51st Annual Scientific and Standardization Committee Meeting

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Biorheology

Chairman: J.J. Zwaginga, The Netherlands
Co-chairs: T. Diacovo, USA ; E. Grabowski, USA ; M. Hoylaerts, Belgium;
G. Nash, UK(absent)

In a well attended meeting (60-80 persons) several new distinguished members of the Subcommittee gave presentations in 3 sessions. In the sessions 2 important issues that will further be addressed by the Subcommittee were clearly reflected. a). the need to define rheologic parameters for intravital microscopy studies in conjunction with the Animal Model Subcommittee and the need to translate available literature on a. flow incorporating devices that test general hemostasis and b). on fundamental biophysical studies on cellular bonding kinetics and associated computational modelling of these interactions, towards better practical use for clinicians and drug development.

On general rheologic aspects, Arnoud Bonnefoy (Inserm, France) gave a comprehensive overview on intravital microscopy; its still present limitations in view of rheologic characteristics were compared to in vitro flow models. Michael King (Rochester, USA) gave and elegant talk on basic rheologic studies incorporating e.g. flowing protein coated microspheres but also intravital observations of leukocytes interacting with blood vessels. Computational modelling of particle interactions was shown to become more and more adequate to explain observations in even complex rheologic systems with multiple cells, combinations of cells and in junctions of blood vessels. Eric Grabowski (Harvard Univ, USA) showed new insights on rheologic aspects involving endothelial microparticles (EC-MP) and their interaction with the vessel wall. Especially the Shiga Toxin important in the pathogenesis of HUS was shown to encrypt tissue factor on EC and cause detachment of especially glomerular (kidney) EC from their matrix with formation of EC-MP.

On drug and receptor aspects, Johan Heemskerk (Maastricht, Netherlands) touched on the considerable differences between flow device studies in respect of the blood used, anticoagulated vs. non-anticoagulated blood, the reactive surface used for bloodcomponent interaction, the flow chamber etc. For (PPACK anticoagulated) human and animal blood he showed comparable results in flow experiments over collagen in respect of alfa2 beta1 and GPVI dependent platelet adhesion. He, however, stressed the importance of differentiating various phases in platelet adhesion (unstable vs. stable as shown by real time intracellular Ca2+ mobilisation) and also the need to observe platelet thrombi stabilisation (by factors like autocrine released ADP) and destabilisation. Concerning the collagen surfaces, it was shown that the type of collagen profoundly influences findings with more or less modulation of the observed platelet adhesion by (therapeutics that block) typical collagen receptors. Marc Hoylaerts (Leuven, Belgium) talked about the often observed cyclic build-up and detachment of thrombi and resulting local flow variations and thus the need for 3D reconstruction/imaging of thrombus growth. He furthermore stressed that drug effectiveness can be modulated by local shear stress by showing the shear-modulated function of the (blockade of the) P2X1 ion channel in collagen dependent platelet activation and thrombus formation. Moreover, combinations of antithrombotic drugs -even with different working mechanisms- were shown not to be necessarily synergistic when studied under physiological flow. Thomas Diacovo (Columbia Univ. NYC, USA) showed that high temporal...
resolution microscopy imaging of vWF A1 domain-coated microspheres under flow interacting with GPIb allow clear conclusions about how on-(attachment) and off-rates (detachment kinetics) are influenced by drugs or snake venoms like botrocetin.

On devices and modelling, Patrick Andre (Portola company, USA) showed an promising combination of a rectangular collagen coated capillary perfusion chamber with real time fluorescence microscopy of FXa inhibitor anticoagulated whole blood. The set-up allowed software aided 3D imaging of platelet thrombus growth and detachment kinetics. The used anticoagulant approach but also the real time imaging of thrombus stability was shown to be critical for measuring the antithrombotic potential of concentrations of drugs like ADP receptor, GPIIbIIIa and Thromboxane inhibitors that are relevant for in vivo effects. He speculated that devices that allow measuring the individual hemostatic potential will be extremely valuable for diagnostics but even more for personalized therapy. Michael King ended the session by showing that serial and time dependent measurements of interaction angles of platelets rolling over the vessel wall allows modelling and understanding of the observed pole-vault like interactions of platelets (discoid shapes) with the vessel wall. These Multiparticle Adhesive Dynamics moreover, allow calculations of interacting and detachment forces of these typical tethering interactions and with it in theory effectivity of drugs especially designed to target these interactions.
Control of Anticoagulation

Chairman: S. Schulman, Sweden
Co-chairs: M. Greaves, UK; J. Harenberg, Germany; C. Kearon, Canada; M. Laposata, USA; J. Olson, USA; G. Palareti, Italy; A.M.H.P. van den Besselaar, The Netherlands

Joint session with the Scientific Subcommittee on Perinatal and Pediatric Haemostasis
Chairs: S. Schulman, P. Massicotte

As a novelty, it had been decided to conduct the first half of our meeting as a joint session with the Subcommittee on Perinatal and Pediatric Haemostasis, the reason being that representatives from each committee felt that there were many topics of common interest, that there are needs to learn from each other and that in case of separate sessions there is a difficulty to fit that into the itinerary. The joint session was designed to contain educational components as well as updates on subcommittee activities of potential mutual interest.

New anticoagulants for pediatric use; lessons from adults

Jeff Weitz gave an overview of the development of new anticoagulants over the past few years, with emphasis on the lessons learnt and possible implications for the pediatric population. He described the development of heparins towards lower molecular weight with problems regarding decreased clearance in case of renal impairment, lack of specific antidotes and long half-life. New, selective anticoagulant agents have usually rapid onset and offset, a wide therapeutic window and no or reduced need for monitoring, but pediatric data is lacking as well as specific antidotes. A comment from the audience was that argatroban and bivalirudin, both approved drugs, are in clinical trials in the pediatric population.

Recommendation: It is important to ensure that some of the new anticoagulants will fit the needs of the pediatric population, for example with parenteral (subcutaneous) administration, no need for monitoring, not contraindicated in case of hepatic failure etc.

Towards a unified definition of major hemorrhage in clinical trials.

I. Non-surgical studies

Report S. Schulman
The process from discussion at the previous SSC in Venice 2004 was recapitulated briefly. The recommendation was published as a full length paper in JTH in April 2005. The European regulatory authority, EMEA, has been contacted and expressed interest in possibly adopting the recommendation but preferred to have the complete set, including recommendations for orthopedic and general surgery studies. Informal contacts have also been taken with FDA, and there is a growing interest there for possible issues of harmonization.

Plan: Encourage the process for similar recommendations in surgical studies and to further develop the contacts with EMEA and FDA.
II. Surgical studies – orthopedic.

Update. G. Raskob

The Working Party on Bleeding Complications in Orthopedic Studies has identified a large variety of definitions used in their field by performing a systematic literature review. Traditional measures of severity of bleeding have limitations in the early postoperative period. There is a definite need to include the surgeon’s assessment of the surgical site bleeding.

Recommendations (preliminary): 1) There is a need for explicit reporting for the surgical site, which must be distinguished from other bleeding. 2) A blinded assessment should be done by a surgeon regarding the clinical importance of the bleeding at surgical site. 3) Bleeding index and clinically important bleeding should be reported independently as separate outcomes. 4) A clinically important bleeding at the surgical site if it leads to wound dehiscence, infection, re-operation, prolonged hospital stay or contributes to myocardial infarction, stroke or death, as assessed by an independent adjudication committee. The WP will accelerate their pace of development of the recommendations and will endeavor to get this published within the next 12 months.

III. Surgical studies – general.

Update. D. Bergqvist.

This issue is even more complicated than the orthopedic procedures, since the surgical procedures are less standardized in general surgery and the severity of the procedures vary greatly. Gynecologic procedures may differ a lot from other general surgery. The transfusion requirements are influenced by more or less conservative policies.

Recommendations (very preliminary): Major bleeding is tentatively defined as 1) leading to death, 2) leading to transfusions or endovascular hemostatic procedures, or 3) occurs in critical organs.

Plan: To form a working party within the next few months to continue the development of a unified definition.

The Use of Heparin in Children. P. Monagle

Unfractionated heparin (UFH) is the anticoagulation of choice in infants and children who are at high risk of bleeding (peri surgery, trauma, chemotherapy) because of ease of reversibility (protamine sulfate) and short half life. During cardiopulmonary bypass and extracorporeal membranous oxygenation, UFH is currently the anticoagulant agent of choice. However, infants and children do not respond to UFH in the same way as adults. The activated partial thromboplastin time (aPTT), a surrogate measure of UFH level, does not correlate to increasing levels of heparin in the same fashion as in adults. In fact, if comparing therapeutic anti factor Xa levels to corresponding aPTT levels, in infants and young children the therapeutic aPTT ranges are much high than those in older children and teenagers. This difference may relate in part to developmental haemostasis differences, but there may be other different mechanisms of interaction compared to adults.
**Recommendations:** The difference response to unfractionated heparin between adults and children will be determined. A subgroup lead by Dr Monagle will determine how best to monitor UFH in neonates and children. This will be submitted as a position paper to the SSC.

**HIT in children A. Greinacher**

The literature in adults with HIT was summarized. In neonates and children, heparin induced thrombocytopenia is rare (2.4% of those exposed). Most infants who develop antibodies have underlying cardiac disease. In children and teens, most who receive heparin have deep venous thrombosis. The testing for HIT in children has not been standardized and cut off values for abnormal must be established.

**Recommendations:** A standardized approach to the diagnosis of HIT in children must be established. Dr Greinacher will lead a subgroup to develop a diagnostic approach in children which will be submitted as an SSC position paper.

**Activity reports from the Subcommittee on Control of Anticoagulation**

**Chairs: J. Harenberg, A.M.H.P. van den Besselaar**

*European Concerted Action on Anticoagulation: A multicentre calibration study of WHO International Reference Preparations for thromboplastin, rabbit (RBT/90) and human (rTF/95).*

**Report . L. Poller, A.M.H.P. van den Besselaar and A Tripodi**

The ECAA study was performed, starting in 2001, to check the stability of thromboplastins over time. If one outlying lab of the 10 participants was excluded, there was a statistically significant difference between RBT/90 and rTF/95. Historically, a PT ratio for rTF/95 of 3.2 gave an INR for rTF/95 of 3.0 but presently the same PT ratio gives an INR of 3.2. Although this difference is statistically significant, it is considered acceptable, being below the 10% limit. WHO does not allow changes to the assigned ISI.

**Recommendation:** It is important to continue to monitor the stability of thromboplastins.

**International collaborative study for calibration of two candidates international standard for thromboplastin, rabbit, plain.**

**Report. A. Tripodi and A.M.H.P. van den Besselaar**

The rabbit brain thromboplastin RBT 90 has been depleted, unfortunately to the extent that nothing remained for calibration versus a successor. Instead, two other rabbit standards were made available for this study and they have in turn been calibrated against RBT 90. Two new candidates for the international standard for thromboplastin rabbit plain have been thoroughly evaluated regarding a) within laboratory precision of calibration, b) between laboratory precision of calibration, c) conformity with the WHO model and d) stability. Important results were, regarding
The distribution of ISI values were overall close to 1.2;

b, c) The overall Cv was 5% and the percent of calibrations with a deviation of normals from patients line: 14.3% with 04/106 and 11.1% with 04/162, which shows the adequacy of the model. Between laboratory precision, measured as Cv of the ISI was 5.0 for both candidates.

d) The stability of the candidates underwent accelerated testing at 45ºC for 3 months demonstrated a minor increase of the PT after storage, equal to what previously was reported for another reference material for thromboplastin, rabbit, plain. Candidate 04/162 was marginally better than 04/102.

The results of this study had been circulated to the co-chairmen (M. Greaves, UK; J. Harenberg, Germany; C. Kearon, Canada; M. Laposata, USA; J. Olson, USA; G. Palareti, Italy; A.M.H.P. van den Besselaar, The Netherlands) before this SSC meeting and the selection of the 04/162 candidate was approved, with the exception that one co-chair suggested that both candidates be approved.

**Recommendation and decision:** It was proposed to the subcommittee that 04/162 should be selected, and this was confirmed by show of hands with an overwhelming majority supporting this candidate. Thus, 04/162 will be recommended to WHO as the new standard for thromboplastin, rabbit, plain. An SSC communication in the JTH is expected within the next year.

**Working Party on Thrombin Generation Test: Survey on current practices and in vitro study on suitability of a general method for the thrombin generation test.**

**Report. E. Gray**

This Working Party reports directly to the Committee on Plasma Coagulation Inhibitors. A survey on the most frequently used methods for TGT had been sent out via the ISTH web site with 57 responses (2.4%) and the UK NEQAS with 35% response rate. A mini-review on the TGT methods has been published on www.bloodmed.com. A pilot study on chromogenic non-sampling method for TGT has been performed. Preliminary results from 4 labs were shown, noise to signal was very high.

**Plan:** An international study on fluorogenic methods for TGT will be carried out and invitation goes out in mid-September. Analysis is planned by the end of December with report to SSC 2006. A suggestion was made to compare samples that are microparticle-free by high speed centrifugation.

(A Boehringer-Ingelheim representative took at this point the opportunity to announce a lunch meeting not found in the program)

**Validation of a thrombin generation test.**

**Report. M. Samama**

A validation of the Thrombogram has been performed. The results show that it is not very important which anticoagulant is used, except that citrate is sensitive to the velocity index. The concentration of
tissue factor (TF) affects the kinetic parameters (lag-time and T-max) more than the endogenous thrombin potential (ETP). Optimum appears to be 6 pM final concentration of TF for Recombiplastin and 8 m M of phospholipids. There was no difference between the PPP reagent from Synapse versus Recombiplastin and synthetic phospholipids. Frozen PPP gives shorter lag-time and reduced T-max versus fresh but no difference in ETP. Interindividual Cv was 25% with fresh PPP and 30% with frozen PPP, independent of experimental conditions and is probably related to individual variations of pro- and anticoagulant factors.

**Thrombin generation as a test for all types of anticoagulants?**

**Report. H.C. Hemker, R. Al Dieri and S. Béguin**

Heparins, direct thrombin inhibitors (DTIs), direct FVIIa and Xa inhibitors and vitamin K antagonists (VKA) were tested. Heparins, including pentasaccharides, affect all parameters of the Thrombogram. Hirudin prolongs particularly the lag-time due to decreased feed-back activation, but melagatran gives a different result. Direct FVIIa and Xa inhibitors affect all parameters. ETP and ETP-inhibition have lower variability to different heparins than to FVIIa and Xa inhibitors. VKA gives increased lag-time. Aspirin has a significant effect on mainly the lag-time (with PRP used in the system). Thus, all anticoagulants diminish the thrombin generation, as measured in this TGT. However, the influence on the test is not necessarily equal to the clinical effect and further investigations with each anticoagulant agent will be needed.

**Overview of the NCCLS (CLSI) document: Procedures for Verification of INR and Local Calibration of PT/INR Systems.**

**Report. D. Adcook**

The guideline on these procedures was developed through the CLSI (NCCLS) in conjunction with DIN, per request from the FDA, with the objective to have some kind of standard before their approval of a PT calibrant product in the US. The ISTH document was the basis for the development of this guideline. It will be approved later this month and published in September 2005.

**Prothrombin-induced Clotting Time (PiCT) in the monitoring of low-molecular-weight heparins.**

**Report. M. Wilmer (Pentapharm) for D. Hoppensteadt**

Pefakit PiCT uses purified components. Clotting times are proportional to concentrations of anticoagulants against F Xa and IIa; are sensitive to the type of low-molecular-weight heparin (LMWH); more sensitive to LMWH than APTT and equal to Heptest; are slightly prolonged by direct Xa inhibitors.

**Standardization of methods to monitor fondaparinux.**

**Report. E. Gray**

This pilot study was reported at the last meeting. In brief: Six laboratories participated. PPP was spiked with 0-2 m g/ml of fondaparinux. Intralaboratory variability was low for commercial kits but high with in-house preparations. There was no effect of additional antithrombin in normal plasma. The inter-
laboratory variability was high, due to the design of the assays. A second study addressing the performance of commercial kits on ex-vivo plasma samples is needed but it is hard to obtain the plasma samples.

Plan: Submission of manuscript for SSC approval in the near future. The next phase of this study is to receive ex-vivo plasmas form clinicians on patients treated with fondaparinux and if possible with direct Xa inhibitors and to compare in different anti-Xa tests such as PiCT, Heptest, chromogenic anti-Xa.

Monitoring of the direct thrombin inhibitors argatroban, angiomax and hirudin. Discordance between the anticoagulation and dosing.

Report. O Iqbal
APTT has been recommended for dose adjustments and is approved by the FDA. Other possible methods are ECT (used for lepirudin in HIT during off-pump aorto-coronary bypass), modified ACT (data from PCI-studies), chromogenic anti-IIa test. There are different effects from the different DTIs (J Cardiol Surg 2005; 20:42 -51).

Importance of quality control of vitamin K antagonist therapy during the initial period.

Report. G. Palareti
The study aimed at assessing the relationship between the quality of laboratory control during anticoagulation for a first event of venous thromboembolism and the risk of recurrence after withdrawal of anticoagulation. Overall 297 patients were included and followed for 21 months. There were 42 recurrences (14.1% or 8.5%/year). The overall quality was worse in patients who suffered a recurrence, the risk of which was significantly higher in those who spent a higher percentage of time at very low INRs (<1.50) during the first 3 months of treatment.

Recommendation: Efforts have to be made to improve the quality of anticoagulation, especially during the first 3 months of treatment, perhaps by more frequent monitoring and dose adjustments and/or bridging with LMWH.
Disseminated Intravascular Coagulation (DIC)

Chairman: K. Hoots, USA
Co-chairs: J. D. Nielsen, Denmark; C-H. Toh, UK; H. Wada, Japan

Part A

The DIC Subcommittee meeting of ISTH met for its annual meeting in the Tumbalong Room of the Sydney Convention Center on Saturday, August 6, 2005. Approximately 90-100 members participated for the session which summarized Subcommittee progress (See Part B, below), discussed areas in need of continued investigation, and reached consensus on important proposed modifications on the DIC algorithm for future consideration. Specifically, the following recommendations were made that were a direct result of discussion of participant members (and were in addition to other proposed alterations discussed in Part B which were likewise affirmed without discussion):

1. The timeline for establishing cutoffs for fibrin marker standards was accelerated.
2. A decision was made based on data presented at the meeting by Drs. Demfle and Toh to consider removing fibrinogen from the DIC algorithms since, in early clinical assessment for DIC, it appears to not add significantly to the overall sensitivity and specificity of the algorithm (Primarily because of its initial rise following insult or injury as an acute phase reactant.)
3. Until more disease specific data is collected prospectively, to maintain the cut-off for DIC determination in the algorithm at 5.
4. Data presented at the meeting concerning longitudinal assessment for DIC utilizing both the OVERT an NON-OVERT Algorithms seem to confer comparable positive and negative predictive value for survival ( despite the fact that individual patients may score differently because of the “negative” scoring permitted in the NON-OVERT algorithm and not utilized in the OVERT Algorithm). Therefore, strong consideration is being given to merging the two algorithms into one which would be modified as appropriate if additional biologic markers are employed in addition to the global markers. Clearly, this will require further validation of longitudinal assessment. However, if this does indeed confer comparable positive and negative prediction of morbidity and mortality risk over time, it would remarkably simplify the utility of the algorithm ( and, perhaps, increase the likelihood of its adoption by physicians in the critical care community).
5. After extensive discussion by Dr. Uri Martinowitz, Professor Nielsen and others, consensus was reached on two subsets of patients where algorithm use requires modification: 1) use of the algorithm in trauma patients be limited to at least 24 hours following injury so that high scores on the algorithm indicating “DIC” do not serve to preclude therapies for life-threatening hemorrhage such as recombinant Factor VIIa (Which use has traditionally been said to be contraindicated in DIC). Since data exist to indicate DIC to be a significant risk later, the algorithm could be used after the first 24 hours. 2) Validation data indicates that patients with severe underlying liver disease are not well-identified with regards to longer term morbidity and mortality by the DIC algorithm. Until further refinements are developed for this population, we will identify such patients for exclusion from use of the algorithm for risk assessment purposes.
6. New assays for assessing global coagulation potential should be investigated for possible future incorporation into the DIC algorithm.

Part B

The DIC Subcommittee of the International Society of Thrombosis and Haemostasis met in Houston, TX, USA on April 29- May 1, 2005 to plan the agenda for the Subcommittee meeting at the ISTH meeting in Sydney in August. In addition, we discussed the accomplishments arising from subcommittee endeavors and assessed future challenges in advancing the diagnosis and therapy of DIC-associated syndromes. The following summarize these latter discussions. Attached to this document are the agenda and a roster of subcommittee chairs and other subcommittee members who participated actively in this planning process.

I. Summary of Subcommittee Accomplishments:

Most of the recent effort of the Subcommittee has focused on validating and refining the DIC Algorithm [1] that was developed by us to be used in the diagnosis of Overt and Non-overt DIC. Further, an important secondary purpose for developing the Overt and Non-overt Algorithms was to develop prospective stratification strategies for therapeutic trials of new (or established) drugs for DIC. We have made significantly greater progress in the former. Specifically, since our publication of the Taylor, et al. article in 2001, no fewer than 6 peer-reviewed manuscripts whose original design was predicated (at least in part) on validating the overt DIC algorithm have been published by members of this Subcommittee.

Specifically, Wada, et al has compared the sensitivity and specificity of our ISTH algorithm with a refined version of the original algorithm developed out of the Japanese Ministry of Health and Welfare. [2] Levi, et al (past chair of this Subcommittee) has published 2 validation studies of the overt algorithm using sequentially larger cohorts (>50 to >240) that indicate that the positive and negative predictive value among consecutive patients admitted to the ICU of a single institution (Univ. of Amsterdam) is extraordinarily high when compared to blinded “expert” clinical assessments of DIC. [3, 4] Further, the presence of DIC by the algorithm was highly predictive of mortality in these cohorts.

Similarly, Dempfle, et al has published similar ICU cohort data that validates the utility of the Overt algorithm in cohorts in Mannheim, Germany, assessing, in particular, the essential role that measurement of fibrin markers (indicative of both fibrin cross-linking and fibrinolysis) play in the Overt DIC Algorithm. [17]

Toh and his colleagues in Liverpool have demonstrated that among subjects consecutively admitted to their ICU, both the Overt and Non-Overt Algorithm predict patients with high mortality, although not typically with the same pathogenetic course. [6] Data from this paper suggest that early abnormalities of coagulant markers parallel those of inflammatory harbingers of bad outcome. This observation is consistent with a basic pathogenetic premise described in the original Taylor paper: that is that quantification of dysfunction of the “microvascular-endothelial” organ provides important predictability of clinical outcome when biologic markers
of inflammation and coagulation are markedly abnormal early in a disease process predisposing to DIC.

Single reports, as important as they have been as markers of Subcommittee progress, however, are not sufficient. It is for this reason that we propose two new submissions as Subcommittee Reports for publication in JTH. This first to be developed this year, will summarize the progress (cited above) to date. The second will suggest refinements to the algorithm based on the studies to date and those that are presently on-going.

As these published reports have indicated, the ultimate clinical utility of these DIC algorithms requires their being married to validated scoring of injury and disease in the critical care setting. This proves to be a significant challenge for the Subcommittee as most of the potential use of the algorithms (and their application to describe the pathogenesis of vascular injury in severely ill patients) lies with physicians whose primary training and affiliation are in Critical Care Medicine rather than Hematology. Our strategies to address this formidable challenge will be described below.

However, before we can export our coagulopathic/inflammatory view of severe disease, the Subcommittee has been working to incorporate the language of vascular injury (which is, again, the theoretical basis to our algorithm development) into the lexicon of severe disease. To achieve this ambitious aim, Subcommittee Chairs, Co-Chairs and members have been striving to incorporate this vascular “world-view” more completely into the concept of DIC for the breadth of the clinical community. One early, but promising strategy has been for us to “re-write” standardized textbooks and reviews of DIC from this vascular injury perspective. Over the last 3-5 years our members have incorporated this biologic view into a neurosurgical journal and textbook, [7, 8] updated classical textbooks of hematology [9-11], in a fundamental discussion in the microvascular research arena [12] and to the broader community of clinicians via a comprehensive review. [13] Further, the concepts on which we have predicated the work of our Subcommittees have been cited in State of the Art discussions of Sepsis [14, 15] and Trauma. [15] These slow, but steady inoculations of new DIC concepts (e.g. Overt versus Non-Overt DIC as clinical spectra of disease) appear to be gradually gaining acceptance in diverse medical subspecialities. However, as a subsequent paragraph will discuss, some areas of medicine (e.g. Obstetrics and Gynecology) have to date largely been ignored in this endeavor. Efforts to fill these gaps constitute significant future work by this Subcommittee. One approach will be to initiate a dialogue with the subcommittee of Hemostatic/Thrombotic Issues of Women chaired by Dr. Marilyn Manco-Johnson. We will focus this initial effort by having Dr. Benjamin Brenner of the University of Illinois-Chicago discuss obstetrical DIC at our Subcommittee Meeting in Sydney. In addition, we have identified several other clinician scientists with both hematologic and obstetrical expertise to work with us in this initiative.

One clinical discipline where we are progressing is pediatrics. Drs. Prasad Mathew (Co-Chair, Pediatrics) and Marilyn Manco-Johnson (Chair, Women’s Health in Thrombosis & Haemostasis Subcommittee) are initiating a retrospective analysis in the Mountain States Region of the U.S. of whether the algorithms (in the present iteration, or perhaps in a modification specifically for very young patients) are as reflective of the biology and outcome as has been demonstrated in adults. Dr. Deborah Brown, a Subcommittee member, will initiate a single institution
retrospective and prospective investigation which will contribute to this effort. Both will examine pre-term and term neonates as well as older children in the Pediatric ICU setting. These efforts constitute a major emphasis of the Subcommittee for 2005-2006.

In addition to efforts by Subcommittee chairs (past/present), Co-chairs and members to extend the clinical capacity to diagnose and treat DIC, we have been working with collaborators whose laboratories are attempting to define more precisely what the vascular response to injury is at the cellular and subcellular level. Okajima has presented at our meeting in Houston and has submitted for publication exciting new work on the role that sensory neurons appear to play in the upregulation of inflammation and importantly, how tight thrombin regulation may be essential to alleviating this effort. Recent work on the relationship between PAR receptor activation and endothelial protein C receptor [16] have intensified efforts to define the cellular and molecular response modifiers of vascular injury-all of which offer the prospect of defining more precisely how therapeutic molecules (e.g. activated protein C, Antithrombin, Tissue Factor Pathway Inhibitor, etc.) may or may not be relevant for clinical subsets of at-risk ill patients prone to develop DIC. In this spirit, members of this subcommittee (e.g. Nigel Key) are initiating a dialogue on our behalf with the Vascular Biology Subcommittee (particularly as it relates to Tissue Factor and platelet microparticles) to explore future opportunities for collaboration.

II. Goals and Challenges

As discussed, we will prepare two Subcommittee Reports for consideration by the SSC for publication in JTH. The first will be a straight-forward report of or progress in validating the Overt and Non-Overt DIC algorithms. The second will make refinements to the algorithms based on new clinical data that we may be able to improve the positive and negative predictive values of the scores by defining parameters more precisely and/or by making categorical distinctions based on the cause of the DIC.

A goal which has preoccupied us for the last 18 months and which has engendered much investigation by Dr. Karl-Ehrich Dempfle (Co-Chair, Fibrinolysis; Member, DIC) has been to establish reliable cut-offs for the fibrin markers. Since most clinical labs employ D-dimers as their measurement of choice, Dr. Dempfle has extensively studied standardizing reagents for the D-dimers test as well as trying to establish quantitative “cut-points” of a “standardized” D-dimer measurement to score in both the Overt and Non-Overt algorithm. [17] Significant progress has been made and, based on this work, new parameters for scoring fibrin marker (D-dimer) in the algorithms should be ready for the next subcommittee report next year. Additionally, we propose to re-examine the contribution of fibrinogen measurements to the algorithms.

Another challenging dilemma has arisen from the work of Wada and colleagues. They have demonstrated that the utility of the overt algorithm may be better for sepsis-induced DIC compared to cancer-associated DIC. [18] This led our Japanese colleagues to modify the JMHW DIC algorithm which appears to make it somewhat more sensitive to cancer-associated DIC. Our approach may be different: one possibility is to utilize a different cut-off in the overt Algorithm (the standard >5) for sepsis and other non-cancer associated DIC states and lower score (e.g. 4) for cancer-related DIC. This approach will require significant further investigation and Dr. Wada is obtaining some preliminary data about such an adaptation.
Although microvascular thrombosis leading to organ failure (MODS) clearly predominates as the morbid and/or mortal outcome of DIC initiated by sepsis and most other causes, there are subsets of DIC patients in whom a hemorrhagic diathesis from procoagulant consumption contributes significantly to clinical morbidity (certain oncologic-associated DIC syndromes are examples). The work by Wada and others will hopefully better define these specific entities. Patients in these unique sub-groups of DIC my benefit from newer hemostatic options (e.g. recombinant Factor VIIa). [19] Precise sub-categorization of The DIC syndrome by the Subcommittee may help identify patient subsets in whom these theoretically risky interventions may confer a positive benefit risk.

Assessing the utility of other biological markers (e.g. Antithrombin, TAT or Protein C levels) for the Non-Overt Algorithm in particular needs further data. Like the D-dimer, these tests suffer from standardization problems. Further, it is still unclear whether measuring them in real time (in laboratories where this is even feasible) contributes significantly to enhanced sensitivity and specificity of the algorithm.

Equally important is the question of whether serial determinations of such biological markers will assist treating physicians in monitoring the impact of therapeutic maneuvers. Assessment of the latter is likely a much longer-term goal and will likely require the implementation of our algorithms in prospective stratification of large clinical trials to answer (see below). Another area of interest which is being pursued in local settings by several of the members (e.g. Dempfle, Escobar) is the use of “point of care” coagulation testing and monitoring in the ICU setting. It is still quite unclear whether we can adapt such measurements for incorporation into the algorithms. Nonetheless, it is a valid area of investigation that the subcommittee will follow with great interest.

Another important pathogenetic clarification that we need to refine in light of recent data [6] is how to discriminate the Overt-Non-Overt spectrum of DIC from a previously employed concept of “Compensated” versus “Uncompensated” DIC. It is apparent that these terms are not entirely interchangeable since MODS and death can occur in some individuals who by scoring have “Non-Overt” DIC, never progress before death to Over DIC but die from organ failure nonetheless. Removing all confusion with regards to these labels will likely require better understanding of individual phenotype. [20]

As was alluded previously, it has always been a primary goal of this Subcommittee to have its algorithms employed prospectively to stratify and then to monitor longitudinally (in the case of Non-overt algorithm) patients randomized in clinical trials of therapeutic interventions. [5] This will require a more proactive approach by the Subcommittee Members than we have previously assumed since most of these trials originate through collaborations between Pharma and Critical Care Physician Investigators. This, however, is consistent with our purposeful effort to insinuate ourselves as hematologists into a more aligned position with these investigators. Our plans for accomplishing this are discussed below. However, even if we are successful, we will need to bring to the table an organized proposal which incorporates expertise in epidemiology, trial design and statistics (skills that have not been traditional emphases for us up to now). However, M. Levy has recent experience in DIC trials and Dr. Hoots has directed a AT-placebo DIC Trauma trial in the past. [8, 21] In addition, we hope to attract individuals from the academic and
Pharma Communities to extend our competencies in these areas. Cogent arguments based on accepted pathogenetic models of DIC definable with our algorithms will likely improve the likelihood that sponsors would be receptive to their use in trial design of new therapeutics. Possibilities for exploring this approach may include proposed trials by Genzyme, ESINAI, APEX, and Chiron.

For the next year, one of our highest priorities is to “sell” our biologic concepts of DIC and our algorithms to the critical care medicine community internationally. We want to work together with them to harmonize disease scoring (e.g. APACHE II and MOD Score) with the DIC Score using published studies and recent data. Dr. Gary Kinesawitz, an academic Critical Care Physician and colleague of Fletcher Taylor at the University of Oklahoma as well as a member of our Subcommittee for the last several years, will lead this effort. He is working with Subcommittee members to create a pre-planned symposium on DIC pathogenesis and prognostic scoring of very ill patients to be offered to organizers of key Critical Care international meetings (e.g. American Thoracic Society, European Critical Care). We hope this will open a dialogue about such harmonization. It may also create opportunities to explore the development of clinical guidelines. This represents an initial, but hopefully significant step towards bridging the gap between the hematologic and critical care view of DIC.

In summary, the DIC Subcommittee continues to push a very aggressive agenda. Summarized are our accomplishments to date and our short-and intermediate-term aims and strategies for their respective pursuits. For the long term, we hope to incorporate new knowledge about individual risk factors as implied by genetic polymorphisms that influence response to vascular injury and new means of measuring biologic processes (proteomics) into an evolving strategy to define DIC more precisely.

References:


Registry of Exogenous Hemostatic Factors

Chair: N. Marsh, Australia
Co-chairs: C. Bon, France; K.J. Clemetson, Switzerland; M.R. Kini, Singapore; F.S. Markland Jr, USA; T. Morita, Japan

- **Welcome and apologies**.
  The Chair welcomed SSC Registry members to the meeting. Apologies were received from Cassian Bon and Takashi Morita.
- **Record of last meeting (Venice, June 2004)**
  The report of the last meeting was approved.
- **Introduction of new Co-Chairmen**
  Professors Ken Clemetson and Takashi Morita were introduced as new co-chairmen.
- **Report on inventory of disintegrins (Mary Ann McLane)**
  A disintegrin inventory with a total of 75 molecules has been prepared. The standardisation of nomenclature was considered particularly with respect to proteins from snakes with the same species name. It was proposed that all disintegrins discovered before 2004 retain their names based on generic and specific Latin names. New molecules from existing species would be numbered. There was some interest in revisiting the publication of a system of snake venom fraction nomenclature.
- **Anticoagulant proteins from snake venoms (Manjunatha Kini)**
  The presentation summarised the anticoagulant activities of snake venoms including metalloproteases, serine proteases, fibrinolytic proteases and phospholipase A2. The site of action of phospholipase A2 on the prothrombinase complex was considered in detail and as well as non-enzymatic C-type lectin anticoagulants and three-finger toxins. Takashi Morita and Ken Clemetson are working on an inventory of C-type lectins.
- **Report on EFATH 2005 Satellite Meeting**
  Neville Marsh provided details of the meeting being held on 13-14 August at University of Technology Sydney. A total of 21 presentations will be given on a range of venom-related topics. The snake venom symposium on Thursday 11 August was also noted.
- **Next meeting**
  The next SSC meeting will be held in Oslo in 2006 and notification of the next Registry meeting will be sent out early in 2006. However, it was suggested that the Registry could meet in Glasgow as part of the IST Congress. This would require SSC HQ approval.

Professor Neville Marsh
Chair
6 August 2005
Factor VIII and Factor IX

Chairman: K. Mertens, The Netherlands
Co-Chairmen: J. C. Gill, USA; C. Lee, UK; J. Oldenburg, Germany;
JM Saint-Remy, Belgium; A. Srivastava, India; HM van den Berg, The Netherlands

The Chairman opened the Subcommittee meeting at 16.00 for an audience of approximately 200 attendants. He explained that, due to the more condensed format of this year’s meeting, not the entire portfolio of Subcommittee activities could be addressed this year. In particular the issue of novel FVIII assays could not be included this time, but the issue will return on the program in 2006.

Completed Reports and Recommendations

In the past year no SSC recommendations have been published. Standardisation activities performed within the Subcommittee have resulted in two full papers in Journal of Thrombosis and Haemostasis:

- Raut S et al. A collaborative study to establish the 7th International Standard for factor VIII concentrate. JTH 2005, 3, 119-126

Section 1: Factor VIII: Clinical Issues: Co-Chairs: C. Lee and H.M. van den Berg

Global PTP inhibitor surveillance study: follow-up: D.M.DiMichele

The international need for an international PTP surveillance effort was established at the FVIII/IX Subcommittee in 2003, and the project ratified by the Subcommittee in 2004. Donna DiMichele presented a progress report on this ongoing project. In an initial effort to develop a consensus data collection tool, a questionnaire was sent in 10/04 to countries known to have national PTP inhibitor databases (UK, France and Germany) to ascertain both the nature and the scope of data collected. Similar information was also solicited from Italy and Canada in 07/05. As anticipated, data presented from the responses received to date indicate variability in both the nature and scope of ongoing or planned national data collection. A working party currently represented by the US, UK, France, Germany and Italy, but seeking all other interested national participants (contact Donna DiMichele at dmdimich@med.cornell.edu), will begin working on a the development of a consensus minimum international data set to satisfy international regulatory requirements. A funded US pilot effort through the CDC to prospectively collect PUP and PTP inhibitor prevalence/incidence data, is in the planning stages and will incorporate the international consensus guidelines for data collection developed through this working party. A progress report is planned for 2006.

Meta-analysis of PUP studies of recombinant FVIII: H.M. van den Berg
Dr. Van den Berg, who reported also on behalf of drs Gouw and Van der Bom, addressed the question whether treatment-related factors like early age at first exposure, early start of prophylaxis and a higher intensity of treatment concurrently with tissue damage would increase the risk of inhibitor development. An individual patient data meta-analysis has been performed on four international, multicentre studies of recombinant human factor VIII products (Kogenate Ö , Kogenate FS Ö , Recombinate Ö , ReFacto Ö ) including 236 previously untreated severe haemophilia A (FVIII:C <0.02 IU/ml) patients. The outcome was clinically relevant inhibitor development, defined as at least two positive inhibitor titres combined with a decreased recovery. The analysis included the effect of ethnicity, family history of inhibitors, age at first exposure, start of regular prophylaxis, periods of intensive treatment, duration between exposure days and dosing of FVIII products on the risk of inhibitor development. The databases contained data on patient characteristics and all first fifty exposure days (dates, reason of treatment, dose, bodyweight) and inhibitor tests. However, FVIII gene mutations were not available. Sixty-seven of 236 patients (28.4%) developed clinically relevant inhibitory antibodies against factor VIII. Inhibitors developed at a median of 10 exposure days (IQR 7.5-17 days) at a mean age of 17.6 months. Forty-four were high titre (65.7%) and 23 were low titre inhibitors (34.3%). A black or hispanic ethnicity and a positive family history were positively related to inhibitor development. Dr. Van den Berg concluded that combination of age and intensity of treatment is highly associated with inhibitor development. Also peak moments, surgery and a higher dosing of FVIII were found to be statistically significant determinants of inhibitor development.

**Prospective evaluation of a uniform factor replacement protocol in surgery: A.Srivastava**

This report was a follow-up to the international survey done over the last 2 years to document current practices of factor replacement strategies for prophylaxis against post-operative bleeding in hemophilia. The data showed that both continuous and bolus infusion techniques were widely used with doses varying up to 5-fold between centers for similar procedures with no apparently significant difference in complications. This was so regardless of the location of the centers in developed or developing countries. The need for some standardization of practice was obvious. From the various protocols received and based of average figures of factor replacement, consensus protocols were evolved for bolus and continuous infusions and lower and higher doses. Centers were invited to comment on the proposal, choose a protocol that they felt comfortable with and join the effort at collecting prospective information on outcome and complications of surgery. Once identified, funding will be sought for data management and the study initiated over the next 6-12 months. In the discussion, the issue was raised whether this design would be compatible with the notion that individual patients may need individualised dosage. In response to this, Dr Srivastava explained that the advantage of his proposal is its prospective study design.

**Assessment of inhibitor risk: new approach for trial design and evaluation: M.L. Lee**

Dr Lee discussed a new analytical method to design and evaluate data from clinical studies of new Factor VIII products where the endpoint is inhibitor development. Current approaches to this problem involve the evaluation of approximately 80-100 patients followed by the calculation of a 95% confidence interval for the inhibitor rate. The upper bound of this interval is then compared with some fixed standard. The basic problem with this approach is that it requires an
underlying rate in the order of 1% to succeed. Since confidence intervals are a function of sample data and not fixed quantities, fundamental analytical problems arise. Dr. Lee proposed the use of a Bayesian paradigm, whereby any and all prior information on the safety of the product (or similar products) is employed. The clinical data from a prospective study is then used to update this prior knowledge, resulting in a final calculation of a “posterior” probability that the product meets some acceptable standard. This approach is clinical intuitive and provides a very interpretable outcome from the study data. Moreover it is shown that this approach provides acceptable conclusions for currently licensed recombinant Factor VIII products, but would reject a product with known inhibitor risk (Bisinact). Details of this proposal have been recently published (Haemophilia 2005, vol 11, pp 5-12)

Molecular characterisation of haemophilia A patients with undetectable mutations in the FVIII gene: A. Srivastava

Dr Srivastava presented also on behalf of Dr. Oldenburg. About 2% of patients with hemophilia A do not have detectable mutations in the FVIII gene. This could be due inadequate sensitivity of current techniques for detecting sequence change in this gene or changes being in parts of this gene or other genes not being analyzed. Significant numbers of such cases have not been systematically analyzed. This proposal intends to initiate an international multicenter effort at identifying such cases, reviewing their clinical and hematological features, reassessing their status for mutations within the FVIII coding regions and then subjecting them to further analysis if found to be negative. This would include analysis of peripheral blood lymphocytic RNA for detection of splice defects or rearrangements. If this was negative, then haplotype analysis would be undertaken for familial cases see if FVIII deficiency was segregating with the FVIII gene. If so, epistatic factors would need to be evaluated in these cases. For the others, one approach could be a genome wide linkage analysis to identify regions of interest (increased lod scores) followed by further analysis / sequencing of these regions. These issues will be discussed further once the participating centers are identified and resources found for this project.

Section 2: Standardisation Issues: Co-Chairs: J.M. Saint-Remy and K. Mertens

FVIII Collaborative Studies: Phase I field study: S. Raut

Dr. Raut reported on a new FVIII SSC Collaborative Study. This project has been on hold for a few years, mainly due to limited improvements achieved during the successive studies. Last year the Steering Committee that oversees these activities decided that the program may be resumed provided that it will be performed in a more controlled format. As a first step the number of samples has been extended which should make some more detailed analysis possible. Four samples (3 recombinants and 1 plasma-derived) have been distributed over 31 participants. Data were received from 30 labs. Methods used were the 1-stage assay (12 labs) and the chromogenic assay (the majority). Standards included the EP/Mega-2 standard, and in-house standards calibrated directly against the WHO standard. Dr. Raut explained that, as in previous studies, the results still were unsatisfactory, with GCVs between 14 and 19%. The data further revealed assay discrepancies, which differed for plasma-derived (1-stage > chromogenic) and recombinant (1-stage < chromogenic) FVIII. This unique data set will be further analysed in more detail. This will further provide an opportunity to analyse potency estimates based on the
EP/Mega-2 standard material, which has two different assigned potencies depending on the FVIII assay method used. The extended data analysis will be reported at the meeting in 2006.

**ECAT study on FVIII inhibitor assays: B. Verbruggen**

Dr. Verbruggen reported on the recently concluded ECAT study. Three samples containing respectively 0, 1.5 and about 16 u/ml FVIII inhibitors were sent to 175 laboratories worldwide, with 135 responded (77%). For the negative sample, 4 laboratories reported a positive result. For the high-titer sample, all the reported results were positive, the coefficient of variation (CV) ranged from 32% for the Nijmegen method to more than 60% for the Bethesda method. For the low-titer sample, 16/131 (8%) results were reported negative with similar CVs as for the high titer sample. Based on these results Dr. Verbruggen concluded that the specificity of the used methods is acceptable but the sensitivity of the reported method is rather low. Further standardisation of the assay method seems needed.

**Factor VIII inhibitor assay standardisation: S. Raut**

Three types of reagents have been used in an attempt to standardize an assay for inhibitor detection, namely rabbit polyclonal antibodies, two different human monoclonal antibodies derived from the memory B cell repertoire of patients with inhibitors (one type 1 and one type 2 inhibitor) and human plasma. The latter was found difficult to use for ethical reasons. Results show large variations between laboratories, the less so with rabbit polyclonal antibodies. The reasons for such variation are being examined, including the source of FVIII, dilution buffers and the method actually used by laboratories to carry out the assay. Confounding factors include the possible interference due to the presence of rheumatoid factor or anti-allotypic antibodies in some of the serums. Based on these findings Dr. Raut concluded that a collaborative study would be useful to address assay standardisation. Currently a Steering Committee is being formed in order to decide on the design of the Working Party and to oversee its future activities.

**SSC working standard (plasma lot 3): summary of calibration: S. Kitchen**

Calibrations of the Scientific and Standardisation Committee (SSC) Secondary Coagulation Standard Lot #3 for Factor VIII:C, von Willebrand Factor Ristocetin Cofactor Activity (VWF:RCo), von Willebrand Factor Collagen binding (VWF:CB) and von Willebrand Factor Antigen (VWF:Ag) were carried out against the h World Health Organisation (WHO) International Standard (IS) for Factor VIII/VWF and IX in plasma (02/150). A total of 19 expert laboratories in 11 countries participated in this collaborative study employing the same assay protocol but with different reagents and instruments. The overall geometric mean potencies (* excluding 1 statistical outlier) were as follows:

- Factor VIII:C* - 0.80 IU/vial (GCV* - 4.7%)
- Factor IX:C - 0.94 IU/vial (GCV - 4.6%)
- VWF :RCo/Activity - 0.90 IU/vial. (GCV - 9.7%)
- VWF: Collagen binding - 1.07 IU/vial (GCV - 11.2%)
- VWF Antigen* - 1.06 IU/vial. (GCV* - 5.7%)
These potencies have been accepted by the participating centres and by the SSC Working Group on Coagulation Standards.

**Human Genome Variation Society: nomenclature recommendations: A. Goodeve**

The Human Genome Variation Society (HGVS) has devised a series of nomenclature recommendations for gene names, nucleotide and amino acid alterations over the past 10 years. Recommendations are available at [http://www.genomic.unimelb.edu.au/mdi/mutnomen/](http://www.genomic.unimelb.edu.au/mdi/mutnomen/). These detail how all sequence variation can be described using a standardised system. A number of journals and genetics EQA schemes are now specifying use of this system. Factor VIII and IX genes are denoted \(F8\) and \(F9\). A standardised start point of the first methionine for proteins and the A of the ATG start codon for nucleotide sequence, using the cDNA sequence where possible are recommended for all genes. Dr. Goodeve mentioned that the haemophilia databases can adopt this new system, if accepted. In the discussion it was acknowledged that this proposal would indeed eliminate many inconsistencies in the genetic literature. At the same time, however, it might raise confusion in the protein literature on the FVIII and FIX proteins. In this regard Dr. Mertens referred to the dual numbering for the FIX serine protease domain (FIX and chymotrypsin numbering) which is already confusing sometimes. As such, the merit of a third amino acid numbering remains unclear.

**Prothrombin complex labelling: R. Seitz**

Dr. Seitz presented in vitro and animal experiments demonstrating that prothrombin overload leads to enhanced thrombin generation and appears to be a major cause of thromboembolic complications during treatment with PCC. The European Pharmacopoeia considers changing the monograph on PCC to make factor II the labelled potency of PCC. Dr. Seitz asked for the input of the subcommittee concerning the following questions: Are licensed PCC products still used for haemophilia B treatment? How would the proposed change of labelling impact on the clinical use? Would there be a need to carry out new clinical studies? During the discussion Dr. Barrowcliffe noted that the prothrombin overload was mainly associated with a single PCC product that is not being used outside Germany. Along the same line, Dr. Mertens suggested that this problem should not impact on other manufacturers whose products do have equivalent FIX and prothrombin content. As PCCs continue to be used for haemophilia B treatment outside Europe and the USA, the Subcommittee does not support changing the current labelling practice.

**Factor V: calibration of 1st International Standard: A. Hubbard**

An international collaborative study involving 22 laboratories in 11 countries has been undertaken to calibrate the Proposed WHO 1st IS FV Plasma for FV clotting activity. Each laboratory estimated FV:C in the candidate preparation relative to a local normal pooled plasma which was arbitrarily assigned a value of 1.0 unit per ml. Most laboratories (21/23) used thromboplastin-based methods rather than APTT-based methods. Estimates relative to the fresh local pools (mean 0.74 IU/ml) were significantly lower than estimates relative to the frozen local pools (mean 0.80 IU/ml) and this could be an indication that some FV:C activity has been lost during freeze-thawing of the frozen pools. It is therefore proposed that the calibration should be based only on the estimates relative to the fresh local pools with a mean value of 0.74
IU/ampoule and inter-laboratory variability (GCV) of 7.6%. Estimation of FV:C in a second freeze-dried plasma (SSC/ISTH Secondary Coagulation Standard) relative to the Proposed WHO 1st IS demonstrated low inter-laboratory variability (GCV 3.5%, n=23); this indicates that the Proposed WHO 1st IS should lead to improved harmonization between laboratories. Estimates of stability based on an accelerated degradation study have predicted a loss of less than 0.01% per year at -20 °C. All of the participating laboratories have agreed to the proposal to calibrate the WHO 1st IS with a value of 0.74 IU/ampoule. Prior to the meeting, the Chairman had distributed the report among Subcommittee members for a vote per email. This has resulted in acceptance of Dr. Hubbard’s proposal by the Subcommittee.

Section 3: Rare Bleeding Disorders: Co-Chairs: A. Srivastava and K. Mertens

SSC Working Party on Rare Inherited Bleeding Disorders: F. Peyvandi

Dr. Peyvandi reported that the main goals of this official SSC-endorsed Working Party are two-fold. The first goal addresses database development, which should serve to (a) identify current national and international databases, (b) identify potential collaborators worldwide, particularly in areas where RBDs could collect data, (c) finalize database tools and data collection protocols and disseminate worldwide and (d) establish a Steering Committee for data evaluation. The second goal relates to product development and licensure. This involves the following step: (a) to finalize common regulatory requirements within FDA/EMEA, (b) to identify already available products (FVII, FXI, FXIII, fibrinogen), (c) to design completed clinical trial in order to get global licensure, (d) to explore new product development (FV) and (e) to design implement post-licensure surveillance. So far an International Database on RBDs (RBDD, www.rbdd.org) has been developed, with the aim of efficiently collecting and extracting available data. Dr Payvandi has contacted National and International Organizations and Treatment Centres registered in the WFH mailing list with the aim to learn how many Centres would like to participate and what type of intervention needs to be done in each region of the world; so far 22 centres have already responded.

Update MASAC Working Party on US situation: D.M. DiMichele

This update was presented by Donna DiMichele on behalf on the Working Party on RBDs of the Medical and Scientific Advisory Council of the NHF, chaired by Amy Shapiro. The report focused on efforts planned by this group in the aftermath of the FDA Workshop on Rare Plasma Protein Disorders held June 13-14, 2005 in accordance with the priorities established by both this meeting and by the FVIII/IX Subcommittee Working Party on RBDs. The efforts planned include:

- National data collection on RBDs (molecular, laboratory, and clinical) through the NATIONAL HEMOPHILIA DATABASE consortium currently being established. The database will be developed and data collection supervised by a subcommittee of vested parties. The aims of this data collection include a) contribution to the International Data Collection effort; and b) database for industry and investigator-initiated clinical trials for designated product licensure.
• Ongoing dialogue with the FDA to a) pursue FDA/EMEA harmonization of regulatory requirements and explore novel clinical trial design paradigms in order to facilitate product licensure for rare disorders; and b) facilitate insurance reimbursement for personal importation and/or off label use of products pending licensure application

Treatment of rare bleeding disorders in Europe; the French organisation: J. Goudemand

Dr. Goudemand reported that patients affected with rare bleeding disorders in France are included in a national project: FranceCoag Network. This is a prospective multicenter national cohort of patients affected with severe and hereditary haemorrhagic disorders. FranceCoag network is funded by the French health Ministry and coordinated by a public health institution: INVS (Institut National de Veille Sanitaire). Beside the epidemiologic objectives, the aim is to set up a surveillance system able to investigate any unexpected events occurring in this population. Inclusion criteria are defect (<30%) in FVIII or IX, severe defects (<10%) in FII, V, VII, X, XI, XIII, afibrinogenemia, severe VWD. At that time 4049 patients registered in 37 French centres have been included in the project and 3439 record forms analyzed. There are 3103 patients (90%) with haemophilia, 239 (7%) with severe VWD and 97 (3%) with other rare bleeding disorders. Clinical and biological data are collected as part of the regular follow up (main bleeding episodes, surgeries, treatments, coagulation tests…) and monitored by the coordinating centre. The project (http://www.francecoag.org) is opened to any interested researcher with the agreement of the steering committee. Regarding the treatment, several specific concentrates (VWF, FVII, FXI, FXIII, Fibrinogen, PCC) are available in France to treat these patients.

Complications of management in rare bleeding disorders: U. Seligsohn

Dr. Seligsohn presented an interesting report on the management of bleeding with minimum usage of coagulation factor products. Plasma, and plasma derived or recombinant factor concentrates are used for management of patients with deficiencies of factors I, II, V, VII, X, XI or XIII during bleeding episodes or prophylaxis during surgery. Major complications include transmission of infections agents, development of inhibitors and thrombosis. The paradigm of severe factor XI deficiency was used to evaluate by retrospective analyses whether treatment by blood components during surgery and labor can be avoided or tailored. For tooth extractions, only tranexamic acid was necessary to prevent bleeding in 19 patients. A relatively low frequency of bleeding in 62 untreated women during vaginal (24%) or caesarian deliveries (17%) advocates an on-demand policy of replacement therapy. A similar policy is recommended in patients undergoing surgery at tissues with no fibrinolytic activity because only 8/121 (6.6%) of such procedures were accompanied by bleeding, compared to 29/48 (60.4%) during procedures at fibrinolytic sites. Retrospective analyses of patients with other deficiency states are warranted to minimize the deleterious effects of blood components.

Profile of rare bleeding disorders in India: A. Srivastava

With a population of about 1.1 billion people and significant practice of consanguineous marriages in major parts of the country, India has potentially large number of patients (250-1000) with the rare bleeding disorders. This presentation described the clinical and
hematological features and the molecular genetics of 27 patients with fibrinogen, 7 patients with prothrombin, 20 patients with factor V, 25 patients with factor VII, 25 patients with factors V and FVIII, 19 patients with factor X, 3 patients with factor XI deficiency and 59 patients with factor XIII defects / deficiency. There were significant differences in the clinical features noted in this study when compared to the data in the literature. The cause for this was not clear. A wide variety of mutations, including many novel ones, were detected among patients with prothrombin, factors VII, X, XI and XIII deficiencies. Further characterization of these cases for better understanding their biology and management is progressing. A collaboration is being established with Dr. Peyvandi’s laboratory.

**Report on FDA workshop “Biological therapeutics for rare plasma protein disorders”: M. Weinstein**

FDA and the Office of Public Health and Science (OPHS) sponsored a workshop entitled, "Biological Therapeutics for Rare Plasma Protein Disorders “, in Bethesda, MD on June 13, 14. The focus of the workshop was to facilitate the development of products to treat patients with very rare plasma protein disorders – affected cohorts on the order of 10’s or 100’s in the US. Presentations from the international, patient, physician, and industry perspectives were made on the need for these products, and challenges to their development. We reviewed opportunities and incentives to foster development that are currently available in the US and Europe. These include regulatory pathways to license products with limited clinical data, orphan drug provisions and incentives, small business and research grant support, and the medicare payment program. Case studies of protein C, factor XIII, antithrombin III, and treatment of Glanzmann’s thrombasthenia and Fabry’s disease were presented as examples of product development for very small patient populations. Several potential opportunities for enhancing product development were identified. These included:

- Expanding data bases and registries to identify patients for clinical trials and to help understand the natural history of the diseases
- Consider adopting alternate and/or internationally harmonized regulatory pathways
- Improving investment analyses for industry
- Reviewing regulatory options for product development for rare plasma protein disorders in one to one meetings of sponsors with FDA.
- Important communication pathways were established among the regulatory agencies, industry, and stakeholders.

Slide presentations from the meeting are at http://www.fda.gov/cber/summaries.htm. When available, transcripts of the workshop will be at http://www.fda.gov/cber/minutes/workshop-min.htm. A docket is being prepared for comments and suggestions for further product development.

The Chairman closed the meeting at 20.00, thanking the speakers and the audience for their contribution.
Factor XIII

Chair: Robert Ariëns, UK
Co-chairs: Paul Bishop, USA, Akitada Ichinose, Japan, Hans Kohler, Switzerland, Rainer Seitz, Germany

Active Members: Laszlo Muszbek, Hungary, Muriel Maurer, USA, Ivaskevicius, Germany

SSC approval sought for:

- Endorsement of International Registry for FXIII deficiency (Ivaskevicius)
- Nomenclature of FXIII (Muszbek)

Ongoing activities:

- Antigen potency estimation for 1st International FXIII Standard
- Development of a standard for concentrates and recombinant FXIII

The FXIII subcommittee held a joint meeting with the fibrinogen subcommittee this year. We had a very busy agenda for both subcommittees, due to which the total joint meeting exceeded its allocated timeslot of 3.5 hours by up to almost 1 hour. Apologies were received from Bishop and Maurer, all other chairs and active members were present. The meeting was attended by around 80-100 delegates.

The FXIII session was opened by Akitada Ichinose (Japan), who provided an overview of the diseases with which transglutaminases are associated. In addition to thrombosis, cardiovascular disease and bleeding, these include neurological disorders, cancer, celiac disease and Huntington disease. Ichinose gave an overview also of the activities of the FXIII standard working party, which has been active since 2002 and has developed the 1st International Standard for FXIII, approved by the SSC and WHO in late 2004.

Ivaskevicius (Germany) presented data from a registry for FXIII deficiencies previously endorsed by ETRO. Currently the registry contains around 100 entries, but the estimated number of cases of FXIII deficiency worldwide is somewhere between the figures of 6,500-19,500. The registry provides important information for the management of FXIII deficient patients. It will also aid in our understanding of genotype-phenotype relationships for FXIII, and structure-function relationships. It is proposed that this registry should be expanded as a true international registry. The SSC is asked to endorse the development of this new, expanded registry for FXIII deficiency.

A new method measuring FXIII activity was presented by the group of Rainer Seitz (Germany), using a biotinylated selection peptide bound to a streptavidin coated microtiter plate. FXIII in a sample is activated outside the plate by thrombin; the reaction is stopped by hirudin. A fluorescence labelled detection peptide is incubated in the plate together with the mixture containing the activated FXIII. The reaction is stopped by EDTA and the plate washed with urea,
before bound fluorescence is measured. Multiple variations of the detection peptide can be used as a powerful tool to study the enzymatic characteristics of FXIII.

The measurement of FXIII activity in concentrates was discussed by Laszlo Muszbek (Hungary). Different FXIII activity assays behave differently with regard to the diluent of the concentrate. Choice of diluent includes buffer, FXIII deficient plasma or FXIII free fibrinogen. The latter two appear to be the materials of choice for the accurate and consistent measurement of diluted FXIII concentrates. It is proposed to use FXIII free fibrinogen at a concentration of 2 mg/ml for future standardisation studies of concentrates.

FXIII nomenclature was discussed by Laszlo Muszbek. Nomenclature of FXIII had been previously considered at the SSC meeting in Florence, 1997. At that time a draft proposal was made and it was decided to test its use by researchers in the haemostasis and thrombosis field. The nomenclature proposal was revisited at this meeting (attached) and it was unanimously accepted in unmodified form by everyone present. It is proposed to seek endorsement of this FXIII nomenclature by the SSC, after which it is planned to submit an SSC brief communication outlining the details to the Journal of Thrombosis and Haemostasis on behalf of the FXIII subcommittee.

Sanj Raut (UK) presented data from a collaborative pilot study by the FXIII standard working party (SWG) for the measurement of FXIII antigen in the 1st international standard for FXIII. During the international collaborative study in 2003-2004 to develop the 1st IS for FXIII, antigen levels had already been determined at 0.93 (GCV 14%). At that time, however, it was decided not to assign this value as yet, as different methods for FXIII antigen determination (anti-A/anti-A, anti-A2B2/anti-A, anti-B/anti-A sandwich ELISA, and A2B2 Laurell) had been employed. As the tetrameric form of FXIII (A2B2) is the prevalent and (potentially) active form of FXIII in plasma, it was decided that this should provide the ‘gold’ standard for FXIII antigen determination. The current SWG pilot study is based on the use of one A2B2 ELISA kit, performed in 5 laboratories, using a protocol developed by the NIBSC. Preliminary data showed an antigen potency estimate of 0.91 (GCV 1.8%), which is in close agreement with the previous study using various antigen assays. The FXIII SWG proposes to await full analysis of the data and, if confirmatory, to pool the estimates from both studies for the assignment of a FXIII antigen potency estimate for the 1st IS for FXIII. Approval for this antigen estimate by the SSC and WHO will be requested in due course.

The effect of polymorphic variants on kinetic activity assays for FXIII was discussed by Robert Ariens (UK). Assays that are based on the kinetic measurement of early pentylamine substrate incorporation into fibrin, can be sensitive to differences in activation rate and hence to FXIII Val34Leu, a common polymorphism in certain populations (25% allele frequency in Caucasians). Ariens presented data of the modification of one such kinetic pentylamine assays into an end-stage, total activity assay for FXIII. The reaction mixture was incubated for 60 minutes rather than 5-10 minutes, and the measurement principle was based on dose-response curves of absorbency against plasma dilution rather than kinetic curves of absorbency against time. The modified assay proved sensitive to FXIII in the ng range with high specificity as demonstrated by parallelism of dose response curves for plasma, purified FXIII, recombinant FXIII-A and a FXIII concentrate for clinical use. Identical dose-response curves were found for
FXIII VV, VL and LL samples with similar A2B2 antigen concentrations. It is proposed that this end-stage assay can be used as alternative to kinetic assays when it is desirable to measure total activatable FXIII.

**TERMINOLOGY TO DESIGNATE DIFFERENT FORMS OF BLOOD COAGULATION FACTOR XIII**
(Laszlo Muszbek)

*Factor XIII normally present in the plasma (tetramer; A2B2):*

**Recommended term:**
Plasma coagulation factor XIII (Plasma factor XIII)

**Not recommended terms:**

*Factor XIII of intracellular localization present in platelets, megakaryocytes, monocytes, macrophages (dimer; A2):*

**Recommended term:**
Cellular coagulation factor XIII, (Cellular factor XIII).

**Not recommended terms:**
Platelet, monocyte, placenta, etc. factor XIII, Cellular protransglutaminase.

**PROPOSED FXIII TERMINOLOGY AND ABBREVIATIONS I.**

Blood coagulation factor XIII: FXIII  
Cellular factor XIII (A2): cFXIII  
Recombinant factor XIII (A2): rFXIII

**Potentially active FXIII subunit:**

recommended term: A subunit of factor XIII (FXIII-A) not recommended terms: factor XIII a or a subunit, (FXIIIa, FXIIIa, FXIII a, FXIII a, FXIII-a, FXIII-a)

**Inhibitory/carrier FXIII subunit:**

recommended term: B subunit of factor XIII (FXIII-B) not recommended terms: factor XIII b, b or S subunit, (FXIIIb, FXIIIb, FXIIIb, FXIII b, FXIII b, FXIII b, FXIII S, FXIII-b, FXIII-b, FXIII-S)
Proteolytic activation of plasma

\[ \text{thrombin} \quad \text{(fibrin)} \]

low Ca\(^{2+}\)

Non-proteolytic activation of plasma FXIII

\[ \text{high Ca}^{2+} \]
PROPOSED FXIII TERMINOLOGY AND ABBREVIATIONS II.

Activated form of blood coagulation factor XIII in general:

activated factor XIII (FXIIIa)

Activation peptide cleaved off from the A subunit by thrombin: factor XIII activation peptide (AP-FXIII)

*Intermediates and endproducts of the activation process:*
Thrombin cleaved inactive form of plasma and cellular factor XIII:

Thrombin-cleaved active factor XIII:

Non-cleaved active factor XIII:
The FXIII and fibrinogen subcommittees held a joint meeting this year. There were more than 150 interested researchers present and the presentations were followed by lively discussions. Due to a busy agenda and discussions the meeting was about one hour over time but it was appreciated that most of the delegates were present till the end.

Michel Hanss described the exhaustive and continuous numbering of published variants has been started on the Internet beginning in the year 2000 with 219 cases thanks to the GEHT (groupe d’etude d’ hemostase et thrombose) website, http://www.geht.org/databaseang/fibrinogen (SSC 2000). It now references 363 entries with a noticeable increase in a/hypofibrinogens and amyloidosis related dysfibrinogens. The referenced cases are identified with affected protein/gene position main symptoms, first author and year of publication. Minimum biological and clinical characteristics have been proposed in order to provide possible on-line registration of new cases. This meeting gave a good opportunity to enlarge the database due to new contacts with researchers in this field such as Wolfgang Miesbach, Shirley Utte de Willige and others.

Susan Lord spoke about recombinant fibrinogen polymerization and Factor XIII binding. In particular, this research project concerned the specific binding of Factor XIII to the \( \gamma' \) chain of fibrinogen. In adding Factor XIII to normal plasma fibrinogen, the final turbidity increased considerably. However, the recombinant \( \gamma \gamma', \gamma' \gamma \) or mixtures of them did not show any effect on turbidity. These results may contribute to the standardization of determination fibrinogen levels.

Leonid Medved presented a review of fibrinogen polymerization and fibrinolysis. As part of his review, he included new x-ray crystallographic data on the binding of thrombin to fibrinogen, which provides evidence on the mechanisms involved. For the process of fibrinolysis, there are epitopes that are cryptic in fibrinogen and exposed through conformational changes and are important for binding of tPA and plasminogen. The new view facilitates the understanding of fibrin clot formation and dissolution now becoming more important for the understanding of bleeding and thromboembolic diseases in patients.

John Weisel spoke about the unique and remarkable viscoelastic properties of fibrin and their clinical significance: clot stiffness is necessary for hemostasis, the strength and integrity of the clot may be more important than parameters more commonly measured in the coagulation lab, clot plasticity may be necessary to prevent obstruction, they determine whether embolization may occur and the response to treatments such as angioplasty, thrombolysis and surgery, and epidemiological studies suggest a correlation between clot stiffness and MI. Fibrin is a polymer with both elastic and viscous aspects, which were outlined, but we know little about the origin of
either of these components. The research required to determine these mechanisms will require interactions between clinicians and basic scientists.

**Wolfgang Miesbach** presented studies of 350 cases involving 170 distinct mutations, of which 56% were asymptomatic, 25% bleeding or easy bruising, and 20% were thrombotic. There were two mechanisms involved in the thrombotic cases, either defective thrombin binding or defective lysis. Arterial thrombosis was overrepresented in these cases. The cases were characterized in terms of clinical symptoms and the molecular defects. In the same families with identical defect there could be a heterogeneous or even opposite manifestation of symptoms (e.g., bleeding and thrombosis). These cases will be further studied to try to determine the mechanism or to find further markers that could explain these unexpected results.

**Jaap Koopman** proposed that we standardize our toolbox used to characterize fibrinogen. There are two general sources of variation, natural variation in structure/function and methodological variation. There is little consistency in the parameters that are studied or in the analytical methods or the reference material. There is much natural variation in fibrinogen that is important for its characteristics. The parameters to be used to characterize fibrinogen need to be standardized. The following methods were suggested: SDS-PAGE, HIC (HMW/LMW ratio), ion exchange chromatography, turbidity curves, FPA/B release, and FXIII, plasminogen and fibronectin impurities.

**Nicodemo Weinstock** stressed that fibrinogen - though it is well known to be the central molecule in the clot formation and shows risk odd ratios comparable or higher than cholesterol - has been almost forgotten by the clinical community. The consequence: the number of fibrinogen determinations is dramatically falling. The reasons are: 1.) poor standardization for fibrinogen measurements 2.) mostly incorrect normal values determined in the 70ties (e.g. 150-450 mg/dl) 3.) strong variation of “normal mean values” (e.g from 230 up to 390 mg/dl) and cut offs(280; 300, 350, 380, 400) reported in large risk studies made in the 90ties. Therefore the opinions of what is normal, what is high or low, who or when persons are at risk, vary from lab to lab. The consequence is confusion and the feeling that fibrinogen is an invaluable parameter. The opinion was that these items should be overcome as soon as possible and that this should be part of the fibrinogen subcommittee work to be done in the next years.

The fibrinogen subcommittee decided to form working parties for further studies e.g.:

1. standardization of nomenclature (Koopman, Medved, Weisel)
2. reference values (Miesbach)
3. introduction of the new high fibrinogen standard (Weinstock)
4. studies on different methods of fibrinogen determination and correlation to CRP in cardiovascular disease study on 15,000 patients starting in October 2005 (Preetz)
5. standardization of methods (Koopman)
Fibrinolysis

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

TAFI/proCPU

Dr Willemse presented a new kinetic assay for measuring proCPU/TAFI levels in plasma. He first gave an introduction about currently available assays and pointed out the major pitfalls of antigen and activity-based assays. The novel assay he presented is based on the quantification of arginine-cleaved from hippuryl-L-arginine by CPU using 3 coupling enzymes (arginine kinase, pyruvate kinase and lactate dehydrogenase) finally leading to the consumption of NADH which can be followed continuously at 340 nm. The assay shows excellent correlation with the HPLA-assisted reference assay and has a high precision. Compared with HPLC, the assay is much easier to perform and it allows a much faster determination of proCPU concentrations. This, combined with the broad linear range of proCPU determination (100-2400U/L) makes it a useful tool for sensitive screening of clinical samples. Because the assay measures the cleaved arginine it can be used with all kinds of C-terminal arginine-containing substrates and can be used as a useful tool for screening different synthetic and physiological CPU substrates.

In discussion the problem of availability of one of the enzymes needed for the coupled assay was highlighted since it must be purified and is not commercially available.

Standardization of fibrinolytic factors

Calibration of SSC Plasmas

Dr. Longstaff reported on the calibration of SSC plasma #2 and #3 to measure t-PA antigen, PAI-1 antigen and activity. Eight labs joined in the collaboration study to assess the feasibility of calibration. GCV results for t-PA antigen in SSC #2 and #3 were variable, and a little less so for 94/730. However, there was some improvement in variability after the removal of outlier and 94/730 was close to the expected value of 25 ng/ml. Normalisation of SSC2 and 3 using 94/730 as calibrator improved the spread of data and suggests the preparation will be useful as a standard for tPA antigen assays.

Table 1 Summary of potencies

<table>
<thead>
<tr>
<th>Sample</th>
<th>geo mean pot</th>
<th>95% lower</th>
<th>95% upper</th>
<th>%gcv</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC2</td>
<td>3.38</td>
<td>1.87</td>
<td>6.12</td>
<td>103.4</td>
</tr>
<tr>
<td>SSC3</td>
<td>3.67</td>
<td>1.89</td>
<td>7.11</td>
<td>120.9</td>
</tr>
<tr>
<td>*94/730</td>
<td>24.45</td>
<td>19.47</td>
<td>30.7</td>
<td>27.9</td>
</tr>
</tbody>
</table>
*One statistical outlier removed

With the same aim, PAI-1 antigen was compared with 92/651, SSC #2 and #3. The mean value in 92/654 was 73.5 ng/ml, though the expected value was 250 ng/ml. SSC #2 and #3 gave consistent and reasonable results. Labs 1 and 6, using the same kit, gave consistently low results for all samples (see table 2). Results from this small study do not allow us to assign a potency for PAI-1 antigen in ng/ml in plasma with confidence.

**Table 2 Summary of results**
Summary of PAI-1 Antigen after removing labs 1 and 6 [Normal: 4 -43 ng/ml]

<table>
<thead>
<tr>
<th>Assay</th>
<th>geo mean pot</th>
<th>95% lower</th>
<th>95% upper</th>
<th>% gcv</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC2</td>
<td>13.16</td>
<td>8.75</td>
<td>19.8</td>
<td>39.0</td>
</tr>
<tr>
<td>SSC3</td>
<td>13.94</td>
<td>9.98</td>
<td>19.46</td>
<td>30.8</td>
</tr>
<tr>
<td>92/654</td>
<td>107.9</td>
<td>70.61</td>
<td>165.0</td>
<td>40.7</td>
</tr>
</tbody>
</table>

In the study to calibrate the SSC plasmas for PAI-1 activity, laboratories split into 2 groups reporting activity in ng/ml or inhibitor units/ml. These results could not be easily compared. Both groups showed large variability, especially the ng/ml group. Further work is needed before standardization of PAI-1 activity can be attempted.

During the discussion with the audience it was generally agreed that some problems with 92/654 may be due to the recombinant nature of the PAI-1 used to spike the plasma in this International Standard. It was suggested that a new standard of plasma containing a high level of (native) PAI-1 would be useful, especially for the diagnosis of elevated PAI-1 in patient samples.

*Standardization of methodology for plasminogen activator activity.*

C Longstaff gave a final report on the methodology study for determination of thrombolytic potency of plasminogen activators. The aim of the study was to investigate the feasibility of a proposed assay for the determination of absolute enzyme activity is SI units (pM/s plasmin production), as an alternative to International Units (IU). This approach would fulfill some recommendations for assay methods and allows different thrombolytics to be compared, which is not the case currently as IU are different for current plasminogen activator IS. A very detailed assay protocol was agreed before the study and all participants were provided with all critical reagents to measure streptokinase, tPA and uPA activity. In spite of this there was a wide spread of final results returned for absolute enzyme activity. Means of all assay results gave satisfactory dose response curves for all activators. Some improvement in variability (expressed as %GCV) was observed if streptokinase was used as a standard for the other plasminogen activators, but variability was still quite large, around 35%. The conclusions of the study were that absolute determination of enzyme activity is very difficult. It is also problematic to provide a new method to laboratories and expect them to perform the method well without training. The traditional method of calibrating International Standards by recruiting as many labs as possible and allowing them to use familiar methods is a pragmatic approach that will be difficult to change in favor of a specific assay if this involves much complexity.
Standardization problems with recombinant streptokinase

Colin Longstaff reported on observations with potency determinations of native and recombinant streptokinase using the current 3rd International Standard (IS) for Streptokinase. The international standards for streptokinase have been used successfully for more than 40 years and international collaborative studies show excellent agreement between consecutive standards. Many companies around the world manufacture streptokinase to treat the global epidemic of cardiovascular disease seen in developing countries and in Eastern Europe. Some of these products are recombinant (rec) and some of these rec products do not behave well against the 3rd IS for streptokinase in different assay formats. For example, using two standardized methods, (1) without fibrin and (2) with fibrin the potency of two recombinant streptokinase products available in India was measured relative to the 3rd IS for Streptokinase. The ratio of potency for normal, native streptokinase is 1.0 comparing these assay methods, but for one rec streptokinase the ratio was 0.3 and for a second rec streptokinase was 1.5. Thus the inclusion of fibrin in the assay can dramatically affect the potency of the product and the dose given to patients. This is especially important since different pharmacopeias recommend assay methods, without fibrin (e.g., European and British Pharmacopoeia) or with fibrin (Indian Pharmacopoeia). Changing the assay format could result in lethal doses of streptokinase being given to patients. Further work is needed to determine the cause and possible solutions to these problems, which may include provision of additional standards or pharmacopoeial methods.

D-dimer

Assay of D-dimer in multicenter trial I. Jennings

The measurement of D-dimer is used for diagnosis of DIC, monitoring the treatment of DIC, diagnosis of DVT/PE and the prediction of recurrence. The multicenter trial for the assay of D-dimer was performed (431 centers participated). For this trial, 4 D-dimer samples were prepared; high D-dimer level (pool 1: ~1000 ng/ml), low D-dimer level (pool 2: ~300 ng/ml), mixture of equal quantities of pool 1 and pool 2 (pool 3) and pool of plasma with ~300 ng/ml of D-dimer (pool 4).

This trial demonstrated the large variation in results between reagent groups. The comparison of findings in different centers was difficult. The calibration curve was made by plotting overall median D-dimer level versus individual laboratory D-dimer levels for low, mix and high sample. Although the calibration curves could be constructed, comparison of results only valid where linearity was good and data was not extrapolated. Difference may exist between different samples that preclude valid comparison between methods.

Harmonization of D-Dimer assays: Results of the FACT4 study C. Dempfle

At first, Dr. Dempfle presented the consensus of D-Dimer antigen. Monoclonal antibodies used in D-dimer antigen assays should display minimal cross-reactivity with fibrinogen, (monomeric) fragment D from fibrinogen, non-crosslinked fibrin, other proteolytic fragments of fibrinogen or non-crosslinked fibrin.
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 sample 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. Distribution of fibrin compounds and matrix should closely match with clinical plasma samples. Since D-dimer antigen is not a homogeneous 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. Therefore, pooled patient plasma samples could be used for harmonization of D-dimer antigen assays. The pools should contain a variety of clinical plasma samples including the target groups DVT, PE and DIC. Different responses of D-dimer antigen assays in different concentration ranges preclude the use of simple conversion factors.

For D-dimer assay, a common calibrator should be need. Fibrin fragment D-dimer is not suitable for D-dimer assay. One standard is sufficient if the standard is a pooled plasma with a large number of donors. It is necessary to evaluate assays individually for determination of the cut off for DVT exclusion. Dr. Dempfle proposed the preparation and validation of a lyophilized reference preparation based on pooled human plasma. Dr. Dempfle also recommended the definition and validation of the procedure for calibration of the reference preparation, and the clinical evaluation of the calibrator.

Problems with the D-Dimer Assay J. Olson

In reporting the D-Dimer level, two units are usually used. One is D-Dimer Units (D-DU), another one is fibrinogen equivalent unit (FEU). Surprisingly, 1 ng/ml (D-DU) of D-Dimer equals to 2 ng/ml (FEU) of D-Dimer. Data from a study in the U.S.A. indicated nine commercial kits for D-Dimer are available, six recommends reporting FEU and three recommends reporting D-DU. Among all methods for reporting the quantitative D-Dimer, there is wide variation in the type and magnitude of units reported.

Nearly 40% of laboratories are converting the analyzed units and reporting in unit other than those recommended by the manufacturer. Many laboratories are unclear about which type of units they are reporting. This is the major problem with the D-dimer assay performance in the U.S.A.

General discussion and activity for next term

Since we have still several questions and issues to be clarified, we would like to keep the studies on TAFI, Standard of fibrinolytic factors and D-Dimer. Requests to the audience were made to suggest new issues that might be appropriate for the committee.
Haemostasis and Malignancy

Chairman: A. Falanga, Italy
Co-chairs: G. Agnelli; C. W. Francis, USA; A. K. Kakkar, UK; A. Lee, Canada; M. Prins, The Netherlands; L. Zacharski, USA.

The first part of the meeting, chaired by A. Falanga and C. Francis, addressed biological issues. A Working Party on TF standardization in cancer was presented. The panel will be composed by the following scientists, who have agreed to collaborate in the project: A. Falanga and T. Barrowcliffe (Coordinators), E. Gray, N. Key, 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 our current knowledge on TF relevant role in cancer, not only for its procoagulant effects, but also for its involvement in angiogenesis, tumor growth and metastasis. 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 party are: to investigate and compare methods for measurement of TF in cancer cells, to make recommendations on methodology of TF assays, to establish one or more reference reagents. The outline work Programme for 2005-2006 has been proposed.

Along this line, N. Key showed the characteristics of three different TF assays: i.e.:1. The commercial plasma TF antigen assay (Sandwich ELISA); this test gives no information about TF activity; it measures both microparticle (MP)-bound TF and TF soluble species; 2. The capture assay for MP-associated TF Procoagulant Activity (TF-PCA) in plasma, and 3. The inhibition assay of TF/FVIIa by Antithrombin (AT) promoted by HSPGs on cell surfaces. The comparison of the results of TF measurement with the above three assays in plasma samples from subjects with hemophilia A, hemophilia B, and age-matched controls, shows no correlation between the plasma levels of TF antigen, MP-associated TF-procoagulant activity, and VIIa-AT antigen complexes.

N. Mackman presented a method for the determination of blood borne TF by a single stage clotting assay. This method can be applied to test different blood cellular fractions, i.e.: mononuclear cells, platelets, and microparticles. He presented results on TF measurement with this assay in patients with different types of cancer.

The issue of circulating thrombotic markers in malignancy was expanded to other blood components and molecules. A. Khorana presented the results of the analysis of a prospective study evaluating the predictive value of pre-chemotherapy platelet count for the development of chemotherapy-associated VTE. The results of this analysis suggest a predictive value for elevated platelet count (>350,000/mmc). A clinical risk model incorporating the pre-chemotherapy platelet count has been proposed for identifying patients at high risk for VTE at time of initiation of chemotherapy.

J. Fareed described the differential down-regulation by LMWH and warfarin of the following thrombotic and inflammatory markers: TNF-alpha, IL1-beta, CRP, MCP-1, CD 40L, TF, TFPI,
ATFI, ADMA, NO, ADAMTS-13. The results indicate that LMWH can down regulate both thrombotic and inflammatory mediators in patients with cancer.

The second part of the meeting, chaired by A. Lee and G. Agnelli, was an update of clinical trials of antithrombotics in patients with cancer. It included trials still ongoing or recently completed, in addition to the designs of new clinical trials that are going to be started.

I. Pabinger updated on the CATS (Cancer And Thrombosis Study), which consists in the identification of predictive parameters of VTE and PE in patients with various malignancies. The study design is a prospective nested case-control study, which includes patients with newly diagnosed malignancy or relapsed after remission. Projected end of the study is October 2007; 497 patients have been enrolled; 58 patients died; 24 VTE events are recorded.

S. Haas updated on TOPIC I and TOPIC II trials on the prevention of VTE by LMWH certoparin in patients with metastatic breast (TOPIC I) or lung cancer (TOPIC II). Both trials are completed.

VTE events in metastatic breast cancer were less frequent than expected (4%). The interim analysis failed to show superiority of LMWH and the enrolment of patients was stopped. VTE in metastatic non-small cell lung carcinoma was more frequent than in breast cancer. There was no significant decrease in the VTE rate in the treatment group. A post hoc analysis for the influence of disease stage provides evidence that LMWH certoparin affects significantly the VTE rate in patients with disease stage IV.

A. Kakkar presented the design 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. 170 patients have been recruited so far.

G. Agnelli updated on the ongoing study named PROTECHT (PROphylaxis of ThromboEmbolism during ChemoTherapy). This multicenter clinical trial evaluates the efficacy of the LMWH nadroparin versus placebo in the prevention of symptomatic venous and arterial thromboembolism in advanced cancer patients during chemotherapy. Types of cancer included: Lung, Breast, Gastrointestinal, Ovarian, Head and neck. Number of patients enrolled so far: 753 (calculated sample size = 1,200). The interim analysis for efficacy and safety (400 patients) will be available on September 2005.

An update on the newest information available on the prevention of central venous catheter (CVC)-related thrombosis was presented by two groups: 1. G. Agnelli showed the analysis of
risk factors for CVC-related VTE in cancer patients enrolled in the ETHICS study, recently published. The original study shows that the risk reduction by Enoxaparin prophylaxis is not statistically significant. Furthermore, the rate of CVC-related DVT was lower than expected. The inconsistency of risk for CVC-related thrombosis across the study population suggested this risk factor analysis. The results lead to the conclusion that an inadequate position of CVC tip and left-sided CVC insertion are independent risk factors for CVC-related thrombosis. Further, age >60 years and metastatic disease significantly increase the risk; 2. A. Kakkar, on behalf of A.Young and MRC nurse study, presented the latest results of the UK Multicentre Prospective Randomised, Controlled Trial of Thrombosis Prophylaxis with Warfarin in Cancer Patients with Central Venous Catheters. The data show that prophylaxis with 1 mg fixed dose warfarin is not effective in preventing CVC related thrombosis. A subsequent random between 1 mg fixed dose warfarin versus adjusted dose warfarin (INR: 1.3 -1.9) demonstrates the efficacy of the adjusted dose.

Finally, H. Buller presented the design of a new study, named IMPACT (IMproving the Prognosis in Advanced Cancer with low-molecular weight heparin Therapy). 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. The recruitment should start on October-December 2005, approx 25 centres. Eligible patients will be randomised to: standard anti-cancer treatment, or standard anti-cancer treatment plus nadroparin. The primary outcome will be death from cancer.
Lupus Anticoagulants/Phospholipid-Dependent-Antibodies

Chairman: Ph. G. De Groot, The Netherlands
Co-chairs: M. Galli, Italy; S. Machin, UK; J. V. Pengo (Italy); H. Rand, USA; G. Reber, Switzerland, R. Roubey, USA (bold = present at the meeting)

The number of attendees at this meeting was > 200 at 16.00 h and about 50 at 19.30 h

Dr. Steven Krilis gave the first presentation. He reported on the results of a consensus meeting on new criteria for the definition of APS. This meeting was held in Sydney, Australia during the XIth International Congress on Antiphospholipid Antibodies, November 2004. The members of the workshop included rheumatologists, immunologists, obstetricians, biochemists, hematologists, and neurologists. This multidisciplinary company thoroughly discussed all the evidence available in the literature on possible criteria that define the antiphospholipid syndrome. The literature on possible criteria is far from complete and often contradictory. The agreement finally reached was not only based on evidence from the literature but also on eminence opinion and will be published shortly.

Dr. Flip de Groot gave the second presentation. He reported on a SSC-mediated multicentre study on the predictive value of a b2GPI specific LAC assay for the detection of a risk for thrombosis. Seven laboratories originally promised to participate, five laboratories, Veronique Regnault & Thomas LeCompte INSERM, Nancy, France, Guidido Reber. University Hospital, Geneva, Switzerland, Jacek Musial, School of Medicine, Cracow, Poland, Bas de Laat & Flip de Groot, UMC, Utrecht, the Netherlands Ricardo Forastiero, Universidad Favaloro, Buenos Aires, Argentinia, actually participate.

Barry Woodhams & Patrick van Dreden, Stago, (Paris, France) prepared ready-to use kits and together with the instructions how to perform the assay, the kits were send to the participants. The results were disappointing. Large variations between the results of different laboratories were observed. The final outcome, a small but significant improvement in the correlation of the new test with thrombosis over the original LAC assay was completely based on the results of two laboratories. In a attempt to understand why there were such large differences between the different laboratories, additional experiments were performed, focused on the possible differences in the performance of the assays in the different laboratories. In Northern Europe, blood is collected in 0.109 M citrate, while in Southern Europe blood is collected in 0.129 M citrate. It turns out that the b2GPI-dependent LAC assay is only functional in 0.109 M citrate and not in 0.129 citrate. These differences were not explained by the small differences in Ca2+ concentration but by differences in Zn2+ concentration. Addition of 50 m M ZnCl2 to the patient plasma not only restored the effect of cardiolipin on the b2GPI dependent LAC in 0.129 M citrate anticoagulated patient samples, it also makes the assay more sensitive in blood anticoagulated in 0.109M citrate. To be continued.

Dr Ian Jenning, also on behalf of drs Elaine Gray and Jef Arnout discussed the development of a standard for LAC testing. The goal is to develop two standards for LAC testing, one based on normal plasma spiked with monoclonal antibodies against b2GPI and prothrombin and one based...
on a mixture of collected patient plasmas. The major progress made the last year was obtaining ethical approval for collecting plasmas from controls and patients. The ethical approval will only be valid for the UK. Hopefully the standard will be there next year.

**Dr Guido Reber** discussed factors that influence the results of an anti-b2GPI antibody ELISA. When anti-b2GPI ELISAs from different commercial sources or different homemade ELISAs were compared, large differences in results are found. To better understand the cause of these differences, the influence of coating buffer (pH), antigen source, microplate brand, washing steps, blocking buffer, ionic strength of the dilution buffer, the role of Ca2+ and the secondary antibody used was tested. The most important factors that influence the results of this ELISA were the brand of the microtitre plate, blocking buffer (only for a selected patient sample), ionic strength of the dilution buffer and the presence of Ca2+ in the dilution buffers. There is at the moment not a strategy to overcome these problems. The only solution that might help at short run is the introduction of a standard to all commercial assays. Dr. Koike (Sapporo, Japan) has made available human monoclonal antibodies (IgG and IgM) for this purpose. Negotiations with the commercial companies will be continued.

**Dr. Eiji Matsuura** discussed the presence of b2GPI-oxLDL complexes in plasmas of patients with the antiphospholipid syndrome and the presence of autoantibodies directed against these complexes. Oxidized low-density lipoprotein (oxLDL), not native LDL, binds *in vitro* to b2 GPl via specific oxLDL-derived ligands to form oxLDL/b2 GPl complexes. Elevated serum levels of oxLDL/b2 GPl complexes are frequently found in patients with autoimmune diseases and antiphospholipid syndrome (APS). The presence of these complexes along with oxLDL/b2 GPl autoantibodies strongly suggests an important atherogenic role autoimmune-mediated atherosclerosis. IgG and IgM antibodies to oxLDL/b2 GPl complexes were measured by ELISA in 45 systemic lupus erythematosus (SLE) without APS, 29 with secondary APS, 53 systemic sclerosis (SSc) and 81 rheumatoid arthritis (RA) patients. Healthy blood donors (n=100) served as controls. Mean ODs of IgG antibodies were: SLE = 0.215 with 40% reacting above the cut-off (mean OD + 3SD of controls), secondary APS = 0.521 with 76% positives, SSc = 0.177 with 20% positives, and RA = 0.185 with 18% positives. SLE, secondary APS and SSc were statistically higher (p<0.001) compared to controls (0.135). Mean ODs of IgM antibodies were: SLE = 0.265 with 20% positives, secondary APS = 0.684 with 41% positives, SSc = 0.279 with 19% positives, and RA = 0.516 with 51% positives. All groups were statistically higher (p<0.007) compared to controls (0.178). IgM results did not correlate with RF activity. Secondary APS had high IgG and IgM antibody levels. However, RA and SSc, to a lesser degree, only had increased IgM antibodies. This suggests, that IgG and IgM anti-oxLDL/b2 GPl antibodies may play an atherogenic role in APS. Whether IgM anti-oxLDL/b2 GPl antibodies in RA are anti-atherogenic remains to be determined.

IgG anti-oxLDL/b2 GPl antibodies were also measured in 93 secondary APS patients: 37 had venous thrombosis, 42 arterial thrombosis and 14 pregnancy morbidity. Mean OD for arterial thrombosis group was 0.802 with 38% of the patients reacting above the cut-off (mean OD + 3SD of controls); 0.544 for venous thrombosis with 19% positives, and 0.137 for pregnancy morbidity with 0% positives. Mean OD for arterial and venous thrombosis groups were not statistically different (p=0.262), but both (arterial and venous) were statistically higher compared to the pregnancy morbidity group (p<0.02). Positive predictive value (PPV) for total thrombosis...
(arterial plus venous) of IgG anti-oxLDL/b 2 GPI antibodies was 92% (p=0.018), for arterial thrombosis 89% (p=0.004), for venous thrombosis 77% (p=0.161) and for pregnancy morbidity 0%. Mean antibody level was higher in APS patients with arterial compared to those with venous thrombosis, but this difference was not statistical significance. However, PPV for arterial thrombosis (89%) was statistically stronger than that for venous thrombosis (77%). These results provide additional support for a pathogenic role of oxLDL/b 2 GPI antibodies in the development of autoimmune atherosclerotic complications.

Dr Monica Galli participated in the WAPS (Warfarin in the Anti-Phospholipid Syndrome) study. Antiphospholipid antibodies include, among others, anticardiolipin (aCL), anti- b 2-glycoprotein I (a b 2GPI), anti-prothrombin (aPT), anti-annexin V (aAV) and anti-protein S (aPS) antibodies. Since their clinical significance in the antiphospholipid syndrome has not been clearly defined, we assessed 112 patients (24 males and 88 females, aged 23-81, median 42 years) enrolled in the WAPS Study. Ninety-one subjects (81.3%) were lupus anticoagulants (LA)-positive according to the SSC criteria established in 1995. Thirty-two (28.6%) suffered from autoimmune diseases, and 87 (77.7%) from antiphospholipid syndrome. Eighty-one (72.3%) had a history of arterial (n=35) and/or venous thrombosis (n=51), and 17 (19.3%) women had suffered from one or more abortions. During a median follow-up time of 4 years, 15 (13.4%) patients experienced arterial or venous thrombosis. Commercially available ELISAs were used to measure IgG and IgM aCL (Asserachrom APA), a b 2GPI (Asserachrom Anti-b 2GPI) and aPT (Asserachrom Anti-Prothrombin) antibodies. Prototype ELISAs were used for the detection of IgG and IgM aAV and aPS antibodies. All ELISAs were kindly provided by Diagnostica Stago. Values of IgG and IgM aCL, aPT antibodies were expressed in units. Values of IgG and IgM aAV and aPS antibodies were expressed in mOD. Values were grouped by tertiles. Odds Ratio and p values were calculated by logistic regression.

The following statistically significant associations were found:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clinical associations</th>
<th>OR (95% CI), p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG aCL High vs low tertile</td>
<td>Antiphospholiid syndrome</td>
<td>3.945 (1.117-13.939), 0.0331</td>
</tr>
<tr>
<td>IgM aCL High vs low tertile</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>IgG a b 2GPI High vs low tertile</td>
<td>Antiphospholid syndrome</td>
<td>16.714 (2.061-135.5), 0.0084</td>
</tr>
<tr>
<td></td>
<td>Total retrospective thrombosis</td>
<td>3.829 (1.121-13.079), 0.0322</td>
</tr>
<tr>
<td></td>
<td>(Recurrent) retrospective abortions</td>
<td>14.492 (1.692-124.1), 0.0147</td>
</tr>
<tr>
<td>IgM a b 2GPI High vs low tertile</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>IgG aPT High vs low tertile</td>
<td>Antiphospholid syndrome</td>
<td>4.128 (1.260-13.529), 0.0192</td>
</tr>
<tr>
<td></td>
<td>Total retrospective thrombosis</td>
<td>3.613 (1.286-10.151), 0.0148</td>
</tr>
<tr>
<td></td>
<td>Venous retrospective thrombosis</td>
<td>2.453 (1.041-5.783), 0.0402</td>
</tr>
<tr>
<td>IgM aPT High vs low tertile</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>IgG aAV</td>
<td>(Recurrent) retrospective abortions</td>
<td>6.250 (1.227-31.838), 0.0274</td>
</tr>
<tr>
<td>IgM aAV</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>IgG aPS</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>IgM aPS</td>
<td>none</td>
<td></td>
</tr>
</tbody>
</table>

No significant association was observed between tested variables and the M isotype, irrespective of the antibody. No significant association with thrombosis registered during follow-up was found, possibly because of the small number of events.

In conclusion, these data suggest to measure IgG a b 2GPI in patients suspected of suffering from APS and raise the possibility that they may replace aCL in the diagnosis of antiphospholipid syndrome. IgG aPT measurement also seems to properly establish the syndrome. The role of aAV and aPS antibodies and, in general, of the IgM isotype remains to be elucidated.

**Dr. Jacob Rand**, from Montefiore Medical Center in Bronx, NY, USA provided an update on the current status of annexin A5 resistance testing in APS. Annexin A5 is a potent anticoagulant protein that crystallizes over phospholipids bilayers shielding them from availability for coagulation reactions. Antiphospholipid antibodies create defects in this shield, and thereby reduce its effectiveness as an anticoagulant. This has been translated to devise a clinical assay for detecting resistance to annexin A5 anticoagulant activity. Thus far several small blinded studies with well characterized patients and control groups have demonstrated the presence of annexin A5 resistance in APS. Additional studies with larger numbers of patients from different centers are needed to test the validity of this assay.
Perinatal/Pediatric Haemostasis

Chairman: P. Massicotte, Canada
Co-chairs: G. Kenet, Israel; P. Mathew, USA; P. Monagle, Australia; W. Muntean, Austria; U. Nowak-Göttl, Germany; N. Schlegel, France

Joint Session with the Scientific Subcommittee on Control of Anticoagulation
Chairs: S Schulman/ P Massicotte

New anticoagulants for pediatric use; lessons from adults

Jeff Weitz gave an overview of the development of new anticoagulants over the past few years, with emphasis on the lessons learnt and possible implications for the pediatric population. He described the development of heparins towards lower molecular weight with problems regarding decreased clearance in case of renal impairment, lack of specific antidotes and long half-life. New, selective anticoagulant agents have usually rapid onset and offset, a wide therapeutic window and no or reduced need for monitoring, but pediatric data is lacking as well as specific antidotes. A comment from the audience was that argatroban and bivalirudin, both approved drugs, are in clinical trials in the pediatric population.

Recommendation: It is important to ensure that some of the new anticoagulants will fit the needs of the pediatric population, for example with parenteral (subcutaneous) administration, no need for monitoring, not contraindicated in case of hepatic failure etc.

Towards a unified definition of major hemorrhage in clinical trials.

I. Non-surgical studies

Report S. Schulman
The process from discussion at the previous SSC in Venice 2004 was recapitulated briefly. The recommendation was published as a full length paper in JTH in April 2005. The European regulatory authority, EMEA, has been contacted and expressed interest in possibly adopting the recommendation but preferred to have the complete set, including recommendations for orthopedic and general surgery studies. Informal contacts have also been taken with FDA, and there is a growing interest there for possible issues of harmonization.

Plan: Encourage the process for similar recommendations in surgical studies and to further develop the contacts with EMEA and FDA.

II. Surgical studies – orthopedic.

Update. G. Raskob
The Working Party on Bleeding Complications in Orthopedic Studies has identified a large variety of definitions used in their field by performing a systematic literature review. Traditional measures of severity of bleeding have limitations in the early postoperative period. There is a definite need to include the surgeon’s assessment of the surgical site bleeding.
**Recommendations (preliminary):** 1) There is a need for explicit reporting for the surgical site, which must be distinguished from other bleeding. 2) A blinded assessment should be done by a surgeon regarding the clinical importance of the bleeding at surgical site. 3) Bleeding index and clinically important bleeding should be reported independently as separate outcomes. 4) A clinically important bleeding at the surgical site if it leads to wound dehiscence, infection, re-operation, prolonged hospital stay or contributes to myocardial infarction, stroke or death, as assessed by an independent adjudication committee. The WP will accelerate their pace of development of the recommendations and will endeavor to get this published within the next 12 months.

III. Surgical studies – general.

**Update. D. Bergqvist.**

This issue is even more complicated than the orthopedic procedures, since the surgical procedures are less standardized in general surgery and the severity of the procedures vary greatly. Gynecologic procedures may differ a lot from other general surgery. The transfusion requirements are influenced by more or less conservative policies.

**Recommendations (very preliminary):** Major bleeding is tentatively defined as 1) leading to death, 2) leading to transfusions or endovascular hemostatic procedures, or 3) occurs in critical organs.

**Plan:** To form a working party within the next few months to continue the development of a unified definition.

*The Use of Heparin in Children. P. Monagle*

Unfractionated heparin (UFH) is the anticoagulation of choice in infants and children who are at high risk of bleeding (peri surgery, trauma, chemotherapy) because of ease of reversibility (protamine sulfate) and short half life. During cardiopulmonary bypass and extracorporeal membranous oxygenation, UFH is currently the anticoagulant agent of choice. However, infants and children do not respond to UFH in the same way as adults. The activated partial thromboplastin time (aPTT), a surrogate measure of UFH level, does not correlate to increasing levels of heparin in the same fashion as in adults. In fact, if comparing therapeutic anti factor Xa levels to corresponding aPTT levels, in infants and young children the therapeutic aPTT ranges are much high than those in older children and teenagers. This difference may relate in part to developmental haemostasis differences, but there may be other different mechanisms of interaction compared to adults.

Recommendations: The difference response to unfractionated heparin between adults and children will be determined. A subgroup lead by Dr Monagle will determine how best to monitor UFH in neonates and children. This will be submitted as a position paper to the SSC.

*HIT in children A. Greinacher*
The literature in adults with HIT was summarized. In neonates and children, heparin induced thrombocytopenia is rare (< 1%). Most infants who develop antibodies have underlying cardiac disease and develop antibodies post cardiac surgery. In adolescents who develop HIT, the most common indication for unfractionated heparin is the treatment of venous thromboembolism. The testing for HIT in children has not been standardized and cut off values for abnormal must be established.

**Recommendations:** A standardized approach to the diagnosis of HIT in children must be established. Dr Greinacher will lead a subgroup to develop a diagnostic approach in children which will be submitted as an SSC position paper.

**Bleeding**

**Treatment of Bleeding**

*FVIIa use in non hemophiliac children: International Registry(ISTH Study)* - P. Mathew/ J. Blatny

The use of FVIIa is increasing internationally in non haemophilac children despite the lack of properly designed studies. The establishment of an international web based registry to record and follow those children is important to provide an estimate of safety and efficacy in the absence of randomized controlled trials.

SevenBleep Registry is a web based International Registry which has just gone live. The Registry can be accessed from the ISTH home page.

**Recommendations:** Encourage international health professionals using FVIIa in non hemophiliac children to enter data in registry to estimate safety and efficacy of the product. Users must ensure that local research ethics boards formally accept this entry of de identified data.

**Antiplatelet Therapy**

*ASA resistance (ISTH study proposal)* - M. Rand/ M. Albisetti

A significant benefit of ASA has been demonstrated in the prevention of arterial thrombotic events in high-risk adult patients. Recurrent thromboembolic events have been reported in 5-45% of patients despite ASA therapy; this has been termed ASA ‘resistance’, but may actually reflect treatment failure. The failure of ASA to affect ASA-dependent laboratory tests has also been termed aspirin ‘resistance’. The Working Party on ASA Resistance of the Platelet Physiology Subcommittee has concluded that a clinically meaningful definition of ASA ‘resistance’ needs to be developed, based on data linking ASA-dependent laboratory tests to clinical outcomes in patients. Studies in children are required to explore ASA ‘resistance’. In an ongoing prospective study of ~100 children with arterial ischemic stroke, 20% are aspirin ‘resistant’ based on laboratory testing. A prospective study in children following interventional cardiac catheterization is undergoing ethics approval, and several other centres with appropriate patient populations have been identified.
**Recommendations:** Encourage international participation in this cohort study to determine the percentage of children who are ASA resistant. Further studies are required to determine alternative anti platelet agents in ASA resistant children.

**Predictors of Bleeding**

*Coagulation tests as predictors of Bleeding in CPB: New ways to predict bleeding / When should we test – G. Kenet / N. Schlegel*

Tonsillectomy and adenoidectomy are the most common surgical procedures performed in children. Despite the progress made in this type of surgery, the operative hemorrhagic risk factors are not clearly defined. However, the occurrence of either peri or post surgery hemorrhage is considered as potentially life threatening due to the anatomical location of the palatine tonsils and the adenoids. Hemorrhage is the most common complication of such a surgery. An estimated 2-3% of patients have hemorrhage and 1 of 40,000 patients die form hemorrhage. Bleeding may occur during surgery or after surgery either within the 24 hours (primary hemorrhage) o between day 2 to 10 (secondary hemorrhage). Pre-surgery physical examination and questioning for personal and familial bleeding history are recognized to be the most informative procedures. By contrast, in absence of controlled trials, there is no consensus of opinion about the practice of pre surgery haemostasis tests. The pediatric specifics: developmental haemostasis peculiarities, risks of coagulation activation due to difficult blood drawing, risks of artifactual results due to heparin contamination, enhance the poor positive predictive value for bleeding of the screening tests for platelet functions and the limitations of INR and aPTT. The inherited haemostasis disorders associated with bleeding risks, which are rare diseases (Von Willebrands disease being more common), are mostly diagnosed early in life, but some defects might be unknown at the time of the surgery. Taking inot account the pros and cons, the most common attitude is to perform screening tests in the following situations: any child before walking age, positive personal and/or familial bleeding history, acquired disease with hemorrhagic risk, drugs associated with bleeding risk, family questioning not reliable, not relevant or not possible.

Official recommendations will be helpful from both the medical and legal points of view. The recommendations will facilitate a pre surgical estimate of the risk of bleeding and appropriate preventative therapies within the expert team (anesthesiologist, surgeon and hematologist). Platelet count and aPTT appear to be the most useful initial pre tonsillectomy and adenoidectomy tests to complete. If there are any concerns re peri surgical bleeding, Von Willebrands Disease testing should be completed pre surgery. Currently, there are no validated platelet function tests that allow a reliable assessment re peri surgical bleeding risk.

**Recommendations:** A subgroup lead by Gili Kenet and Nicole Schlegel will explore the institution of pre surgical questionnaires that indicate a potential peri surgical bleeding risk. The subgroup will liase with Francesco Rodeghiero and the European Union re the validated and pre existing questionnaire for bleeding associated with Von Willebrands Disease. This established questionnaire may have potential application to tonsillectomy and adenoidectomy bleeding risk.

*Cardiopulmonary Bypass (CPB) and Stroke: Update – A. Chan / P. Massicotte*
Arterial ischemic stroke (AIS) is likely to be an important complication arising from cardiopulmonary bypass in children. The best estimated incidence of AIS at the present time is 0.4%. The three parameters that have been associated with an increase incidence of AIS are older age at the time of CPB, longer bypass time and lower pre-op activated partial thromboplastin time. Forty-eight percent of the patient with AIS had severe to moderate neurological deficit. However, large multicentre prospective studies are necessary to determine the incidence, risk factors and long term outcome of such complications. Optimization of anticoagulation therapy and cardiopulmonary bypass techniques, and development of neuroprotective agents are necessary in order to prevent the devastating long term complications associated with CPB, such as AIS.

Recommendations: The subcommittee has submitted a position paper to the SSC and awaits comments/approval. This position paper will facilitate properly designed incidence studies.

Thromboprophylaxis of mechanical heart valves: Point of Care Testing (ISTH position paper) – M. Bauman / F. Newall

Children with mechanical prosthetic heart valves require oral antithrombotic therapy using vitamin K antagonists. Monitoring vitamin K antagonists in children is difficult as they usually have complex underlying health problems, are on multiple medications and are often difficult to venesect. The cohort of children requiring primary thromboprophylaxis for the management of mechanical prosthetic heart valves is further challenging due to their lifelong need for thromboprophylaxis. This position paper will discuss the rationale for point-of-care capillary monitoring of vitamin K antagonists and provide an overview of POC monitoring. Clinical recommendations will be made regarding the use of POC testing generally, and use of this technology in home monitoring programs. POC INR monitoring, including home INR monitoring, in children has been demonstrated to be safe and efficient with the setting of dedicated paediatric anticoagulant clinics. Whether such monitoring remains safe and efficient when implemented within other models of care has not been determined. Implementation of strategies to reduce the risk associated with reduced reliability of POC INR results greater than 5.0 can optimize this management strategy. Consideration should be given to the education program upon which home INR monitoring programs are built.

Recommendations: The position paper is ready for comments/review by the subcommittee members. This will facilitate properly designed studies to determine safety and efficacy, quality of life and cost effectiveness of POC monitoring in children.

Risk factors for Venous Thrombosis/Thromboprophylaxis

Factor VIII, lipoprotein (a) and other risk factors in Thrombosis Recurrence in children. Update of Studies U. Nowak Gottl/ M. Manco Johnson

In children FVIII assays must have cut off values for abnormal which are dependent upon age and blood group. If FVIII is persistently increased, there is controversial data as to whether this is associated with an increased risk of VTE. Goldenberg et al show an increased risk of
recurrence while Nowak Gottl et al demonstrate no increased risk of recurrence (unpublished data).

In children lipoprotein a cut off values for abnormal are age related. Increased lpa is associated with an increased risk of recurrent stroke. It is unclear whether recurrent VTE is associated with increased lpa.

**Recommendations:** More studies to determine the relationship of FVIII to the development of VTE in children are necessary.

**Antiphospholipid Antibodies: Risk of Thrombosis in Children with APLA+: Status of manuscript (ISTH position paper)**

C. Male

Antiphospholipid antibodies (APLA) occur in children with a variety of conditions, most frequently following infections. However, APLA associated with thrombotic events (TE) are essentially limited to children with underlying autoimmune disease. The best evidence on the risk of TE associated with APLA comes from studies in children with systemic lupus erythematosus (SLE). Multiple laboratory tests for APLA are available but there are conflicting reports which test best predicts an increased risk of TE.

A recent cohort study of 58 consecutive children with SLE determined lupus anticoagulants (LA), anticardiolipin antibodies (ACLA), anti- b 2 -glycoprotein-I (anti- b 2 GPI), anti-prothrombin (anti-PT) in serial samples and prospectively (and retrospectively) assessed patients for symptomatic TE, confirmed by objective radiographic tests. Ten TE occurred in 7 patients (12%). Lupus anticoagulant showed the strongest association with TE since no LA-negative patient had TE. LA remained the only significant factor in multivariable analysis. Persistent ACLA, anti- b 2 GPI, and anti-PT were all significantly associated with TE. Considering all positive (persistent and transient) antibodies, the strength of association remained similar for LA and anti- b 2 GPI, while ACLA and anti-PT were no longer associated with TE. Positivity for multiple APLA subtypes showed substantially stronger associations with TE than for individual APLA subtypes because of improved specificity.

In conclusion, the criteria to best predict the risk of TE associated with APLA in children are: 1. underlying autoimmune disease, e.g. SLE; 2. persistent presence of APLA; 3. presence of LA, and 4. positivity for multiple APLA subtypes.

**Recommendations:** The risk of thrombosis in children with APLA+ will be submitted to the SSC for publication.

**Open Prospective International Registry: Infants of mothers with APLA syndrome**


The aim of this register is to evaluate the correlation between maternal disease / treatments and the clinical events, neuro-developmental features and immunological status of the babies. The prevalence, type and kinetics of disappearance of antiphospholipid antibodies (APLA) in infants
and children born to mothers with primary or secondary APS can be determined, according to Sapporo criteria. The potential effects of these antibodies will be evaluated in children until the age of 5. In 54 babies already included, we observed a higher prematurity rate than in the normal neonatal population. All types of APL antibodies were found in 39% of the neonates and in 5/54 neonates, new specificities of antibodies, different from maternal antibodies, were present. The time of disappearance of these antibodies is prolonged to 6-18 months. Furthermore, we observed 8 children, negative at birth who subsequently became positive. The registry is expected to recruit 300 neonates and mothers within the next 3 years.

To download the data base and to send the form please contact: philippe letoumelin@avc.ap-hop-paris.fr

Treatment and Prophylaxis of Thrombosis

Treatment of venous thrombosis - E. Chalmers/ H. Van Ommen

There are no new studies determining the safety and efficacy of medical therapy for venous thrombosis in infants and children. The differing treatment practices for venous thrombosis in infants and children were determined in an international survey (see below).

Recommendations: The results of the survey to be published will facilitate the design of treatment studies in infants and children with venous thrombosis.

Central Venous Lines: Thromboprophylaxis: Status of manuscript (ISTH position paper) S. Revel-Vilk / L. Mitchell

The two high risk groups with CVL related thrombosis that have been identified are children with ALL and children with congenital heart disease (CHD). Need summary

Recommendations: The recommendations for thromboprophylactic studies in these high risk groups will be submitted as a position paper to the SSC.

Report on Diagnosis and Treatment of DVT in children: Survey of the Pediatric Perinatal SSC (ISTH publication) L. Bomgaars

The Pediatric Perinatal subcommittee (30 members) was surveyed re the diagnosis and treatment of VTE in infants and children. There were 17 questions in the survey which was completed by 72% of the members (18 centres, 9 countries). The conclusions are:

1. LMWH as part of therapy in neonates is considered standard of care by all surveyed.

2. Objective testing schedule and duration of therapy is highly variable.

2. Thrombophilic work ups are more often performed in children as compared to neonates and most commonly included the measure of Protein C, S, Antithrombin, Factor V Leiden, and prothrombin 20210 defect.
3. Further RCTs are needed to define optimal therapy and evaluation in children.

**Recommendations:** The determination of international expert practice for the diagnosis and treatment of thrombosis in infants and children will facilitate the design of proper safety and efficacy studies. The results of this survey will be submitted as a position paper to the SSC.

**Antiphospholipid Antibodies**

**Risk of thrombosis in children with APLA – C. Male**

There is good evidence of a high risk of thrombosis in children with SLE and APLA. The association of APLA with TE found in pediatric cohorts are stronger than those found in adult studies. Few children with SLE who are negative for APLA develop TE. Lupus anticoagulant is a stronger predictor of the risk of TE than anticardiolipin antibodies, anti beta 2 glycoprotein antibodies and anti prothrombin antibodies.

In children without underlying SLE case reports describe associations of APLA and severe thrombotic complications (primary APLA syndrome). Currently, it is unknown what the risk of TE is in well children with APLA. Increased prevalence of APLA are found in children who suffered from stroke compared to controls. However, recent evidence suggests, in general, APLA presence is not associated with an increased risk of recurrent stroke.

**Recommendations:** The subcommittee recommends that the data on stroke and APLA in children be published in a Position paper with the recommendations that more studies are urgently needed in this area especially in primary prophylaxis of patients with APLA and SLE.
Plasma Coagulation Inhibitors

Chair: E. Gray, UK
Co-chairs: F. Bernardi, Italy; F. Church, USA; C.J. Jackson, USA; D. Lane, UK; K. Suzuki, Japan; H. Whinna, USA

WHO International Standards: Chair: F Bernardi

International Genetic Reference Panel for Prothrombin G20210A Mutation. E Gray

An international collaborative study to validate the WHO 1st International Genetic Reference Panel for Prothrombin G20210A Mutation was presented. The panel included 3 preparations of human gDNA: wild type, homozygote and heterozygote for G20210A mutation. The study involved 45 laboratories from 23 different countries, employing a total of 22 different methods against in-house known patient or commercially available controls. The majority of the participants correctly genotyped (error rate 0.7%) and therefore confirmed the validity of the panel. It was therefore recommended that the panel (05/130) should be considered by the SSC and the ECBS of the Who to be established as the international genetic reference panel for prothrombin G20210A mutation. The subcommittee approved the recommendation.

International Standard for Protein C, Plasma and Concentrate. E Gray

E Gray announced the forthcoming international collaborative study to replace the 1st International Standard for Protein C, Plasma. The study will also serve to establish the 1st International Standard for Protein C, Concentrate. The same exercise will also calibrate SSC secondary plasma standard Lot #3 for Protein C activity and antigen. The samples and protocol will be dispatched to the participants in August 2005 and the results of the study will be presented at the next SSC meeting in June 2006.

International Standard for Protein S, Plasma. AR Hubbard

The current WHO 1st International Standard for Protein S, Plasma was established in 1995 and the stocks have fallen to approximately 700 ampoules. It is therefore necessary to calibrate a replacement preparation. The candidate WHO 2nd IS Protein S Plasma (03/228) has been prepared from a pool of 24 plasma units from normal healthy donors. The variability of the liquid fill into ampoules was extremely low with a CV of only 0.09%; the mean fill weight was 1.0063 g and the residual moisture was only 0.064 %. Preliminary tests have indicated a level of 0.92 IU total Protein S antigen per ml. These results indicate that the preparation (03/228) is suitable for calibration as the Proposed 2nd IS Protein S Plasma. The calibration exercise will involve comparison with the current WHO 1st IS and with locally collected normal plasma pools in order to check on the continuity of the International Unit and the possible drift of the IU from the original calibration of the WHO 1st IS. As with the WHO 1st IS three parameters will be measured: total antigen, free antigen and function. The collaborative study is planned to commence in October/November 2005 with a view to submission to WHO and establishment in November 2006.
Protein S Multimers Chair: F Church and F Bernardi

*Another look at protein S monomers/multimers and their direct anticoagulant activity*. MJ Heeb

Plasma protein S has activated protein C-independent, direct anticoagulant activity (PS-direct). It was reported that monomeric purified protein S has only weak PS-direct, that multimeric purified protein S has good PS-direct in the presence of limiting phospholipids (0.1 µM), but that plasma contains only monomeric protein S, leading to the possible conclusion that either plasma has no PS-direct or that active purified protein S containing multimers has artifactual PS-direct. Dr Heeb showed that conventionally-purified protein S prepared with MonoQ-Sepharose had poor PS-direct and poor phospholipid affinity. Monomers, dimers, trimers and higher forms of affinity-purified protein S were identified by analytical ultracentrifugation. Multimers were not dissociated by Ca 2+ or promoted by EDTA alone, but may be concentration-dependent.

On a mass basis, monomers and multimers separated from affinity-purified protein S had the same specific PS-direct in the presence of saturating phospholipids (25 µM) and the same ability to compete with prothrombinase components for limiting phospholipids (2 µM). She concluded that Protein S monomers and multimers were detected in citrated plasma that had PS-direct, and in whole and fractionated hirudin-plasma. Thus, protein S multimers are naturally-occurring and plasma PS-direct is represented by affinity-purified protein S but not by some conventionally-purified protein S.

*Should we bother about protein S multimers?* T Hackeng

Dr Hackeng addressed the history and current status of protein S multimers and concluded that these multimers are in vitro artefacts that can seriously affect results of experiments and conclusions drawn. The APC-independent activity observed in model systems using purified proteins at low phospholipid concentrations could be completely tracked back to the presence of protein S multimers. The multimers were not present in plasma although APC-independent activity of protein S was observed in plasma. This activity therefore must follow a different mechanism than the APC-independent activity of purified protein S in model systems using purified proteins.

Global Coagulation/ Haemostatic Tests. Chair: H Whinna

*Pre-clinical validation of the Calibrated Automated thrombogram (CAT)*. HMH Spronk

Dr Spronk presented data on setting reference ranges for thrombin generation test performed on the CAT. He compared results obtained from blood samples collected by half and full draw and found that there was no significant difference between these samples. Reference ranges for female and male were presented. He found that there is no need for reference range for different age groups, but there was a correlation between age and endogenous thrombin potential (ETP).

*Recombinant tissue factor (rTF) and tissue plasmingon activator (t-PA) used in a new global assay to determine the combined effect of coagulation and fibrinolysis in plasma*. S He
A global assay involving the addition of r-TF and tPA to platelet poor plasma was discussed. Overall coagulation potential (OCP) was obtained by the addition of rTF to platelet poor plasma (PPP), while the addition of t-PA gave results on overall fibrinolytic potential (OFP). The balance of OCP and OFP yielded the overall haemostatic potential (OHP). Results on application of this assay to haemophilic plasmas and patient samples (coronary heart disease and DVT) were discussed.

*The Nijmegen Haemostasis Assay (NHA). W van Heerde*

Dr van Heerde presented an assay that measures thrombin and plasmin generation simultaneously in a single well, using two different fluorogenic substrates. To validate the NHA several conditions were tested. Titration of TF varied the thrombin generation lagtime, the total thrombin generation (thrombin potential) and the plasmin generation lagtime. Total plasmin generation was not affected. Corn trypsin inhibitor did not interfere in the NHA indicating that initiation via the intrinsic pathway does not occur. Dilution of cephalin resulted in a diminished thrombin potential. Hirudin completely blocks thrombin and plasmin generation suggesting the requirement of fibrin for plasmin generation. Addition of carboxy-peptidase inhibitor diminishes clot lysis time indicating thrombin-activatable-fibrinolysis inhibitor (TAFI) activity. TAFI activity is increased by low thrombomodulin (TM) concentrations. High concentrations of TM and active protein C affect thrombin potential. e-aminocaproic-acid inhibits plasmin generation. Ultimately, increased concentrations of t-PA decrease thrombin generation lagtime suggesting interplay between fibrinolysis and coagulation. Titration of plasmin proved that an increased fibrinolytic activity results in an increased thrombin generation. In conclusion, the NHA allows the detection of coagulation and fibrinolysis and the interplay between both and may be suitable for screening haemostatic disorders.

*Thrombin Dynamics Test (TDT) and ROTEM whole blood coagulation analysis potential value for the assessment of inhibitors and thrombophilic states. A Calatzis*

The principle of ROTEM was described and it is based on the kinetic analysis of clot formation using whole blood. It allows the assessment of factors affecting thrombin generation, fibrin formation and polymerization as well as fibrinolysis. The principle of TDT was also described and it is an assay for the kinetic measurement of thrombin formation based on platelet poor plasma and optical detection on routine coagulation instrument. Results on the sensitivity of TDT to factor deficiencies and anticoagulants as well as procoagulant treatment were shown.

*The possible use of the TDT in monitoring hypercoagulability. B Woodhams*

Dr Woodhams reported results from a preliminary study to evaluate the use TDT to discriminate between normal subjects and hypocoagulable samples. Thrombin activity in patient citrated plasma was monitored using a 'fast' thrombin chromogenic substrate with added Gly-Pro-Arg-HydroxyPro to inhibit fibrin polymerisation. The assay can be performed easily on most automated routine haemostasis analyser. The data showed that there were some overlap between normal ranges and hypercoagulable plasma samples, but was useful for plasma samples from hypocoagulable patients.
**Working Party on Thrombin Generation Test (TGT): Results on current practices survey and progress on working party activity. T Henckle**

Dr Henckle reported on the activity of the Working Party. The survey results on current TGT practices is now available. The most widely used methods are the non-sampling chromogenic and fluorogenic assays. A mini review on TGT was published by the Working Party. A pilot study on a chromogenic non-sampling method has been initiated and an international collaborative study is now being planned to assess the comparability of current fluorogenic method for TGT.

**Reference Materials and Methods for Antithrombin. Chair: E Gray**

**Structure and activities of the ISTH/IFCC Joint Committee on Standardisation of Coagulation Tests (C-SCT). E Gray**

The structure and activities of the C-SCT is still to be decided. Activity such as reference methods and materials for antithrombin will continue to be carried out and reports on the activity of the C-SCT will continue to be presented at this subcommittee.

**Assays for detection of antithrombin Type I and Type II deficiencies. S Kitchen**

Dr Kitchen presented important differences between results of antithrombin (AT) assays in which human thrombin, bovine thrombin or factor Xa are used as the target enzyme for inhibition. In the case of AT Cambridge II the median results obtained by groups of UK NEQAS participants were 77 IU/dl, 81 IU/dl and 87 IU/dl for bovine thrombin human thrombin and factor Xa respectively. Both AT Cambridge II and AT Denver appear as normal or type I defects if Xa is used, but are phenotypically type II if bovine thrombin is employed. Furthermore the results of AT assays in some subjects are critically influenced by the incubation in the assays. It was concluded that there are a number of issues related to AT assays which must be taken into account in the standardisation of AT testing.


The remit of the Working Party was reported and they are to develop a primary reference method for measurement of antithrombin with SI traceability, to establish reference materials using the primary reference method and to subsequently establish secondary reference materials for antithrombin type I and II deficiencies. A pilot study has now been set up to identify matrix effects and influence of different concentrations of reactants on the proposed primary reference method.
The SSC on plasma kallikrein-kinin meeting was well attended with approximately 40 participants. The meeting was active, with much interesting discussion from most participants. Presentations covered a wide variety of topics ranging from standardization to reviews of basic science topics to clinical and human studies.

A brief review of the topics presented is described below:

**Update on FXI Standardization (Dr. Elaine Gray)**

Dr. Gray presented recent work focused on gaining acceptance of the WHO standard for FXI activity. At the same time, the SSC Lot 3 was assayed for FXI. This study involved 27 labs in a number of countries. The studies have been designed to compare the FXI levels in the Lot 3 standard with the current WHO international standard (IS) and “in-house” standards prepared in different labs participating in the study. Relevant results include the observation that the Lot 3 standard and IS are virtually identical in their FXI activity (0.88 vs 0.86 IU/ml) and that there is excellent interlaboratory agreement with these results. In comparison, the in-house standards showed significantly more variability. The potential sources of this variability was discussed, and may reflect ethnic differences in the local pools as well as other variables. A vote was taken as to whether the Lot 3 standard was felt to be acceptable for presentation to the ISTH for approval for submission to the WHO as consideration as an IS, and all attending were in favor. Endorsement was specifically provided by Dr. Gray, Dr. Keith McCrae, Dr. Robert Colman, Dr. Alvin Schmaier and Dr. Zia Shariat-Madar.

**Prolycarboxypeptidase (PRCP): A new assay to assess PK activation on cells (Dr. Zia Shariat-Madar)**

Dr. Shariat Madar reviewed work performed leading to the identification of PRCP as an endothelial cell PK activator, including the purification process leading to a 27019-fold purification that allowed identification of the enzyme. He also reviewed data demonstrating colocalization of PRCP with known HK receptors on endothelial cells, as well as functional data using PRCP antisense constructs. Future directions for this work, as well as possible ways to standardize PK activations assays were discussed by the group.

**The intrinsic coagulation pathway is essential for arterial thrombus formation in mice (Dr. Thomas Renne’)**

Dr. Renne discussed recently published studies from his own lab as well as that of Dr. David Gailani which have focused on coagulation and thrombosis in FXI and FXII deficient mice. Discussions included the importance of feedback activation of FXI by thrombin in FXI -/- mice, in which platelets adhere at the site of a wound but have deficient aggregation. Interestingly,
FXII deficient mice were found to have no bleeding phenotype, but to have deficient thrombus formation in that occlusive thrombi did not form in these animals under circumstances in which they formed rapidly in wild-type mice. Possible reasons for this observation were discussed at length by the group. The use of FXII inhibitors as thromboprotectants was also considered, and thought to be worthy of future consideration.

**Update on development of thrombostatin (Dr. Alvin Schmaier)**

Dr. Schmaier discussed thrombostatin, the RPPGF product derived from bradykinin. This agent has been shown to effectively inhibit coagulation in mice, including thrombin induced platelet aggregation. Studies that have led to further identification of thrombostatin mechanisms were reviewed, including its ability to bind to peptidic thrombin cleavage sites of PARs (1 and 4) and inhibit PAR cleavage. Role of specific amino acids in these interactions were defined, in particular a specific arginine residue and proline 46 of PAR 4. Finally, the development of new thrombostatin peptidomimetics, including FM19, was reviewed. The potential clinical utility of these agents was discussed at length by the group.

**Kininogen in inflammatory bowel disease and reactive arthritis (Dr. Robert Colman)**

Dr. Colman reviewed the pathogenesis of IBD and arthritis and the animal models used to study these disorders. The broad range of activities of BK and HK in the pathogenesis of inflammatory disorders was described in detail. The marked efficacy of a kallikrein inhibitor P8780 in preventing IBD and the systemic symptoms of inflammation which accompany gut disease was presented and discussed by the group, and the reasons for differences in disease susceptibility between Lewis and Buffalo-Fisher rats, which relate to the susceptibility of HK cleavage due to an HK polymorphism between these strains was described. Dr. Colman also described the development of an HK deficient rat (and wild type control) by back crossing of B/N Katholiek HK deficient rats with common rat strains was presented. Studies with these animals demonstrated an important role for HK in the development of inflammatory disease.

**Kininogen and angiogenesis: A review (Dr. Keith McCrae)**

Dr. McCrae discussed several areas relating to the role of HK/HKa in regulation of angiogenesis. The antiangiogenic activity of HKa and HK domain 5 was reviewed, as well as the current mechanisms that have been proposed to account for this activity. Studies focused on means by which the active domain 5 structure may be defined were discussed. Characterization of the murine kininogen gene locus was also reviewed, including the recent finding that two kininogen genes occur in the mouse and rat. The development of a mouse deficient in the murine kininogen gene 1 was discussed, as well as preliminary data suggesting that this gene, KNG1, is the source of plasma kininogen.

**ELISAs for FXIIa and kallikrein-C1INH complexes: preliminary data from the second NorthwickPark Heart Study (Dr. Jose’ W.P. Govers-Riemslag)**

Dr. Govers-Riemslag discussed several aspects of the measurement of these complexes in patients enrolled in the NPHS, who were healthy men aged 52-61 without known heart disease.
Extensive detail concerning the development and standardization of these assays was provided, demonstrating linearity of log-transformed plots using standards developed specifically for this purpose. The study was a nested case control study involving 829 men, 231 with CHD, 56 with stroke). Many results were presented, but among these one of the most important was that the highest amounts of CHD occurred in patients in whom the levels of FXIIa:C1INH levels were in the lowest tertile. There was not a strong correlation between these levels and free FXIIa. The relationship of these findings to the findings of Dr. Renne’ in FXII deficient mice was discussed by the group.

**Detection of activated FXIIa in humans (Dr. David Pritchard)**

Dr. Pritchard discussed the use of a specific monoclonal antibody that recognizes FXIIa with >10 8 fold sensitivity greater than FXII to study plasma FXIIa. Most of FXIIa is found in the fluid phase of blood, with some distributed to lipids and cells. Interestingly, significant amounts of FXIIa occurred on cells even in FXII deficient patients. Other molecules with which FXIIa associated included ApoA1 and E, C1INH, kallikrein and kininogen. The fact that the 78 kDa form of FXIIa increases after MI/thrombolysis was discussed, and the potential role of thrombolysis per se in inducing these elevations was discussed.

At the conclusion of the meeting, Dr. McCrae thanked all participants, and encouraged communication of new ideas and proposals for more discussions at future SSC meetings. A major goal of this subgroup is to expand and make others aware of the broad reaching physiologic importance of the kallikrein-kinin system.

Submitted 8/9/05

Keith R. McCrae, M.D.
Platelet Immunology

Chairman: T Warkentin, Canada
Co-Chairs: JB Bussel, USA; BH Chong, Australia; D. Cines, USA; A. Greinacher, Germany; V. Kiefel, Germany; H. Kroll, Germany; M.F. Murphy, UK; G.P. Visentin, USA

Committee Co-Chairs (in attendance): J. Bussel, B. Chong, A. Greinacher, H. Kroll
Committee Co-Chairs (not in attendance): D. Cines, V. Kiefel, M. Murphy, G. Visentin

The program was divided into three parts: (I) Alloimmune Thrombocytopenia, (II) Autoimmune Thrombocytopenia, and (III) Drug-Induced Thrombocytopenia.

ALLOIMMUNE THROMBOCYTOPENIA (Chairs: C. Kaplan, H. Kroll)

W. Ouwehand (Cambridge, UK): Collaborative study to establish the first international standard for quantitation of anti-HPA-1a. W. Ouwehand, speaking on behalf of Paul Metcalfe, summarized the development and validation of a reagent, NIBSC code 03/152, for the quantitation of anti-HPA-1a alloantibodies in patient serum. The process by which the ISBT approved this reagent was reviewed, which included evaluation using this reagent of three sera of varying anti-HPA-1a titers by 39 laboratories in 24 countries. These included 8 laboratories represented at the Platelet Immunology SSC (Aster/Curtis; Greinacher/Eichler; Kaplan; Kelton/Smith/Warkentin; Kiefel; Kroll/Santoso; Murphy; Ouwehand), all of whom had one or more members that voted in favor of the proposal (and none opposed). The final vote was 11 in favor (none opposed) for recommending that the ISTH approve the reagent (NIBSC code 03/152) as an anti-HPA-1a (anti-Pl A1) standard. This matter was to be considered at the meeting of the Standardization committee later in the Sydney ISTH meeting.

G. Bertrand (Paris, France): Quantitation of anti-HPA-1a alloantibodies using the MAIPA procedure. A method to quantitate anti-HPA-1a alloantibodies using the monoclonal antibody immobilization of platelet antigens (MAIPA) method was summarized, which constructed a standard curve using diluted high-titer serum (from 1:1 to 1:128), and employed computerized fitting of the data for analysis. It was concluded that this method was useful for quantitating antibody titer, thus permitting studies of correlations between antibody titer and fetal/neonatal thrombocytopenia.

A. Greinacher (Greifswald, Germany) on behalf of V. Kiefel (Rostock, Germany): Treatment of NAIT: update on experience with random donor platelets for treatment of NAIT. Anecdotal evidence was provided suggesting that most newborns with severe NAIT may benefit from transfusion of random donor platelets. (Current recommendations are that these infants be transfused with platelets negative for HPA-1a/5b alloantigens, and/or intravenous gammaglobulin and/or maternal platelets.) Sixteen cases were reviewed from 7 centres (6 German, 1 Canadian) in whom platelets increased rapidly to >40 in 15 of the infants (the platelet count rose to >80 in 11 of 16 infants). No side effects were observed. It was concluded that treatment of NAIT with random platelets is a potential option for managing this situation, which has the advantage of avoiding delays from some of the other options. Discussion included the comment that there are limited data in the literature indicating that this treatment can be
beneficial. It was noted that in the U.K. 8 blood centre locations have HPA-1a/5b-negative platelets available, allowing for platelets to be available usually within 2 hours.

**H. Kroll [Presenter]/S. Santoso (Giessen, Germany): Use of novel cell lines for diagnostics of NAIT/Proposal for the distribution of reference material.** It would be useful to have target cells available for alloantibody testing that bear uncommon/rare platelet alloantigens. EBV-transformed cell lines have been prepared for HPA-1-5,6,7,8,9, and 15. Two laboratories (Giessen, Germany; Potters Bar, UK) act as repository laboratories to ensure storage, viability, etc. of these cells. Laboratories that define new platelet alloantigens should ensure immortalization of B cells, or make available of material for expert laboratories to perform this. There is a need for development of stable cell lines. **PROPOSAL:** to establish a registry of alloimmunization to alloantigens other than HPA-1-5/15, i.e., low frequency alloantigens (coordinator: H. Kroll). This would provide information on antibody frequency and heterogeneity, situations of non-immunization despite alloantigen mismatch, variability in clinical severity, which methods for detection are appropriate, and clinical information on items such as outcomes of previous pregnancies, duration of survival of transfused platelets, etc. In discussions, an issue arose as to whether patient informed consent was required even when non-identifying information would be placed into a registry.

**H. Kroll [Presenter]/S. Santoso (Giessen, Germany): Heterogeneity of alloantibodies in alloimmune thrombocytopenia.** Factors contributing to variable severity of NAIT were reviewed. Questions were raised about whether anti-HPA-1a antibodies impair fibrinogen-gpIIb/IIIa interactions. A notable finding was that in fibrinogen-mediated cell adherence experiments most CHO cells (HPA-1a/1b) did not have impaired adhesion in presence of anti-HPA-1a antibodies, but some did, especially using PTP sera (blood obtained at time of severe thrombocytopenia). In the case of PTP, these features may be associated with disease pathogenesis. Another issue is heterogeneity of anti-HPA-3a alloantibodies, with variable binding (despite evident disease) that appears to be assay-dependent (in some cases, only whole blood assays show binding). Issues such as platelet handling/storage and lysis appear to be relevant. Reactivity is optimized if HPA-3a/3a platelets are used. Some conclusions were that most anti-HPA-3a alloantibodies were detectable by flow cytometry (though there are problems with HLA interference). All anti-HPA-3a alloantibodies were detectable on day 0 by MAIPA, with decreased reactivity on longer storage. Thus, the MAIPA is a suitable assay when fresh, homozygous platelets are used. **PROPOSAL:** to establish a registry of alloimmunization involving anti-HPA-3a. The aim of this project would be to develop recommendations for optimal testing for anti-HPA-3a alloantibodies.

**C. Kaplan (Paris, France): Heterogeneity of anti-HPA-9bw alloantibodies.** NAIT due to anti-HPA-9bw was summarized, which accounts for about 2% of NAIT, usually quite severe, and often with poor platelet count increments following random donor platelet transfusion. It was noted that no one monoclonal antibody has been identified that permits detection of all anti-HPA-9bw alloantibodies.

**AUTOIMMUNE THROMBOCYTOPENIA (Chairs: J. Bussel, B. Chong)**
J. Bussel (New York, USA): Definition of autoimmune thrombocytopenic purpura: preliminary report of a working party. A working party is being organized to define better ITP, including an algorithmic approach for its diagnosis that includes certain aspects of laboratory testing. Some issues with existing definitions were reviewed; e.g., it was noted that the ASH guidelines on ITP indicate that “no other causes of thrombocytopenia” is a criterion, but details on how exactly to ascertain the absence of other causes of thrombocytopenia are not listed. The presentation focused on two interrelated issues, beginning with what investigations might ‘rule out’ ITP. Various infections as causes of apparent immune thrombocytopenia (or ITP-mimicking illnesses) were listed (HIV, HCV, Helicobacter pylori, CMV, dengue, malaria), as were clinical and laboratory features suggesting hereditary thrombocytopenia (family history, small/large platelets, hearing abnormalities, renal failure, cataracts, mental retardation, leukemia/lymphoma, thalassemia trait or myelodysplasia in X-linked inheritance, absent radii/other orthopedic problems). The possibility of an ITP-mimicking drug-induced AITP syndrome was raised. Early myelodysplasia can resemble ITP. Also, how does one deal with related disorders that historically have conferred the term “secondary ITP”, such as certain infections (HIV), humoral immunodeficiency (antibody, complement), Hashimoto’s thyroiditis, Evan’s syndrome, neoplasia (CLL, Hodgkins lymphoma, NHL, SLE, APS, etc.). Then, an algorithmic approach was discussed in regards to how one might ‘rule in’ ITP. Three distinct approaches could include (a) positive test for platelet-reactive autoantibodies; (b) platelet count response to IVIgG or anti-RhD; or (c) evolution of illness over continuing follow-up (say, a 6-month period). In summary, the following approach was suggested as a first step for discussion: (a) development of diagnostic “check list” to improve diagnosis by listing those features of history, examination, and laboratory testing that improve specificity of diagnosis; (b) study of these features could help to identify those laboratory tests that should be performed as a routine (?HCV ?immunoglobulin levels ?anti-phospholipid antibodies ?H pylori); (c) evaluate various laboratory tests for anti-platelet antibodies, TPO, etc., that might be helpful diagnostically.

T. Kuehne (Switzerland) on behalf of P Imbach and D Provan: PARC Registries and EHA Working Party update. An update was given on existing registries of pediatric and adult chronic ITP. The first registry provided information on the natural history of ITP. Subsequent registries deal with such issues as disease heterogeneity, biological and genetic markers, and so forth. This presentation was made as a matter of information for committee members.

DRUG-INDUCED THROMBOCYTOPENIA: D-ITP (Chairs: B. Chong, H. Kroll)

A. Greinacher (Greifswald, Germany) (on behalf also of R Aster, B Chong, B Curtis, H Kroll, S Santoso, T Warkentin): Proposal for definition of what is required by the SSC to accept putative drug-induced immune thrombocytopenia (D-ITP), including proposal for a registry of those drugs agreed to cause D-ITP, e.g., web-based database.

Both scientific and drug safety reasons were listed for aiming to improve the diagnosis of D-ITP. Careful analysis of patients with HIT and GPIIb/IIIa thrombocytopenia have shown that they are different from “classic” D-ITP. As the majority of drugs currently listed in textbooks as inducing D-ITP has been selected by clinical criteria only, the SSC favors a rigorous approach in which both clinical and laboratory criteria need to be fulfilled to establish a drug as causing D-ITP. This may allow better definition of common pathogenetic mechanisms or development of
improved techniques for detecting the relevant antibodies, thus providing a positive impact on improving diagnosis. **PROPOSAL:** The Working Party proposes to generate a set of clinico-pathologic criteria for establishing a drug as definitively causing D-ITP. Additionally, the Working party proposes to generate a website on which those drugs (and the relevant criteria met) are listed. Committee members discussed some of the potential inclusion criteria, such as the number of well-documented cases needed to establish a drug as causing D-ITP (?1 ?3, etc.), sharing of serum among a minimum number of laboratories (?2 ?4), the minimum platelet count nadir (e.g., <20, <100), and other considerations. These will be developed by the Working Party. The audience voted in favor of this proposal. A question arose as to whether the ISTH has special requirements for establishing such a website.

**DRUG-INDUCED THROMBOCYTOPENIA: HIT (Chairs: Y. Gruel, T. Warkentin)**

A. Greinacher (Greifswald, Germany): Frequency of anti-PF4/heparin antibodies of the IgG, IgA and IgM class: the Greifswald experience. Sera from 755 patients with clinically-suspected HIT were studied over a 9-month period. Only about 15% of all referred sera tested positive in any of the assays (HIPA, EIA). Only about 40% of these positive sera had platelet-activating antibodies (positive HIPA). About 70% of all positive sera tested had anti-PF4/heparin antibodies of IgG class. Of those testing positive, only 3% were only positive in the HIPA, indicating that a negative EIA is good at excluding HIT. Analysis of cases suggest that in patients who only have IgA and IgM class antibodies, other clinical reasons for thrombocytopenia and/or thrombosis were generally evident. The data suggest that there may be considerable over-diagnosis of HIT.

T. Warkentin (Hamilton, ON): Relevance of anti-PF4/heparin antibodies of the IgM and IgA class. The Hamilton prospective trials. Data were presented evaluating the systematic serologic investigation (by serotonin release assay [SRA], EIA-IgG, EIA-IgM, EIA-IgA, EIA-GTI) of patients who participated in large prospective trials of heparin therapy for orthopedic surgery. The data show that the SRA, EIA-IgG, and EIA-GTI all have high sensitivity for detecting clinically-significant antibodies. However, specificity was SRA > EIA-IgG > EIA-GTI. It was further shown that median levels of IgA and IgM class antibodies in patients with clinical HIT were indistinguishable (in terms of their overall degree of reactivity) from patients who formed a non-HIT immune response (as judged by a positive reaction in the EIA-GTI). The data suggest that the operating characteristics of the EIA for diagnosis of HIT likely would be improved by detecting only antibodies of IgG class.

A. Leyte (Amsterdam, Netherlands), Y. Gruel (Tours, France), T. Warkentin (Hamilton, ON): Scoring system for clinical HIT. Experience using a clinical scoring system for HIT (the 4 T’s) was presented. The first speaker (Leyte) presented experience in an intensive care unit (ICU) population. Using the 4 T’s plus criteria presented by B Chong at the Paris meeting, the authors concluded that only 0 to 7% of thrombocytopenic patients in their study had probable or definite ICU. Moreover, the discrepancy between clinical criteria and results of EIA testing was large. It was suggested that the ICU population is a special one that will require special attention. The second speaker (Gruel) showed data from a prospective multi-centre evaluation of the 4 T’s in which several laboratory assays (SRA, EIA, and rapid particle gel assay [Diamed]) were assessed. The scoring system showed high (100%) negative predictive
value, based upon the SRA. However, 9/42 low score patients tested positive in the EIA (but with a negative SRA). This indicates that the SRA provides much greater diagnostic specificity than does the EIA. All three assays were positive in the 5 patients that had a high clinical score for HIT. The third speaker (Warkentin) showed that the 4 T’s scoring system had high negative predictive value for excluding HIT. The positive predictive value depended on the setting in which it was performed, with high positive predictive value of a high clinical score seen in Hamilton (performed by a hematologist) whereas it was lower in Germany (used by multiple physicians when requesting laboratory testing for HIT antibodies).
This year’s Platelet Physiology Subcommittee Program in Sydney Australia was divided into 3 parts:

1. **Aspirin and Clopidogrel Resistance (co-chairs Alan Michelson and Marco Cattaneo)**


2. **Inherited Thrombocytopenias (co-chairs Alan Michelson and Paquita Nurden)**

Dr. Carlo Balduini (Italy) spoke on "Classification Of Inherited Thrombocytopenias", Dr. Catherine Hayward (Canada) spoke on "Clinical Challenges With Assessing Inherited Platelet Disorders Associated With Thrombocytopenia”, and Dr. Amy Geddis (U.S.A.) spoke on “A Registry And Shared Resource For Congenital Thrombocytopenias”. A general discussion followed. Drs. Amy Geddis and Jim Bussel have set up a Non-immune Thrombocytopenia Registry.

3. **The Platelet Transcriptome (co-chairs Willem Ouwehand and Koneti Rao)**

In addition to those listed above, the following manuscript is undergoing SSC review prior to submission to the *Journal of Thrombosis and Haemostasis* as an official communication of the SSC: Hayward CPM, Harrison P, Cattaneo M, Ortel TL, Rao AK. Closure time in the evaluation of platelet disorders and platelet function: a report of the Working Party on the PFA-100.

**Working Parties of the SSC Platelet Physiology Subcommittee:**

- **Platelet Proteome**
  - Chair: Steve Watson
  - Members: Wadie Bahou, Desmond Fitzgerald, Andrew Leavitt, Willem Ouwehand, Koneti Rao
- **Platelet Transcriptome**
  - Chair: Willem Ouwehand
  - Members: Wadie Bahou, Dermot Kenny, Koneti Rao, Steve Watson, Andrew Weyrich
- **Evaluation of Platelet Function Disorders**
  - Chairs: Marco Cattaneo, Cathy Hayward, Koneti Rao
  - Members: Carlo Balduini, Amy Geddis, Alan Michelson, Diane Nugent, Paquita Nurden, Koneti Rao, Steve Watson
- **Aspirin Resistance**
  - Chair: Alan Michelson
  - Members: Marco Cattaneo, Chiara Cerletti, John Eikelboom, Paul Gurbel, Kandice Kottke-Marchant, Thomas Kunicki, Fabio M. Pulcinelli, Koneti Rao
- **Clopidogrel Resistance**
  - Chair: Alan Michelson

**Registries of the SSC Platelet Physiology Subcommittee:**

- **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 Haemostatic Variables in Cardiovascular Disease

Chairman: P.J. Grant, UK
Co-Chairs: G. Lowe, UK; G. Palareti, Italy; V. Salomaa, Finland; A. Tosetto, Italy

Audience: between 80-100 participants

Update on meta-analyses of fibrinogen, CRP, IL-6, vWF, tPA and D-dimer in prediction of vascular risk. Professor G.D.O Lowe, Glasgow, UK.

This talk reviewed a metaanalysis of 31 studies of fibrinogen and vascular risk involving ~154,000 individuals. The methods involved have been previously published (Eur J Card Prev Rehab, 2004). A 3 fold increase in CV risk was seen in the top 20% of fibrinogen levels. Similar relationships existed between fibrinogen levels and both stroke and non-vascular deaths. Neither the fibrinogen assay used by the investigators, nor time to assay affected the results.

CRP had a weaker association with vascular risk than fibrinogen and seemed to show more variation with storage than fibrinogen. Other variables had weaker associations with CV risk.


The relative information gained by examining the genome, transcriptome and proteome was discussed. The plasma proteome is difficult to characterise, there is a wide dynamic range of proteins with albumin alone accounting for 55% of proteins in plasma. The Human Proteome Organisation was discussed and the aims and objectives of the plasma proteome project outlined. Standardisation of methodologies is a problem and reference samples are being developed and sent out to participating laboratories.

Increased numbers of circulating endothelial cells predict poor outcome in acute coronary syndromes. A. Blann, Birmingham, UK.

The potential for measurement of circulating endothelial cells (CECs) as a marker of vascular disease was discussed. CECs are recognised by the presence of CD146 and generally exist in concentrations of ~1 cell/ml of venous blood. In acute coronary syndromes this may rise to ~20 cells/ml of venous blood. Increased CECs have also been described in a variety of inflammatory states.

Methodological issues were discussed, in particular the problem of differentiating CECs from circulating progenitor cells, a problem that doesn’t appear to have been entirely resolved.

The adipocyte and cardiovascular risk. J. Prins, Brisbane, Australia.

Recent work on the paracrine and endocrine effects of the fat filled visceral adipocyte was presented. The cardiovascular system was described in novel terms of obeying the messages sent out by the adipocyte and the brain. The obese adipocyte secretes 3 times as much TNF alpha and
also secretes increased renin angiotensin activity, PAI-1, leptin, with suppressed adiponectin. The structure function of adiponectin and its receptors was discussed.

**Multiple Environmental and Genetic assessment of risk factors for venous thrombosis, the MEGA Study. C. Doggen. Netherlands**

The MEGA study design was presented with preliminary data from this cohort. Interactions between FV Leiden and contraceptive use was presented and the analysis of the data set was discussed.

*P.J. Grant Sydney, August 2005*
Summary of Approvals and Working Parties:

1. Continuation of WP on VWF Assays in VWF in VWD Diagnosis: The lyophilized samples were sent out to labs and the results will be collected by September 2005 (A. Hubbard, A.B. Federici, C.A. Lee, R.R. Montgomery)

2. Continuation of the WP on Standardization of Multimeric Analysis, with more laboratories (U. Budde and C. Mazurier)

3. Novel suitable reagents for VWF:CB (collagen binding assay) have been prepared and will be tested during the next year (L. De Marco, E. Favaloro and A. Hubbard)

4. The WP on VWD classification has approved that the final report will be presented during the next ISTH-SSC meeting in Oslo (E. Sadler & the panel of VWD experts)

5. The WP on Standardization of methods for mutation and expression studies will continue and will prepare a written report before Oslo (A. Goodeve, D. Lillicrap, J. Eikenboom, R. Schneppenheim)

6. The WP on development of new improved assays for ADAMTS-13 will continue and report update results in Oslo (J.E. Sadler & R. Schneppenheim)

7. The WP on requirements for shear-stress related VWF assays to be used in clinical diagnosis of VWD and drugs interfering with VWF-platelet interactions decided to postpone the report in Oslo (Y. Ikeda & Z.M. Ruggeri)

8. A specific Web site for an updated version of International Registry on Acquired Von Willebrand Syndrome is now available and the first interim report will be present in Oslo (A.B. Federici, U. Budde, H. Mohri, , J.H. Rand, I. Sussman)

A) Progress reports on different Working Parties
(Chairs: J. Eikenboom and E. Favaloro)

The session started with a report on VWF assays in VWD diagnosis by A. Hubbard & C.A. Lee. The objective of the working party is to determine the best diagnostic repertoire for VWD diagnosis and thereby produce guidelines for the minimal requirements for correct VWD diagnosis. This will be achieved through a review of methods currently in use and a multi-center international study in which plasma samples covering a range of VWD variants will be dispatched for diagnosis. Trial fills on VWD plasma have indicated that lyophilisation does not affect the estimation of VWF. A panel of plasma samples from patients with known VWF mutations (and viral marker negative) were lyophilised in 2004. Thirty four laboratories from 17
different countries have agreed to participate and dispatch of the coded samples is almost complete. Participants are requested to return information on their methodologies as well as laboratory data to support their diagnosis of the lyophilised samples. Analysis of the results is planned for the last quarter of 2005.

L. De Marco & E. Favaloro reported on the results of the WP on different collagen reagents. It is intended that the VWF:CB working party undertake a study to help evaluate the utility of different collagen preparations in the diagnosis of VWD, and in the discrimination of qualitative VWD Types, as well as for identification of functionality of VWF in therapeutic factor replacement products. The VWF:CB study will entail (i) retesting of an identical plasma set by a smaller select number of expert laboratories using methodologies currently existing in those laboratories as well as by additional methodologies, including commercial options, (ii) additional testing of in-house well characterised plasma material with all methods, (iii) testing of therapeutic VWF concentrates. Companies producing commercial VWF:CB kits have agreed to participate and different collagen preparations will also be evaluated. Proposed members and participants of this exercise will be Dr Favaloro, Dr De Marco, Dr Hubbard, Dr. Federici & Dr Mazurier, plus other interested parties to be identified. It is proposed that this study commences after completion of the above mentioned diagnostics study. In the interim, the members of the VWF:CB working party will formulate a study protocol that identifies inclusions and requirements.

U. Budde & C. Mazurier reported on the progress of the WP on multimeric analysis. Since the session in Venice we had requests from 3 more laboratories to analyse our samples. Only 1 of these did send results and one laboratory analysed their results in a way compatible to the others. This brings up seven results that can be evaluated statistically for most of the 10 samples. While for the 4 samples with a full or near full content of large multimers the coefficient of variation for the content of >10 multimers was acceptable (below 15%), it was much too high for those samples that missed greater parts of the large multimers: between 52% and 81%. Thus the method is still far from standardized. We await results from a WP of VWF assays (results from 30 laboratories) and from the type 1 study before proceeding further in our aim to standardize the multimeric analysis.

The WP on VWD classification has produced a report with recommendations for VWD classification, which was presented by J.E. Sadler. As of the SSC meeting in Venice in 2004, the Working Party on the classification of VWD had evaluated recent published advances in our understanding of the pathophysiology of VWD and made significant progress in revising the classification to reflect this progress. Substantial changes were made to the definition of VWD and of VWD type 1. At this time, a preliminary manuscript for the revised classification has been written and extensively revised. Before it is submitted for publication, the Working Party would prefer to include the major findings of the Canadian and European studies of VWD type 1, which will be relevant to the changes proposed for VWD type 1. The first papers from these studies should be submitted for publication with the next few months. During the next year, the working party will review the published results of Canadian and European studies of VWD type 1, and other ongoing studies of VWF assays and multimer analysis. With the benefit of these data, a manuscript entitled "Update on the classification of von Willebrand disease" will be submitted for publication no later than the next SSC meeting in 2006.
The session ended with a report by L. Hilbert & D. Lillicrap on a new WP on VWF molecular biology and expression studies. The aim of this working party is to complete a review on the methods used for the identification and expression of molecular abnormalities in VWD. A questionnaire was sent to laboratories involved in two parallel projects on type 1 VWD: the European MCMDM-1VWD project and the Canadian project. The major conclusions from this pilot survey were presented and indicated that there is some consensus in the methods used for the identification of a VWF gene abnormalities and for the construction of mutated expression vectors harbouring a VWD mutation. However, transient transfections were performed using different procedures, in different cell types and this results in recombinant VWF antigen levels that may vary by a 10-fold factor according to the laboratory. In order to conduct a second larger survey, this questionnaire will be sent to other laboratories willing to participate. Those interested should send an e-mail to hilbert@lfb.fr or lillicrap@cliff.path.queenu.ca.

B) Progress reports on ADAMTS13 assays & clinical applications
(Chairs, Anne Goodeve & Reinhard Schneppenheim)

1. Role of chloride ions in the VWF-ADAMTS13 interactions

R. de Cristoforo reported on the kinetics of VWF cleavage by ADAMTS13 at different concentrations of NaCl. Low concentrations of NaCl significantly enhance cleavage of VWF compared to physiologic NaCl concentrations. Testing of different kations and anions, respectively, revealed that the inhibitory effect of higher salt concentrations was dependent on anions rather than on cations. It was suggested that the inhibitory effect was due to conformational changes of VWF that could be reversed by ristocetin. Accordingly, anions should stabilize a more closed structure of the VWF A2 domain, whereas shear should stabilize an open form.

2. The fluorogenic substrate for ADAMTS13

H. Kokame reported on normal values for ADAMTS13 of a large Japanese control population of 3,822 individuals obtained by the commercially available ADAMTS13 FRET assay developed by the group. They observed a slow decrease of fluorescence at zero activity. The lower limit of detection was estimated as 3 % of normal, and the assay was able to differentiate between values of 3 %, 0 % and 5 %. The minimum value was 43 %, and the reference range was 52 – 172 %.

3. Novel ELISA assay for ADAMTS13 activity using MoAbs directed against the N-terminal decapetide of VWFA2 domain

M. Matsumoto reported on a new ELISA based on MoABs directed against the N-terminal decapetide that is exposed after ADAMTS13 digest of VWF. This assay is very sensitive and specific with a detection limit of 0.5 % which would be superior to all other assays to date. The correlation with classical assays like the multimer method was good.

4. ELISA for ADAMTS13 autoantibodies in TTP patients
J. Kremer-Hovinga reported on her experience measuring the titer of autoantibodies against ADAMTS13 in 74 patients with TTP/HUS by a new ELISA (Technozyme ADAMTS13 Inh, Technoclone, Vienna). The group detected antibodies in 93% of cases with ADAMTS13 activity < 10%, in 39% of cases with ADAMTS13 between 10 and 25%, in 8% of cases with ADAMTS13 between 26 and 50%. The limit of detection was estimated as < 1BU which would be an improvement compared to the standard Bethesda method.

5. Action on ADAMTS13 on different VWF mutants

R. Schneppenheim and U. Budde showed experimental data about the action of ADAMTS13 on different VWF mutants. The group reproduced the enhanced susceptibility of classical VWD type 2A (IIA) mutants for ADAMTS13 proteolysis not only for the group 2 mutations, previously defined as being susceptible for enhanced proteolysis but also for a group 1 mutation in the A2 domain (S1506L) for which previously intracellular retention of large VWF multimers was made responsible for their observed lack. The group could also show by in vitro mutagenesis involving single and multiple substitutions of amino acids that Y1505 and M1506 flanking the VWF proteolytic site are not essential for recognition by ADAMTS13.

6. Clinical experience on Italian Registry of TTP

F. Peyvandi reported on the Italian experience of TTP in 140 patients. Plasma level of ADAMTS13 was reduced to < 10% in only 57% of patients in acute phase. Measurements of ADAMTS13 in remission phase of recurrent patients more often revealed lower levels than in patients with a single episode. Severe ADAMTS13 deficiency (<10%) was associated with a high prevalence of neutralizing inhibitors (61%). 12% of patients without measurable neutralizing antibodies had non-neutralizing antibodies on Western blotting. In about 1/3 of patients with severe ADAMTS13 deficiency no neutralizing antibodies were found which could not be explained by diagnosis of the inherited form of TTP predisposition (Upshaw-Schulman syndrome, USS) alone, since only 5/140 (3%) patients had USS.

C) Other reports and proposal for projects and surveys
(Chairs: J. Di Paola and D. Lillicrap)

Update of the Working Party on DDAVP in VWD: Dr. Lethagen summarized the current status of this project. He introduced the project web site and re-iterated the main points of the project. In addition to the aim to enroll 150 VWD patients for the main biological response and clinical efficacy part of the study, there is also a plan to assess a detailed pharmacokinetic profile in 40 patients.

Long-term prophylaxis of VWD: Dr Abshire introduced this new activity. He described the rationale, organization, objectives and entry criteria for the proposed VWD prophylaxis project. This project will be organized by the newly created VWD Prophylaxis Network and will be sponsored by an investigator-driven grant from ZLB-Behring.
Update of the Registry on Acquired von Willebrand Syndrome: Dr. Budde presented a brief update on the activities associated with this registry. He provided details on the new Registry web site (www.intreavws.com) and the information that could be entered at this site.

Protocol on non-immune thrombocytopenia and type 2B VWD: Dr. Bussel introduced this project and discussed the details of a proposed questionnaire that would be sent to interested investigators. He encouraged anyone with an interest in this area to contact him.

US NHLBI VWD Document: Dr. Nichols presented information about the recently completed US NHLBI “clinical guidelines” project on VWD.
**Thrombophilia Issues in Pregnancy**

Ian Greer presented a systematic review of thrombophilia in relationship to pregnancy outcomes. He emphasized important variables in screening that the health problem be significant, with a known natural history and identifiable before onset of disease; that the predictive test be safe and acceptable; and finally that an effective intervention be available. It was noted that we are at a time of fragile equipose regarding attitudes toward anticoagulant prophylaxis during high-risk pregnancies, and that women are not willing, in some circumstances to allow randomization to placebo or no treatment. B. Eni Brenner presented his scoring system for severity of thrombophilia syndromes and severity of pregnancy outcomes. The scoring system is intended to predict potential outcome of pregnancy based upon personal and family history of thrombosis, past pregnancy outcome and thrombophilic traits identified. S. Eichinger presented data regarding factor V Leiden and thrombosis outcomes, concentrating on oral contraceptives.

The Scoring System of Dr. Brenner will be compiled to be submitted from this Subcommittee publication in JTH. A collaborative activity will be developed to apply the scoring system prospectively to pregnancy, as an observational study, with or without clinically ordered intervention, in order to validate the scoring system. In particular the strength of a previous history of thrombophilia-related thrombosis in predicting thrombophilia-related non-thrombotic complications of pregnancy needs to be analyzed. S. Eichinger will develop a scoring system for thrombophilia and OCP outcomes modeled on the thrombophilia/pregnancy outcome scoring system. Recommendations regarding issues for women with FVL around oral contraceptive and hormone replacement therapy will be developed. If appropriate, the OCP, HRT and thrombophilia risk scoring system would be later extended beyond FVL to other thrombophilias.

Dr. M. Nijkeuter is continuing to organize the project on diagnosis of pulmonary emboli during pregnancy. This will be reported at the Oslo meeting, next year.

**Reports on Registries**

*Pregnancy Outcomes in Women with Prosthetic Heart Valves*: Andra James This registry is developed and three patients have been enrolled. The data base will reside at Duke University, Durham, US. The protocol, consent and data forms will be listed on the ISTH SSC website along with Dr. James contact information.
Thrombotic Outcomes in Women Undergoing Ovarian Stimulation: Barbara Konkle has established this registry on the ISTH SSC website.

Pregnancy Outcomes in Women with Antithrombin Deficiency: J. Conard has developed this registry. It will be expanded to include women with deficiencies of protein C or protein S. Forms, consents and contact information will be included. Dr. Conard can be reached by email: jacqueline.conard@htd.ap-hop-paris.fr

New Business:

Validation of the usefulness of global assays to detect hormonally-related alterations in coagulation. This project is in development. The Women’s Subcommittee will collaborate with the Thrombin Generation Questionnaire that has been developed by the SSc on Plasma Coagulation Inhibitors. All interested potential participants employing global hemostasis assays are encouraged to participate. Contact marilyn.manco-johnson@uchsc.edu.

Position Papers:

Evaluation of Women with Menorrhagia, Claire Phillip, 1st author: This paper has been developed and has been circulated among SSC Subcommittee members. It will be forwarded to the ISTH Council shortly.

Pre-analytic variables affecting testing of hormonally-affected coagulation proteins. Margareta Blomback, 1st author: This paper has been completed and is receiving comments from co-chairs.

Scoring system for thrombophilia and pregnancy outcomes, Benjamin Brenner, 1st author: This paper has been developed and will be circulated among subcommittee members promptly.
Working Group on Vascular Biology

Chairman: Peter J. Newman, (USA)

SSC Organizing Committee: Michael C. Berndt (Australia), John Griffin (USA), Irène Juhan-Vague (France), Klaus T. Preissner (Germany)

The program was divided into two parts: (I) Detection and characterization of circulating endothelial cells and their progenitors, and (II) Determination and characterization of (circulating) microparticles.

Approximately 160 people attended the session.

Session I: Detection and characterization of circulating endothelial cells and their progenitors (Chairs: K.T. Preissner & P.J. Newman)

Andrew Blann (UK): “Circulating endothelial cells (CECs) and endothelial progenitor cells (EPCs): Two sides of the same coin or two different coins”? That endothelial cells detectable in blood are not a homogeneous population, but rather represent more than one species of endothelial cells was discussed. CECs are thought to arise from the vessel wall, whereas EPCs are mobilized from the bone marrow. Thus, although originally defined according to different criteria, there are also some common characteristics. The lack of consensus regarding definition and methodologies remains an important area of future work for this Working Group. The use of CD146-coated magnetic beads to identify and purify CECs was discussed, as well as the extent to which the presence of CECs reflects endothelial cell damage. The origin of CD34+ EPCs remains controversial as they are identifiable in peripheral blood and capable of forming in vitro colonies. Improving the array of surface markers allowing to discriminate CECs from EPCs remains an important problem for further study.

Alexander Woywodt (Germany): “Detection of circulating endothelial cells by immunomagnetic separation (IMS) assays.” That circulating endothelial cells are a novel marker of microvascular damage was reinforced. CD146 driven-immunomagnetic isolation appears to be the technique of choice to isolate and enumerate these cells. However, several variables influence isolation of CECs by IMS (both at the pre-analytical and analytical levels). In this respect, a standardized methodology represents an important step towards consensus regarding CECs. Moreover, given the variable phenotype of these rare cells in peripheral blood, this technique still has several pitfalls, and precautions taken to avoid them were discussed. The second part of the talk focused on the clinical utility of detecting circulating endothelial cells as a marker of ANCA-associated small-vessel vasculitis.

Françoise Dignat-George (France): “Detection of circulating endothelial cells in the vascular compartment.” A historical perspective of the development of CD146 mAbs as selective markers for detecting CEC was provided, followed by a description of clinical disorders that have been associated with increased circulating CECs, including infection, malignancies, transplantation, and immune disorders like TTP. A working definition of CECs was proposed, and the clinical utility of CECs as biomarkers of endothelial damage was discussed. It was concluded that a consensual definition of the most appropriate technique is a key issue to be addressed in order to
validate CECs in large cohorts of patients. The future potential for proteomic analysis to provide selective markers allowing to discriminate CECs (from damaged vessels) versus EPCs (bone marrow) was also discussed.

Session II: Determination and characterization of (circulating) microparticles (Chairs: J Griffin & J-M Freyssinet)

Françoise Dignat-George and Jean-Marie Freyssinet (France): “Questionnaire on microparticle detection and characterization: a retrospective analysis”. Since MP are increasingly being viewed as markers for various pathophysiological processes, and may in addition have therapeutic applications, Drs. Dignat-George and Freyssinet have undertaken the large, important, and challenging task of surveying how investigators in this growing field prepare and characterize MP. The results of their ISTH-sponsored questionnaire were presented, and the results divided into pre-analytical and analytical procedures. Relative consensus was reached that blood should be anticoagulated with citrate, and that minimal manipulation should be involved to avoid cellular activation and inadvertent production of MP. Outstanding issues remaining on the pre-analytical side include whether whole blood or plasma is used to prepare MP, and if/how MP should be stored prior to analysis. On the analytical side, standardization of methods of quantitation, surface markers, and functional properties of MP remains an important goal. Current technologies used in this regard include flow cytometry, ELISA, determination of pro-coagulant and anti-coagulant activities, and the beginning of adoption of proteomic technologies.

Rienk Nieuwland (Amsterdam, The Netherlands): “Detection and characterization of microparticles by flow cytometry”. Circulating cell-derived microparticles most often have procoagulant properties due to exposure of negatively charged phospholipids. Most studies on microparticles, however, have been performed on microparticles after in vitro manipulations, e.g. pelleting/resuspending or freezing/thawing, thereby possibly influencing microparticle structure. This group investigated whether non-manipulated microparticles expose phosphatidylserine (PS), whether this exposure is affected by in vitro manipulations, and if so, whether this changes their procoagulant properties. Surprisingly, in their hands only very few non-manipulated microparticles from venous blood of healthy individuals, or from pericardial blood of patients undergoing cardiac surgery exposed PS, as evaluated by annexin V binding. Upon pelleting/resuspending, freezing/thawing, or both, however, the fraction of PS-exposing microparticles increased, which was accompanied by fragmentation, change in the size of MPs, and/or loss of particular microparticle populations. Interestingly, the extent of PS exposure did not affect the procoagulant activity or the mechanism of coagulation activation, allowing them to conclude that even low exposure of PS is sufficient to support coagulation. They cautioned that microparticles in fresh samples should not be quantified based on PS exposure. In the discussion, however, it was emphasized that the conditions for using annexin V have to be better defined with respect to the biochemistry of this PS probe.

Johan W. M. Heemskerk (Maastricht, The Netherlands): “Phosphatidylserine-dependent procoagulant potential of microparticles”. Tissue factor-induced thrombin generation with PRP and with platelet-derived MP similarly relies on phosphatidylserine exposure. Thrombin generation in PRP is enhanced by integrin αIIbβ3-mediated shedding of MP. Platelets shed
phosphatidylserine-exposing MP in the absence of activation (coagulation). This shedding is thought to be (1) secondary to F actin degradation, (2) mediated by integrins, and (3) negatively regulated by PKA (cAMP). It was concluded that integrin αIIbβ3 signaling accomplishes destabilization of the membrane cytoskeleton, negatively controlled by PKA, and resulting in MP shedding from the plasma membrane.

Yasushi Ozeki (Japan): Described a new ELISA method for detecting platelet-derived microparticles that utilized an anti-GPIX mAb for capture, and an anti-GPIb mAb to detect. This commercially-available assay might be detecting very small MP that flow cytometric analysis misses.

Nigel S. Key (Minneapolis, USA): “Tissue factor-dependent procoagulant potential of microparticles”. Evidence for TF-dependent procoagulant activity on MP derived from platelets and monocytes was presented, and the concept of encrypted versus de-encrypted TF exposure and its associated pro-coagulant activity was discussed, as were assay variables of TF procoagulant assays and the need for standardization and normalization.

Thomas Exner (Sydney, Australia): Presented the so-called XACT assay aimed at detecting procoagulant phospholipids in plasma, based on factor Xa-activated clotting time. One advantage of this assay is that it can be performed with whole plasma samples and is insensitive to the presence of most lupus anticoagulants. Hence, it can be anticipated that procoagulant MP are also detected.

Cheng Hock Toh (Liverpool, UK): “Anticoagulant potential of microparticles”. Dr Toh highlighted the fact that microparticles, depending on their cellular source, are able to display not only procoagulant properties, but also/instead express EPCR-bound activated protein C, which often functions to initiate anticoagulant pathways. The quantitation and functional analysis of these particular microparticles were described.

Bruce Furie (Harvard, USA): “Impedance-based flow cytometry for measuring microparticles: New instrument, new answers”. TF-bearing, PSGL-1-bearing MP, likely derived from monocytes, bind to laser-damaged vessels in a P-selectin-dependent manner, and deliver TF in such a way as to promote fibrin deposition and thrombus growth. This model is currently thought to reflect inflammatory injury. Quantitation and detection of at least a sub-population of very small MP is unfortunately complicated by the fact that the particle size is on the same order of magnitude as the wavelength of light (488 nm) used for their detection. To overcome this limitation, an impedance-based instrument, which measures electronic volume and has a 10X signal:noise ratio has been developed, and found to be able to detect up to 1.6 x 10^6/ml TF-bearing MP in the blood of individuals with certain forms of cancer, including pancreatic, colon, breast, and ovarian. It is postulated that delivering TF in this way might contribute to the incidence of thromboembolism prevalent in the later stages of many cancers.

Eric F. Grabowski (Boston, USA): Microparticles in flowing blood”. The hemolytic uremic syndrome (HUS) results from Shiga-toxin-producing strains of E. coli, and causes acute renal failure in children. Though Shiga-toxin is able to increase TF activity 2-3 fold on the surface of
activated endothelium, it does not appear to be able to similarly activate TF on EC-derived MP, perhaps due to downregulation of MP TF activity by TFPI.

Pudur Jagadeeswaran (San Antonio, USA): “Zebrafish microparticles from thrombocytes and their role in hemostasis”. Thrombin and collagen were found to induce the formation of annexin V-positive MP from zebrafish thrombocytes, and these MP were capable of accumulating at sites of laser-induced arterial injury.

Jean-Marie Freyssinet (Paris & Strasbourg, France) - “Round table discussion of an action plan for the standardization of the determination of microparticles”. There was broad agreement that microparticles can generally be defined as 0.1-1 μM cell-derived vesicular structures that lack a nucleus or synthetic capability. They can, and often do, however, contain a membrane skeleton. Microparticles have their origins in a variety of blood and vascular cell types, and mAb and proteomic analysis is likely to shed important clues as to their varied origins – much work remains to be done in this regard. MP contain varying amounts of surface-exposed PS, depending on their origins and mode of preparation/shedding. Both the phenotype and function of MP vary according to cellular origins and inducers of vesiculation. Hence, MP can be pro-coagulant or anti-coagulant, but when the balance is disrupted in favor of the former population, this reflects an increased thrombotic risk. Preferential approaches to their preparation and analysis was felt to be premature, but remains a worthy goal of future VB Workshop activities.
Use of SSC Lot #2 in QA exercises

Drs Brandt and Kitchen presented the results of EQA exercises in which SSC Lot #2 was tested as an anonymous sample. Results were encouraging in the good agreement between the mean values obtained and the values assigned to SSC Lot #2. One noticeable exception was the value for FVIII:C which differed from the assigned value and might be an indication that some manufacturers of calibrant plasmas have still not assimilated the re-alignment of the IU which occurred with the calibration of the WHO 4th IS FVIII Plasma.

Status and calibration of SSC Lot #3

SSC Lot #3, consisting of 54,800 vials, was received from the manufacturer (Technoclone, Vienna) by NIBSC in November 2003. Good progress has been made in the calibration of both existing and new parameters. Dr Hubbard reported on the calibration for Fibrinogen, FVII, Protein S and new parameters (FV and FXIII). Dr Gray reported on the calibration of FII, X, Antithrombin and a new parameter FXI; the calibration exercise for Protein C will be carried out over the next few months. Dr Kitchen reported on the calibration for FVIII, FIX and VWF, including the new parameter VWF:CB. Some responses from participants regarding the proposed assigned values are still outstanding. However, with the completion of the Protein C calibration in 2006 there will be a total of 19 assigned values on SSC Lot #3. It was agreed that the calibration reports should be circulated to the Executive Committee for consideration regarding formal assignment.

The calibration assays for SSC Lot #3 also included SSC Lot #2; in all cases there were no significant differences between the estimates for SSC Lot #2 obtained in the current study and those in the original calibration in 1999. This is a good indication of the continuity of the IU between Lots #2 and #3 and also supports the good stability of Lot #2.

Dr Declerck presented the results of an assessment into the feasibility of calibrating SSC Lot #3 for fibrinolytic parameters (tPA antigen, PAI-1 antigen, PAI-1 activity). Several laboratories estimated tPA antigen using different methods and standards and found reasonable agreement (with the exclusion of one outlier). Normalisation of the results relative to an NIBSC reference reagent was also encouraging in terms of inter-laboratory variability. A larger multi-centre study with a view towards formal calibration of the NIBSC reagent and SSC Lot #3 could be performed subject to approval by the Fibrinolysis sub-committee. Estimates for PAI-1 antigen were more variable and were not improved by normalisation relative to the WHO 1st IS PAI – this may reflect differences between plasma PAI-1 and purified PA-1 used to spike the reference materials. This will be discussed further in the Fibrinolysis sub-committee. Estimates of PAI-1 activity were extremely variable and calibration was not considered feasible.
Dr Hubbard suggested that a more comprehensive “Instructions For Use” should be prepared for SSC Lot #3 to cover safety considerations, details of storage and reconstitution and bulk filling details. The label for Lot #3 was also discussed: it was agreed that the manufacturer should be consulted regarding the recommended storage temperature and that a decision on the expiry date should be made once the initial stability testing has been performed. There was overall agreement that the stability testing (accelerated degradation) should also include FV and FXI as well as FVII and FVIII.

**Status of SSC Lot #2**

Dr Hubbard reviewed the despatch of SSC Lot #2. Between May 2001 and June 2005 approximately 38,000 vials have been despatched leaving approximately 7,300 vials in stock. Use in proficiency studies by CAP and UK NEQAS accounted for over half of the total vials despatched. The stock level should be sufficient to maintain supply up to the labelled expiry date of June 2006.

Dr Hubbard presented the results of the stability testing of SSC Lot #2. The accelerated degradation study has been carried out over the last 6 years and is now completed. The residual potency (for FVII and FVIII) of vials stored at elevated temperatures has been measured at NIBSC and the Royal Hallamshire Hospital, Sheffield, UK (Dr Kitchen). The predicted % loss per year for vials stored at -20 °C did not exceed 0.011 % per year which indicates that SSC Lot #2 is extremely stable at the bulk storage temperature. The predicted stability at +20 and +37 °C supported the shipment of vials at ambient temperature. In addition, an objective real-time measure of the stability of vials stored at -20 °C was obtained by comparison with vials stored for 6 years at -70 °C; no difference in FVII or FVIII was found in assays performed by NIBSC and the Royal Hallamshire Hospital, Sheffield.

**Joint Committee on Traceability for Laboratory Medicine**

Dr Gray reviewed the need for SSC Lot #3 to be placed on the JCTLM database as an internationally certified reference material. The need to demonstrate commutability, with respect to the assay of normal plasma and/or patient plasma samples, as part of this process was also discussed. It was considered that the inclusion of SSC Lot #3 in QA surveys may satisfy this requirement. In accordance with Dr Gray’s recommendation Lot #3 will be nominated for inclusion on the JCTLM database.