically severe disease. With plausible biological basis for these polymorphisms affecting clinical phenotype, these data suggest that the clinical heterogeneity of severe hemophilia is not only determined by a balance of various coagulation proteins but also by polymorphisms in inflammatory cytokines. This work is on-going.

**Section III. Factor III / IX. Inhibitors. Co-Chairs: C.R.M Hay / J. M. Saint-Remy.**

This section of the SSC included reports of completed, ongoing and planned studies of the incidence and risk factors for factor VIII inhibitors in PUPS, MTPS and PTPs. Past research has primarily focussed on the incidence and genetic and non-genetic risk factors for inhibitor development in PUPs. However, the risk factors and incidence of inhibitors in PTPs are poorly understood and require further study. Since regulators are using PTPs as their model to test the immunogenicity of new products, it is against this yardstick that these products need to be measured.

**Global PTP inhibitor surveillance study: follow-up – D. DiMichele / C.R.M. Hay**

Dr. DiMichele outlined the development and growth of the US registry. Data on 529 patients in 9 sites are being retrospectively and prospectively collected and the database is growing. Inhibitor and genotypic testing is being done centrally and 240 patients have been genotyped so far. Dr. Hay described the UK registry, the planned European Adverse event network and the planned German registry. The UK registry has networked all 108 UK haemophilia centres, covering 6500
patients with haemophilia A and a total of >23000 patients with bleeding disorders of all types. Adverse events, such as new inhibitors, and new diagnoses are reported electronically in real time. Most patients have already been genotyped. A wider dataset is about to be collected prospectively to explore risk factors for inhibitor development in PTPs. A European adverse event surveillance system (EUHASS) is the subject of an EU grant application and is hoped to be operational by early next year. This will involve 45 haemophilia centres across Europe serving a total of 14,500 patients. The German Registry (national data) has also been set up and is due to open later this year. It is expected that these four registries will collaborate closely. Harmonization of datasets is being negotiated and it is hoped to present a common dataset in 2008.

International ITI study update – C.R.M. Hay.

Dr Hay reviewed the current status of the International Immune tolerance Study. This is an open randomized comparison of low and high-dose ITI in good risk patients. This study investigates factors influencing outcome, morbidity and cost-effectiveness. The study also forms a framework for several other satellite studies. The power calculation indicates that 90 patients are required to demonstrate a 20% difference between treatment arms and 150 patients to demonstrate equivalence. About 80 patients have been recruited and an interim analysis is expected later this year.

Rodin study: Update – H. M. van den Berg.

Dr. van den Berg explained that this study has been developed to determine the risk factors which cause or prevent inhibitor development in PUPs with severe hemophilia and may have important implications for eventual future prevention of inhibitors in these patients. The RODIN study aims to study potentially modifiable treatment related factors that affect the risk for inhibitors in these patients. It will include a cohort of patients with severe haemophilia who are treated in one of 30 participating European centres for the first 75 exposure days to factor VIII. Clinical data is collected from patients with severe (<1%), moderate (1-5%) and mild (5-25%) haemophilia A or B born after January 1st, 2000. Data will be collected from patients born between 1-1-2000 and 1-1-2008. In total, 400 patients with severe haemophilia will be collected through the PedNet registry.

Section IV. FVIII / IX Standardization issues. Co-chair: K. Mertens

FVIII collaborative studies: Phase II field study. M. Lee

Dr. Lee reported on the statistical analysis of the SSC 9th field study. This study included both recombinant and plasma-derived FVIII products. All participants had been asked to perform testing using two different operators on two separate occasions. The aim of this was to find an explanation for the high interlaboratory variation in the field studies, including the 9th. Multivariate analysis revealed that variability was mainly due to the assay as such, and not to other factors such as operators or days. As in the 8th SSC study, the main factor introducing variability was the prediluent (FVIII-deficient plasma or buffer) used. This study confirms the previous SSC recommendation that predilution in FVIII deficient plasma should always be
applied. In the discussion, Dr. Lee mentioned that the current activities are in full support of the previously published SSC recommendations for the assay of FVIII in concentrates. As such, it seems appropriate to work toward completion of these field studies, if appropriate, by publishing the current conclusions.

**First International Standard for Factor VIII inhibitor - update. S. Raut**

Dr. Raut provided an update on the collaborative study on proposed reference standard for FVIII inhibitor. Preliminary data of this study had been presented in 2006 and a final report was subsequently distributed to participants, requesting feedback from them. The study highlighted that although one candidate preparation Y (pooled inhibitor patient plasmas- 05/206) had the lowest overall CV (17.7%) with a mean Bethesda titre of 8.2 BU/vial, inter-laboratory variability within the study was relatively high (CVs 17-33%). The intra-laboratory variability also gave high CVs (0.5-36%). Furthermore, when the results were recalculated relative to the 5 candidate preparations, only a slight improvement in inter-laboratory CVs was observed for the patient test plasmas and only relative to sample Y, with minimal further improvement for the Nijmegen modification and hybrid inhibitor methods compared to the classical Bethesda assay when assaying patient / plasma inhibitor samples. Major improvement in the inter-laboratory variability was also observed when assaying residual FVIII activity using the chromogenic assay (CVs: 2.5-20.1%) compared to the one-stage assay (CVs: 17.9-32%), although only 3 laboratories used this method. Following further discussions at a FVIII Inhibitor SWP meeting in Amsterdam (May 2007), it was decided to defer finalization of these results till the reasons for such large inter-laboratory variability have been precisely delineated.

**Replacement of the 7th International Standard FVIII concentrate – S. Raut**

Dr. Raut further announced the forthcoming replacement of 7th FVIII concentrate standard. Stocks of the current WHO 7th IS (99/678) are running low and could be exhausted by 2009. This standard is used for the potency measurement/estimation of FVIII in therapeutic concentrate products, both recombinant / plasma derived FVIII by manufacturers and clinical laboratories. Approximately 600 - 800 ampoules are despatched each year from NIBSC. The current 7th IS is a plasma derived material. Material (both plasma derived FVIII and recombinant FVIII) for its replacement will be sourced from product manufacturers. The materials to be selected as candidates will be discussed and decided after carrying out in-house comparative assessments (trials fills, accelerated degradation studies and potency estimations). Calibration will be performed by clotting assays and chromogenic assays relative to the current WHO 7th IS in an international multi-centre study involving manufacturers, clinical laboratories and regulatory authorities. Objective will be to submit to ECBS in October, 2009.

**Replacement of the International Standard for Factor VIIa – A. Hubbard**

Dr. Hubbard reported that the stocks of the current WHO 1st IS Factor VIIa concentrate (89/688) are low and a replacement preparation is required. Candidate materials are currently being collected and definitive fills should be completed by the end of 2007. The original calibration of the 1st IS was performed by one-stage clotting assay, relative to the WHO 1st IS Factors II, VII, IX, X, plasma (84/665), using the same thromboplastin reagent in all
participating laboratories. This was necessary since potency estimations of FVIIa concentrate relative to plasma FVII show considerable variation depending on the thromboplastin reagent used. Since the original thromboplastin reagent used to calibrate the WHO 1st IS FVIIa concentrate is no longer available it is proposed that calibration should rely on a direct comparison of the proposed 2nd IS relative to the 1st IS FVIIa concentrate. This approach is supported by accelerated degradation and real-time stability studies which have indicated that the 1st IS FVIIa concentrate has not degraded since it was calibrated. Depending on the availability of suitable candidate materials it is planned to complete the multi-centre collaborative study by spring of 2008.

**Thrombin generation tests- Report of the 2nd collaborative study – E. Gray**

On behalf of the SSC Working Party (WP) on Thrombin Generation Tests, Dr. Gray referred to the fact that this WP was set up in 2004 under Plasma Coagulation Inhibitors subcommittee with the remits to investigate, standardize and validate methodologies for the quantitation of results to facilitate good intra and inter laboratory agreements. As the results from the first study in 2006 indicated, the use of a reference-plasma would lower both intra- and inter-laboratory variability, this second study was carried out to investigate the feasibility of establishing a reference plasma for thrombin generation tests. Six freeze-dried samples including three candidate normal pooled plasmas were sent to 110 laboratories and 128 sets of results were returned for analysis. The majority of the labs used commercial kits (CAT, Dade-Behring-ETP, Technothrombin and In-TDT). Four labs used in-house methods. The results confirmed data from the first study and show that calibration against reference plasma improves intra- and inter- laboratory agreement. All 3 candidates reduce variability, but 2 of these materials were better. The WP is now discussing how a reference plasma should be used and also how it can be established as a SSC reference plasma for thrombin generation tests. The next task for the WP is to investigate the application of thrombin generation tests for use in the study of haemophilic plasma. The WP is requesting collaboration with experts from the FVIII/FIX Subcommittee who are interested in the standardization of thrombin generation tests.

In his concluding remarks, the chairman thanked all the co-chairs, speakers and the audience for their participation and closed the meeting at 19:45 hours.
Fibrinogen and Factor XIII

Chairs: M. de Maat (The Netherlands) and R. Seitz (Germany)
Co-Chairs: R. Ariens (UK), P. Bishop (USA), A. Ichinose (Japan), H. Kohler (Switzerland), J. Koopman (The Netherlands), M. Maurer (USA), L. Medved (USA), N. Weinstock (Germany), J. Weisel (USA)

This was the first meeting of the combined Fibrinogen & Factor XIII subcommittee in a single session of 4 hours and some experiences were shared: A common concern is that the time for the meeting is short, and that several aspects could not be discussed with sufficient detail (especially the nomenclature and the laboratory aspects).

PART 1: Fibrinogen

In the first presentation, dr. W. Koenig showed us that the biological variation of fibrinogen levels in plasma affects the relationship between plasma fibrinogen levels and risk of cardiovascular disease. The reliability index of fibrinogen measurements is around 0.5-0.7, which means an 30-50% underestimation of the risk estimate in epidemiological studies. Multiple measurements (2-3 samples collected at least 2 weeks apart) and exclusion of samples collected during inflammatory conditions improves the risk estimation.

In the next presentation, dr. L. Medved and dr. J. Weisel gave an update on the nomenclature of the fibrinogen molecule and on the nomenclature of the fibrin formation. A major point to consider is the numbering of the amino acids, since it is common use for fibrinogen to use a numbering system, based on the mature protein, while the recommendations of the Human Genome Variation Society use the transcription initiation site as +1. This approach is now also being discussed for other (hemostasis) proteins. Since everybody is very much used to the old numbering system, it has been suggested to use double numbering to avoid confusion. A report is being prepared which is expected to be finalized in 2008.

The next presentation was also on nomenclature, now of the fibrinogen variants, and dr. M. de Maat and dr. J. Koopman prepared and presented a suggestion for a clearer nomenclature. A report is now being prepared that will be circulated among the fibrinogen investigators and will also be finalized in 2008.

Since it was noticed last time that it is difficult to compare different publications on the characteristics of fibrinogen mutations, Dr. M. Neerman-Arbez presented a minimum set of assays that should always be performed. Of course, different types of mutations (resulting in afibrinogenemia or dysfibrinogemia) require different approaches. This list will be included in a SSC report and several investigators that were present agreed to participate in the discussions.

The next two presentations focused on the effects of measuring fibrinogen with different assays in the association with cardiovascular risk. First dr. A. Silveira showed that in the recent meta-analysis of the Fibrinogen Research Collaboration, there is no indication that the association with risk is different for the different types of assays. Also, in a recent QTL analysis, they showed that the association between genetic markers and fibrinogen levels is similar for the Clauss assay and...
a nephelometric assay. In the next presentation dr. D. Peetz presented first results of the Gutenberg Heart Study, in which 5 different fibrinogen assays were used, and here the correlation between the assays varied between 0.5 and 0.9. A common concern is that fibrinogen assays need to be further standardised.

PART 2: FXIII

Session I. International Standardization and Registry Issues.

A. Ichinose gave a report about the FXIII SWP business meeting in Dresden in February 2007:
A) Political/Social issues: The current chair of the FXIII subcommittee explained that he initially could not accept the chair of the merged subcommittee, but was able to accept a joint chairmanship. The FXIII Standardization Working Party (SWP) decided to continue to work under the FXIII subcommittee of SSC/ISTH, and to centralize to the FXIII SWP all activities regarding the standardization of Factor XIII to ensure formal and consistent practices. B) Credit issues: On petition of the chair of the FXIII SWP, ISTH/SSC headquarters opened a homepage in their website in order to publicize/announce the major contributions of the FXIII SWP for the establishment of the 1st International standard for plasma (ISP) of FXIII. In addition, one FXIII SWP member was asked to complete a paper on the 1st ISP of FXIII. In order to avoid the recurrence of complications, the FXIII SWP decided to make written agreements for confirmation in advance and when needed. C) Financial issues: Financial difficulties were reported individually, and possibilities to raise funds for the standardization activities were discussed. The FXIII SWP decided to estimate the amounts of money required for direct research costs and travel expenses to attend the FXIII SWP meetings, and agreed to seek possible financial supporters. D) Scientific & Technical Issues: Difficulties in reproducible measurements of XIII A were considered carefully. The FXIII SWP decided to perform a pilot study to explore possible solutions to this problem. The requirements for good FXIII-deficient plasma and antibodies against various forms of FXIII were also confirmed. E) Clinical issues: the FXIII SWP addressed the necessity of standards for plasma FXIII concentrates and rec. FXIII preparations, for clinical management of FXIII deficiency.

A Report on standardisation of FXIII Concentrate on behalf of the FXIII SWP was presented by Sanj Raut. Following ISTH/SSC FXIII & Fibrinogen Subcommittee & SWP meetings where the need for a FXIII concentrate reference standard was established, it was proposed to carry out a pilot study (activity & antigen) on all available FXIII concentrate materials. Aims of the study was to evaluate candidate materials for the establishment of the 1st IS for FXIII concentrate, to investigate the relationship between measurement of FXIII in the concentrate vs plasma and to investigate the relationship between measurement of FXIII activity and FXIII antigen levels. Trial fills & accelerated degradation stability studies of candidate materials have been completed and samples & assay kits have been shipped out to the SWP participating laboratories together with protocol and assay design. Study is currently on hold whilst a number of issues are being resolved but it is envisaged that the study will resume later in 2007. 5 materials were provided: (X) FXIII Concentrate (02/170), activity potency ~ 40 IU/amp; (Y) WHO 1st IS FXIII Plasma (02/206): activity potency 0.91 IU/amp; antigen potency 0.93 IU/amp; (Q) rFXIII Concentrate (06/021PM), activity potency ~ 40 IU/amp; (R) rFXIII Concentrate (06/022PM), activity potency ~ 40 IU/amp; (J) FXIII Concentrate J, potency ~ 40 IU/vial. Laboratories were asked to
use routine and/or provided FXIII activity and antigen (A2B2-pdFXIII; A2 subunit-rFXIII) assays. They were requested to carry out 4 independent assays on each sample (on 2 separate days) using preparation Y as standard and following assay instructions/design as described in the protocol. They were to pre-dilute in FXIII deficient plasma and submit all raw data for analysis (by Nov 2007). It is envisaged that results will be analysed and presented at the SSC in June 2008. Based on this, selection of materials for definitive fills will be carried out and a full international collaborative study would be initiated. The objective is to submit study report to WHO/ECBS for establishment in Oct 2009.

V. Ivaskevicius focused on the progress of the international FXIII registry. Recently the data summarizing the former FXIII Registry of ETRO Working Party was published in Throm Haemost (2007, 97;6:914-21). A new on-line Questionnaire for patients affected by FXIII deficiency was presented. This questionnaire is available on the www.f13-database.de website. Further, Standardization of Genetic Terms were discussed in relation to FXIII genes and of clinical issues. Advantages and disadvantages were shown of new nomenclature supported by Human Genome Variation Society and old traditional nomenclature. Proposals were provided regarding classification of degree of severity depending from FXIII activity and bleeding symptoms.

Session II: Scientific and Clinical Issues:

W. Korte showed data confirming previous publications that a seemingly moderate intraoperative decrease of FXIII to levels below ca. 60% is associated with subsequent bleeding. A prospective study on intraoparative FXIII substitution was terminated early after 22 patients already, since a significant reduction of bleeding was observed. Further studies on the postoperative setting will follow.

Concerning diagnosis of FXIII deficiency, five cases were presented by H.P. Kohler, where FXIII levels had been overestimated by the most widely used Berichrom assay finding up to 15% FXIII activity despite non-detectable FXIII antigen; a problem already described in recent literature. Notably, it has been shown that overestimation can be amended by the subtraction of the blank; however manufacturer and user do not appear to be aware. L. Muszbek presented an algorithm for the laboratory diagnosis and classification of FXIII deficiencies: 1/ Screening test: A quick functional assay for the determination of plasma FXIII activity; 2/ Mixing study for the detection of neutralizing antibody; 3/ If FXIII activity is <5%, further functional test for the precise assessment of FXIII activity in the low activity range (amine incorporation assay, evaluation of fibrin cross-linking by SDS PAGE); 4/ Determination of FXIII A2B2 complex (R-ELISA); 5/ If the concentration of the complex decreased determination of individual FXIII subunits in the plasma; 6/ Determination of platelet FXIII activity and FXIII-A concentration; 7/ Detection of non-neutralizing antibodies against FXIII subunits by binding assays; 8/ Molecular genetic investigations. Guidelines on diagnosis and monitoring of FXIII therapy are missing, and it is proposed to publish an SSC position paper. This proposal was endorsed by the attendees.

Dr. Kitano discussed genetic and molecular bases of phenotypes of the B subunit of Factor XIII. Three major protein phenotypes of FXIII-B (FXIIIB*1, FXIIIB*2, and FXIIIB*3) are determined by isoelectric focusing and immunoblotting. FXIIIB*1 is the most common
phenotype among Europeans, while FXIIIB*2 and FXIIIB*3 are common in Africans and Asians, respectively. FXIIIB*4 is a rare phenotype. Recently, we determined amino acid residues responsible for each phenotype by nucleotide sequencing analysis using genomic DNAs. Assuming that FXIIIB*1 would be a basic phenotype, FXIIIB*2 had an amino acid substitution of codon 95 in exon III from His to Arg, and FXIIIB*4 had an exchange of codon 368 in exon VII from Glu to Val. For FXIIIB*3, we discovered a C-to-G change in intron K. This nucleotide substitution would create a better splicing acceptor AG dinucleotide, result in differential splicing of intron K, and produce a totally new exon. The presence of this message was confirmed by RT-RCR using hepatic mRNA. As a result, FXIIIB*3 has a 15 residues-longer carboxy-terminal than other phenotypes as well as two additional basic and one extra acidic amino acid residues. Accordingly, all four phenotypes contain variable numbers of charged residues, which ultimately contribute to their differential isoelectric points.

N.T.P. Bakker spoke about the hypothesis that chronic changes in blood flow and blood pressure induce an adaptation of vascular calibre, and that this remodelling depends on the cross-linking enzyme tissue-type transglutaminase (tTG). Blood pressure-dependent and flow-dependent remodelling was studied in wild-type (WT) and tTG-null mice using a surgically imposed change in blood flow in small mesenteric arteries. WT mice showed inward remodelling after 2 days of low blood flow, which was absent in arteries from tTG-null mice. Yet, after continued low blood flow for 7 days, inward remodelling was similar in arteries from WT and tTG-null mice. Studying the alternative pathways of remodelling, we identified monocytes/macrophages as a source of factor XIII and backup mechanism in tTG null mice.

R. Ariens addressed in his presentation the role of gamma dimer formation in determining clot elasticity and lysis rates is discussed. Cross-linking of fibrin by FXIIIa occurs between gamma-gamma and alpha-alpha chains. The relative contribution of gamma-gamma chain cross-linking is poorly understood. We made mutations in the gamma chain cross-linking sites and investigated fibrin structure and function. We found that gamma-dimer formation contributes significantly to clot rigidity. Gamma-alpha hybrid cross-links did not effectively increase clot rigidity. Differences in fibrin degradation products were observed in fibrinogen cross-linking mutants. However, no differences in fibrinolysis rates were observed when gamma-gamma cross-linking was eliminated. We conclude that gamma chain cross-linking plays a major role in determining clot rigidity but not lysis.
Fibrinolysis

Chair: C. Longstaff (UK)
Co-Chairs: C. Dempfle (Germany), D. Hendriks (Belgium), O. Matsuo (Japan), M. Nesheim (Canada)

TAFI/CPU Chaired by D Hendriks

M Nesheim presented work on a new assay for TAFIa in plasma based on modified plasminogen with a fluorescently labelled active site and a chemically-derivatized FDP containing a quencher molecule. Removal of C-terminal lysines by TAFIa is measured by increasing fluorescence. The assay was sensitive down to 10-15 pM TAFIa with good results for intra- and inter assay variability of 6.3 and 8.3%. There was no interference from normal plasma plasminogen in the normal physiological range or the TAFI polymorphism at position 325. Several normal plasma samples from volunteers were tested and an average of 20 pM TAFIa determined (range 4-32). Data were presented from animal experiments following injection of procoagulant FXa and PCPS demonstrating a dramatic, transient increase in TAFIa levels with the expected half-life. Sub-lethal doses of *E.coli* injected into a baboon as a model of sepsis were able to activate up to 30% of available TAFI zymogen. Conditions suitable for collection of plasma samples to optimise TAFIa stability were discussed.

J Willemse discussed approaches for the measurement of proCPU and CPU using specific small chemical substrates with the structure Bz-Xaa-Arg (where Xaa represents a naturally occurring amino acid). These assays are complicated by the presence of constitutively active CPN, the activity of which must be subtracted to determine CPU activity. A total of 15 synthetic substrates were screened with CPU and CPN to determine which amino acids optimised the ratio of activity (expressed as kcat/Km) for CPU/CPN. Aromatic residues or chemically modified aromatic residues were optimum for maximising the ratio of CPU/CPN activity. A summary of results from a pilot study measuring CPU levels generated during thrombolytic therapy for ischaemic stroke was presented demonstrating significant generation of CPU activity. Conditions for the minimisation of ex-vivo proCPU activation were discussed.

A Gils discussed the application of 3 different ELISA approaches to investigate the extent of TAFI activation in clinical studies. It was hypothesised that not the total amount of TAFI protein but the amount of activated TAFI may play a critical role in the interference with fibrinolysis. Therefore, two ELISAs were developed measuring either the activation peptide or activated TAFI (TAFIa). Intact TAFI and TAFI fragments were determined in three different groups of patients i.e. patients with hyperlipidemia, patients with stroke and patients with sepsis.

From the data obtained it was concluded that the ELISAs that measure the extent of TAFI activation are more sensitive markers in studies on the relationships between TAFI and cardiovascular diseases, but assessment of all possible markers may be needed and should be assessed on a case by case basis. Standardisation of the assay was discussed and it was confirmed that recombinant active peptide did not react in the same way as native peptide found in plasma and could not be used as a standard, however it may be possible to express results in pM of peptide with further work.
T Lisman discussed 3 epidemiological studies on venous and arterial thrombosis in which thrombosis risk associated with hypofibrinolysis was investigated. Hypofibrinolysis was assessed with a plasma-based global fibrinolysis assay where plasma is clotted with tissue factor in the presence of calcium and phospholipid vesicles and fibrinolytic potential estimated from clot lysis curves. Three studies were discussed. LETS (leiden thrombophilia study), a case/control study on venous thrombosis (421 pts/469 controls) where hypofibrinolysis was associated with a 2-fold increased risk for a first venous thrombosis. MEGA (multiple genetic and environmental assessment of venous thrombosis), a case/control study on venous thrombosis (2913 pts/2129 controls), where increased thrombosis risk was associated with hypofibrinolysis, and showed an interaction of hypofibrinolysis with factor V Leiden. SMILE (study of myocardial infarction leiden), a case/control study on myocardial infarction in men (426 pts/646 controls), where hypofibrinolysis was associated with an increased risk of MI only in men below the age of 50. The SMILE study also showed elevated TAFI levels (measured by activity assay, Pentapharm) to be protective against MI.

During the general discussion D Hendriks proposed that a number of common samples should be compared using the different approaches described for measuring active CPU/TAFIa.

D-Dimer Chaired by C-E Demple

I Jennings reported on two separate UK NEQAS for Blood Coagulation exercises to explore the degree of precision amongst laboratory D-Dimer measurements, and the degree by which inter-method agreement could be improved using a calibration curve model. The first exercise demonstrated generally good within-centre precision, with 82% centres reporting results for two identical but differently coded samples within 10% of each other. However, 6 centres reported results which would have excluded DVT for one sample but failed to exclude DVT for the other, identical sample. In the second exercise, overall between-method precision of D-Dimer results for two samples was shown to improve markedly when a calibration model was applied, using the consensus median values obtained by all participants for three “calibration plasmas” to recalculate D-Dimer values. For centres reporting results in fibrinogen equivalent units (FEUs), between-centre coefficients of variation (CVs) fell from 25.9% to 11.6% and 22.4% to 7.7% respectively for the two samples. For centres reporting in ng/ml, CVs fell from 45.3-21.6% and 40.8-11.6% respectively. Improved harmonisation of D-Dimer results by different methods may be achieved by a calibration model and common calibrant plasmas.

P Meijer reported results from the latest ECAT study involving 600 participants and covering 25 methods, although 8 methods account for 90% results. More participants report results in FEU rather than ng, in contrast to UK NEQAS studies. Issues of repeatability and the importance of repeat testing for results around the cut-off level were discussed as an approach to minimise false positives and negatives. Sources of variability in assay results were discussed which include the usual reasons of differences between antibodies and reference standards used in different methods but also additional problems from different lots of reagents in the same kits.

Both NEQAS and ECAT quality control studies highlight the large discrepancies of numerical D-dimer values reported, which add to the complications surrounding interpretation of assay results in the clinical context of VTE exclusion. The main cause of discrepancy is the different
calibrations used by the manufacturers of the assays, but problems for clinical decisions arise from choice of cutoff values. These may be taken from package inserts but may be in-house evaluations. Even for individual assays, the cutoff values used may vary considerably between laboratories and VTE exclusion cutoff values have been validated in appropriate clinical trials only for a minority of D-dimer assays. Assay results in the high concentration range are also highly discrepant, which makes it difficult to use D-dimer assays in scoring systems, such as the ISTH-DIC score, or establishing cutoff values for other indications apart from VTE exclusions.

P Meijer also discussed harmonisation of immuno-assay in general with particular relevance to possible approaches to measurement of plasma tPA and PAI-1 antigen. Ideally a standardisation hierarchy should be adopted with a SI units and a primary reference methods at the head, which also requires clear definition of the entity to be measured. This is often not possible for complex biological mixtures (“soups”) and approaches to harmonisation may propose the use of consensus values, but it should be recognised that this approach is “unstable”. A number of proposals were made to improve standardisation and harmonisation of immuno-assays including the organisation of collaborative studies with samples having a range of values to be measured, inclusion of all available methods and assessment of clinical samples where possible and clear understanding of the entity being measured.

C-E Demplfe discussed applications of D-dimer assays for purposes other than VTE exclusion including DIC, aortic aneurysm exclusion, monitoring of anticoagulant therapy (including detection of heparin-induced thrombocytopenia), and monitoring of intensive care patients and risk stratification after stopping anticoagulant therapy. Reporting on a common scale is particularly important in these cases as is the ability to measure a wide range of D-dimer values.

A summary of a consensus statement issued at a meeting on D-dimer hosted by the ECAT was presented which included the following points:

- Fibrin fragment D-dimer is a terminal product of plasmin proteolysis of fibrin containing crosslinked C-terminal gamma-chains.
- D-dimer antigen indicates antigenic material detected by use of monoclonal antibodies generated by immunization with fibrin fragment D-dimer or related compounds.
- The minimal structure detected by D-dimer antigen-specific monoclonal antibodies is fibrin fragment D-dimer. Larger compounds containing dimerized D-domains are detected as well.
- In clinical plasma samples, fibrin fragment D-dimer represents only a portion of the total D-dimer antigen. A major portion of D-dimer antigen in clinical plasma samples has a higher molecular weight than fibrin fragment D-dimer. Based on these findings, fibrin fragment D-dimer is not a primary candidate for a calibrator.
- Since D-dimer antigen is not a homogeneous entity and monoclonal antibodies against D-dimer antigen react with different antigenic sites of the D-dimer antigen structure, a primary reference standard cannot be formulated.

It was suggested, that pooled plasma from patients with high D-dimer antigen concentration be used for harmonization, applying a series of dilutions of the pooled plasma as used in NEQAS and ECAT quality control.
C-E Dempfle showed results from the Fibrin Assay Comparison Trials (FACT) parts 4 and 5. FACT4 compared assay reactivity of fibrin fragment D-dimer and pooled plasma from patients with DIC, using serial dilutions in plasma from healthy blood donors, and in buffer. Some D-dimer assays displayed identical dose-response with fibrin fragment D-dimer and the pooled plasma samples, some assay reacted considerably better with the low molecular weight fibrin degradation product than with the predominantly higher molecular weight fibrin contained in the plasma, and other assays responded poorly to fibrin fragment D-dimer. For the pooled plasma, assay reactivity was similar for dilutions with plasma and buffer. It was concluded that a single pooled plasma with high concentration of D-dimer antigen would be sufficient as common calibrator, with no need to prepare sets of dilutions, or pooled plasma with different levels of D-dimer antigen.

In the recently completed FACT5 trial, reference laboratories of assay manufacturers received a pooled plasma with high concentration of D-dimer antigen for preparation of serial dilutions with assay-specific diluents, and a set of 50 pooled plasma samples with different levels of D-dimer antigen. The correlation of assay results for all 30 assays included was excellent, with a mean regression coefficient of 0.946±0.054 (range 0.703 – 0.999). Common calibration with the pooled plasma reduced the coefficients of variation from nearly 60% to approximately 20%.

Since the procedure for harmonization based on consensus values produced variable results depending on the set of assays included, a new procedure was suggested by C-E Dempfle to generate D-dimer antigen values on a common scale for all D-dimer assays. This procedure is based on the distribution of a pooled reference plasma with high concentration of D-dimer and an assigned D-dimer level to the assay manufacturers. The D-dimer concentration is assigned by a procedure involving ‘homogenization’ of the D-dimer antigen by extensive plasmin digestion of the reference plasma and quantitation of the amount of D-dimer generated with a calibrator consisting of terminal plasmin digest of a cross linked fibrin clot prepared from a known amount of fibrinogen. By the plasmin digestion, all D-dimer antigen present in higher molecular weight form is transformed to fibrin fragment D-dimer, which is a homogeneous analyte. This allows the preparation of successive plasma pools with constant levels of D-dimer antigen.

Future activities will include quality control issues of the procedure used for assigning D-dimer concentration values, and investigations on the effect of lyophilization of the plasma on the results as part of the process of making long term stable standards. The effect of common calibration on future quality control exercises, and the performance of diagnostic algorithms involving D-dimer antigen will be other topics.

Plasminogen Activators and Plasmin chaired by O Matsuo

C Longstaff reported the conclusions of a collaborative study to determine tPA antigen in 4 samples: (1) SSC/ISTH secondary coagulation standard lot 2; (2) SSC/ISTH secondary coagulation standard lot 3; (3) NIBSC Preparation 94/730; (4) NIBSC Preparation 86/670. In total 14 sets of results comprising 48 independent assays were analysed using 8 different methods: 6 commercial kits and 2 in-house methods. Results for the 2 SSC/ISTH plasma samples were similar and within the expected range at 2.9 and 3.0 ng/ml for lot 2 and 3, respectively. The overall mean antigen value for 94/730 was close to 25 ng/ml, the expected
value based on the formulation of this preparation and on past studies. Data were also analyzed using local standards and a common standard for all assays: 94/730 with an assigned value of 25 ng/ml. In this analysis the mean antigen values for the SSC plasmas were not changed from the analysis using local standards but there was a modest reduction of up to 7% in inter-laboratory gcv. Analysing data according to method, grouping different methods or kits, highlighted significant differences between methods. However, it was possible to correct for these differences and harmonise results for normal plasma pools using data from the SSC plasma samples which produced significant reductions in % gcv, and left the antigen values unchanged. Sample 94/730 (recombinant tPA in plasma) would make a satisfactory reference preparation for tPA antigen determinations in plasma with a consensus value of 25 ng/ml. It was recommended that 94/730 be proposed as the WHO 1st International Standard for tPA antigen in plasma. SSC coagulation plasma lot 3 can be assigned a consensus value of 3.0 ng/ml tPA antigen. The process of approval developed by SSC/ISTH was followed to prepare a report for the WHO Expert Committee on Biological Standards to recommend establishment of 94/730 as an International Standard for tPA Antigen. Approval statistics and comments from the collaborative study participants (10/13), a panel of experts with a background in fibrinolysis standardisation issues (9/13) and Fibrinolysis Subcommittee co-chairs were summarised. Among the responders no one disagreed with the proposals that 94/730 should be established as the WHO 1st IS for tPA antigen in plasma with a value of 25 ng/ampoule or the ISTH/SSC coagulation plasma lot 3 should be calibrated at 3.0 ng/vial. The only comments received suggested possible improvements to the calibration process which would include the use of expert laboratories rather than collaborative studies and consensus values. Another respondent pointed out a possible source of variability in the data obtained not identified in the report which could be due to matrix effects and “cryptic” tPA. No further comments or objections were received from the meeting.

C Longstaff reported results from a collaborative study to measure PAI-1 antigen in plasma recently completed with an aim to investigate the possibility of harmonising results obtained for PAI-1 measurements using different methods. Participants were provided with 5 different samples comprising 3 freshly collected frozen small plasma pools containing Low (L), Medium (M) and High (H) levels of PAI-1 and 2 lyophilised plasma preparations from pools of donors, which were the SSC/ISTH secondary coagulation standard lot 3 and a sample prepared at NIBSC coded 06/053. Twelve sets of data were returned comprising 7 methods, designated A-G. As expected, results for the 5 samples were highly variable between methods. The 5 samples were assigned a consensus value for PAI-1 antigen content as the arithmetic mean value from the 12 sets of data. A regression equation was then calculated from plots of each laboratory’s results for the 5 samples versus the consensus value. Conversion factors for slope and intercept were calculated for each method to make it possible to convert results from each method into values on the consensus scale. The harmonisation process worked well for most methods except one, which determined a different ranking for the PAI-1 content of the 5 samples from all other methods. The harmonisation procedure allows expression of results on a common scale so results can be compared. However, the consensus approach does not allow determination of PAI-1 antigen content in absolute units of real ng/ml and further work is required to achieve this. The results obtained raised questions about the normal range of circulating PAI-1 antigen in plasma.

C Thelwell reported on the possible usefulness of a standard of 4-nitrophenol for the standardising active site titrations using NPGB. Traditionally IS have been calibrated IU
following an international collaborative study using laboratory’s own in-house methods. There has been a recent movement towards introducing SI units for standard preparations. Such a standard might be useful in conjunction with active-site titrants available for a range of proteases including plasmin, thrombin, urokinase and factor Xa, and associated inhibitors. NPGB, a suitable active-site titrant for trypsin, and an example of a collaborative study to establish the 1st IS for Alpha-1 antitrypsin (proteinase inhibitor) in units of moles of active inhibitor was briefly described. Trypsin cleaves NPGB to release 4-nitrophenol, which can be measured by absorbance and converted into moles of active enzyme based on a 4-nitrophenol calibration curve. This approach relies on the accuracy of generating the calibration curve. This accuracy could be improved if a standard for 4-nitrophenol was available to eliminate variation introduced in the preparation of stock solutions. Fluorimetric active-site titrants also exist, such as MUGB, which offer greater sensitivity. A standard for 4-methylumbelliferone could be used to calibrate titrations with MUGB. This approach would allow new and replacement protease (and associated inhibitor) standards to be calibrated in molar concentrations as well as assigning SI units to existing IS. This was recommended as a possible approach for standardising plasmin as it will soon be necessary to work on replacing the existing 3rd IS.

P Vandeberg presented work on the use of active site titration for the value assignment of a reference standard for plasmin, used during development of therapeutic plasmin which is being investigated as a direct acting thrombolytic. This standardization was done for the purpose of maintaining consistent dosing. The method used is essentially the same as what was published by Chase and Shaw (Biochem Biophys Res Commun. 1967 Nov 30; 29(4): 508-14.), but has been adapted for use in 96 well plates for manual or automated execution. Work involving crossover testing with the International Reference Preparation for Plasmin (97/536) using an amidolytic assay (chromogenic substrate S-2403) was also covered, although concerns were raised over the long term stability of the WHO IS for Plasmin which mitigated against over-reliance on this preparation.

Fibrinlisis Subcommittee Announcements by C Longstaff

C Longstaff closed the meeting with several short announcements related to Subcommittee activities. These included a request from the European Pharmacopoeia for a standard for plasmin inhibitor (alpha-2-antiplasmin) which is needed to measure the remaining inhibitor activity in virus inactivated human plasma used therapeutically. The feasibility of making such a standard will be explored and a request was made for groups interested in taking part in a collaborative study should contact C Longstaff.

An update on the modification of the Instructions for Use accompanying the 3rd IS for Streptokinase 00/464 was provided. It is proposed to recommend that the IS only be used for native streptokinase or for recombinant streptokinase that has been checked for suitable activity in fibrin and non-fibrin-based assays. The presence/absence of fibrin can lead to discrepant results using the 3rd IS for Streptokinase with some recombinant products and this is potentially dangerous. Where discrepant results are obtained there is currently no way of assigning a potency with the 3rd IS for Streptokinase.
An update was provided on the status of the WHO 1st IS for Streptodornase. This IS will now likely need to be replaced following earlier consideration that it might be discontinued.

There was one Fibrinolysis SSC publication in the past year briefly reporting the outcome of a study on fibrinolysis methods for potency determinations of streptokinase, tPA and urokinase, C Longstaff et al, J Thromb Haemost 5(2) 412-4: 2007.
Hemostasis and Malignancy

Chair: M. Prins (The Netherlands)
Co-chairs: G. Agnelli (Italy), A. Falanga (Italy), C. Francis (USA), A. Lee (Canada), L. Zacharski (USA)

M. Prins opened the meeting and the first session on laboratory aspects was chaired by A. Falanga and L. Zacharski.

E. Gray (UK) reported on the progress of the Working Party on TF standardization in cancer. A first set of 6 samples has been sent to 5 laboratories. There was a considerable variation, in part related to the type of samples sent to the laboratories (cell lysates vs recombinant) and assays used. E. Gray presented an update for the Working Group on TF standardization in cancer. The panel of this Group is composed by: A. Falanga and T. Barrowcliffe (Coordinators), E. Gray, N. Key, B. Osterud, K. Mann, S. Butenas, B. Osterud, K. Mann, S. Butenas, N. Mackman, J. Morrissey, F.R. Rickles. The need for creating a task force to standardize the procedures for TF measurement in malignant tissues comes from the knowledge of TF relevant role in cancer. There are many methods to measure TF in tissues as well as in circulating blood. However, the sensitivity and specificity of the available assays are variable. The specific aims and proposed activities of the TF working group are: 1. To compare measurements of TF with a variety of methods in different laboratories; and, 2. To improve intra- and inter-laboratory reproducibility by development of standardised protocols, appropriate reagents and reference materials. Gray presented the results of the pilot collaborative study aimed to investigate the suitability of a panel of candidate TF reference materials (freeze-dried): purified tissue factor, recombinant, cell lysates (THP-1, NB4). The participants of the pilot study were the 7 Working Party laboratories. Assay methods utilised were: clotting, chromogenic and antigen. So far 6 out of 7 participating labs have returned results. A report of the pilot study to participants will be done by August 2006. Future plans (Time frame – 2007/2008): 1- to assess suitability of current batch of purified recombinant TF (may be able to share a batch with Working Party on Thrombin Generation Tests), assess suitability of freeze-dried cell lysates: cell numbers, stimulated or unstimulated, excipient. Prepare large scale batches of tissue factor and cell lysate; 2- to initiate main international collaborative study on proposed candidates; 3- dependent on collaborative study results, establish candidates as ISTH/SSC references –; and 4. standardise assay methods.

N. Key (USA) reported on the measurements of circulating TF. It is possible to measure both antigens and activity and to measure this in different compartment of the blood (plasma, microparticles, cells). He concluded that measurement of activity of whole blood components would be most ideal.

M. Marchetti (Italy) reported, also on behalf of and A. Falanga on testing for the non-anticoagulant effects of heparins. She described several assays used to quantify and assess differences between specific types low molecular weight heparins.

S. Osanto (The Netherlands) reported on MP-associated TF activity in cancer patients. She showed that there was a clear link between MP-associated TF activity and cancer stage and
between the development of VTE complications in cancer and higher MP-associated TF activity. Finally MP-associated TF activity seemed highly related to cancer survival.

**L. Zacharski (USA)** forwarded a hypothesis Iron stores as a link between malignancy and coagulation disorders. Based on a common epidemiology in relation to age and mechanisms, excess iron inducing procoagulant as well a mutagenic potential iron stores were hypothesized to be a common link by triangulation. Several studies on lebotomy to lower ferritin concentration provide evidence that indeed this is linked to a lower incidence of arterial thrombosis and cancer.

The next part of the meeting addressed clinical issues in the relation between cancer and thrombosis.

**G. Meyer and P. Girard (France)** gave on behalf of the steering committee of the TILT study a presentation of the design of this study that assesses the Effect of low molecular weight heparin on survival of stage I, II, or IIIA non-small cell lung cancer. A multicenter, open, randomized controlled trial. Low molecular weight heparin will be given during the entire period of chemotherapy. The primary outcome is survival. It is planned to include 800 patients.

**A. Lee (Canada)** gave an update on the FOCUS study. The general objective is to identify a potentially efficacious and safe dose of dalteparin as an adjuvant agent in women receiving standard chemotherapy for newly diagnosed ovarian cancer, for phase III investigation. The study wants to determine the effect of 3 selected doses of dalteparin on CA-125 response in women receiving standard chemotherapy for extended ovarian cancer, in addition to the determine incidence of symptomatic VTE in this group of women and establish the safety (bleeding) of dalteparin when given with chemotherapy over a 3-month period. Finally the study will determine the feasibility of once daily subcutaneous injections and women compliance with self-injections over a 3-month period. Also a substudy to explore the relationships between tumour biology and activation of coagulation will be performed. Currently 60 patients are included.

**H. Büller (The Netherlands)** gave an update on the INPACT study. It is a prospective, randomised, open-label, multicenter study to evaluate the survival in patients with Lung (NSCLC, Stage III-B), Prostate (Hormone refractory), or Pancreatic (locally advanced) cancer. Eligible patients will be randomised to: standard anti-cancer treatment, or standard anti-cancer treatment plus nadroparin. All patients will have standard anti-cancer treatment: 14 days weight-adjusted “full therapeutic” dose, followed by 4 weeks half dose, then a 4-week wash-out period, finally 2-week once daily full dose + 4-week wash-out period for several cycles. This last cycle can be repeated up to 6 times in the absence of contraindications. The primary outcome is death from cancer. Currently 200 patients are included.

**A. Kakkar (UK)** presented a study on LMWH in gastric cancer. This trial will be conducted in India and will include patients with stage III/IV gastric cancer. Patient are randomized to usual care with or without added LMWH. The trial is open and plans to include 600 patients.

**H.M. Otten** provided an update on the Trousseau study (Screening for Occult Malignancy in patients with idiopathic VTE). Trousseau study: “Screening on malignancy in patients with an
idiopathic VTE: Effect on mortality”. It is a prospective, multicenter, cohort study. The objective is to see the effect on mortality of screening with CT chest/abdomen + mammography versus routine in patients with idiopathic VTE. Inclusion criteria are: Objectified VTE, No risk factor, 40 years, First VTE, No signs of malignancy at routine examination. Trousseau is ongoing and reached approximately 500 patients and an interim anterim analysis will be presented at the ISTH 2007.

A. Falanga (Italy) presented a study on the incidence of VTE in cancer patients receiving adjuvant chemotherapy. Trghwe overal incidence of VTE during chemotherapy was 8.0%. There were no additional risk identificators.

A. Khorana and C. Francis (USA) reported on the development and validation of a predictive model for chemotherapy-associated thrombosis. Leucocyte count, platelet count age, high BMI and cancer types were included in the model. Based on the risk score the incidence of thrombosis was as low as 2% in the lowest risk group and reached 7% in the highest risk groups in the validation stage.

M. Monreal (Spain) reported on potential differences in efficacy and safety of different LMWH’s for the s econdary prevention of VTE in cancer patients. The data were based on the RIETE registry. It was remarked that the study was not randomized and that dosing regimens between these LMWH’s differed considerably.

I. Pabinger updated on the CATS (Cancer And Thrombosis Study). Aims of this study are to evaluate the incidence of venous thromboembolism in cancer patients and to identify predictive parameters for the development of venous thrombosis and pulmonary embolism in patients with malignancies. It is a prospective nested case control study. Patients with newly diagnosed cancer of the central nervous system, breast, lung, kidney, the gastrointestinal or genitourinary system, sarcoma or haematological malignancies (multiple myeloma, high and low grade lymphoma) or progression of disease after complete or partial remission, are enrolled into the study. At today enrollment has been completed. Dr. Pabinger reported that surgery, radiotherapy and high platelet count at baseline were risk factors for VTE. Full results are presented at the ISTH 2007.

A. Kakkar presented the first results of a Prospective Registry of Cancer and Events Involving Venous Thromboembolism (PERCEIVE). In this prospective multicentre study of newly diagnosed malignancy (Pancreas; Lung; Prostate; Breast; Colon and rectum; Ovary), patients will be treated according to local best practice, no additional tests or procedures will be required. Selected data will be collected from the patients’ clinical records. Patient progress will be monitored for up to 1 year, with special attention to medical history, VTE risk factors, treatment and outcome. Primary objective is to collect data on the clinical incidence, treatment and outcome of VTE; secondary objectives are to produce evidences to help set standards of practice to improve patients’ clinical care and expected outcome in terms of both prevention and treatment of VTE, and to identify areas of interest for future studies to investigate specific related issues. PERCEIVE registry. The overall incidence of thrombosis was somewhat lower than expected (approx. 3%), and mainly occurring close to the diagnosis of cancer.
S. Schulman called for active participation in the registry of Recurrent VTE in cancer patients. This registry represents a collaborative project of the two subcommittee, on “Control of anticoagulation” and on “Haemostasis and Malignancy”. Recurrence of VTE occurs in spite of adequate anticoagulation in 7-27% of cancer patients per years. There is also a high risk of major bleeding on vitamine K antagonists in patients with cancer (5-13%/year). There is no guideline for the treatment of such recurrences. This registry collects data on 200 events of this type, the treatment provided and the effect and safety thereof. The data will create a basis for future trials. Currently there are only 10 reported events and participation was highly recommended.
Lupus Anticoagulant/Phospholipid-Dependent Antibodies

Chair: V. Pengo (Italy)
Co-Chairs: P. de Groot (The Netherlands), M. Galli (Italy), T. Ortel (USA), J. Rand (USA), G. Reber (Switzerland), R. Roubey (USA), A. Tripodi (Italy)

Vittorio Pengo (Italy) opened the session reminding speakers to be concise and that their presentation will last 10 min with 5 min discussion. No discussion if the presentation lasts 15’.

He then started his presentation on the survey on Lupus Anticoagulant LAC diagnosis made by central evaluation of positive plasma samples. Main results were that a false positive diagnosis of LAC is significantly more frequent in older patients, in those first diagnosed with LAC, in those in whom LAC is mild or on oral anticoagulant treatment. Knowledge of these characteristic may help physician and clinical pathologist to suspect false positive LAC.

Armando Tripodi (Italy) proposed the preparation of reference material for LAC determination. The aim of the project was to prepare certified lyophilized plasmas with assigned potency and to develop a system of standardization/quantification based on the above certified plasmas. Phase I consists in preparation of candidate certified plasmas, in their certification with potency assignment and definition of the scheme. Phase II will be a field study to assess the value of the scheme.

Ian Jennings (UK) confirmed the need for reference material for the diagnosis of LAC and illustrated the strategy and progress on the production of a candidate International reference plasma panel.

KM Devreese (Belgium). LAC activity due to β 2 glycoprotein (β 2GPI) antibodies shows a high correlation with thrombotic events. Methods to discriminate between β 2 GPI dependent LAC and LAC caused by antibodies to various phospholipid-binding proteins could be a promising tool in the diagnosis of APS.

Guido Reber (Switzerland) stressed the need for standardization of anticardiolipin (aCL) and anti b 2Glycoprotein I (a b 2GPI) at the level of companies.

Silvia Pierangeli (USA). Cut-off values in aCL and a b 2GPI were calculated in a huge number of patients and confirmed to be similar to those proposed by most companies.

Doruk Erkan (USA) reported on the real world experience with antiphospholipid antibody and how stable are the results over time. aPL results remained stable for at least three quarters of subsequent tests, regardless of the laboratory performing the test; the small amount of variation that occurred did not appear to be caused by aspirin, warfarin, or hydroxychloroquine use.

Philip de Groot (The Netherlands). The risk of thrombosis among healthy persons who have antiphospholipid antibodies have to be established. Ideally, a prospective study in which healthy persons with incidentally found positive antiphospholipid antibodies are followed, should give the answer. The second best is large population based case-control studies that are now available.
and that can be used to establish the thrombotic risk in patients with antiphospholipid antibodies who had experienced a first arterial or venous thrombosis. These studies have now been performed and they showed that when only elevated levels of anticardiolipin antibodies are considered, anticardiolipin antibodies were not related to venous or arterial thrombosis in patients with a first event but without any sign of autoimmune disease. For a 2GPI antibodies, not enough studies are available to draw a firm conclusion. There is evidence that lupus anticoagulant is an independent risk factor in patients without signs of autoimmunity.

**Bas de Laat (The Netherlands)** Through the years the validation of the anti-beta2GPI antibody ELISA has been proven to be a point of concern. In addition, several groups have shown that a conformational change in beta2GPI is needed before antiphospholipid antibodies are able bind beta2GPI. The different purification methods of beta2GPI result in beta2GPI preparations with different conformations which might explain a part of the variations in the anti-beta2GPI antibody ELISA. We and others have shown that the pathological antibodies are directed against domain I. By performing the ELISA with domain I, this change in conformation is not needed as domain I is already accessible for antibodies. This might be one of the reasons for its high correlation with thrombosis compared to regular anti-beta2GPI antibody ELISA’s. In addition, it has been shown that anti-domain I antibodies are better correlated with thrombosis even when beta2GPI is properly folded. In conclusion, the anti-domain I ELISA might be a better assay for the detection of pathogenic antiphospholipid antibodies than the anti-beta2GPI antibody ELISA.

**Monica Galli (Italy).** To assess the clinical significance of lupus anticoagulants (LA) and antiphospholipid antibodies (aPL) towards thrombosis and abortions, we measured them in 112 patients whose samples were available at enrolment in the WAPS study. When considered separately, IgG antiß2-glycoprotein I (aß2GPI) and prothrombin (aPT) antibodies were associated to anamnestic arterial and venous thrombosis, respectively, and those to annexin AV (aAnAV) with abortions. IgM antibodies to protein S and the lupus ratio of the dilute prothrombin time were associated with prospective thrombosis. LA-positive patients who carried IgG aß2GPI and aAnAV antibodies were at risk for both anamnestic abortion and prospective thrombosis.

**Ingrid Pabinger (Austria).** We performed a study in 4,756 individuals, in whom anticardiolipin antibodies were determined, and evaluated their mortality during follow up. Anticardiolipin antibodies were associated with cancer mortality but not with vascular mortality. Therefore anticardiolipin antibodies are not predictive to identify patients with high cardiovascular mortality.

**Robert Roubey (USA).** An update on the Antiphospholipid Syndrome Collaborative Registry (APSCORE). Collaborative studies on collected plasma are wellcome.

**Waander van Heerde (The Nederlands).** AnxA5 form 2D lattices on physiological cell membranes. Anti-β 2GP1 and β 2GP1 disturb only the 2D lattice formation. The prothrombotic tendency in patients with antiphospholipid syndrome may be explained by interference into 2D lattice formation of AnxA5.
**Luis R Lopez (USA).** aPLs, specially a b 2GP1 occur frequently in patients with chest pain-ACS and are associated with higher rates of vascular complications. The presence of oxLDL/ b 2GPI complexes are significantly associated with severity and poor outcome of coronary artery disease.

**QUESTIONNAIRE PROPOSED TO 13 SPEAKERS**

1. Would you like to eliminate anticardiolipin ELISA from screening tests for antiphospholipid syndrome? 9 NO 4YES
2. Would you like to eliminate IgM isotype from ELISA tests for antiphospholipid syndrome? 8 NO 5YES
3. Do you agree with Sydney consensus recommendations that testing for anticardiolipin antibodies alone an APS diagnosis can be made? 7 NO 6YES
4. Do you agree with Sydney consensus recommendations that testing for antibeta2GPI antibodies alone an APS diagnosis can be made? 6 NO 7YES
5. Guideline for LAC diagnosis (Brandt T&H 1995): should be they updated? 5 NO 8 YES
6. Would you like to change the lab classification categories (category I=multiple positivity; Category IIa=LAC positive only; Category IIb=aCL positive only; Category IIc=abeta2GPI positive only) for the diagnosis of antiphospholipid syndrome? 7 NO 6 YES
Perinatal/Pediatric Hemostasis

Chair: G. Kenet (Israel)
Co-Chairs: J. Journeycake (USA), P. Massicotte (Canada), P. Mathew(USA), P. Monagle (Australia), W. Muntean (Austria), N. Schlegel (France)

A: Pediatric thrombosis- chairs: P Massicote, U Nowak-Gottl

1. Recurrence of venous thromboembolism in pediatric population: A Chan

Recurrence is a major end-point in VTE studies, yet there are few evidence based data about this issue in pediatric patients. Comparison between neonates and children was emphasized, focusing on outcome studies available for neonatal RVT. Neonates exhibit a very low risk of reported recurrence as compared to older children. Spontaneous VTE, prothrombotic risk factors and presence of co-morbid states have been shown to increase recurrence rate, whereas D-Dimer elevation and higher factor VIII have been associated with adverse outcome of pediatric VTE. The duration as well as intensity of anticoagulant treatment to prevent recurrence in children still need to be elucidated.

Recommendation: More data should be collected on recurrence rate among neonates and children, including impact of anticoagulation therapy and presence of risk factors.

2. Factors affecting recurrence of stroke and CSVT in pediatric patient: U Nowak-Gottl

Data on recurrent stroke and Cerebral Sinus vein thrombosis (CSVT) from pooled international databases held at the hospital for sick kids, Toronto, University of Munster, Tel Hashomer hospital- Israel, and Great Ormond Street hospital, UK were presented. The impact of thrombophilic risk factors upon risk of recurrent stroke was evaluated. Data were available from 678 stroke patients (age range: 1 month to 21 years) followed for a median of 36 months. Recurrence rates were significantly higher among patients with cardiovascular diseases and increased lipoprotein(a) levels. The presence of any prothrombotic risk factor doubled the risk for recurrence after adjustment for presence of cardiovascular disease, whereas the use of either antiplatelet or anticoagulant therapy significantly reduced it.

For CSVT recurrence, 396 children were prospectively followed. Age at onset (>2 years, non-administration of antipagulants, persistant venous occlusion and presence of FIIG20210A variant were independently associated with higher recurrence risk.

Recommendations: Due to the paucity of information regarding the risk of recurrent stroke in children, pooled international data collection and further collaborative studies are strongly encouraged

B: Therapy of pediatric Thrombosis. Chairs: W Mountean, M Bonduel

The differences between children and adults treated with unfractionated Heparin (UFH) regarding bleeding tendency, monitoring tests, the mechanism of action and half life of UFH were discussed.

Recommendations: Further specific and targeted studies of pharmacokinetics, dosing schedules and monitoring strategies in children should be done. A position paper, which recommends the desired strategies for assessing UFH therapy in neonates and children has been suggested.

2. TPA in pediatric stroke: K Leofond

TPA is being given for childhood stroke according the adult guidelines with small deviation, despite the differences in physiologic and fibrinolytic systems of children as compared to adults. Preliminary data of TPA therapy in pediatric patients with stroke were presented.

Due to minimal information concerning the safety and appropriate dosing of tPA in childhood stroke, a multi-centre cohort safety and dose-finding study has been designed to assess tPA in childhood stroke by the International Pediatric Stroke Study (IPSS).

Recommendation: International collaboration is encouraged.

3. New Anticoagulants in children: G Young

Newer anticoagulant agents are available and licensed, with potential advantages over heparin, low molecular weight heparin and warfarin. Summery of bivalirudin pilot study and preliminary data on argatroban study in children with thrombosis were presented. Laboratory studies utilizing thromboelastography with five different anticoagulants and their potential in-vitro reversibility by rFVIIa were discussed.

Recommendation: Randomized controlled clinical trials are recommended since the use of anticoagulation recently increased in children despite lack of information.

4. Thrombolysis in pediatric DVT-new study proposal: M Manco Johnson

A proposal for a new international multicenter study has been made, based upon better outcome with lower rate of post-phlebitic sy in children reported treated with systemic thrombolysis as compared to standard anticoagulant therapy.

Recommendation: Studies are required to asses the role of thrombolytics (either systemic or regional vs heparin alone) in pediatric VTE.

C: Pediatric bleeding –chairs: N Schlegel, M Rand

1. Towards a standard bleeding score in pediatric patients: M Rand

Bleeding history evaluation in children is often diffuculat and has been challenged before. A specific adjusted bleeding questionnaires has been validated in Sick-kids hospital, Toronto.
Pediatric-specific bleeding questionnaire based on the ISTH Bleeding questionnaire for the diagnosis of Type 1 von Willebrand Disease (VWD) was applied in collaboration with colleagues in: Kingston, Canada; Vicenza, Italy and Oakland, USA.

**Recommendation:** Use of standard bleeding questionnaires should be practiced, leading to better diagnosis and treatment for children with bleeding disorders

2. **Bleeding score and questionnaire adopted for children:** N Schlegel

The feasibility and efficacy of questionnaire and bleeding score in neonates, infants and children who were scheduled to undergo various surgeries, was presented.

A proposal for use of bleeding score composed of combined questionnaire results, lab tests and surgical bleeding risk assessment was made.

**Recommendation:** Collaborative international data collection of bleeding score results and outcome is proposed

3. **Experience with VW questionnaire in pediatric patients:** C Bidingmayer

A study that evaluated peri-operative PTT screening was discussed. Retrospective chart analysis of 492 consecutive patients (age 1-17 yrs., Median 5), referred for hemostatic assessment, after evaluation of PTT screening and history, for standardized laboratory workup was presented. Only in 35% of patients prolonged PTT was confirmed, 2.4% suffered from significant bleeding disorder (BD). The positive predictive value (PPV) to detect BD for prolonged PTT in combination with positive bleeding history was higher.

**Recommendation:** Since PTT screening yields many false positive results, an effort of the ISTH Pediatric SSC to validate standard questionnaires and scores for evaluation of bleeding risk in children undergoing surgery is urgently required.

**D. Perinatal Hemostasis – chair: J Journeycake**

Neonatal IVH: past, present and future perspectives: P Mathew

Neonatal IVH is responsible for many adverse sequelae including post hemorrhagic hydrocephalus, cerebral palsy and death. Reducing the risk of progression to higher grades of IVH in VLBW infants and thereby reducing adverse long term outcome by proper intervention therapy should be attempted.

A study to investigate the natural history of early germinal matrix-(GM-IVH) in very low birth weight (VLBW) infants and to evaluate the safety of using rFVIIa in preterm infants was presented

**Recommendation:** A prospective study of the natural history of neonatal IVH has been proposed and submitted for funding.
Plasma Coagulation Inhibitors

Chair: E. Gray (UK)
Co-Chairs F. Bernardi (Italy), S. Kitchen (UK), H. Whinna (USA)

WHO international Standards

Proposed international standard for protein C, concentrate. E Gray (UK)

In 2006, a collaborative study was carried out to value assigned a replacement international standard for protein C, plasma and a new international standard for protein C, concentrate. The candidate plasma preparation, 02/342 was subsequently established by the Expert Committee on Biological Standardisation (ECBS) of the World Health Organisation (WHO) in October 2006 as the 2nd International Standard for Protein C, Plasma. It was value assigned against the 1st International Standard (IS) for Protein C, plasma (86/622) and the labelled values are 0.85 and 0.84 IU/ampoule for function and antigen respectively.

For the proposed concentrate standard, 04/252, all participants and the SSC subcommittee approved the proposed assigned antigenic value of 14.3 IU/ampoule against the 1st plasma IS. However, based on their in-house experience, one participant did not agree with assigning the candidate with an overall functional potency ie combining the values obtained for chromogenic and clotting assays. As this has implications for the comparability of the proposed candidate with clinical products, further consideration and study was required to clarify this issue and so the recommendation to establish the 1st IS for Protein C, Concentrate was deferred until more discussion and/or data are available.

NIBSC and the participant who raised this concern have now carried out a joint study involving assays of a number of their production batches by chromogenic and clotting methods and have found that there is a distinct discrepancy between the potencies obtained by chromogenic and clotting assays. Taking into consideration that 02/342 has been established as the 2nd IS, the following new proposals and options were presented to the participants of the study and a panel of SSC experts: the candidate concentrate standard, 04/252 to be assigned with potencies relative to the 2nd International Standard for Protein C, Plasma, 02/342 with the two options. The first option is to label with chromogenic and antigenic value and the second option is to label with functional chromogenic and clotting potency and antigen value.

All participants agreed with the proposed assigned value for antigen. All participants agreed with the proposed assigned value for functional chromogenic activity. Five out of the 20 participants also agreed with labelling with functional clotting potency. All SSC experts agreed with the assigned values for antigen and functional chromogenic activity only. Therefore it will be recommended to the ECBS of the WHO to establish 04/252 with an antigenic and a functional chromogenic value of 14.5 and 15.0 IU/ampoule respectively.

Replacement of the 2nd international standard for antithrombin, concentrate. E Gray (UK)
Twenty-one laboratories participated in a collaborative study to establish a replacement for the 2nd International Standard for Antithrombin, Concentrate (96/520). There was excellent agreement between laboratories, as indicated by low inter-laboratory % GCV for the 3 candidate materials which consisted of one recombinant and two plasma derived clinical concentrates. In terms of performance, stability profile and physical characteristics of the candidates, all 3 materials are very similar. However, there is a larger number of ampoules of sample C, 06/166 available. It is therefore proposed that sample C, 06/166 is considered as the 3rd International Standard for Antithrombin, Concentrate, with labelled potencies for both functional (4.4 IU/ampoule) and antigenic (4.5 IU/ampoule) activities. All participants have agreed with this proposal. All participants and SSC experts agreed with this proposal. Therefore it will recommended to the ECBS of the WHO that 06/166 to be established as the 3rd iS for Antithrombin, Concentrate, Human.

**Protein S and protein C**

**Protein C and protein S assay discrepancies experience from UK NEQAS for Blood Coagulation. I Jenning (UK)**

Accurate diagnosis of PC and PS deficiency depends on precise and reliable laboratory methods. UK NEQAS for Blood Coagulation exercises have identified discrepancies in results for PC and PS assays between users of different sources of commercial kit. For PC assays, marked differences (>10%) have been observed over the last 2 years between the two kits most widely used by participants in the programme, both employed by >60 laboratories.

In-house investigations used the SSC secondary plasma standard lot #3 as a calibrator for the two kits, and results for both lyophilised and frozen plasma demonstrated good agreement when this calibrator was used. Data indicated that the potency for one commercial reference plasma was incorrectly assigned by approximately 6%, which contributed to the discrepancy in the NEQAS exercises. A further UK NEQAS exercise in which a patient sample and the SSC secondary plasma standard were distributed demonstrated improved agreement in the patient sample results when results for both methods were cross-calibrated against the SSC plasma standard.

Discrepant results continue to be observed between PS kits, and the importance of locally-determined reference ranges is demonstrated by a >10% error rate in diagnosis of PS deficiency if a local range is not utilised.

**Protein C and protein S assay discrepancies - North America experience. R Marlar (USA)**

Often there is a lack of correlation between thrombophilia diagnostic test result and correct clinical phenotype. This could be due to numerous interfering substances in the tests or other conditions that may affect the test and interpretations of results. For example, genetic deficiency of PC does not correlate with clinical phenotype (venous thrombosis). Plasma level of protein C or genetic mutation do not always predict clinical phenotype. Age was not found to be a factor for thrombosis and major life events do not affect phenotype. Therefore other non-protein C factors must influence the development of thrombosis. Interfering substances or conditions can cause inaccurate test results. For example, heparin therapy can cause decrease of factors utilizing
or influenced by heparin and warfarin causes decrease of vitamin K-dependent protein levels (and increased Antithrombin levels). The interpretation of protein C and protein S test results should take into account that 1. expression of clinical phenotype is not consistent with genotype, 2. thrombophilia phenotype is a multi-mechanism disorder based on multiple genes & acquired RF, 3. inconsistencies of test results can be due to patient, pre-analytical variables, interfering substances and/or assay problems and 4. cost effective protocol of diagnostic tests should be ordered based on prevalence and understanding.

Global coagulation/haemostatic tests

Progress on the activities of the working party on thrombin generation tests.
E Gray (UK) on behalf of the Working Party on Thrombin Generation Tests

The Working Party (WP) on Thrombin Generation Tests (TGT) was set up in 2004 under the auspice of the Plasma Coagulation Inhibitors subcommittee. The main remit is to investigate, standardise and validate methodologies for the quantitation of results to facilitate good within and between laboratory agreement. In 2004 a survey on current TGT methods was carried out and the WP published a mini review (on line publication www.bloodmed.com The Thrombin Generation Test (TGT) by Lawrie et al.). In 2005 the survey results and a pilot study results within WP on chromogenic non-sub-sampling methods were presented at the SSC. In 2006 a study on “Fluorogenic Methods for Thrombin Generation Tests” was initiated and completed. It is the intention of the WP to publish the data on the findings of the fluorogenic method study in 2007 and also to initiate and complete a study on the feasibility of establishing a reference plasma for thrombin generation tests. The WP also has plans to investigate the application of thrombin generation tests in the study of haemophilic plasma.

Report on the international collaborative study on thrombin generation tests.
E Gray (UK) on behalf of the Working Party on Thrombin Generation Tests

The main aim of this study is to investigate the feasibility of establishing a reference plasma for thrombin generation tests. One hundred and ten labs returned results and in total there was 128 sets of data available for analysis. Six coded freeze-dried samples including 3 candidate normal pooled platelet poor plasmas, the SSC Lot#3 (included for comparative purpose only) and 2 abnormal plasmas were provided. Four commercial methods (CAT, Technothrombin, Dade-Behring-ETP and In-TDT) were used by the participants and results were also obtained for 4 in-house methods. This study confirmed results from the previous study that a reference plasma would improve intra- and inter-laboratory variability. The Working Party therefore recommends the establishment of a reference plasma for Thrombin Generation Tests. Further discussion will be required to determine how the reference plasma should be used. The Working Party will also make a proposal to the SSC to establish the reference plasma as a SSC reference material.

Inter-laboratory evalutation of the TGA Assay. P Meijer (NL)

The first survey on the Technothrombin TGA concluded that the inter-laboratory variation observed depends upon the read-out variable and the level of thrombin generation. There is a difference in quantification of thrombin generation between laboratories and that this difference
is affected by the type of instrument used. This second survey aimed to investigate if there is improvement in the inter-lab variation, are there still quantification differences, does the effect of instrument still exist and could harmonization improve result comparability. Forty-one participants were involved and a set of 8 plasmas including normal and abnormal samples were sent to each lab. The inter-laboratory variability for the measurement of thrombin generation in a normal pooled plasma is comparable in both surveys. The quantification differences between laboratories still exist and that these differences are more systematically higher the higher the thrombin generation. There are small but not statistically significant differences between fluorimeters. There are obvious differences in the inter-laboratory variability between fluorimeters. Harmonisation is only possible if there is a good correlation between two samples. Samples with low to very low thrombin generation did not show a good correlation with a normal sample. Harmonisation of absolute thrombin generation did not improve the inter-laboratory variation. Harmonisation by expressing readouts relative to a refrence plasma shows a small improvement of the inter-laboratory variation.


Four manufacturers of Thrombin Generation Tests (TGT) Kits took part in a wet workshop to demonstrate their details of their techniques on 9 plasma samples provided by ECAT. The 4 kits were CAT (Thrombinoscope), Technothrombin, TGA (Technoclone), Dade-Behring-ETP (Dade Behring) and In-TDT (Pentapharm). Two variants of two of the kits were also included, so in total there was 6 different methods.

The samples included plain pooled plasma, pooled plasma spiked with hemolysed cell material, alpha-2-macrogloblin (alpha-2-M), argatroban, unfractionated heparin, an ultracentrifuged pooled plasma with low level of microparticle, protein S congenital deficient plasma, a factor VIII congenital deficient patient and a plasma with lupus antibodies.

The absolute data could only be compared when expressed relative to the pooled plasma. It was observed that the 9 plasmas showed quite different TGT profiles and read-out. Method specific effects were also noted. Some of these effects on a single sample were further investigated by ECAT, others will also be follow-up to fully define the differences between the methods which are clearly present. It was identified that ultracentrifugation of a plasma highly reduced thrombin generation in some methods. This was not corrected by exogenous lipids, but only by reconstitution with microparticles (MPs). MPs are a major determinant of some methods. It was observed that increase in alpha-2- M had a strong effect on some read-outs of some methods and further investigation is carried out on this observation. It was observed that argatroban (a direct thrombin inhibitor) reduced the read-out on thrombin activity, but did not properly identified what happened with prothrombin conversion when compared with F1+2 generation. It is recommended that each method should be considered as different. This will provide opportunities for specific applications, with refinement by selecting specific read-outs.

Thrombin generation induced by cancer cells. G Gerotziafas (FR) on behalf of GT Gerotziafas, C Prengel, E Verdy, I Elalamy, J-F Bernaudin
Several lines of evidence show that thrombotic risk is different in patients suffering from different histological types of cancer. Experimental studies have shown that cells from some histological types of cancer express tissue factor (TF) which is implicated to their metastatic and angiogenetic potential. However, the influence of cancer cells on blood coagulation has not been adequately studied. The procoagulant potential of pancreatic and breast cancer cells (BXPC3 and MCF7 cell lines respectively) when they are in contact with human platelet-poor plasma (PPP) were evaluated. In addition the procoagulant activity of cancer cells using a specific anti-TF antibody was titrated. The contact of cancer cells with recalcified PPP resulted in acceleration of TG as compared to the control. This effect was manifested by a significant decrease of the lag-time, and time to Peak of thrombin (ttPeak) and by significant increase of the mean rate index (MRI) of the propagation phase of TG as compared to the control experiment. Cancer cells induced a slight increase of thrombins’ Peak but they did not significantly influence the endogenous thrombin potential (ETP). Both cell lines when issued from cultures with 40% confluence showed higher procoagulant activity as compared to that manifested by cells from cultures with 90% confluence. BXPC3 had significantly more potent procoagulant activity compared to MCF7 cells. BXPC3 manifested maximum procoagulant activity at the concentration of 2 cells/ml whereas MCF7 manifested maximum effect at the concentration of 200 cells/ml. The incubation of cancer cells with an anti-TF antibody resulted in a concentration dependent inhibition of their procoagulant effect mainly on the lag-time of TG. Significantly higher concentration of the anti-TF antibody was required for 50% inhibition of the effect of BXPC3 on thrombin generation as compared to that required for 50% inhibition of MCF7 procoagulant activity. In conclusion, pancreatic cancer cells (BXPC3) and breast cancer cells (MCF7) accelerate thrombin generation of human plasma in a TF dependent manner. BXPC3 have a significantly more potent procoagulant activity than MCF7 probably due to increased TF expression. The number of cells suspended in plasma and their proliferative status according to the level of confluence, are important determinants for the procoagulant potential of the studied cancer cell lines. Chronometric parameters of thrombogram (lag-time and ttPeak) and the mean rate index of the propagation phase of TG seem to be more sensitive than ETP and thrombin’s peak to detect the TF dependent procoagulant potential of cancer cells. A specific anti-TF antibody might serve as calibrator for the evaluation of the TF-dependent procoagulant potential of cancer cells from different histological types of tumors.

Behaviour of different anticoagulants in thrombin generation tests. M Samama (FR)

Three different patterns of thrombograms measured by the CAT were reported. Type 1 was typical of irreversible thrombin inhibitor, hirudin. Hirudin prolonged lag time, but had no effect on peak and ETP. There was an artifact at low dose which was due to mathematical inadequacy in the software to resolve the thrombin generation curve produced by hirudin. Type 2 was typical of reversible thrombin inhibitors such as dabigatran, argatroban, melagatran. These inhibitors all prolonged lag time, decreased velocity and lower peak thrombin and ETP. Type 3 was typical of danaparoid and fondaparinux. These inhibitors had minor influence on lag time, but dramatic decrease in velocity and lowering of peak thrombin. Dermatan sulphate had almost no effect on lag time but decreased peak thrombin and ETP.
With regards to low molecular weight heparins, the importance of antithrombin activity on the thrombograms has been presented. Full detail on the effects of low molecular weight heparins on thrombogram has been published by Gerotziafas et al (JTH, 2007; 5, 955-962)

**Thrombin generation in patients with arterial and venous thrombosis.**

H Spronk (NL) on behalf of HMH Spronk, AWJH Dielis, AJ ten Cate – Hoek, M Marchetti, R van Oerle, MH Prins, A Falanga, K Hamulyák and H. ten Cate

Thrombin generation (TG) has been shown useful to detect a hypercoagulable state in individuals at risk of venous thrombosis. In the current study we investigated the applicability of TG by means of the Calibrated Automated Thrombogram (CAT) under conditions of hypercoagubility in plasmas from patients suffering from one of the following conditions: acute myocardial infarction (AMI), deep vein thrombosis (DVT), or a chronic myeloproliferative disorder (MPD, such as essential thrombocytosis (ET)).

For the AMI group, mean age was 62 years, 74% was male (n=100). TG was increased and shortened at 0d (lag time (LT) 0.86, ETP 1.07, peak height (PH) 1.19), but decreased and prolonged at 4d (LT 1.43*, ETP 0.99, PH 0.80*) when all patients received heparin/LMWH. Patients with heparin levels >0.05 U/mL at 4d had prolonged LT (1.43), and decreased ETP (0.81) and PH (0.64) compared to patients with levels below 0.05 U/mL (LT 1.04*, ETP 1.26*, PH 1.51*). Heparin concentration correlated with LT, ETP and PH (R=0.37*, -0.82* and -0.87*). Between 4d-3m, TG changed to levels comparable with 0d (no differences between 3m and 6m).

The DVT cohort consisted of 72 males (46%) and the total group had a mean age of 56.1 years (17.5-82.6), with no difference between males and females. TG slightly increased with age. At all visits lag time, ETP and peak height at 1 pM TF were significantly increased in patients compared to healthy subjects. In patients, TG measured at 1 pM TF changed between V1 and V3 with a shortened lag time (-26.1%), increased ETP (+11.2%) and peak height (+13.7%). TG measured at 5 pM showed the same changes (lag time -20.4%, ETP +9.2%, peak height +13.7%). Healthy subjects showed no changes over time. Addition of TM to the 1 pM TF assay reduced ETP in all patients at all visits (between -8.8% and -19.50%) - but considerably less than in normal pool plasma (-48%).

ET-patients had shorter LT (2.10±0.48 vs 2.39±0.33 min*) and time to peak (4.08±0.73 vs 4.50±0.43 min*) compared to controls for TG with 5 pM TF, while no differences were observed in ETP and PH. Similarly, at 1 pM TF, patients showed shorter LT (3.69±0.88 vs 4.56±0.68 min*) and time to peak (6.5±1.4 vs 8.2±1.3 min*). In addition, PH (279±55 vs 244±62 nM*) and slope (106±36 vs 75±35 nM/min*) were increased in MPD. Among MPD, ET patients had higher ETP (1521±219 vs 1290±369 nM.min*), PH (290±49 vs 245±63 nM*), and steeper slope (112±37 vs 87±32 nM/min*) compared to PV patients.

In conclusions, as compared to healthy individuals, the TG shows alterations in time that may indicate systemic hypercoagulability in patients after AMI, DVT, or with ET. The sensitivity of the assay differs per patient population, such that test conditions may have to be adjusted per indication (eg. for DVT, TG assessed with 1 pM TF is more sensitive than 5 pM TF). Finally,
TG is very sensitive to the effects of anticoagulant treatment. Prospective follow up of these patient cohorts should establish the predictive values of TG for recurrent arterial and venous thrombosis.
The Kallikrein-Kinin subcommittee met on July 7, 2007 in the Mont-Blanc room of Palexpo. The session was extremely well attended, with more than 80 participants, surely a new record for this subcommittee. The session was introduced by the current chair, Dr. McCrae who reviewed the procedure for an ISTH publication, and suggested the possibility of the group preparing a manuscript on the role of the KKS in thrombosis. Follow up with specific group members will be obtained after the meeting.

The meeting then proceeded with a number of scientific publications, each of which was followed by a lively discussion. A brief synopsis of these presentations is provided below.

Dr. Keith McCrae discussed the role of kininogen in the regulation of angiogenesis, particularly with respect to the newly developed mouse model in which one of the two kininogen genes has been deleted. These animals display abnormalities in angiogenesis and tumor growth, though the underlying mechanisms have not yet been defined.

Dr. Alvin Schmaier discussed the mechanisms by which BKB2 receptor deficient mice are protected from thrombosis. These include the increased generation of the BK derived peptide RPPGF, which inhibits thrombin and thrombin signaling through PARs, as well as increased generation of NO and PGI2 mediated through the AT2R. He also described preliminary studies in the PRCP deficient mouse, which is prothrombotic.

Dr. Thomas Renne discussed a number of studies relating to the KKS and thrombosis. A number of studies were presented demonstrating that deficiency of FXI and FXII reduced cerebral damage in stroke models. He also discussed the role of naturally occurring polyphosphates as physiologic activators of FXII, and described studies demonstrating that FXII deficiency was protective in a murine model of polyphosphate-induced lethal pulmonary embolism.

Dr. Heiko Herwald discussed the role of the KKS in gram positive and gram negative bacterial infections. Bacteria activate several KKS system proteins, including factor XII and HK, and elevated levels of activated factors and BK are observed in sepsis. Moreover, HK derived peptides, including the HKH20 peptide are potent antibacterial agents, with killing activity similar to that of defensins.

Dr. Jonas Emsley discussed structural aspects of FXI and prekallikrein. Several aspects of the crystal structures of these proteins account for their similarity and differences. For example, a gly(FXI)-cys (PK) difference at position 321 explains the ability of FXI, but not PK, to dimerize. Activation of PK by prolylcarboxypeptidase was difficult to explain on a purely structural basis given the location of the putative PRCP cleavage site on PK.

Dr. Robert Colman discussed the role of HK domain 5 on angiogenesis and inflammation. Studies in a 3D collagen-fibrin gel system suggested that the major antiangiogenic mechanism of
HKa reflects its ability to inhibit endothelial cell migration rather than induce endothelial cell apoptosis. This may be mediated through ERK activation. In terms of inflammation, HKa induced the secretion of multiple cytokines from monocytes, including MCP-1 and IL-8. The receptor for these activities has not been defined, but signaling through JNK, NFKB and p38 MAPK, but not ERK, appears to be involved.

Dr. David Pritchard discussed studies using a new antibody that is highly specific for FXIIa. Whether this antibody reacts with FXIIa from other species has not yet been determined, though it stains fixed tissue strongly for FXIIa. The relevance of FXIIa measurements in a number of ACS studies were also described. Interesting, the fourth quartile of FXIIa is an independent risk factor for death in most of these studies, and has strongest prognostic activity in the subgroup of patients presenting with chest pain, but without increased tropomyosin in plasma—i.e. those in whom acute MI was ruled out.

Dr Jose’ W.P. Govers-Riemslag discussed the topic of whether the KKS system is relevant to clinical thrombosis. Discrepant results for this question have been described in a number of clinical studies. The hypothesis that a U-shaped curve may account for these abnormalities was presented. At low FXIIa levels, fibrinolysis may be decreased, while at high levels coagulant processes are significantly stimulated. For FXII in the middle quartiles, risk may not be significantly changed.

A brief discussion was held at the end of the meeting on whether the KKS subcommittee would be the appropriate subcommittee to champion a C1 esterase inhibitor standardization project. It was felt that this would be appropriate.
Platelet Immunology

Chair: T. Warkentin (Canada)
Co-Chairs: B. Chong (Australia), A. Greinacher (Germany), Y. Gruel (France), V. Kiefel (Germany), H. Kroll (Germany)

Committee Co-Chairs (not in attendance): None

The program was divided into several parts: (I) Autoimmune Thrombocytopenia, (II) Alloimmune Thrombocytopenia, (III) Drug-Dependent Thrombocytopenias, (IV) Autoimmune HIT, and (V) Heparin-induced Thrombocytopenia.

Information item: Our committee received as an information item a proposed standard for human antibody against HPA-1a (minimum potency preparation) NIBSC code 05/106 (report of Paul Metcalfe & Mathew Breirley) and this information was transmitted to Dr. Koen Mertens (Chair, WHO-ISTH Standards Liaison Group). This item is for information only as the ISBT is primarily responsible for this study.

AUTOIMMUNE THROMBOCYTOPENIA (Chairs: B. Chong, V. Kiefel) 15:45-16:15h

An update of the activities of ICIS were presented. Several unresolved issues in ITP diagnosis and management were listed. Four prospective studies were described, two completed (Registries I and II), and two ongoing (Splenectomy Registry, PARC-ITP). Certain interesting results were highlighted (e.g., marked male predominance in very young ITP children <1 yr of age). Various substudies ("Trees in the PARC") were listed, e.g., Genetics/Single Nucleotide Polymorphisms, Severity of Bleeding, Quality of Life, Refractory ITP, Secondary ITP, and ITP during Pregnancy. Publications of ICIS were listed. The ICIS ITP Expert Meeting (Sep 2006) in Yverdon Switzerland was discussed, with six categories of ITP defined (primary, prolonged, chronic, intermittent, secondary, Evan's syndrome), with staging and response criteria summarized.

F. Rodeghiero Standardization of terminology and definitions in ITP: an update. A summary of the recent efforts to standardize terminologies and definitions for ITP was presented. The heterogeneity of the current literature in this regard was emphasized. Outcomes of the recent meeting of the European Hematology Association (Vienna, June 2007) and information on a planned consensus meeting (Vicenza, October 2007) of ~25 experts with predefined program and structured questionnaires were provided. A relevant website regarding these activities was provided (www.tcpeha.org). In the Discussion, the Platelet Immunology SSC Chair commented upon the potential value of including a laboratory component (autoantibody detection) within the framework of definitions in order to help spur relevant research into laboratory testing for these disparate conditions.

ALLOIMMUNE THROMBOCYTOPENIA (Chairs: Y. Gruel, V. Kiefel) 16:15-17:15h
J.A. Peterson, M.L. Gitter, B.C. Pietz, S.C. Johnson, B. Curtis, R.H. Aster: Simultaneous testing by Multicode â -PLx of DNA for all known platelet alloantigens. After listing the various known platelet alloantigen systems, a technique known as Multicode â -PLx was described, which is a 3-step assay for multiple alleles in a single tube. The technique utilizes multiplex PCR, allele-specific extension, with adherence to "addressed" Luminex beads for detection. The use of a synthetic nucleotide (iC) allows for complementary binding of biotin-iG, for detection by streptavidin. Forty-two different alleles (21 allele systems) could be tested using just 3 wells per patient assayed, with 100 patient samples per day being analyzed. The technique allows for clear discrimination of homozygous and heterozygous allele status.

B. Curtis, RH. Aster: Do ABO blood group alloantigens ever cause NAIT? The authors presented a case in which considerable evidence was provided indicating that the cause of neonatal thrombocytopenia might well have been maternal anti-B alloantibodies. A minority of blood group A and B individuals are "high expressors" of A and B antigens, including on platelets (on PECAM, GPIIb/IIIa). In particular, the "type II" hyperexpressors have a sharp peak with greatly elevated blood group antigen on platelets. In the case presented, the data supported the concept that maternal anti-B resulted in thrombocytopenia in the affected neonate (3rd pregnancy) who was a high expressor of blood group B antigen on platelets. Evidence presented included high-titer anti-B in mother, reactivity of mother's serum with father's GPIIb/IIIa by MACE, absorption of antibody using B red cells, low H expression in father and children, absence of identifiable "specific" platelet alloantibodies, and lack of thrombocytopenic in a subsequent (4th) pregnancy in whom the baby was blood group A, and so forth. The authors suggest considering ABO incompatibility as a possible explanation for NAIT when no other cause can be found, and a relevant ABO discrepancy exists.

I. Socher, C. Andrei-Selmer, G. Bein, H. Kroll, S. Santoso: Severe neonatal alloimmune thrombocytopenia caused by low-avidity anti-HPA-1a alloantibodies: detection by surface plasmon resonance. In 15-25% of cases of suspected NAIT due to anti-HPA-1a alloantibodies, such antibodies cannot be detected. The technique of surface plasmon resonance (SPR) technology permits a rapid assessment in real time without the need for monoclonal antibodies and washing techniques, as it relies on detection of antibodies based upon kinetic (association/dissociation) and affinity considerations. In this technique, HPA-1a and -1b alloantigens are immobilized using HPA-1a-specific monoclonal antibodies. Antibodies can be distinguished by SPR based upon their avidity of binding. The authors propose that antibody avidity may represent a parameter to predict bleeding complications in NAIT.

V. Kiefel, H. Kroll, A. Reil, J. Bux: External quality assessment in platelet serology—conclusions from the German experience. The results of an external quality assessment (EQA) in German labs of platelet serology were reported. Beginning in 2007, two assessments per year are planned (2002-2006, once/yr), involving 2 sera (obligatory), 2 DNA samples (HPA-1,2,3,5 obligatory, HPA-15 optional), and 2 platelet samples (optional). Various problems were described (e.g., difficulty in obtaining certain test samples in sufficient quantities, the expectation of laboratories that only antibodies that are easily detectable using commercial reagents would be tested). Specific frequencies of correct reporting of results for various serologies (including presence of anti-HLA alloantibodies) and DNA testing in various years were shown. The presentation listed the reactivities that are expected to be correctly assessed in the future (HPA-1,
2, 3, 5, 15; presence of anti-HLA alloantibodies; platelet autoantibodies; platelet cross-matching,
antigen testing (DNA and serological for HPA-1), and quality of counseling by lab staff).

C. Ghevaert, P. Stafford, K. Campbell, C. Proulx, G. Smith, L. Williamson, E. Ranasinghe,
N. Watkins, J. Huntington, W. Ouwehand: Immunological and structural analysis of ten
novel domain-deletion β3 integrin probes designed for detection of HPA-1 and HPA-rare
antibodies. Given the importance of the GPIIIa (β3) domain as a target for several platelet
alloantibodies implicated in NAIT (both common and rare) the strategy of using domain-deletion
β3 integrin probes was described. Of note, some alloantibodies reacted with all 4 peptides,
whereas some reacted only against the longer fragments. This approach could be used for
detecting anti-HPA-1a alloantibodies, though it remained unclear to what extent antibodies not
detected by routine assays would be detected using this strategy. Other peptides were generated
with rare alloantigen targets.

C. Kaplan and G. Bertrand: NAIT and rare platelet alloantigens. This presentation listed
some of the rare platelet alloantigens and the various pitfalls involved in establishing the role of
putative rare platelet alloantigens in cases of NAIT.

DRUG-DEPENDENT THROMBOCYTOPENIA (Chairs: H. Kroll, T.E. Warkentin)
17:15-17:45h

R. Aster, D. Bougie: Sensitivity and specificity of lab testing for drug-induced immune
thrombocytopenia: quinine and vancomycin as models. Dr. Aster noted that the J. George
criteria for evidence for drug-induced thrombocytopenia do not include lab testing. There is little
information on sensitivity and specificity of lab testing for drug-dependent antibodies in the
literature. Results of screening the normal population for quinine-, sulfamethoxazole-, and
ceftriaxone-dependent antibodies showed that antibodies were rare with quinine and sulfa, but
11/466 were positive (4 strong) with ceftriaxone. Some of the difficulties in obtaining such data
from hospitalized patients receiving drugs (e.g., vancomycin) were listed. However, of 25
patients who received vancomycin without developing thrombocytopenia, none had detectable
antibodies. Patients suspected of having vancomycin-induced thrombocytopenia but in whom
antibodies were not detected generally had other explanations for thrombocytopenia found. The
authors suggest that sensitivity and specificity of testing for vancomycin-dependent antibodies is
probably high. Brief mention was made of a murine model under development utilizing SCID
mice in which it may be possible to show in vivo pathogenicity of human drug-dependent
antibodies (thus avoiding re-challenge in patients).

D. Bougie, B. Curtis, R. Aster: Naturally-occurring anti-GPIIb/IIIa antibodies. The speaker
reviewed the frequencies of thrombocytopenia with six different GPIIb/IIIa receptor antagonists.
The frequency of naturally-occurring antibodies with three clinically-used agents (abciximab =
1.6%; tirofiban = 1-2%; eptifibatide = 0.5-4%) correlates with the approximate frequencies of
abrupt-onset thrombocytopenia seen in clinical practice. For abciximab, the situation is
somewhat more complex, in that 20% of normal individuals have antibodies that react with
abciximab-coated platelets, of which 92% of these react against the antibody's papain cleavage
site in abciximab, and the remainder (1.6% overall) react against murine sequences incorporated
into abciximab that confer specificity for GPIIb/IIIa.
A. Greinacher: Amegakaryocytic thrombocytopenia occurring with anti-GPIIb/IIIa inhibitors. This presentation was waived by the presenter (Committee Co-Chair, A. Greinacher) in the interests of keeping the session on schedule.

AUTOIMMUNE HIT (Chair: A. Greinacher) 17:55-18:15h

T.E. Warkentin, R. Jay, M. Makris, J.G. Kelton: Spontaneous HIT. Three cases were presented that appeared to have clinical HIT (thrombocytopenia, thrombosis or other known sequelae of HIT, presence of strong platelet-activating anti-PF4/heparin antibodies) yet without a plausible history of preceding heparin treatment (either in the recent or remote past). Of note, the patients had one or more recent (or concurrent) inflammatory events. The concept that on exceptionally rare occasions patients could develop "spontaneous" HIT—perhaps as a result of associated inflammation—was suggested.

T. Warkentin, B.T. Maurer, R.H. Aster: Fondaparinux-associated HIT? A case was presented of HIT with thrombosis (bilateral adrenal necrosis, DVT, moderately-severe thrombocytopenia) that occurred on about day 7 of fondaparinux thromboprophylaxis post-orthopedic surgery, in the absence of apparent perioperative exposure to heparin. The serologic feature was the presence in patient serum of platelet-activating antibodies that caused 90% serotonin release in the absence of heparin, although serotonin release increased to 96% release in the presence of therapeutic heparin (with 0% release in the presence of high heparin), i.e., serological features of "delayed-onset HIT". Since fondaparinux therapy is known to be associated with formation of anti-PF4/heparin antibodies (some with platelet-activating properties), the concept is that on rare occasions a patient could form antibodies in association with fondaparinux therapy that might cause HIT irrespective of whether any anticoagulant (including fondaparinux) is given/continued some days later. Some implications of the case, and of this model, were presented, i.e., low-dose fondaparinux might not prevent thrombotic events in the setting of severe HIT-induced hypercoagulability; the occurrence of fondaparinux-associated thrombocytopenia should not be taken to infer that fondaparinux might not be an effective treatment of HIT—indeed, by the model proposed, even such a case of apparent fondaparinux-associated HIT might have been treated effectively by use of (therapeutic-dose) fondaparinux.

R.H. Aster: Delayed-onset HIT: musings on pathogenesis. The speaker reviewed the literature on delayed-onset HIT, emphasizing that administration of further heparin (after presenting with thrombosis/thrombocytopenia) can be associated with catastrophic thrombi, and the presence of unusually high titers of HIT antibodies, many of which showed considerable platelet activation in the absence of added heparin. Investigators have shown that residual heparin is not the explanation for the lack of heparin requirement. Animal studies by the Philadelphia group show that the monoclonal antibody KKO binds to human platelets without requirement for heparin when high levels of platelet-associated PF4 are present. It remains uncertain whether delayed-onset HIT is due to high-titer antibodies in the setting of high PF4 expression by platelets, or whether other unusual characteristics of these antibodies are present.

HEPARIN-INDUCED THROMBOCYTOPENIA (Chairs: T. E. Warkentin) 18:15-19:45h
When should HIT be suspected after cardiac surgery?

C. Pouplard, Y. Gruel: Post-cardiac surgery HIT: Temporal profiles. Two presentations of HIT in post-cardiac surgery patients were presented: type 1 pattern (biphasic), and type 2 pattern (monophasic). The previous evidence that the type 1 profile is more common than the type 2 pattern, and more predictive of HIT (as judged by a positive serotonin-release assay [SRA]), was summarized. New data were shown indicating that the predictive values of these patterns for HIT remain true: 6/7 type 1 pattern patients were shown to have HIT, and 1/8 type 2 pattern patients were shown to have HIT (data from May 1/2005 – Jul 1/2007). The importance of a functional test (e.g., SRA) was emphasized for diagnosis in this patient population.

S. Selleng, A. Greinacher: Post-cardiac surgery HIT: Greifswald study. A study of HIT in post-cardiac intensive care unit (ICU) patients was presented. Both type 1 and type 2 patterns were recognized, though the type 1 pattern was not as specific for HIT as seen in the French study (perhaps reflecting the ICU patient composition in the Greifswald study). A new finding was that a further fall in platelet count in the day 5-10 "window" increased the specificity for HIT in patients with the type 2 pattern.

What tests for HIT are being performed?

J. Zehnder, E.A. Price, C. Hayward, T. Warkentin, K. Moffat, J. Moore: NASCOLA Survey of laboratory practices regarding testing for heparin-induced thrombocytopenia. In this survey of 44 specialized labs that perform testing for HIT antibodies, two objectives were stated: to determine current practice in laboratory diagnosis of HIT, and to identify areas in need of standardization and improvement. A wide variety of specimen types/pre-analytic variables were accepted, with two "concerns" being heparin contamination within samples (78%) and that the timing of the blood draw could be too early for antibody detection (75%). Antigen assays (e.g. EIA [ELISA]) are most commonly performed, especially the assay from GTI (71%) versus the one from Stago (27%), with a wide variety of reporting strategies and test cutoffs utilized. A minority of labs had tried the "rapid" assays (particle gel immunoassay and particle immunofiltration assay). Platelet activation assays were performed in only about 1/3 of labs, with similar numbers using "washed platelets" as platelet-rich plasma. Most labs used prescreened donors known to react well in the activation assays. Again, a wide variety of test conditions (e.g., heparin concentrations) were used. The most common pattern of practice (55% of labs) involved screening with a commercial EIA, and referring out of samples to a reference lab for platelet activation assays. A number of items that might benefit from standardization were presented.

Rapid assays for HIT: DiaMed

Y. Gruel, C. Pouplard: Prospective evaluation of 4T's score and rapid particle gel immunoassay specific to H/PF4 complexes (Pa GIA â ID H/PF4 to exclude heparin-induced thrombocytopenia. The aim of the study was to combine the 4T's score and a rapid assay for HIT—the particle gel immunoassay (PaGIA) from DiaMed—in excluding the diagnosis in emergency situation. This prospective study evaluated 213 patients in 4 hospitals with suspected HIT over 11 months. HIT was diagnosed based upon positive EIA/SRA. About one-third (34.7%) of patients had low 4T's score: none had HIT, though 5/74 had a positive PaGIA.
Among the 22 patients with HIT, 21 were positive in the PaGIA, and all 22 in the EIA. In contrast, among the 191 non-HIT patients, 8.4% had a positive PaGIA, whereas 18.3% of 191 non-HIT patients had a positive EIA. The presenters also showed Bayesian approach, and concluded that a negative PaGIA with a low/intermediate score essentially excludes HIT. Also, a high probability 4T's score with a positive PaGIA had a high probability (>90%) of HIT. In other combinations (e.g., high 4T's score, negative PaGIA; intermediate 4T's score, positive PaGIA), the diagnosis of HIT required the SRA. The authors conclude that a low 4T's score is useful for ruling out HIT, and that a negative PaGIA is useful in ruling out HIT in a patient with an intermediate 4T's score. The SRA is useful in many situations for ruling in the diagnosis of HIT.

S.J. McRae, M. Al Muslahi, E.M. Duncan, R. Tadros, J.V. Lloyd, T.E. Warkentin: Evaluation of a pretest clinical score for the diagnosis of HIT. Consecutive patients referred over a 3-yr period with suspected HIT in Adelaide, Australia, were investigated. The DiaMed PaGIA and the platelet aggregation assay (using citrated platelet-rich plasma) were performed prospectively. The 4T's scoring system was applied retrospectively by 2 hematology registrars. Three additional assays—the SRA, in-house EIA-IgG, and EIA-GTI were performed retrospectively by the McMaster Platelet Immunology Laboratory. Presence of HIT antibodies was defined as SRA>50% release plus positive EIA-GTI. Of 115 patients studied, 24 (21%) met the criteria for HIT antibodies. The mean platelet count was 48. Using the 4T's scoring system, almost half the patients (48%) had a low score, 35% had an intermediate score, and 20% had a high score. The incidence of HIT antibodies by test score was 0% (low score), 15% (intermediate score), and 90% (high score). The PaGIA was positive in 17/24 (71% sensitivity) patients with HIT antibodies, with 98% specificity. The platelet aggregation assay was positive in 20/24 (83% sensitivity), with 100% specificity. The EIA-IgG was positive in 24/24 (100% sensitivity), with 94% specificity. Using a Bayesian diagnostic approach, a low 4T's score essentially ruled out HIT, an intermediate score with a positive and negative PaGIA had 85% and 5% probability of HIT, respectively, and a high 4T's score with a positive and negative PaGIA had 99% and 73% probability of HIT. The authors concluded that a low 4T's score ruled out HIT (thus, further lab testing may not be required), that a high 4T's score plus positive PaGIA essentially ruled in HIT, but that in all other situations, confirmatory testing beyond that of the PaGIA was required. Other conclusions were that the EIA-IgG was very sensitive for HIT, and the platelet aggregation test quite specific for HIT.

Rapid assays for HIT: PIFA

J. Francis, A. Drexler, M. Duncan, H. Desai, M. Amaya, T. Robson, T. Meyer, E. Reuyes, A. Amirkhosravi: Performance of the PIFA test for anti-PF4/H antibodies in a prospective study of patients with suspected HIT. This study prospectively compared a Particle ImmunoFiltration Assay (PIFA), from Akers BioSciences, that is FDA-approved based upon >90% agreement with the EIA (from GTI). However, these authors did not find any significant correlation between the GTI and the PIFA among consecutive samples tested using both assays: 32/73 sera testing positive in the EIA were positive in the PIFA, and 68/101 sera testing negative in the EIA were negative in the PIFA, resulting in 57.5% level of agreement (essentially random). Low agreement levels with the EIA from Stago (57.4%) and SRA (59.1%) were also seen. Problems identified with the assay included difficulty distinguishing pos (white) vs neg
A. Greinacher, R. Raschke, J.I. Sheppard, T.E. Warkentin: The operating characteristics of the PIFA test for HIT antibodies. Two centres evaluated the PIFA: the first (McMaster Platelet Immunology, Hamilton, Canada) utilized 93 previously-frozen sera that had been evaluated in a prospective study of the 4T's scoring system and 3 assays (EIA-GTI, EIA-IgG, SRA); the SRA (>50% release) was used to define presence of HIT antibodies. Three different volumes of serum (20, 10, and 5 uL) were used so as to mimic the effect of 2- and 4-fold sample dilution. The second study (Greifswald, Germany) tested fresh (never frozen) serum from 199 consecutive patients referred for diagnostic testing for HIT antibodies, as well as 137 fresh (never frozen) samples obtained in ongoing prospective studies of anti-PF4/heparin antibody formation during heparin therapy. For these studies, 20 uL of patient serum was used. A positive heparin-induced platelet activation (HIPA) test with lag time <30 min was used to define the presence of HIT antibodies. All samples were processed within 24 hr of collection (21.4% within 5 hr). Both the McMaster and Greifswald studies showed that the sensitivity/specificity tradeoffs—plotted as receiver-operating characteristic (ROC) curves—were that of a non-informative assay. In contrast, the EIA's assessed in both centers (EIA-GTI and EIA-IgG in Hamilton, EIA-IgG/A/M in Greifswald) showed excellent operating characteristics, with optimal cutoffs of approximately 1.40 and 0.60 for the EIA-GTI and EIA-IgG/A/M in Hamilton and Greifswald, respectively. The presenter concluded that the assay cannot be recommended.

EIA Controversies

T.E. Warkentin, J.I. Sheppard: Relationship of EIA positivity in relation to onset of HIT. The authors addressed an interesting paradox, namely that the EIA is claimed to have a very high sensitivity for clinical HIT (~99%), yet some advocate testing a later blood sample when an initial EIA result is negative. The authors studied 10 patients who developed clinical HIT during prospective clinical studies to determine to what extent EIA's were positive at the beginning of the platelet count fall marking an episode of HIT. The authors found that the median EIA was strongly positive (~2.20 OD units), with the lowest measuring about 0.70 units, at the onset of HIT. These data were interpreted as indicating that the view that an EIA is initially negative at the time of onset of thrombocytopenia due to HIT is likely to be incorrect.

T.E. Warkentin, J.I. Sheppard: What is the appropriate cut-off for a positive EIA? The authors reviewed the EIA OD distributions of heparin-treated patients, both with and without clinically-significant HIT antibodies (as per SRA >50%), to determine what OD cutoff(s) were appropriate for diagnosis of HIT. Considerable overlap in OD values between the two groups was shown. The authors proposed a reporting system in which the physician is given data on the usual distributions/ranges of OD values for patients testing positive and negative in the SRA, e.g., 90% and 95% of patients with HIT have OD values >1.5 and >0.95, respectively; whereas, for heparin-treated patients, 90% and 95% of patients without HIT have OD values <0.70 and <1.10, respectively (these values are shown for illustration purposes, and do not necessarily reflect the experience of a particular lab).
R.H. Aster, B.R. Curtis: Diagnostic utility of high concentrations of heparin in "confirming" a positive EIA—pro. From >5000 patient samples tested, 5.3% showed a positive EIA that was not inhibited by high heparin. The authors compared the 4'Ts score among 21 patients whose sera tested positive in the EIA and was inhibited by high heparin, versus 31 patients with a positive EIA that was not inhibited. The distributions of probability for HIT (as indicated by "low/intermediate/high" 4T's score) were: heparin-inhibited (2/4/15) vs non-heparin-inhibited (22/6/2). These data suggest that a sample that is not inhibited by high heparin is unlikely to be HIT, and thus useful diagnostic information is provided by this maneuver.

J. Francis: Diagnostic utility of high concentrations of heparin in "confirming" a positive EIA—con. The presenter observed that 12/75 samples were not inhibited by high heparin concentrations, of which 7 and 5 were low and intermediate probability for HIT, respectively. However, 2/12 were positive in the SRA. The presenter also stated that the term "confirmatory" testing was misleading, as it might suggest "confirmation" of the diagnosis of HIT rather than for the detection of anti-PF4/polyanion antibodies. The presenter also observed that performing the high heparin assay will either double the test costs (by requiring high heparin with each sample tested) or increase the turnaround time (by requiring a second assay with high heparin in case the initial test is not performed with high heparin). The lack of published data in support of performing the high heparin step was also commented upon.

J. Amiral: Evaluation of a new EIA for detecting heparin-dependent antibodies. A new assay for pathologic heparin-dependent antibodies (Zymutest) was described that utilizes a heparin-coated surface to which patient serum/plasma as well as platelet-leukocyte lysate is added. The expectation is that non-PF4-dependent (e.g., anti-IL-8/heparin antibodies) antibodies as well as anti-PF4/heparin antibodies would be detected. The assay allows for separate detection of IgG, IgA, and IgM antibodies. A high correlation with antibody detection by standard EIA was reported (r 2=0.890).
Platelet Physiology

Chair: A. Michelson (USA)
Co-Chairs: J. Bennett (USA), M. Cattaneo (Italy), P. Harrison (UK), C. Hayward (Canada), D. Kenny (Ireland), D. Nugent (USA), P. Nurden (France), S. Watson (UK)

This year’s SSC Platelet Physiology Subcommittee Program was held on July 7, 2007 from 8:00 am – 12:00 pm in Geneva, Switzerland. The meeting focused on the standardization of turbidometric platelet aggregation. The topics and speakers were:

2. *The Molecular Basis of Platelet Aggregation*, Alan Nurden, Pessac, France
5. *Clinical and Laboratory Standards Institute (CLSI) Standardization of Platelet Aggregation*, Alvin Schmaier, Cleveland, U.S.A.
7. *Statistical Analyses of Reference Ranges for Platelet Aggregation*, Catherine Hayward, Hamilton, Canada
8. *SSC Working Party on Platelet Aggregation Survey*, Marco Cattaneo, Milan, Italy

These speakers were followed by a general discussion on the road forward with regard to the standardization of turbidometric platelet aggregation.

Registries of the SSC Platelet Physiology Subcommittee are:

- Bernard-Soulier Syndrome (Dermot Kenny)
- Glanzmann Thrombasthenia (Debbie French)
- Non-immune Thrombocytopenia (Amy Geddis/Jim Bussel)

Alan D. Michelson, M.D.
Chair, Platelet Physiology Subcommittee, SSC/ISTH
Predictive Variables in Cardiovascular Disease

Chair: G. Lowe (UK)
Co-Chairs: P. Grant (UK), V. Salomaa (Finland), A. Tosetto (Italy)

Drs Lowe and Tosetto presented a summary of the draft report, prepared by the Chair/Co-Chairs, on Haemostatic Variables in Prediction of Arterial Cardiovascular Events in healthy persons. This was discussed. A further draft will be prepared in October, incorporating the discussion points as well as comments from invited experts. A final draft will be submitted to SSC by end 2007.

Dr Salomaa presented a review of thrombomodulin (antigen, activity and genotypes) and their associations with cardiovascular events. Further work was recommended especially on activity and genotypes.

Dr R Tait presented a study of fibrin D-dimer and other haemostatic variables in prediction of embolic events in persons with atrial fibrillation. D-dimer appeared to add predictive value to the CHADS clinical score. Further management studies were suggested.

A further report on haemostatic variables in prediction of arterial cardiovascular events in persons with established cardiovascular disease was discussed as a possible future Subcommittee activity.

Dr G Palareti reviewed published studies on D-dimer as a predictor of recurrent venous thromboembolic events after discontinuation of oral anticoagulants; and gave an update from the ongoing PROLONG-2 trial. Dr M Verhovsek presented a meta-analysis of the published studies of D-dimer as a predictor of such events. Dr P Meijer reviewed progress with harmonisation of D-dimer assays in management of VTE, including an update from the Fibrinolysis Subcommittee. After discussion, it was recommended that the Predictive Subcommittee should draft a brief report on the predictive value for recurrent VTE of D-dimer (perhaps including an updated meta-analysis, possibly including individual participant data) and other haemostatic variables, in liaison with the Subcommittee on Fibrinolysis. A further report on haemostatic variables in prediction of first VTE events was discussed as a possible future Subcommittee activity.
Vascular Biology

Chair: J-M. Freyssinet (France)
Co-Chairs: M. Berndt (Australia), F. Dignat-George (France), J. Griffin (USA), I. Juhan-Vague (France), P. Newman (USA)

The session was divided into three parts, addressing key issues in vascular biology and related disorders. None of the three topics does specifically belong to the scope of ISTH, which emphasizes the efforts to be made to maintain a leadership in the field.

Regarding microparticles (MPs), the new feature emerging from several presentations was the incidence of MPs in several types of cancers (B. Furie, N. Mackman, A. Weltermann), and more particularly with respect to the presence of tissue factor (TF). In a context where the elevation of MPs has been widely documented in cardiovascular diseases (R. Nieuwland, Y. Ahn), this considerably reinforces their pathophysiologic significance and therefore highlights the need of methodologies for better determination and characterization. A first step was precisely proposed as the “MP challenge” that would consist in the distribution of appropriate materials for the standardization of flow cytometry analysis (B. Furie, F. Dignat-George). Calibrated microbeads should enable to standardize instrument settings, then biological samples could be distributed provided shipment problems are solved with respect to specific national security regulations. The search for correlation with the procoagulant potential(s) of MPs could also be included. If the samples to be tested could be prepared by a single laboratory, pre-analytical conditions would not require particular attention at this stage. For feasibility purpose, suggestions and input from investigators interested in participating in this challenge are welcome, please contact Françoise Dignat-George at: Francoise.Dignat-George@mail.ap-hm.fr. Although it is generally agreed that MPs stem from the plasma membrane of stimulated or apoptotic cells, it has however to be kept in mind that MPs have still to be better defined, e.g. on standardized bases since their physical properties, and probably a proportion of associated biological effects, mainly depend on the method used for their isolation. This is the other main goal of this subcommittee because it is essential to avoid confusion of functions with respect to other membraneous vehicles such as exosomes.

Receptor shedding and shed receptors as plasma biomarkers of vascular injury was the second topic. The fate of three platelet receptors was reported, GPV (J. Clemetson), GPVI (B. Nieswandt, P. Smethurst) and semaphorin-4D (L. Brass). This underscores the significance of the platelet “sheddome” in pathology. Not only the shed receptors have to be considered by themselves but also the pathways associated with, or accounting for, the shedding process. For instance, GPVI can be released as a true soluble truncated form or as a probable full-length membrane protein in MPs, depending on the conditions of platelet activation. In case studies in patients show the value of these, and perhaps other, shed receptors as biomarkers, standardized ELISA methods based on the availability of appropriate antibodies could be proposed for diagnosis and/or for the assessment of treatment efficiency.

Circulating endothelial cells (CEC) are believed to reflect endothelium damage or degeneration whereas endothelial progenitor cells (EPC) are viewed with regenerative potential, at least in cardiovascular disorders knowing they can also be mobilized in tumor development. Regarding
CEC, definition and markers appear consensual. There is a general agreement on CD146-dependent immunomagnetic separation as a reference method, and owing to cell scarcity, there is a trend to combine a first step of enrichment and flow cytometry (F. Sabatier, P. Goon). The situation is somewhat more confusing for EPC as there is no consensus on the definition itself and no appropriate methodologies taking function and phenotype into account (P. Gaussem, J. George, N. Saunders). Hence, definition of EPC constitutes an essential next goal for this subcommittee.

In summary, in its first year of existence, the Scientific Subcommittee on Vascular Biology has identified three main topics to be further investigated at methodological and standardization levels, the interest being demonstrated by the importance of the attendance with up to ~300-350 participants.
von Willebrand Factor

Chair: D. Lillicrap (Canada)
Co-Chairs: T. Abshire (USA), G. Castaman (Italy), J. DiPaola (USA), J. Eikenboom (The Netherlands), E. Favaloro (Australia), A. Federici (Italy), A. Goodeve (UK), B. Lämmle (Switzerland), R. Schneppenheim (Germany)

335 people in attendance

Working Group on VWF assays in the diagnosis of VWD (Christine Lee, UK)

Plasmapheresis of 6 patients with different types of VWD. Prospective study involving 32 laboratories worldwide. Laboratories from 17 countries participated. Laboratories reported a median 6 correct diagnoses. 16 laboratories could perform all of VWF tests. The Lyophilized plasma samples collected for this study maintained their multimeric patterns. All 5 assays are required for optimal diagnostic evaluation. The more tests performed the better. At low levels of VWF the RCo:Ag ratio is problematic.

VWF collagen binding assays (Emmanuel Favaloro, Australia)

A number of laboratories performed VWF:CB in the recent prospective assessment of diagnostic tests for VWD. Discussed the relative merits of the VWF:CB in the VWD diagnostic samples. Bovine and equine tendon material is preferred (type 1 collagen). Can give results that are more sensitive than the VFW:RCo. Discussion of the potential of alternative forms of collagen.

VWF propeptide (Bob Montgomery, USA: Jan van Mourik, Netherlands)

The VWF propeptide can identify patients with accelerated clearance of VWF. Should the measurement of the VWF:pp and the VWFpp/VWFAg be standardized? Prior assessment of the VWF:pp with other existing ISTH and WHO standards for VWF has shown good consistency. The pp:Ag ratio is also consistent. There is an influence of the ABO blood group on the pp:Ag ratio. There is a need to involve more laboratories to extend the assessment of the VWF:pp assay. We need to agree upon the accepted nomenclature for the VWF:pp. Measurement of the VWF:pp and VWF:Ag also allow assessment of endothelial cell perturbation. The respective half-lives of the VWF:pp and VWF:Ag are 2.5 and 12 hrs. The assays of VWF:Ag and VWF:pp can be used to diagnose acquired VWD where VWF is cleared prematurely from the plasma. The plasma level of VWF:pp is 5.5 nmol/L.

VWF Standards (Tony Hubbard, UK)

The current VWF plasma standard is the 5 th WHO FVIII/VWF plasma standard (202/150 established in 2003). 700 ampoules of this standard are distributed each year. There is a need to replace this plasma standard by end of 2009. The 1 st WHO VWF concentrate standard (00/514) was established in 2001. There have been problematic discrepancies using collagen binding assays with the VWF concentrate standard. Collagen binding assays are very sensitive to the HMW multimers. It is important to correlate concentrate and plasma units for diagnosis and
therapeutic purposes. The requirement of the highest MW VWF multimers is still uncertain in therapy. Proposal for a 2nd VWF concentrate with a lack of HMW multimers to try to address the issue of the collagen binding assay discrepancies.

Standardization of VWF propeptide using SSC Lot#3 standard. It is proposed that this initially be undertaken by a small number of laboratories – 5/6 labs. Future international assessment of the VWF:pp standardization. There are currently also two different assays that have been developed in the laboratories of Drs. Montgomery and Van Mourik. There appear to be some pre-analytical factors that will alter the assay results. Working groups need to be established for propeptide measurement and for the use of collagen binding assays in standardizing VWF concentrates. The committee voted in favor of the formation of these working groups.

Acquired von Willebrand Syndrome - aVWS (Augusto Federici, Italy)

There was an initial discussion of the diagnostic background and the associated diseases and the assays needed to make the diagnosis. There is a need for better assays to identify anti-VWF autoantibodies. A working group is already in place to evaluate this diagnosis. An online registry has been established for Acquired VWS. 3 centers have enrolled 12 patients into the online registry. There is an interest in using the registry for assessment of the frequency of aVWS. MGUS and ET were the most frequent accompanying conditions. Also need to assess incidence of aVWS in association with anti-phospholipid syndrome. Finally, there is a need to evaluate novel therapies for aVWS.

ADAMTS13 assays (Koichi Kokame, Japan)

There was a discussion of the various ADAMTS13 assay methodologies that have been developed. All are difficult to use in the clinical laboratory. An international interlaboratory study has been performed and reported but there still needs to be advances for the reliability of this test. There was a description of the VWF 73mer substrate assay using a 73mer peptide sequence around the ADAMTS13 cleavage site. This assay incorporates a FRET readout and is a single step assay. In a large population study, women had higher levels of ADAMTS13 than males. The cause of this variance is as yet unexplained.

ADAMTS13 resistance (Reinhard Schneppenheim, Germany)

The effect of variation of the VWF substrate on ADAMTS cleavage was discussed, with a consideration of the concept of a more resistant VWF molecule. Sequence variation in the A1 region has been shown to produce ADAMTS13 resistance. Type 2B rVWF appears to be ADAMTS13 resistant. In some type 2B mutants this ADAMTS13 resistance is profound. The type 2B New York/Malmo variant is ADAMTS13 resistant. There is a possibility that this could also enhance VWF clearance. This phenotype should be considered in TTP patients in whom ADAMTS13 deficiency is not present. In these patients, A1 domain VWF sequencing should be performed.

TTP - ADAMTS13 mutation registries (Johanna Kremer Hovinga and Bernhard Laemmle, Switzerland and Yoshihiro Fujimura, Japan)
There was a description of the Berne and Japanese experiences with Upshaw-Schulman Syndrome and other forms of thrombotic microangiopathy. Both presenters summarized the clinical experience they had documented. There is now an extensive molecular genetic experience with this condition. There is a proposal for an international registry to improve international awareness, to improve genotype-phenotype correlations and to learn more about the natural history of TTP. Beware the re-reporting of the same patients in the literature. Some patients are studied repeatedly. The committee voted in favor of establishing an international TTP registry. There was further discussion of thrombotic microangiopathic pathologies in 783 patients from Japan. There had been extensive investigation to evaluate the causative pathologies in these cases. Note that acquired TTP can occur in children. The outcome of these cases is poor. Diagnosis is not easy and sometimes wrong.

**Standardized Bleeding scores (Francesco Rodegheiro, Italy and Paula James, Canada)**

There was a review of the history of bleeding scores by the SSC and other bodies. There have been previous publications on this issue from the SSC. There is already a bleeding score questionnaire available at the VWF ISTH website. Ideal features of a bleeding score were described. There is no current ‘gold standard’ for mild bleeding in particular. The aim of this initiative is to improve diagnosis, treatment and communication about bleeding within the community. In Canada, the original Vicenza survey was condensed to be able to administer this in 5-10 mins. This maintains a -1 to +4 scoring system and has positive scores of >=+3 in males and >=+5 in females. Data on >230 subjects was presented from 3 ongoing studies to assess the application of this bleeding score. There was a proposal for the establishment of a joint working group (with the pediatric SSC) to develop guidelines for a quantitative bleeding score. The committee voted in favor of this proposal.

**Report on DDAVP study in VWD (Giancarlo Castaman, Italy/Stefan Lethargan, Denmark)**

The initial part of this international prospective study has been completed. 245 patients have been enrolled. The study will continue as the enrolled patients are followed with therapeutic administration of DDAVP. There are all types of VWD enrolled in the study

**Report on Prophylaxis in VWD (Tom Abshire, USA and Eric Berntorp, Sweden)**

This is a prospective international multicenter study. Central data collection center will occur in the USA and there is a central laboratory site in Sweden. The study will evaluate the effects of prophylactic therapy on joint bleeding, mucosal bleeding, epistaxis and menorrhagia. This is a prospective non-randomized study. Any licensed VWF product can be used for the prophylactic therapy. The treatment strategy will involve a dose-escalation approach. Dose escalation will be carried out based on defined bleeding events. IRB review currently in progress at several centers. Enrolment will start later in 2007.

**Report on type 3 VWD Registry (Augusto Federici, Italy and Paul Giangrande, UK)**
There is need for a much better estimate of the prevalence of severe type 3 VWD. We also have no idea of how many of these patients have anti-VWF antibody development. In some of these antibody +ve patients episodes of anaphylaxis have been reported with the infusion of VWF concentrate. There is a significant need for a standardized anti-VWF antibody assay. Proposal for a multicenter, multinational study involving both developed and developing countries. This will involve an outreach study in which larger expert centers will assist smaller centers in the diagnosis and therapy of this condition. There is an aim to enroll approximately 500 patients for analysis.

VWF mutation and polymorphism database (Anne Goodeve, UK and Dan Hampshire, UK)

A summary was provided of the updated web-based VWF mutation database. There has been a recent significant increase in the reporting of VWD mutations. These include all types of VWD. Mutations are found across the VWF gene and involve all types of mutation. Some new categories of sequence variation have been added to the database. There was a call for the submission of newly acquired data.

VWD type 2B and Platelet type VWD Registry (Maha Othman, Canada)

There was a description of the background of the problem. The clinical implications of the two diagnoses was highlighted. There is no idea what the frequency is for PT-VWD. Discrimination can be done with phenotypic studies or by genotype. A proposal was made for a new international registry for PT-VWD. The committee voted to support the establishment of this registry.
Women’s Health in Thrombosis & Haemostasis

Chair: Andra H. James (USA)
Co-Chairs: Margareta Blombäck (Sweden), Benjamin Brenner (Israel), Jacqueline Conard (France), Sabine Eichinger (Austria), Ian Greer (UK), Barbara Konkle (USA), Claire Philip (USA)

Minutes:

Introduction and Overview:

In the introduction and overview Dr. Andra James presented the

- Purpose and function of the scientific subcommittees
  - to develop standards, methods, nomenclature
  - to address issues of practical importance to the research community
  - to complete a specific program of work
  - to create international collaborations for the purpose of planning, executing and completing projects of benefit to the international community
  - to generate, publish and distribute reports, recommendations and other documents concerning the above

- Route to publication of SSC reports, recommendations and documents
  - development
  - approval by a “vote” of international experts within and possibly outside of the SSC
  - approval by 2 external reviewers
  - approval by the chair of the scientific subgroup who forwards the document to the Chair of the SSC and ISTH headquarters
  - approval by the Chair of the SSC and the ISTH

- Options for publication
  - on the ISTH Website
  - in the Journal of Thrombosis and Haemostasis
  - short communication (1 ½ pages) – usual route for SSC publication – requires approval by editors of JTH
  - full-length article – requires usual peer review and approval by editors of JTH
  - (official SSC documents may not be published elsewhere.)

- Development of standards (similar process to development of document)
  - After approval by ISTH, a recommendation is forwarded to the Expert Committee on Biological Standardization (ECSB) of the World Health Organization (WHO)

Dr. Marilyn Manco-Johnson presented an overview of the history of the Women’s Health in Thrombosis and Haemostasis including its origins and accomplishments.

Educational Activities
Dr. Benjamin Brenner reported on the successful 2nd Symposium on Women’s Issues in Thrombosis and Hemostasis held in Vienna the first week of February 2007 and announced the next symposium to be held February 6-8, 2009.

Report on the Women’s Health in Thrombosis & Haemostasis Subcommittee Inspired or Sponsored Publications

Dr. Brenner reported on a Scoring System for Pregnancy Outcomes. Application of this scoring system to patients at his institution has shown a strong correlation between the score and the level of anticoagulation required during pregnancy. Publication of these results will emanate from his institution. Future directions include, possibly, application to women who have and have not received anticoagulation and an international, multi-center validation of the study.

Dr. Margareta Blomback summarized the Women’s Issues SSC publication, Blomback et al, Preanalytical conditions that affect coagulation testing, including hormonal status and therapy. JTH 2007; 5 (4), 855–858.

Report from the 8th American College of Chest Physicians’ Conference on Antithrombotic and Thrombolytic Therapy

Dr. Shannon Bates summarized her group’s recommendations for the chapter, “Use of antithrombotic agents during pregnancy,” and highlighted the differences from the last version published in 2004. Of interest to the audience were expanded options for the management of women with mechanical heart valves and the absence of a recommendation to screen women with a history of poor pregnancy outcome for thrombophilia. The reasons cited for the latter were weak associations between thrombophilia and poor pregnancy outcome and limited data on the benefits of anticoagulation in these women.

Update on Registries

Pregnancy outcome in women with antithrombin deficiency:
Dr. Jacqueline Conard presented a summary of the incidence of thrombosis during pregnancy among women with antithrombin deficiency. The only contributors to the registry have been from France.

Pregnancy outcome in women with mechanical heart valves:
Dr. James reported that only one subject has been added to the registry this year. Dr. Claire McLintock has been recruited to assist with enrollment. Dr. McLintock reported on 15 cases from New Zealand that she expects will be added to the registry.

Thrombosis and thrombotic risk in women receiving ovarian stimulation for pregnancy:
Dr. Barbara Konkle reported that to date no subjects have been enrolled in this registry and it will be withdrawn.

Update on New and Proposed Registries:
Mirena IUD for menorrhagia in women with bleeding disorders:
Dr. Rezan Kadir reported that she and Dr. Peter Kouides have completed a questionnaire that can be used to gather data about women with bleeding disorders and their experience with the Mirena IUD. The questionnaire is ready to be added to the website.

Application of pregnancy scoring system to neonatal thrombosis – joint activity with the Perinatal/Pediatric Scientific Subcommittee
Dr. Manco-Johnson reported that the concept for this study is evolving into a case-control study. The study will likely not take the form of a registry.

Update on Other New and Proposed Projects:

Monitoring anti-Xa activity during pregnancy:
Dr. Sandy Duncan spoke to the benefits of monitoring anti-factor Xa levels in pregnancy. In the ensuing discussion, questions were raised about the methodological problems in trying to answer the relevant research questions using a registry. An outcome of the discussion was the recognition of the need for a registry for women who have received fondaparinux during pregnancy. Dr. James will pursue the establishment of such a registry with Dr. Duncan.

New Opportunities for Collaboration: Studies of Reproductive Tract Bleeding in Women with Bleeding Disorders:

Dr. Althea Grant summarized the past and current research on women with bleeding and clotting disorders being conducted at the United States Centers for Disease Control and Prevention. Her presentation was followed by a presentation by Dr. Flora Peyvandi on her group’s proposal of a study on menorrhagia in women affected by bleeding disorders. Data would be collected from multiple international sites. There was general consensus regarding the merits of the study and the feasibility of an international collaboration.

Future Meetings:

There was support for the new congress meeting structure that will include educational sessions. The Women’s Health SCC will propose that 1 to 1.5 hours at their next meeting be devoted to education. The two topics that will be proffered are, “Anticoagulation during Pregnancy,” and “Reproductive Tract Bleeding in Women with Bleeding Disorders.”

There were approximately 100 persons in attendance.
Working Group on Coagulation Standards

Chair: A. Hubbard (UK)

Review of Lot #3 (A Hubbard, NIBSC, UK)

Background details for Lot #3 were presented to the WG (source and filling, assigned values, despatch conditions). The SSC/ISTH Secondary Coagulation Standard Lot #3 became available in July 2006, following expiry of Lot #2, and 12,990 vials have since been despatched. The majority of Lot #3 was despatched to manufacturers (7480 vials to 21 different companies in 9 different countries) and a lesser but still considerable amount was ordered by EQA schemes (total of 4900 vials to UK NEQAS and the College of American Pathologists). A relatively small amount was used in calibration exercises and research projects (610 vials). The use in the first year exceeded the annual average for Lot #2 (approx 9,000 vials) and will have a significant impact on the lifetime of Lot #3 if it continues at this rate. Representatives from the EQA scheme UK NEQAS indicated that they will require less vials over the coming year. At least 24 months, but preferably 30 months, notice will be needed to complete the processes of tender and calibration for a replacement to Lot #3. The stability of Lot #3 is under continuous assessment through an accelerated degradation study. Results from testing performed by NIBSC and the Royal Hallamshire Hospital, Sheffield have indicated that Lot #3 is extremely stable with predicted losses per year at -20 °C of less than 0.1% for coagulation factors V, VII, VIII and XI.

Calibration of Lot #3 for t-PA antigen and PAI-1 antigen (C Longstaff, NIBSC, UK)

The results of an international collaborative study to calibrate the proposed 1st International Standard t-PA antigen (94/730) were presented; this study also included the calibration of Lot #3. A mean value of 25 ng/ml (n=12) was obtained for 94/730 (spiked plasma) and a value of 3.0 ng/ml (n=14) for Lot #3 (normal plasma). The study participants and the Fibrinolysis sub-committee have agreed that the mean values should be assigned to 94/730 and Lot #3. The proposed IS (94/730) will be submitted to the Expert Committee on Biological Standardisation of WHO for establishment in October 2007. Following the establishment of 94/730 the proposal to assign a value of 3.0 ng/ml to Lot #3 will be circulated to the Executive Board and to SSC. It was noted that the level of t-PA antigen in Lot #3 was close to the lower limit of detection for some methods and this may compromise its use as a reference standard.

Estimates for PAI-1 antigen in Lot #3 from a study in 2005 and a more recent study (2007) were presented. The recent study included 7 different methods and also included 3 freshly collected plasma pools (low, medium, high) as well as a second freeze-dried, pooled, normal plasma (06/053). Lot #3 and 06/053 were found to have higher PAI-Ag content than the 3 fresh pools and there was considerable inter-laboratory variability for all samples (GCV range 49 – 78%; Lot #3 GCV 53%). After harmonisation of the individual laboratory results, relative to a consensus mean regression curve, the inter-laboratory variability was reduced to a GCV range of 6 – 35% (Lot #3 GCV 14%). There was some discussion over the definition of the “normal” level for PAI-1 antigen. In the absence of an International Standard it was considered premature to assign a value for PAI-1 antigen to Lot #3.
Experience of EQA schemes with Lot #3

UK NEQAS (S Kitchen, UK)

Lot #3 was distributed as an anonymous sample in 2 surveys in 2006 for the testing of analytes FII, FV, FVII, FXI, Protein C, Protein S and Antithrombin. Median FV and FXI estimates differed from the assigned values by 6 – 21 % and 0 – 14 % respectively (depending on reference plasma) and this probably indicates that the recently established International Standards for FV and FXI have not been used for assignment to commercial calibrant plasmas. It is expected that this deviation should be reduced in the future as the IS and Lot #3 are used. Median FII estimates were close to the assigned value (-3 to +4 % depending on reference plasma). Median estimates for FVIII:C relative to 3 reference plasmas were close to the assigned value whereas estimates relative to 2 others differed by 12 and 20 %. Similar discrepancies were also found with other test samples and investigations concluded that this was not caused by mis-calibration of Lot #3. One of the manufacturers has since re-labeled the FVIII:C content of their reference plasma. Median estimates for Antithrombin, by method, recovered the assigned value (difference -1%). Median free Protein S antigen estimates differed by -1 to +8 % of the assigned value depending on method. Overall median Protein S activity differed by 3% from the assigned value and by less than 10% for 3 individual methods; however, one method differed by 19% and was a cause for concern. The median estimates for Protein C activity (chromogenic) differed from the assigned value by 4% overall and by 10% or less depending on the reference plasma used. Out of 168 centres performing a full phenotypic thrombophilia screen 85.5% reported that Lot #3 was normal.

College of American Pathologists (M Cunningham, USA)

Lot #3 was included in a CAP Coagulation Education Survey in 2007 where analytes are measured at the discretion of the participating laboratories. A total of 33 analytes were measured. Mean estimates differed by less than 5% from the assigned value for most analytes. A larger difference (7.1 %) for FV may indicate that the assigned values to the IS and Lot #3 have not been fully assimilated by the manufacturers. Larger overall mean differences from the assigned values (13% and -16%) were also observed for Protein C function and VWF:CB respectively; in the latter case it should be noted that the mean was derived from only 3 estimates. Numerous unlabeled analytes were also measured and these were generally associated with the highest inter-laboratory variability (eg. Prothrombin fragment 1.2, CV 201%; Thrombin-Antithrombin complex, CV 196%). It was noted that this information could be extremely useful to other sub-committees as an aid in the prioritisation of future standardisation needs.

Manufacturer’s perspective

Use of International Standards and SSC Plasmas at Instrumentation Laboratory
(K Trumbull, Instrumentation Laboratory, Lexington, MA, USA)

This presentation described how four departments used International Standards and the SSC Plasma standard. House standards are calibrated directly against the relevant WHO IS with inclusion of Lot #3 to verify the assignment. Different Lots of control plasmas are calibrated relative to the House standard with inclusion of Lot #3 for verification. Lot #3 is also included in
the development/verification of new or modified reagents or parameters in specific instrument systems. Lot #3 may also be used in complaint investigations which could be related to shipping stress or incorrect use of products.

**Additional uses for Lot #3 (A Hubbard, NIBSC, UK)**

Three additional uses for Lot #3, approved by the Executive Board, were presented.

- Lot #3 will be included in a small multi-center study to assess the inter-laboratory variability of VWF propeptide estimation and to also provide a common source of calibration for local references with the objective of improving harmonisation of results including normal and pathological reference ranges.
- Lot #3 has been included in a large multi-centre study on the standardisation of the thrombin generation test (TGT). Although Lot #3 is not proposed as a TGT reference preparation its characterisation in the study could be useful in ensuring continuity between different lots of TGT reference plasmas. Additionally Lot #3 has been used to characterise the proposed TGT reference plasmas by measuring numerous analytes.
- It has been agreed that Lot #3 can be used by EQA schemes for trouble-shooting purposes, specifically the investigation of anomalous results from laboratories identified through EQA surveys. EQA schemes will purchase Lot #3 for this purpose and be responsible for the despatch to laboratories. This use will be limited to a maximum of 1,000 vials of Lot #3 in the first year and will be reviewed at the next WG meeting in July 2008.

**Lot #3 and the JCTLM database (E Gray, NIBSC, UK)**

As an internationally certified reference material Lot #3 is exempt from the need for “CE marking”. In order to maintain this status it is necessary to include Lot #3 on the JCTLM (Joint Committee on Traceability in Laboratory Medicine) database of higher order reference materials. Lot #3 has been submitted for inclusion and this will reviewed in October 2007. There is also a requirement to demonstrate commutability for Lot #3 in the measurement of analytes in patient samples. It is hoped that this can be addressed by reference to the inclusion of Lot #3 in EQA surveys.

**Thrombin Generation Test Reference plasmas (E Gray, NIBSC, UK)**

A large multi-centre study on the Thrombin Generation Test has demonstrated that inter-laboratory variability can be considerably reduced when results are calculated relative to a common reference plasma. This study will lead to a proposal to establish a reference plasma for the TGT, possibly as a SSC reference. Subject to this approval the WG on Coagulation Standards may be requested to act as “curator” for the reference plasma and to oversee the storage, despatch and stability requirements.