48th Annual Scientific and Standardization Committee Meeting

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Animal, Cellular & Molecular Models

Chairman:  P. Carmeliet, Belgium
Co-chairs:  L. Drouet, France; P. Jagadeeswaran, USA; G. Johnson, USA; N. Maeda, USA

Business:  The meeting was chaired by Subcommittee CoChairs, G. Johnson and P. Jagadeeswaran. It was attended by approximately 100 persons.

Dr. G. Johnson proposed that the Subcommittee authorize a Working Party to develop a comprehensive data base on murine hemostatic parameters and models of thrombosis. The Working Party will tabulate published and volunteered unpublished data and prepare a manuscript for approval as an official SSC publication at the 2003 meeting. This proposal was approved by the Subcommittee. Dr. Johnson will Chair the Working Party.

Scientific Program:
Justin Hamilton, University of California at San Francisco, described studies of PAR 4 and PAR 3 knockout mice. Both knockouts manifested prolonged bleeding times, impaired thrombin-induced platelet aggregation and protection from experimental thrombosis. These studies demonstrate the important cofactor function of PAR 3 in vivo.

Mieke Dewerchin, Center for Transgenic Technology and Gene Therapy, Leuven, presented results of studies of antithrombin R47C mutant mice. This mutation resulted in a high rate of neonatal mortality. Survivors developed spontaneous thrombi in multiple organs. Thromboses were not prevented by the administration of pentasaccharide. These studies emphasize the critical functional role of the heparin binding site of antithrombin.

Brian Peterson, Scripps Research Institute, San Diego, spoke about the consequences of low tissue factor (~1% in brain) in mice. These animals had increased post-partum uterine hemorrhage, fatal hemorrhage in 20% and tissue fibrosis resulting in impaired cardiac function. These mice were resistant to experimental arterial thrombosis that was not corrected by transplantation of wild type bone marrow. Thus deficiency of vascular tissue factor appeared to be more responsible for hemorrhage and resistance to thrombosis than blood cell tissue factor.

Pudur Jagadeeswaran, University of Texas, San Antonio, presented an overview of his laboratory's extensive characterization of hemostatic parameters and thrombosis in zebrafish. The similarity of blood coagulation factors, platelets and thrombosis in zebrafish to those of mammals, combined with their utility as genetic models, make zebrafish a valuable model for the study of mechanisms of hemostasis and thrombosis.

Laveena Sharma, Monash University, Australia, presented evidence from studies of mice with a targeted deletion of the cytoplasmic domain of tissue factor that the carboxyl terminus plays an
important role in inflammation. Animals with the deletion challenged with LPS demonstrated improved survival, decreased pulmonary neutrophil accumulation and cytokine production, and they manifested impaired delayed hypersensitivity.

Ed Conway, Center for Transgenic Technology and Gene Therapy, Leuven, described studies that demonstrated the importance of the lectin domain of thrombomodulin in inflammation. Deletion of the lectin domain resulted in normal development and fertility, and normal interaction with protein C, but mice with this deletion had exaggerated responses to LPS and experimental arthritis, increased experimental myocardial infarction size and increased neutrophil adhesion to endothelial cells.

Anne Angellilo, University Medical Center, Geneva, spoke of studies of the role of Gas 6 in mouse platelet function. Gas 6 knockout mice had normal plasmatic coagulation, bleeding time and fibrinolysis, but they demonstrated impaired platelet aggregation and secretion. These mice were protected against experimental thromboembolic death. The impaired platelet aggregation was restored by addition of exogenous Gas 6. These mice also demonstrated impaired inflammatory responses.
INTRODUCTION:

Dr. Diamond introduced the prevailing accepted assays in biorheology that allow study of adhesion on a surface (parallel-plate and tube flow) or bulk aggregation (cone and plate or concentric cylinder). Flow forces are intrinsically important in understanding bonding function in thrombosis. As an example, platelets have difficulty bonding to surface-sorbed vWF A1 domain while flow greatly facilitates bonding with a "hydrodynamic threshold" at ~100 1/s. Additional examples of flow regulation of thrombosis were illustrated with fibrin formation around adherent neutrophils due to prothrombinase activity when Factor XIIa is not inhibited by corn trypsin inhibitor (CTI). CTI may see increasing use when working with recalcified whole blood or plasma in order to avoid unintended contact activation. Membrane mechanics can play an important role in adhesion of real cells, e.g., platelet P-selectin-neutrophil PSGL1 bonding under flow where long membrane tethers can be pulled from the neutrophil to shield the bond from force loading. Differences between bead systems and cells can often be fully reconciled by accounting for membrane deformation. The additional point was made that recombinantly expressed receptors may not have the same kinetic/mechanobiology as intrinsic receptors. Issues of sulfation and precise glycosylation structure are proving very important in the selectin literature. Adhesive interactions and mechanisms may depend on prevailing shear rates with respect to bonding pairs. Dynamics of rolling to arrest and associated signaling may be shear dependent. For example, at very low flow (< 100 1/s) normal RBC can form receptor mediated adhesion with activated platelets or neutrophils. The exploration of the full flow range from static conditions to low shear (10 1/s) to higher shears (1000s 1/s) for surfaces ranging in low to high densities of adhesive ligands or cells provides a full characterization of bonding dynamics important to thrombosis.

As a summary, the subcommittee should be prepared to address emerging "omic" trends in (1) single bond mechanics (functional proteomics enabled by detachment assays, AFM, laser tweezer, micromanipulation studies); (2) cell adhesion under flow (functional cellomics or "hemodynamic phenotyping"); (3) integrative systems biology for combined study adhesion/coagulation/inflammation biology; and (4) high throughput assay (2D/MS, lab-on a chip, and "rheomics").

PRESENTATIONS:

Dr. Klaus Peissner
"The contribution of the urokinase receptor (uPAR) to the dynamics of inflammatory reactions."
An overview of uPAR interactions with uPA, vitronectin, b2-integrins sets the opportunity to study neutrophil uPAR regulation of Mac1 (CD18/11b) function in neutrophils. Notably, in patients with acute myocardial infarction (AMI) but not angina or in healthy individuals, the
level of leukocyte uPAR is elevated. These cells are more adhesive to endothelium, an adhesion blocked by 20 µg/ml of anti-uPAR. This adhesion was reduced by uPA. In a flow assay (concentric cylinder), fMLP/Zn activated neutrophils were allowed to bind to fibrinogen coated beads. Zn, which activates uPAR, caused a marked increase in heterotypic aggregation which was reduced by uPAR antibody. This suggests a role for uPAR activation in elevating the avidity state of Mac1. Platelets are an important source of Zn in the microdomains of a thrombus. The D5 domain of kininogen, a cationic domain enriched in histidine, glycine, and lysine, has activity against the neutrophil-endothelium adhesion in a thioglycollate model. GST-D5 protein or peptide 483-502 of D5 were anti-adhesive. D5 may reduce uPAR-Mac1 interactions or uPAR-vitronectin interactions. D5 increased the tail bleed time. The role of uPAR as a Mac1 ligand on neutrophils during homotypic aggregation can be explored in the future.

Mony Frojmovic (Past Subcommittee Chair)
Evaluation of shear-dependent drug efficacy on inhibition of platelet aggregation
Dr. Frojmovic highlighted the opportunity for evaluation of drug activity as a function of prevailing hemodynamic forces. Notably, the IC50 of a drug may change depending on the prevailing flow. The time-dependent, binding affinity of fibrinogen for active GPIIbIIIa was a focus of study and may be shear dependent. This phenomenon, long observed with platelets, can be observed with purified IIbIIIa on beads where bound fibrinogen is easily desorbed with Ro44883 when the drug is added early on, but after 20-30 min fibrinogen is not easily displaced by Ro. The conclusion is that fibrinogen can go deeper into the binding pocket of IIbIIIa after a period of about 30 minutes. Under shear conditions, however, Ro disaggregated fibrinogen-GPIIbIIIa interactions, regardless of the age of the aggregates. The conclusion is that under flow conditions, the fibrinogen cross-bridges may not be able to access the deep pocket, irreversibly bound state. Again the equilibrium properties of the binding interaction in a biacore or binding assay with zero force (other than thermal motions) may not be predictive of performance of a system under hemodynamic forces.

Scott Diamond
Normal erythrocytes: Not so passive and a little sticky during coagulation
Dr. Diamond noted that DVT is a low shear pathology whose prevention includes vascular compression therapy to elevate blood flow. A study at depressed venous flow conditions under 100 1/s indicated that normal RBC can adhere to collagen adherent platelets via GPIb and CD36 mechanisms, but that this adhesion was not blocked by antibodies against IIbIIIa, TSP, vWF, or Pselectin. RBC-platelet adhesion was blocked by fibrinogen or EDTA. RBC also adhere at these low flow conditions to fMLP-activated neutrophils through a CD1811b mechanism. One of the RBC mac1 ligands was found to be ICAM4 (LW antigen). The other ligand is not known. RBC were found to bind under low flow conditions to fibrin formed from plasma.

Jaap Jan Zwaginga
Platelet adhesion decreases to lysed fibrin whereas neutrophil adhesion remains intact under conditions of flow
Dr. Zwaginga discussed neutrophil receptors (PSGL1, ESL1, Lselectin and Mac1, LFA1, and VLA4) in the context of endothelial counter-ligands (Pselectin, Eselectin, CD34/Madcam/sLeX, and ICAM1 and VCAM1). While neutrophils had little adhesion to endothelial matrix (200/mm2 which drops to near zero adherence at about 300 1/s), neutrophils can adhere to ECM
in the presence of platelets (1000/m2 which drops to low levels at about 800 1/s. Neutrophils were noted to roll on surface spread platelets, roll and arrest on platelet/fibrin, and arrest on fibrin alone (at low shear rate < 100 1/s). The platelet ICAM2-neutrophil LFA1 interaction was noted to be fairly minor. Accumulation of neutrophil strings/clusters on fibrin was found to be dependent on Lselectin, presumably via Lselectin-PSGL1 neutrophil-neutrophil mediated capture. Neutrophils also adhere better to fibrin that is aligned in the direction of flow, via improved cell-fibrin contact and not via a new epitope in aligned fibrin fibers. Finally it was observed that neutrophils adhere nicely to partially degraded fibrin (tpa + plasminogen) while platelet adhesion is lost. This may have importance to clot remodeling and wound healing. The mac1 binding epitopes on fibrin gamma chain are preserved during fibrinolysis.

Marc Hoylaertz
Shear controlled platelet rolling over vWF is blocked by antibody shielding and by site directed mutagenesis of the A1 domain binding site for platelet GPIba
Dr. Hoylaertz explored the function of the GPIb-vWF interaction under flow conditions. This interaction, once thought to be long lived, is now recognized to be a short lived bond with a life under 1 sec under shear conditions. A number of loss of function mutants of the A1 domain were explored with special emphasis on conserved amino acids between 563 and 622. Binding to wt A1 was found to increase with shear stress. Antibody AjvW2 appears to bind position 563, 566, 570 which are on the binding face of A1. AjvW2 competes with heparin for these sites on A1. Notable loss of function mutants were D570A and R616. Notable changes in platelet attachment trends with shear rate (200, 1000, 1500 s-1) were noted with these mutants. Videos of rolling demonstrated fast rolling and transient short lived translocation on these mutants that have loss of function. A full analysis of rolling velocity, pause distribution, and catch efficiency will help define the mechanical properties of the binding face as a function of bond loading. These issues are critical in understanding platelet recruitment under arterial flow conditions and the role of heparin as an antiadhesive.

RECOMMENDATIONS:

1. The committee will review and submit a report providing guidelines for the interpretation of single-molecule bond mechanics in the context of thrombotic/inflammatory reactions under hemodynamic conditions. This includes a summary of kinetic data and their analysis to provide kinetic parameters.
2. The committee will review and submit a report providing guidelines for the interpretation of pharmacological agents (IC50) as a function of hemodynamic environment.
3. The committee will highlight emerging trends in lab-on-a-chip, miniaturization, systems modeling, and proteomics that relate to functional phenotyping of blood function under hemodynamic conditions.
Contact Activation

Chairman:  R. A. DeLa Cadena, USA.
Co-Chairs;  A. H. Schmaier, USA; I. Schousboe, Denmark; H. Saito, Japan;
M. J. Gallimore, UK; Keith R. McCrae, USA; A. Zivelin, Israel

The meeting was well attended with approximately 50 people in the room. The Chair introduced the Co-Chairs present in the room and acknowledged the list of individuals who were listed in the program. The Chair in turn communicated with the audience the message from Dr. Francis, namely the mission of the Subcommittee including new methods, new topics, predictive values and standardization of plasma samples for contact factors.

The session started with the first presentation by Dr. Pixley entitled "Genetic alterations of high molecular weight kininogen modulates inflammation" and then followed by other speakers listed below.

- Antibodies Directed Against Factor XII and Recurrent Fetal Loss (Dr. Wynne Jones)
- Antiphospholipid Murine Antibodies that Cross-react with HK and TSP1.(Dr. Marc Monestier)
- Plasma Prekallikrein Activation on Endothelial Cells: Reality or Illusion (Dr. Zia Shariat – Madar)
- DX-88, A novel Therapeutic Peptide Inhibitor of Kallikrein (Dr. Ian Mackie)

All presentations were highly focused and methodological in nature.

A full sixty (60) minutes were devoted to issues related to:

- Standardization Procedures: Including the Prekallikrein Activator that has been available since 1982 and that Dr. Pixley at Temple University School of Medicine will compare to two other commercially available plasmas. Dr. Schmaier’s laboratory will examine by Western blotting technique the plasma stocks available from the ISTH. It was emphasized by Dr. Schmaier that "no preparation seems to be suitable for the 'so called' Contact System". Dr. Gallimore reminded everyone that a few years ago standardization of plasma required at least twenty (20) laboratories to test the samples. A question was raised about being able today to get twenty (20) laboratories to participate.

- Re-naming of the Subcommittee was discussed with two suggestions from the floor. One from Dr. Schmaier, namely "Kallikrein-Kinin System" and the second one from Dr. Pixley entitled "Kininogen Dependent Systems". The Chair then encouraged everyone to be vocal with suggestions and to pass the word and to ask colleagues for suggestions. The Chair and Co-Chairs in turn will research at the SSC Business Meeting the mechanism for the change of a Subcommittee name.

- Plans for the future:
1. - Continue the mission of the Subcommittee.
2. - New Methods.
4. - Predictive Values.

Respectfully prepared by Dr. DeLa Cadena
Control of Anticoagulation Subcommittee

Chairman: M. Greaves, UK
Co-chairs: H. Bounaumeaux, Switzerland; J. Harenberg, Germany; C. Kearon, Canada; F. R. Rosendaal, The Netherlands;
S. Schulman, Sweden; A. Tripodi, Italy; A.M.H.P. van den Besselaar, The Netherlands

Working Party on Duration of Anticoagulant Therapy in Venous Thromboembolism

Chair: C Kearon [S Schulman assisted in the design of the programme]

Introduction C Kearon
Dr. Kearon introduced the session by reviewing the current evidence base for duration of anticoagulation after a first VTE:

- 6 weeks treatment is not adequate for proximal DVT or PE
- If there was a transient major risk factor, the recurrence rate is lower and 3 or 6 months of treatment is adequate
- If the first event was unprovoked the risk of recurrence is higher.
- Anticoagulation to a target INR of 2.5 results in a risk reduction for recurrence of at least 90%
- The lowest achievable risk of recurrence after discontinuation of anticoagulant therapy is after 6 to 12 months of treatment
- Heterozygosity for factor V Leiden and prothrombin G20210A are not predictive of higher recurrence rate
- Antiphospholipid antibody, homozygosity for factor V Leiden and doubly heterozygous states for heritable thrombophilic disorders [but only possibly deficiencies of proteins C/S and antithrombin] are probably risk factors for recurrence
- Cancer is an important risk factor for recurrence

Influence of presentation (DVT or PE) on risk of recurrence

Dr J Douketis reviewed the evidence for difference in recurrence rates after initial DVT or PE [submassive]. A meta-analysis of 25 studies [JAMA 1998, 279:458] and some additional reports were cited. Overall 3 month recurrence after first PE was not significantly different from that after first DVT, at around 5% for both. However the meta-analysis indicates that the impact of recurrence after initial PE is greater with an approximately three-fold greater mortality. At least some of this is attributable to co-morbidity, especially cardiorespiratory disease. There is also evidence for an approximately two-fold higher recurrence rate after first iliofemoral DVT in comparison to femoral or popliteal DVT.

Dr J Heit reported on the Mayo Clinic VTE study which is based on the Rochester Epidemiology Study. An overall recurrence rate of 30% at 10 years was reported, being highest early. Independent predictors of recurrence were greater age, higher BMI, malignancy and neurological disease. Lower recurrence rates were found when the presenting VTE was associated with oral contraceptive or HRT use or gynaecological surgery, supporting other reports of lower recurrence rates in subjects with transient risk factors.
Dr R White reported on his study based on linked hospital discharge data in California [In press, *Thrombosis and Haemostasis*]. Similar rates of recurrence to those previously reported were found and a higher prevalence of cardiorespiratory disease was noted among patients with PE compared to those with DVT. It was confirmed that those presenting with DVT were much more likely to manifest DVT as the recurrent event whereas those with PE were more prone to PE recurrence [60-80% of patients with recurrent VTE as PE had index PE]. An analysis of time to second event did not support diagnostic bias as a cause of this phenomenon.

**Does extending duration of treatment reduce subsequent cancer incidence?**

Dr G Agnelli reported on the WODIT study. This addressed the question whether extending the duration of anticoagulant treatment after first VTE from 3 months to one year reduces the chance of a new diagnosis of cancer. In a total of 429 cases randomised to one or other group there was no significant difference in number of new cancer diagnoses between the two arms. This study provides no evidence for a reduction of cancer risk with oral anticoagulant therapy.

**Can markers of thrombosis after stopping anticoagulant therapy predict recurrent VTE?**

Dr G Palareti referred to 3 early studies which suggested a rise in plasma D-Dimer concentration after discontinuing oral anticoagulation in some subjects treated for VTE. He went on to review his recently published study which was designed to expand on these observations. In a cohort of around 400 patients, D-Dimer measured after discontinuing oral anticoagulant was significantly higher in those subsequently suffering VTE recurrence. The negative predictive value of normal D-Dimer after 3 months of treatment was 96%. Subgroup analysis suggested that this was also the case in subjects with identified heritable thrombophilia. The higher D-Dimer levels did not seem to relate to poorer recanalisation, although the highest recurrence rates were in the subgroup with no recanalisation and raised D-Dimer concentration. It was noted that Faltorini et al [Thromb. Haemost. 2002; 88:162] have also recently reported a high negative predictive value for D-Dimer measured during anticoagulation.

**Are residual venous abnormalities predictive of recurrent VTE?**

Dr C Kearon and Dr F Piovella reviewed data on residual vessel occlusion and recurrence risk. In the DURAC study it was found that there was a higher recurrence risk in the contralateral than the ipsilateral leg on long term follow-up. This does not suggest importance for local factors in the originally affected veins. In two small studies there was no evidence for significantly higher recurrence risk associated with persistence of abnormal ultrasound or plethysmography findings; however, in a more recent study by Prandoni et al normalisation of ultrasound findings was associated with lower risk of recurrence. Dr Piovella reviewed his recently published study [Haematologica 2002, 87:515] in which normalisation of ultrasound findings predicted lower risk of recurrence. As a result a study in which ultrasound findings and D-Dimer assay are combined is about to commence.

**Is factor V Leiden more of a risk factor for DVT than PE?**

Dr M Prins reported on evidence that heterozygosity for factor V Leiden carries a relatively higher risk of DVT than of PE. He noted that several studies have been remarkably consistent in demonstrating an around 2-fold higher risk of DVT than of PE in carriers when compared with VTE patients without V Leiden. In one study there was no such effect of heterozygosity for G20210A. It was postulated that V Leiden may be associated with less friable clots and/or more local inflammation.
Outcome from the Working Group.
Dr Kearon summarised these presentations and this allowed the following clinically useful conclusions to be drawn:

- Subjects presenting with DVT carry a similar recurrent event risk as those presenting with submassive PE.
- Those presenting with PE are more likely to suffer PE than DVT at recurrence and the case fatality rate is higher for PE than DVT.
- These observations support longer or indefinite treatment for those presenting with PE, but this has not been subjected to clinical trial and bleeding risk and patient preference must also be considered.
- Longer treatment with oral anticoagulants probably does not reduce cancer risk and is not a strategy to be supported at present.
- Factor V Leiden is more strongly associated with DVT than with PE but heterozygosity for V Leiden should not influence treatment duration.
- It appears likely that D-Dimer can be used to predict recurrence, but the role of D-Dimer measurement in determining treatment duration in individual patients is not yet clear, and it is also unclear whether anticoagulation must be discontinued prior to measurement.
- Prospective validation of this use of D-Dimer assay is required before application to routine clinical practice. Independence from other clinical risk factors should be verified also.
- There is probably a lower risk of VTE recurrence in those in whom there is complete resolution of the initial vessel occlusion, but it is unclear if this is independent of the recurrence-free interval.

Chair: AMHP van den Besselaar/A Tripodi

The ECAA multicentre calibration of the CoaguChek Mini and TAS PT-NC monitor systems
A Tripodi
Reference was made to 2 published papers assessing the possibility of switching to use of whole blood for calibration and to assess simplified calibration using recalcified plasma in place of whole blood. The CoaguChek Mini and TAS analysers were assessed. It was concluded:

1. The proposed modified ISI calibration method is reliable for both monitors.
2. For both monitors the CV of the calibration for both whole blood and plasma is lower than 5% but higher than the recommended 3%.
3. Plasma may be suitable for determination of ISI in relation to both monitors.

The use of ECAA lyophilised plasmas for calibration of the CoaguChek Mini and TAS PT-NC monitor systems
Dr A van den Besselaar
Results of experiments with 3 types of plasmas were reported: Artificially depleted [n=60]
Coumarin plasmas [n=60]
Normal \[n=20\]

Two monitors were assessed but not identified in the presentation. With one lyophilised coumarin samples ISI agreed with whole blood ISI, with the other there were discrepancies. On attempting to simplify procedures by reducing the number of lyophilised plasmas for ISI calculation, it was found that a minimum of 30 coumarin and 10 normals sufficed for one machine and 20 and 7 for the second.

**The ECAA system for quality control of the CoaguChek Mini and TAS PT-NC monitor systems**

**Dr Michelle Keown**

The need for methods to facilitate the QC of individual monitors was stressed. An ECAA study at 10 national centres has been performed on TAS and CoaguChek monitors. Sixty artificially depleted plasmas and 60 coumarin plasmas were employed. Five and 3 of these, respectively, were selected which gave good INR distribution in both systems and CV <15%. There were considerable INR differences between centres on the full plasma set and reflected in the selected smaller set. There was little difference in results between sets of 3 and 5 plasmas. There was no clear advantage in use of coumarin plasmas over depleted plasmas. Examples of the use of plasmas for individual QC were given and it was concluded that provision of sets of 3 ECAA plasmas is a simple and practical method for checking reliability of INR measurements on individual monitors of both types.

**Overview of the results of the ECAA Normalisation and Standardisation of Home Monitors PC Study**

**Dr L Poller**

An overview of the group’s publications on this topic was presented. Additional work in press was reviewed. This has demonstrated significant instrument variability over 14 monitors [both TAS and CoaguChek]. There was obvious greater dispersion of results with one monitor type than the other [types not identified]. In a second study equivalence in quality was shown between NPT and laboratory testing with computer assisted dosing employing a randomised cross-over design. Finally the reliability of the displayed INR on the two monitors has been assessed in 10 centres using six hundred coumarin patients’ whole blood and plasma IRP manual PT tests. Displayed INR averaged around 20% higher on one system than the other. This was accounted for by consistent overestimation of the ‘true’ INR on one system and consistent underestimation on the other. It is concluded that extra steps in ISI calibration and/or QC described in the ECAA Technology Implementation Plan are needed.

**Outcome from the Working Group:**

*The ECAA Technology Implementation Plan incorporating ISI calibration and QC of POCT monitors has been accepted by the European Commission.*

*It was noted that the activities of the group had spanned many years and provided invaluable data. Although it is accepted that more work is needed it was agreed that an SSC report should be prepared at this stage in order to provide state of the art information to interested parties. Professor Preston agreed to liaise with the other members in the drafting of a document with a deadline of January 2003.*
Influence of sample optical clarity on INR determinations on fresh, frozen and lyophilised test samples
Dr S Kitchen

A study of the effects of sample optical clarity on INR determination was reported. INR was determined using Innovin/Sysmex CA 6000, PT Rec and PT Fib HS S Plus, both on ACL. Local MNPT and verified manufacturers’ instrument specific ISI were used. Fresh, thawed frozen and reconstituted lyophilised plasmas were examined. Opacity was determined by light scatter and expressed as an ‘h’ value. A significant proportion of samples from subjects on warfarin were found to have a high h value [2% >70]. The study clearly demonstrated that freezing and freeze drying increase opacity and this can raise the measured INR with PT Rec but not with the other 2 reagents tested. Such an effect is seen in fresh samples on occasions. These observations are highly relevant in proficiency testing and should be noted by scheme managers. There may be implications for INR calibration plasmas also.

Status of international reference preparations for thromboplastin
Dr A van den Besselaar/Dr A Tripodi

The MNPT of rTF/95 and RBT/90 between 1995 and 2000 was noted to be stable. The residual stocks of these reference preparations were illustrated, indicating that, based on previous usage rates, there should be sufficient rTF95 for over 5 years but only 3 years for RBT/90. It was concluded that plans should be made and implemented for the replacement of RBT/90 and proposals were presented for the mechanism to achieve this under the auspices of the SSC. Outcome: The proposals were accepted by the Subcommittee and are to be implemented forthwith.

A new algorithm for computer-assisted dosing: Presentation and demonstration
Dr F R Rosendaal

The need for more accurate dosing was identified, especially as bleeding risk is increased with greater fluctuation in the INR in a treated individual as well as through overtreatment. A new model was described which incorporates an estimate of the subject’s previous dose sensitivity. This has been applied to a dataset of over 200 and gave a small predictive error. The algorithm is to be tested prospectively in a randomised study of 500 patients with time in therapeutic target range as the primary endpoint and clinical events as a secondary endpoint. Outcome: Dr Rosendaal indicated that the system would be made available to interested parties should the potential advantages be confirmed in the prospective study.

WHO Working Group on Unfractionated Heparin and Low Molecular Weight Heparin / Working Party on Monitoring of Low Molecular Weight Heparin
Chair: T Barrowcliffe/M Greaves

Progress on global harmonisation of heparin assays
Drs A Padilla/E Gray
There was nothing new to report

Progress on the 2nd WHO standard for LMW heparin
Dr Elaine Gray
The pilot study was reviewed. Anti-Xa and anti-IIa chromogenic assays were employed in 11 laboratories in the study. It had been concluded that UFH should not be used as a calibrant for LMWH due to poor interlaboratory agreement and nonparallelism. All LMWH were comparable in the study, but 2 samples tended to non-parallelism. The study allowed selection of two samples with the lowest interlaboratory variation to be selected for further study.

The protocol for this new study was described. The two candidates [above] are to be compared with the 1st International Standard. Participants will be recruited in July/August 2002. It was noted that there is a need for volunteer clinical laboratories, and those interested should contact Dr Gray.
There will be a report of the findings to the SSC in 2003 and a replacement standard will be recommended.

**Status of Paediatric Guidelines**
**Dr. P Massicotte**
It is considered that evidence-based guidelines cannot yet be formulated. There is a need for more safety, efficacy and monitoring studies.

**Anti-Xa levels during prolonged therapy with LMW heparin in cancer**
**Dr M Kovacs**
A study was designed to determine whether accumulation of the anticoagulant effect of LMWH occurs when administered long term in patients with cancer. Twenty-four patients from a single centre were evaluated. The results were reassuring in that anti-Xa levels measured at 4 to 6 hours after injection were comparable at days 7 and 28 of therapy [1.11 and 1.03 respectively, p 0.37]

**Problems in the evaluation of anticoagulant activity of new direct and indirect factor Xa inhibitors**
**Dr M Samama**
The need for accurate monitoring of some patients treated with these new agents was acknowledged. A study of assays of Fondaparinux [pentasaccharide] and DX9065a [a synthetic propanoic acid derivative] was made. The questions of preferred anti-Xa method, most appropriate expression of results [activity or gravimetric] and heterogeneity of results in global tests were addressed. It was concluded that expression of anti-Xa on a gravimetric basis has some advantages and standardisation of anti-Xa measurement for these new drugs is required. Also the heterogeneous response in more global tests was confirmed and requires further study and explanation. Work is ongoing on these issues.

**CTAD (Citrate, Theophylline, Adenosine, Dipyridamole) as a superior anticoagulant to citrate for heparin monitoring**
**Dr S Kitchen**
A study was performed to examine the practical significance of previous reports that CTAD [citrate, theophylline, adenosine, dipyridamole ] is useful in the prevention of loss of heparin over time in clinical samples, through platelet stabilisation and limitation of PF4 release. Samples were taken into citrate or CTAD and analysed immediately and after mixing for 4 hours. PT/INR was assessed using Innovin and PT Fib HS Plus, APTT with Actin FS and
anti-Xa by chromogenic assay. Samples included 32 normals, 32 warfarinised, 30 on UFH and 30 on LMWH. A 32% loss of anti-Xa activity with UFH was noted in citrate, with around 25% shortening of APTT. This was abolished by use of CTAD. Samples in CTAD were suitable for LMWH treated subjects. There were some differences in results with PT reagents on CTAD samples from warfarinised subjects, and INR values were slightly higher using either reagent in CTAD compared with citrate. It is concluded that CTAD is the anticoagulant of choice for UFH monitoring. For general use in the coagulation laboratory for anticoagulant monitoring, it is not yet clear whether the heparin protection effect is sufficiently advantageous to overshadow the small effect of CTAD on INR.

Working Party on Standardisation of Methods to Determine Direct Thrombin Inhibitors
Chair: M Greaves

Results of the International Interlaboratory Thrombin Inhibitor Study
Dr Elaine Gray
The report of the collaborative study of assay methods for hirudin and argatroban was presented. Spiked plasmas were examined using APTT [local and common reagent~Actin FS, 10 laboratories], Anti-IIa chromogenic assay, Ecarin clotting time by wet and dry chemistry methods, ELISA for hirudin. Four(4) sets of plasma samples were examined in each method. In summary, the APTT and ECT methods gave low inter-laboratory variability [%GCV under 5 for most labs]. There was greater variability using anti-IIa and hirudin ELISA. In interlaboratory analysis common APTT and dry ECT performed well. There was relatively higher variability on hirudin samples with local APTT. In the wet ECT and anti-IIa assays, the variability increased with concentration of analyte. The hirudin ELISA performed poorly. In terms of sensitivity all methods gave linear dose-response and were able to detect 0.31µgm/ml. Overall, although APTT gave good reproducibility within and between labs, the sensitivity was lower than the other methods.

Outcome:
There will be a written report to participants in August 2002 and a report to subcommittee members in October/November 2002. An SSC communication will be submitted for publication early in 2003.
It is acknowledged that further studies are needed, especially those on ex vivo samples from treated patients. Possible collaboration in these further studies with relevant pharmaceutical companies was raised.

Working Group on Calibrated Plasmas for INR Determination
Chair: T Barrowcliffe

NCCLS Guideline on procedures for validation and calibration of the International Normalised Ratio using the Prothrombin Time test.
Dr Dorothy Adcock
A brief update on the NCCLS Subcommittee on PT Calibration and the forthcoming guideline was presented. The major points and recommendations were reviewed. The group were congratulated on the progress achieved.
Discussion of final report of the SSC Working Group on Calibrated Plasmas for INR Determination
Dr T Barrowcliffe

Outcome from the working group:
After detailed discussion of the problems, a vote was taken on the need for preparation and dissemination of a guideline on this topic rather than the review manuscript which had been proposed as a solution to the lack of consensus among the working group. The result was in favour of a guideline in which areas of lack of consensus would be acknowledged. It was agreed that Dr van den Besselaar would take over Chairmanship of the working group and undertake drafting of the guideline alongside the other members. It was suggested that Dr Greaves could assist as a non-cognate facilitator in this process.
The meeting of the DIC subcommittee consisted of three parts: (1) presentations from various research groups on their experience and prospective validation of the scoring system for overt DIC that was accepted by the SSC in 2001, (2) an overview on the use of molecular markers for the diagnosis of DIC, and (3) an update on clinical studies for the treatment of sepsis and DIC.

In the past year several research groups have used the newly adopted SSC/ISTH scoring system for overt DIC and now report here on their experience.

Cheng-Hock Toh (Liverpool, UK) reported on his experience with the DIC scoring system for both overt and non-overt DIC. His data indicate that the non-overt template is workable. He proposed to prospectively collect data to complete and refine the scoring system for non-overt DIC in particular. Hideo Wada (Mie, Japan) compared the ISTH DIC score with the score for DIC of the Japanese Ministry of Health. He demonstrated a good overall agreement between the two scoring systems, with a somewhat higher sensitivity and lower specificity of the Japanese score, mostly due to the high incidence of a diagnosis of DIC in Japanese cancer patients. Marcel Levi presented two prospective studies from Amsterdam comprising 50 and 217 patients, respectively. In consecutive ICU patients the incidence of DIC was 32%, and the DIC score had a sensitivity of 91% and a specificity of 97%. The score correlated well with molecular markers for DIC. Dilys Rapson (Kingston, Canada) discussed the practical issues of assessing the DIC score in critically ill patients.

In subsequent discussion the need for a collaborative trial to prospectively validate DIC was considered. Such a study could benefit from a standardised ‘gold-standard’ for DIC and eliminate inter-center differences. It was concluded that the subcommittee will organise a multicenter study including tentatively 500-1000 consecutive ICU patients from at least 5 centers world-wide. In the coming year the protocol will be developed and centers can enlist with the subcommittee’s chairman.

The second section of the meeting was devoted to the use of molecular markers in the diagnosis of DIC. Michio Matsuda (Tokyo, Japan) presented an overview on the use of fibrin-related markers, such as D-dimer and soluble fibrin, that play a central role in the scoring system for overt DIC. Their specific role in the scoring system for non-overt DIC will need to be worked out. Gary Kinasewitz (Oklahoma, USA) presented data on the use of the SSC/ISTH scoring system in the successful Prowess trial of recombinant activated protein C in patients with sepsis.
In the discussion that followed the direction of the development of the non-overt DIC score was discussed. The subcommittee decided that in the coming year this system will be further developed during a working party meeting in December-January and will be discussed during the subcommittee meeting in Birmingham in 2003.

The last part of the meeting consisted of an overview of the most recent clinical studies in patients with sepsis and DIC with anticoagulant factor concentrates. Jørn Dalsgaard Nielsen (Copenhagen, Denmark) reviewed the clinical experience with antithrombin concentrate replacement therapy. Despite the negative phase III clinical trial there might be benefit to be expected of high-dose antithrombin infusion due to its postulated anti-inflammatory effect and when it is not combined with heparin. Jean Francois Dhainaut (Paris, France) highlighted the positive clinical results with recombinant human activated protein C in patients with sepsis. This agent was shown to lower mortality and is actually one of the first therapeutics in sepsis that was shown to be effective. Fletcher Taylor (Oklahoma, USA) focused on the use of recombinant TFPI in sepsis and DIC in preclinical and clinical settings.

The attendance was about 100 people (at some points during the meeting exceeding the number of seats available). There was adequate discussion opportunity during the meeting.
Factor VIII and Factor IX

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

Completed and Submitted Reports-
Chair: D. DiMichele

The following report was published in 2002:


ISTH website publication:

The Design and Analysis of Pharmacokinetics: Studies of Coagulation Factors.
M. Lee, M. Morfini, S. Schulman, J. Ingerslev

Completed studies/Publications in progress:

The Design and Analysis of Pharmacokinetics: Studies of Coagulation Factors-- Summary.
M. Lee, M. Morfini, S. Schulman, J. Ingerslev

International Registry: Congenital FVII Deficiency
G. Mariani

Dr. Ingerslev reported on behalf of Dr. Mariani and the FVII Registry Study Group . Currently closed to new subject accrual, the registry now contains 514 subjects from 59 centers. Of these 198 are asymptomatic. Among the symptomatic patients, clinical manifestations are classified as follows: 29% severe; 42% moderate and 30% mild.

There have been DNA samples submitted on 335/514 subjects. All but one mutation have been characterized.

The planned analyses of the data include: 1) clinical phenotype genotype correlation; 2) association of FVII deficiency with thrombosis;3) phenotypic characterization of the severe deficiencies including the incidence of CNS bleeds; 4) natural history of disease; and 5) treatment practices. The first publication on the association of FVII deficiency with thrombosis has been submitted to Blood. Manuscripts in preparation are on the topics of genotype/phenotype correlation and CNS bleeds. The subcommittee will continue to be apprised of this group’s excellent productivity.

Registries and Studies in Progress
Co-Chairs: M. van den Berg and A. Srivastava
Registry on FIX inhibitors associated with anaphylaxis- J. Lusher

Dr. Lusher gave an update on the FIX inhibitor registry of Dr Warrier. The most recent update in July 2002 included 85 patients were included. Demographics (mean and range) of the group at the time of inhibitor development are as follows:

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<tr>
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<th>Mean</th>
<th>Range</th>
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<tbody>
<tr>
<td>age</td>
<td>19.5 months</td>
<td>(9-156)</td>
</tr>
<tr>
<td>exposure days</td>
<td>11 days</td>
<td>(2-180)</td>
</tr>
<tr>
<td>peak inhibitor titer</td>
<td>30 BU/ml</td>
<td>(1-960)</td>
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<tr>
<td>complete gene mutation</td>
<td>17/32</td>
<td>(53%)</td>
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All FIX products have been associated with inhibitor development. Among the 32 patients on whom these data are available, 21 attempts at ITI (66%) are noted with only 2/21 successes (9.5%). Data on the other patients are not yet available, so this figure could increase slightly; nevertheless, this is a much lower success rate for immune tolerance than achieved for hemophilia A.

Part of the reason for this registry was to document the prevalence of nephrotic syndrome occurring during ITI. So far three cases demonstrated a clear association. In all, 13 cases have been reported. The nephrotic syndrome occurred after a median duration of ITI of 9 months. Eleven of 13 cases had documented prior allergy to factor IX. Renal biopsy was performed in 2. Both biopsies revealed membranous glomerulonephritis. Immunohistochemical staining was negative in one patient studied.

It was concluded that the prevalence of inhibitors with an allergic phenotype in hemophilia B is low (2.3%), and only 10% of the patients achieved tolerance after ITI. Nephrotic syndrome has been reported in 13 cases. Professor Mannucci commented that although the Milan protocol to study the immunology of this phenomenon is available on the ISTH website, no samples had been received to date. Physicians are again urged to participate.

Update on the Gene Therapy Registry- K. High

Dr. K. High gave a gene therapy registry update with respect to the development of databases produced by authorities. The current NIH database on gene transfer is now on the OBA website: [http://www.4.od.nih.gov/oba/rac/clinicaltrial.htm](http://www.4.od.nih.gov/oba/rac/clinicaltrial.htm) and contains information on 10 trials on gene transfer in X-linked recessive disorders.

During the third and fourth quarters of 2002, the GeMCRIS database - intended for all but with restricted access to the public and non-investigators through graded built-in firewalls - will come into operation. It is currently being piloted by the NIH Clinical Research Center. This database will serve MDs with access to rapid reporting of AE's.

Data exchange with the GTPTS (longterm follow up of gene therapy trial subjects) tracking system of the FDA will occur.
International IT study update- D. DiMichele

Dr. DiMichele gave this presentation on behalf of her two co-principal investigators, Drs. Charles Hay and Evelien Mauser-Bunschoten. Most importantly, after a delay due to the recombinant factor shortage, the study has now officially started (July 2002) and is open for enrollment. A broad outline of the study was presented. It is a two-arm prospective randomized trial comparing IT success in subjects treated with low-dose arm ITT (50 IU/kg 3 times a week) to those treated with a high-dose regimen of 200 IU/kg per week. The anticipated enrollment is 150 subjects. The hypothesis is that the high-dose arm will achieve more rapid tolerance, but may not yield a higher overall success rate. Subjects can be included if they have severe HA, are < 8 years old, have had an inhibitor for < 12 months, have historical peak titers of between 5-200 BU/ml, have a confirmed current titer of < 10 BU. Patients with current titers > 10 BU/ml can be enrolled but will not be immediately randomized until the titer falls below 10 BU/ml in the ensuing 12 months. More information about the trial is available on the Hemophilia Research Society website as well as on the study website: www.itistudy.com. There was a question about whether the study should not also randomize for product type. Dr. DiMichele remarked that the study is not powered to determine the influence of this parameter on IT success; however, using a minimization strategy, both arms would include an equal number of subjects using vWF-containing, monoclonal and recombinant products so that this variable did not confound the analysis.

Proposed registry: FVIII inhibitors developing on continuous infusion- J. Oldenburg

Dr. Oldenburg briefly reviewed the increasing use of continuous infusion (CI) for factor replacement in hemophilia. He also mentioned that so far, 11 cases of inhibitors developing in such patients had been reported. All except one of them had >50 factor exposure days before developing inhibitors. Most of these patients had mild hemophilia. The basis for this phenomenon is not clear but could be related to altered immunogenicity of the FVIII molecule during the process of infusion. He proposed that it was therefore worth establishing a registry of these cases to gather data in an attempt to better understand its pathogenesis and its natural history.

In the discussion that followed, it was pointed out that unless there were control data available on similar patients treated with bolus infusion, it would be difficult to conclude whether these events were indeed due to CI. Suggestions were also made for modifications to the questionnaire so as to include the time scale in which these inhibitors appeared and their subsequent profile.

Challenges in gene based-diagnosis: Proposed working group on standardization- J. Oldenburg

Dr. Oldenburg mentioned that gene-based diagnosis of hemophilia was being increasingly used in recent times. Even though a very large number of a variety of mutations had been documented in the databases, one-third of all new reports still included novel mutations. In spite
of the many different techniques being used for screening for mutations, including sequencing of the gene, the success rate for mutation detection varies between 85-98%. Establishing causality of disease due to these mutations requires expression studies, which are not widely available. Carrier detection in families with large deletions can be difficult to detect using molecular genetic methods. Dr. Oldenburg presented data on the use of fluorescent in-situ hybridization (FISH) with probes for exons 5, 25, 26 of the FVIII gene for detection of such defects. FISH was also useful in documenting somatic mosaicism.

Because of these problems and the need to establish a uniform diagnostic approach, Dr. Oldengurg proposed that a network be established for standardizing the methodology for the gene-based diagnosis of hemophilia. The aim of such a group would be to define the criteria for assigning causality to a particular mutation and also to exchange samples that prove difficult to analyze.

In the discussion that followed, it was mentioned that while this was a good idea, the logistics of establishing such a network, particularly its funding, needed careful planning. It was suggested that a working party be formed to look into the feasibility of this proposal.

**Standardization Issues**

*Co-chairs: K. Mertens/JM Saint-Remy*

**The Mega 2 Standard. N. Kirschbaum**

Last year an extensive calibration program was completed on the new common working standard of the FDA and the European Pharmacopoeia. 100,000 vials of this material are available as an international, common working standard. In view of this major step toward harmonization in this area between US and Europe, it is unfortunate that it proved impossible to assign one single potency for both 1-stage and chromogenic assays. In Europe, this material is available as BRP batch 3 with a potency of 8.6 IU per vial for the chromogenic assay. In the US the same material is called Mega 2 and has an assigned potency of 8.6 IU per vial for the chromogenic assay and 11.3 IU per vial for the 1-stage assay. Dr. Kirschbaum presented stability studies conducted by the FDA on this material, demonstrating excellent longterm stability in both assay methods. She further reported on the standard performance of Mega 2 in the lot release of 43 batches of plasma-derived FVIII and on various lots of two recombinant products. In general there was good agreement with the labelled potency using Mega 2 in both chromogenic and 1-stage assays, with 90% of batch testing yielding potencies within a range of ±10% of the label value. The exception involved one of the recombinant products, for which 86% of batch testing by the chromogenic assay was within the ±10% range, but the use of the 1-stage assay resulted in only 45% of batches testing within this range and another 45% testing in the range of ±10-20% of the label potency. Dr. Kirschbaum concluded that the introduction of Mega 2 will not imply any shift in product potency. Upon questions from the audience, she further explained that the situation of a dual potency for Mega 2, though apparently confusing, seems workable without causing major inconsistencies.

**Proposal to replace WHO concentrate standard – T.W. Barrowcliffe**

Together with the calibration of the US/European working standard, another batch of the same
plasma-derived product has been processed and filled in ampoules (N) to serve as the 7th WHO standard for FVIII concentrate. Dr. Barrowcliffe presented a report of the calibration study involving 38 laboratories. All labs were instructed to follow the ISTH/SSC recommendations, including the use of FVIII deficient plasma as a prediluent. Of the participants, 27 obtained data with the 1-stage assay, while 31 used the chromogenic assay. The candidate standard did not display any significant discrepancy (<2%) between 1-stage and chromogenic assays, thus allowing the data to be averaged according to the general WHO policy. Interlaboratory agreement was much better than in the calibration of the current 6th International Standard, with an overall GCV value of 8.29%. The new candidate standard has been calibrated against the current 6th IS (recombinant) as well as its predecessor, the 5th IS (plasma-derived) and the two secondary standards, the US Mega-1 and the European BRP-2. Against these four references, the potency of the new IS was found to be 11.40, 10.66, 10.60 and 10.09 IU per ampoule, respectively. For the potency of the new IS, Dr. Barrowcliffe discussed three options: (1) 11.4 IU based on the current WHO 6th IS, (2) 10.8 IU based on all four references, and (3) 11.1 IU based on the mean of options 1 and 2. Option 1 seems preferable in terms of continuity of the FVIII concentrate unitage, but other options allow greater harmonization between other standards that are being used. The various options have recently been sent to the participants as well as to the FVIII-IX subcommittee members for an opinion. Dr. Barrowcliffe expects that a final recommendation will be submitted to the Subcommittee for a vote prior to the Birmingham meeting.

Effect of standard and assay method on FVIII measurement in recombinant and plasma-derived concentrates – J.D. Schreiber

A manufacturer's view on FVIII working standards was presented by Dr. Schreiber from Baxter (Vienna). One study compared the company's recombinant and two plasma-derived products employing 1-stage and chromogenic assays against several standards, including the Mega 2 working standard. Good agreement was found using Mega 2 in chromogenic assays at its established potency of 8.6 IU per vial. In the 1-stage assay, however, values were found suggesting that the potency of Mega 2 seems 15% higher than its established value. In a second study, therefore, an in-house standard was calibrated by six different Baxter laboratories. Good agreement between 1-stage and chromogenic assays could be achieved when the calibration was based on the average of Mega 1 and WHO 6th IS, but not on Mega 2. Dr. Schreiber concluded that this in-house standard can be used within Baxter worldwide with one single potency for both assay methods, and with reasonably low (approx. 8%) interlaboratory variability.

Second Refacto lab field study – J. Ingerslev

This study has been recently concluded and focused on the use of Refacto Lab Standard (RLS) to facilitate the measurement of B-domain deleted FVIII in post-infusion samples. In this study, which involved 35 labs worldwide, hemophilic plasma was spiked with Refacto at levels of 0.2, 0.6 and 0.9 IU/ml, and these samples were assayed against normal plasma and RLS as a standard. In the chromogenic assay (7 labs) the expected FVIII levels were found irrespective of the standard used. In the 1-stage assay (31 labs) FVIII was underestimated against the plasma standard, and this was totally corrected by using RLS as a reference. Dr. Ingerslev concluded that this approach should eliminate most of the discrepancies reported so far in assaying plasma of
patients treated with this FVIII product.

Focus of future SSC concentrate studies – K. Mertens

The SSC concentrate studies have been running since 1995 and serve to inform participants on the performance of their in-house assay method on a variety of FVIII samples. Seven studies have been performed involving 12 FVIII samples ranging from intermediate and high purity plasma-derived products to recombinant full-length and B-domain deleted FVIII. In these "field studies" inter-laboratory variability was much larger (overall CV's 10-20%) than in controlled calibration studies. A significant number of participants do not follow the SSC guidelines which imply using the chromogenic assay, FVIII deficient plasma as a prediluent and 1% of albumin for all other dilutions. Given the limited extent of improvement over time, the Subcommittee decided at its meeting in Paris to reconsider these activities. A new Steering Committee has been formed, consisting of Drs. U. Oswaldsson, J.D. Schreiber, M. Lee, S. Raut and K. Mertens (chairman). This group proposed to develop a more systematic study design, if possible. The participants' views will be sought as to potential options to expand the program in a way that should allow for the identification of major sources of variability. In the meantime the studies will be resumed and further developments will be discussed at the Subcommittee Meeting in 2003.

General discussion on concentrate issues

In the general discussion Dr. Kotitschke (Biotest, Germany) presented some data on a collaborative study that will start shortly. This study will focus on the use of prediluents in a standardized chromogenic assay. For this purpose various FVIII deficient plasmas will be compared in the assay of a variety of FVIII products. The main question is how much von Willebrand factor the FVIII deficient plasma should contain in order to be a suitable prediluent. Finding an equivalent to hemophilic plasma would facilitate following the SSC guidelines. Dr. Kotitschke mentioned that 4 additional labs can still join this interesting study.

Proposal to replace WHO plasma standard – A. Hubbard

Upon the establishment of the current 4th IS, the Subcommittee has expressed some concern on the low FVIII activity content of this material (0.57 IU/ml). Dr. Hubbard presented extensive stability data showing that this plasma displays a FVIII activity loss of < 0.1% per year. Thus, the 4th IS is fully appropriate to be used, together with fresh normal plasma, in the calibration of the next IS. A candidate 5th IS has already been filled, and preliminary estimates indicate that its FVIII activity is approx. 0.7 IU/ml. Recruitment of study participants has recently started, and Dr. Hubbard expects that results will be available at the Subcommittee Meeting in 2003.

FVIII unitage and in vivo recovery – K. Mertens

This report involved a pharmacokinetic analysis of two plasma-derived products in a bioequivalence study. The study was designed according to the SSC guidelines, including the recommended sampling scheme, and a cross-over design. Post-infusion patient samples were analysed in a rigorously standardized manner in one single lab using both 1-stage and
chromogenic assays, employing plasma and product standards in parallel. FVIII levels expressed in plasma units were consistently higher than in concentrate units, the difference being 12%. Results of chromogenic and 1-stage assays were virtually identical, irrespective of whether plasma or the product was used as a standard. As expected, the unitage had no impact on determination of half-life, but did affect the AUC and Cmax values. For some batches there was a slight discrepancy between the label potency and the re-assayed potency according to the SSC recommended method. These discrepancies, though minor, were reflected by apparently different in vivo recoveries in the cross-over analysis. Although these discrepancies are minor and without any clinical relevance, this study illustrates the impact of label potency assignment for pharmacokinetic studies, particularly if the infused product is to be used as a reference for post-infusion FVIII assays.

**An improved method for assaying FVIII following B domain-deleted FVIII infusion - C. Wiseman & J. Lusher**

A comparison was run using freshly prepared or frozen ReFacto Laboratory Standard (RLS). It was shown that more consistent results were obtained with the frozen preparation. For instance, intra-assay CVs of 7.7% were obtained with the frozen RLS as compared to up to 10.8% with the freshly prepared RLS. An inter-assay CV of 6.5% was obtained with the frozen preparation, while 7.5% was observed with the freshly prepared RLS. In addition, using the frozen RLS (kept at –80°C) saved considerable time. The use of a frozen RLS could reduce the discrepancy between the one-stage coagulation and the chromogenic assay and is now planned to be used to evaluate plasma samples of patients under ReFacto infusion.

**Standardization of FVIII assays in post-infusion plasma – T.W. Barrowcliffe**

A few studies have assessed FVIII assay discrepancies in post-infusion samples. A comparison of results obtained from different centres showed CVs of up to 15% for the one-stage coagulation assay and up to 20% when the chromogenic assay was used. One confounding variable could be the use of either plasma or FVIII concentrate standards, which are shown not to be interchangeable. For instance, in a FXa generation assay, it is shown that FVIII concentrates increase the rate of FX activation as compared to plasma, while the reverse is true in a thrombin generation assay. Several draft recommendations were put forward for consideration. For pharmacokinetic studies, the product may be diluted in FVIII deficient plasma in order to serve as a standard that facilitates like-versus-like analysis. For monitoring treatment in clinical situations, plasma standards may be used for patients treated with plasma-derived products, and possibly also with full-length recombinant products. As also suggested by Drs. Ingerslev and Wiseman, product standards may be particularly useful for B-domain-deleted FVIII; however, final recommendations await further discussion of these options at the 2003 Subcommittee meeting.

**UK NEQAS study of FVIII measurement: a 92 laboratory survey - E. Preston**

The evaluation of FVIII:C varies greatly from one laboratory to the other. A number of factors can play a role in this variation: use of different reference plasma samples, calibration and standard curves, as well as the coagulometer to cite but a few such variables. A survey was
organized in the UK involving 92 laboratories. A standard reference preparation of FVIII was sent to centres which were asked to run the assay as "field evaluation," namely, using their own reagents and methodology. Large variations were observed. CVs of up to 70% were obtained whenever stored reference samples were used, while CVs of 50% were seen with freshly prepared reference curves. A second survey involved the sending of three plasma samples containing different titres of FVIII to 81 centres in the UK. Again, large variations were observed in FVIII with ± 30% of the laboratories giving results out of the normal variation range. In addition, the interpretation of the results show that in many cases laboratories do not comply with the ISTH classification of haemophilia A patients as severe (less than 1% FVIII), mild (1 to 5%) or moderate (more than 5%). This misinterpretation of results adds further confusion to diagnosis and may possibly exert an influence upon treatment. The reasons why laboratories do not follow the recommendations of the ISTH subcommittee were discussed, as well as possible remedies to this situation.

**Need for Bethesda assay standardization** - A. Macartney & G. Savidge

Many variables can affect the titration of inhibitors in plasma samples. Two of such variables are the source of FVIII and the source of diluent for plasma samples. An assessment of Bethesda titre was carried out using different FVIII preparations, either recombinant (B-domain deleted (BDD) FVIII), or plasma-derived products. Significantly higher titres of inhibitors were obtained using the BDD FVIII in particular. Next, the question of the diluent was examined by comparing reagents obtained from different sources, i.e., plasma of severe haemophilia A patients or plasma artificially depleted in FVIII. It was shown that such diluents vary greatly in terms of content of von Willebrand factor (VWF), and remarkably also of residual FVIII antigen. The IL diluent which contained no detectable functional or antigenic VWF, and no FVIII antigen, gave the highest inhibitor titres using the BDD FVIII preparation. Addition of VWF significantly reduced the inhibitor titre in a dose-dependent manner. This suggests that BDD FVIII might be particularly suitable for the evaluation of inhibitor titres and that VWF may play a role in the detection assay for inhibitor. Further studies are required to understand the precise relationships between these parameters, including the specificity of inhibitor antibodies. The presenters recommended that the Subcommittee pursue the standardization of the Bethesda assay.

**Standardization of inhibitor assays.** - S. Kitchen

An NIBSC attempt at standardization of inhibitor assay was carried out by distributing two potential standards, each consisting of a human monoclonal anti-FVIII antibody, and one consisting of a polyclonal rabbit antibody against human FVIII. The two human antibodies were derived from the peripheral memory B cells of two haemophilia A patients with inhibitor. The rabbit antiserum was generated by repeated SC injections of human recombinant FVIII in adjuvant. Three plasma samples from patients with inhibitors were assayed. All three inhibitor samples were sent to 15 centres which were asked to run a standard Bethesda assay. Results demonstrated an intralaboratory variability of between 30 and 50% using each of the two human monoclonal antibody standards, regardless of whether the one-stage or chromogenic assay was used. The rabbit polyclonal antiserum resulted in decreased interlab variability of ± 25%. The reasons for such variations were discussed and probably relate to the use of different reagents, such as dilution buffer. Dr. Kitchen asked for feedback as to whether a larger study should be
launched using the polyclonal rabbit antibody inhibitor standard. If so, he asked for feedback as to the optimal titer for such material. The chair asked Dr. Kitchen to prepare a report containing background information accompanied by his specific questions. The chair will distribute this report to Subcommittee members for feedback in the next few months so that further study can be undertaken prior to the 2003 meeting.

**Factor VIII Measurement: Is a New Paradigm Possible?**

*Co-chairs: J. Ingerslev and J. Oldenburg*

Dr. K. Mann summarised his work on models of coagulation by introducing the audience to the three principal substrates and models adopted in his lab: 1) purified systems, 2) whole blood, and 3) computerised simulation of coagulation. The series of events in coagulation recorded through the quantification of thrombin activation markers (TAT complexes) all occurred before less than 5% of the clotting reaction had taken place. Levels of anticoagulants such as TFPI and AT modified the time-course of thrombin formation considerably. Dose titration, with the addition of exogenous factor VIII to haemophilia A plasma, demonstrated a tendency toward normalisation of thrombin generation with the addition of very small amounts of FVIII. Early data suggested differences in TAT generation profiles with the addition of similar concentrations of different recombinant clotting factor concentrates. Dr. Mann postulated that, since the clotting response was the composite outcome of multiple genetic and environmental factors, modeling could be used to tailor therapy to the needs of an individual hemophilia patient and alter the current practice of uniform global factor replacement strategies.

Dr. J. Ingerslev, on behalf of his colleague B. Sørensen, introduced their dynamic whole blood clotting method which is based on thrombelastography using a novel software system for the handling of continuous data during coagulation. Coagulation is activated by small amounts of TF. In this system severe haemophilia A and B patients display a severely diminished coagulation signal; however, heterogeneity is found among patients with less than 1% FVIII clotting activity, some patients demonstrating minimal clot formation late in the process and others showing a much earlier signal of coagulation of higher velocity. The incremental addition of small amounts of FVIII ranging from 0.5% to 5% of normal plasma concentration dramatically improved the coagulation profile to close to normal. Dr Ingerslev was asked about the longterm stability of clots formed in his assay at low FVIII concentrations. He did not have any data at present, but plans to study this question in the future.

Dr. T. Barrowcliffe reported on the results of a joint study from NIBSC and the Royal Free Hospital. The group studied thrombin generation in FVIII deficient plasma, with and without the addition of varying amounts of FVIII concentrate. In general, this model was also sensitive to very low concentrations of FVIII which were capable of generating large amounts of thrombin. The subsequent addition of higher factor VIII concentrations primarily influenced the time to thrombin generation rather than altering the maximum thrombin generation potential. The concentration of FIXA significantly affected this assay.

Dr. A. Giles (Biomerieux), on behalf of Drs. Shima and Yoshioka, updated the Subcommittee on the use of waveform PTT analysis, including a summary of the published studies on hemophilic plasma using this technology. In principle, waveform analysis consists of extracting the
photometer data from a clot-based PTT and calculating the first and second derivatives of the signal. Using this technique, derived profiles of coagulation in haemophilia could be established. A large phenotypic variation in the second derivative was seen among severe (<1%) haemophilia plasmas. Dose-response relationships were established using F VIII deficient plasma spiked to various theoretical levels. A small study on the use of this method in the study of the hemostatic response in patients with hemophilia A and inhibitors was also presented. Dr. Giles concluded by saying that with the newest coagulation instruments one can obtain more information than with just an APTT or a PT. Although he agreed that whole blood models of coagulation may prove to be more physiologic in the evaluation of hemostasis in hemophilia, both at baseline and after factor replacement, he believes that there is also a role for the waveform PTT technology in the assessment of the clotting response.

In conclusion, the different methods presented were all consistent in demonstrating that 1) severe haemophilia A plasmas display a heterogeneous pattern of clot formation and thrombin generation; 2) clot formation/thrombin generation is significantly improved by very small incremental increases in F VIII, with some clotting profiles returning to normal or near normal with the addition of less than 5% of factor VIII. All methods demonstrate potential as possible alternative strategies to the currently used plasma–based assay methods. A workshop is planned prior to the next subcommittee meeting to coordinate the systematic study of these techniques. More data on their use in the evaluation of hemostasis in hemophilia is expected in 2003.

**Factor VIII Dosing: Developing Future Strategies**

Co-Chairs: C. Lee and D.DiMichele

**Report on the WHO/WFH workshop on the delivery of treatment in hemophilia - C. Lee**

Dr. Lee reported on the proceedings from the joint WHO/WFH sponsored meeting held in London in February, 2002. The goal of this meeting was to identify the barriers to and development of strategies for extending hemophilia therapy to the developing world. Participants included Drs. C. Lee (UK), E. Berntorp (pharmacokinetics)(Sweden) and S. Schulman(continuous infusion)(Sweden), K. Fischer (prophylaxis)(Netherlands), M. Escobar (historical data on dosing)(US) and D. DiMichele (immune tolerance dosing)(US), A. Chuansumrit (Thai strategies in health care delivery)(Thailand), A. Srivastava (global needs and economics)(India), V. Boulyjenkov and J. Emmanuelle (WHO) and Line Robillard (WFH). Much discussion at the meeting focused on defining optimal and minimum effective dosing. The rationale for not necessarily normalizing factor levels included 1) redundancy in the clotting cascade; 2) factor shortages; and 3) national economics and health care budget appropriations. The goal of therapy in the developing world was discussed and suggested by Dr. Srivastava to be functionality, not perfect joint health. Identified strategies for optimizing dosing included 1) individual pharmacokinetic analysis; 2) better assays for assessing hemostasis in vivo; 3) more frequent dosing; and 4) the concomitant use of antifibrinolytics. IT was determined not to be an ethical use of clotting factor resources in the developing world. The identified barriers to optimizing therapy included 1)unavailability of PK variability relative to ethnicity; 2) vial size limitations; and 3) limitations of factor delivery systems. Although no formal recommendations were possible at this time, ongoing meetings on this topic are planned and will be reported regularly to the ISTH as part of a coordinated project with the FVIII/IX Subcommittee.
A dose-finding study in dogs using B-domainless FVIII- M. van den Berg

Although this study will proceed, it was unable to be initiated prior to this meeting. Dr van den Berg plans to present preliminary results at the 2003 Subcommittee meeting.

Dose response in hemophilia: Orthopedic outcome in the developing world- A. Srivastava

Dr. Srivastava presented his proposal for a study to be conducted in the developing world. The study proposes to document orthopedic outcomes using existing factor replacement strategies currently practiced in the developing world. The optimal orthopedic outcome identified by this study was functional independence. The goals of the study are 1) to document orthopedic outcome on the various dosing regimens used to treat children in a representative group of participating countries; and 2) to develop a cost effective model for treatment in the developing world. Eligibility criteria will include 1) severe (<1%) hemophilia; 2) age of 2-10 yrs. (currently still being defined however); 3) no inhibitor; and 4) well documented care. An enrollment of 250-500 subjects is expected with a 5 yr. follow-up period planned. Participating countries currently include 1) India, 2) Malaysia, 3) Singapore, 4) Iran, 5) Brazil, 6) Venezuela, 7) Thailand, and 8) South Africa. Dosing strategies among these countries vary from 50-500 u/kg/yr. Data to be collected include 1) clinical joint scores (to be standardized by distributing the CDC video), 2) radiologic joint scores (Pettersson), and 3) functional independence scores (WeeFIM modification). After some discussion, the subcommittee endorsed Dr Srivastava’s proposal. An update on the progress of this study will be presented in 2003.

Proposed international survey of FVIII replacement in surgery- A. Srivastava

As a follow-up to data presented last year, Dr Srivastava reviewed the published literature on the data supporting the 1995 WHO/WFH guidelines on factor support required for surgical peri- and post operative hemostasis. (Haemophilia, 1995 (suppl 8-13)). He found the data lacking and proposes to conduct an international survey to establish current practices relative to surgical morbidity and mortality, and to ultimately develop a consensus on acceptable outcomes and the dosing strategies needed to achieve these outcomes. The questionnaire will have two components:

Component A: Current standards of practice with respect to a) factor dosing in surgery, b) use of CI vs bolus dosing, c) duration of factor replacement, d) target factor levels for surgery and post-op period, e) frequency of factor level monitoring, and f) variation in practice relative to type of surgery being performed.

Component B: Outcome data using these practices. He received input from the group with respect to monitoring thrombotic complications and inclusion of data on mild/carrier patients. He will modify the data collection tool and resubmit it to the Subcommittee for final comment prior to sending it out. He will seek external funding for data analysis. An update is expected in 2003.

International prophylaxis group- V. Blanchette
After a review of the published literature on prophylaxis, Dr. Blanchette reported on the activities of the newly established International Prophylaxis Group, the goals of which are 1) to acquire further data on the optimal delivery of prophylaxis; 2) to disseminate this information worldwide; and 3) to continue to define standards for cost effective prophylactic factor dosing and outcome measurement. Annual meetings on specific topics are planned. These topics will include 1) development of a unified MRI scoring system, 2) development of a unified clinical scoring system, 3) further development of QOL instruments and studies. A Steering Committee and Expert Advisory Group have been identified. The Hospital for Sick Children (HSC) in Toronto will function as the Study Coordinating Center. The group is funded through a multi-industry grant. Dr Blanchette will continue to report on the group’s activities to both this Subcommittee as well as to the Pediatric Subcommittee of the WFH.

Dr DiMichele concluded the meeting.
Factor XIII

Chairman: A. Ichinose, Japan
Co-chairs: RAS Ariens, UK; P. Bishop, USA; C. S. Greenberg, USA; L. Muszbek, Hungary

The session was opened with apologies of absence from Drs. Ichinose, Bishop, Greenberg and Muszbek. Drs. Ariens (UK) and Seitz (Germany) resided as Acting Chairs. There were approximately 75 people present and the presentations were followed by lively discussions.

1. Dr. Kohler (University of Bern, Switzerland) "Determination of FXIII activity using different methods. Influence of FXIIIA Val34Leu".

   This presentation was opened with a reminder that there is no clear perspective on which type of FXIII activity assay is the best to use for any given situation. Two particular assays were discussed (i) biotin incorporation assay (PefaKit by PentaPharm) and (ii) photometric assay (Berichrom). Genetic polymorphisms of FXIII such as the Val34Leu polymorphism affect the rate of FXIII activation. The Leu allele increases the rate of FXIII activation and this may be detected when using the PefaKit assay, but will only be detected with the Berichrom assay if low levels of thrombin are used to activate FXIII. When FXIII is fully activated there is no longer any discrimination in specific activity measured with genotype variation. The question therefore is how much thrombin is generated in vivo? Previous work by Kohler et al, Thrombosis and Haemostasis, 1995, has shown that low levels of thrombin are likely to be generated (approximately 0.1u/ml) in vivo, and that there is only partial activation of FXIII. Thus, the best assay to achieve high sensitivity that can discriminate for FXIII polymorphisms is a biotin incorporation assay using low levels of thrombin to activate FXIII. To determine antigen levels of FXIII, an ELISA approach may be taken; alternatively it is possible to achieve full activation of FXIII using high levels of thrombin concentration and then assessing FXIII activity using either a photometric or biotin incorporation assay.

2. Dr. Jennings (University of Sheffield, UK) "Performance and variability of FXIII screening".

   Dr. Jennings was representing the UK NEQAS for blood coagulation screening which is currently using approximately 150 laboratories to screen for FXIII assays. Currently only approximately 20-30 laboratories use more direct FXIII assays but the majority of labs (approximately 120-130) use the clot solubility test to assess FXIII levels. In theory the clot solubility test is meant to be sensitive to approximately 1% of FXIII in plasma. The laboratories involved were given 3 plasma samples (i) normal plasma (ii) FXIII deficient plasma and (iii) FXIII deficient plasma at trough level from a patient that was about to receive prophylactic treatment. Each of the laboratories reported the level of FXIII measured and how they interpreted their result. There was a great variation of levels reported, particularly in the sample from the FXIII deficient patient at trough level (sample iii) where 70% of the laboratories classified this patient in the "normal" category. It was found that labs using a calcium-based clot solubility assay misclassified the result as normal whereas those using thrombin in their assay detected low levels. Other parameters used in the clot solubility test that affect measurement of FXIII were the lysing reagents urea or acetic acid. In May 2002 a further screening exercise was performed. The samples were (i) FXIII deficient patient (prior to treatment at trough level) plasma taken into citrate and (ii) a pool of normal donor plasma taken into both EDTA and citrate. Again there was great variation in levels reported from the laboratories. It has been found
that many variables influence the reported FXIII level; these include calcium, thrombin preparation, urea, volume of plasma/reagents, incubation time, the source of the method and normal range. Different FXIII assays also report variations for a given sample especially the Berichrom assay which reported a range of between 0-55U/dl of FXIII for a given FXIII deficient sample. There is clearly a need for a good standardization method for FXIII measurement as there is too much variation with the current methods employed. The conclusions drawn from this presentation were (i) thrombin-based clotting assays are more sensitive than calcium-based clotting assays but care must be taken to ensure that thrombin preparations do not contain calcium, (ii) there is a lack of accuracy and precision evident in FXIII assays, and (iii) the clinical relevance of ‘mild’ FXIII deficiency is unresolved.

3. Dr. Ariens (University of Leeds, UK) "Genetics of fibrin structure/function".

Dr. Ariens opened with an introduction to FXIII and fibrinogen and showed the vast number of polymorphisms present in both FXIII and fibrinogen. Two polymorphisms of FXIII, Val34Leu in the A-subunit and His95Arg in the B-subunit, alongside two polymorphisms of fibrinogen, AaThr312Ala on the alphaC domain of the alpha-chain and BbArg448Lys on the beta-chain of fibrinogen, were expanded upon in greater detail. These polymorphisms to a certain degree affected fibrin structure (with the exception of His95Arg which was not examined). Fibrinogen level also plays a role in the outcome of fibrinogen structure, so there is an environmental effect also acting on the genotype effect. Fibrin structure was also shown to be affected (compared to controls) in plasma from first-degree relatives of patients with 2- or 3-vessel coronary artery disease. The heritability of fibrin structure was examined and showed 39% heritability of permeation(ks) in a twin study. This is lower than that observed from the heritability of fibrinogen levels (approximately 50%) and other coagulation zymogens (61-75%). This has been attributed to environmental factors which have been shown to affect fibrin structure (e.g. dimethylbiguanide used for treatment of diabetes, calcium ions and ionic strength and thrombin). The conclusions of the presentation were that genetic polymorphisms affect cross-linking and fibrin structure and that environmental effects also play a role.

4. Dr. Barrowcliffe (NIBSC, UK) "Pilot study for standardization; presentation and discussion".

Dr. Barrowcliffe introduced the process for preparing standards
(1) Preliminary investigation of materials
(2) Trial fills
(3) Stability studies and assays on trial fills
(4) Large-scale fills
(5) International collaborative study
(6) Report to WHO

Phases 1-3 have now been completed and the organization of phase 4 is beginning. The types of materials assayed for FXIII standards are plasma, concentrates, fibrin sealants (fibrinogen components) and recombinant FXIII is also being considered. In the SSC meeting last year Dr. Barrowcliffe reminded us of the stability of two FXIII concentrates which were ampouled. Preparation B was shown to have a stability of 0.05% per year at -20°C, which was better than preparation A; therefore, further work will continue with preparation B. Proposals for the materials to be used include: plasma (NIBSC and normal pool), concentrate (one or two
preparations), fibrinogen concentrate and recombinant FXIII (although this may need further consideration). Participants will be manufacturers of preparations, national control labs and clinical/academic labs. The methods to be employed will be chromogenic and antigen assessment. A preliminary collaborative study has begun with four laboratories with the aims of comparing dilution of samples in FXIII deficient plasma with buffer, comparing the Dade and Pentapharm assays, and to look at studies using fibrinogen concentrates. The samples include two FXIII concentrates, a fibrinogen concentrate and plasma. This preliminary study will be scaled up to include 10-20 labs. Persons wishing to collaborate with this study should please contact Dr. Barrowcliffe.

5. Dr Seitz (Paul-Ehrlich-Institut, Germany) "Preliminary Results of PEI Pilot Collaborative Study"

Dr Seitz presented preliminary results as a participating laboratory, following on from Dr. Barrowcliffe’s introduction to the preliminary collaborative study that has started with four laboratories. The aims were to compare FXIII concentrates versus plasma that are diluted in either FXIII-deficient plasma or buffer. The samples were plasma (1U/ml of FXIII content), FXIII concentrate (30U/ml FXIII content) and FXIII concentrate (50U/ml FXIII content). Preliminary results show that pre-dilution of FXIII sample in FXIII deficient plasma gives a better result than dilution in buffer containing 1% BSA. Low levels of FXIII have been found in the fibrinogen concentrates and there is a need for a comparison of methods.

6. Dr. Seitz (Paul-Ehrlich-Institut, Germany) "Preliminary Results: Novel FXIII Assay Based on Cross-linking of Peptides"

Dr. Seitz gave an introduction to the development of a novel assay to measure FXIIIa cross-linking ability. The concept relies on the use of two peptides that are cross-linked to each other by FXIIIa. One of the peptides contains a His-tag that may be bound to Ni-NTA, and the second peptide is conjugated with FITC to allow detection. Preliminary data show that this assay is feasible but requires further development. Dr. Seitz was interested in looking for a collaborative study with a manufacturer to aid in the development of this novel assay system.
Fibrinogen

Chairman: S. Lord, USA
Co-chairs: J. Koopman, The Netherlands; R. McIntosh, UK; N. Weinstock, Germany

Dr. Nicodemo Weinstock presented the summary of the evaluation procedure for a high fibrinogen standard. The project was started at the XVIIth ISTH Congress in Washington (1999), where there was considerable support for the type of standard presented. The main problem is that fibrinogen is a risk factor for CHD, but studies show large discrepancies in fibrinogen levels measured.

The results of a large European study were presented and discussed at the SSC Annual Meeting in Maastricht (2000), where those attending the Subcommittee meeting voted for establishment of a high fibrinogen standard.

The complete approved report of the international study (19 laboratories in 10 countries) was presented at the XVIIIth ISTH Congress in Paris (2001), where the participants voted for the introduction of the presented standard. In addition, the results were evaluated by scientists and clinicians not involved in the study, reaching general agreement. Stability of the plasma preparation was extensively evaluated. Comparison with 1st International fibrinogen standard (WHO) showed acceptable agreement. The audience agreed to present the data to the SSC Business Meeting and to recommend it as the first International High Fibrinogen Reference Plasma with a potency of 5 g/l.

Dr. Colin Longstaff reported on a collaborative study of thrombin standards in fibrin sealants. Two thrombin preparations were prepared, 10000 Ampoules each, approximately 100 units per ampoule (similar to current 1st international standard).

The goal was to replace the currently used standards that are running low and to re-unite IU and NIH-U to a single unit. Preliminary results from 25 participating laboratories were presented.

Each lab received four ampoules of A (current international standard), two ampoules of B (US standard), and four ampoules each of the two new preparations, C and D.

Overall variability was 8.8 % in clotting and 6.5 % in chromogenic assays. There were no differences between human and bovine fibrinogen as substrate concerning activity and the value for plasma as substrate lower than with fibrinogen was not significant. Chromogenic assays displayed a trend towards higher levels of thrombin activity. Candidate C seemed to be quite similar to B, whereas D was close to A concerning amount of thrombin activity in clotting and chromogenic assays.

Sample D was selected as best candidate for determination of final potency. Geometric mean is 110 units per ampoule using A and B as standards and human and bovine fibrinogen as well as plasma.

There are no stability data yet, but since there are only very small quantities of preparations A
and B left, replacement is urgent. Dr. Barrowcliffe commented that differences between IU and NIH U are up to 15% in earlier studies, therefore, a common standard is a great achievement.

**Carl-Erik Dempfle** proposed a collaborative study on measurement of fibrinogen in animal plasma. Results of different studies on fibrinogen in various animal models showed a high degree of fibrinogen levels reported. A specific problem is imposed by the fact that current fibrinogen assays are optimized for use with human plasma, not with animal fibrinogen, and human plasma/fibrinogen is used as calibrator. Reactivity with enzyme (bovine thrombin,...) may be different from human fibrinogen, and the fibrin polymerization rate may be different from human fibrin. Presence of fibrin(ogen) degradation products influences clotting rate (underestimation of fibrinogen concentration). In optical methods, turbidity of fibrin clots may be different from the human system. Clot turbidity is also influenced by presence of fibrin(ogen) degradation products (overestimation in PT-derived fibrinogen). For PT-derived fibrinogen: Differences in PT influence rate of thrombin formation, and fibrin structure (overestimation of fibrinogen concentration in prolonged PT / high INR). It is suggested to study mouse, rat, rabbit and pig, and dog fibrinogen.

Study proposal consists of Phase 1: collection of plasma samples using defined protocol for blood collection, plasma preparation and storage. Fibrinogen concentration is measured by clot recovery method. Assays are screened. In Phase 2, aliquots of 3 plasma samples per species are given to participating laboratories for analysis. Phase 3 includes normal range studies for various animal species and subspecies, using the pooled plasma material of same species for calibration. Audience and readers are asked for support with collection of animal plasma samples, and analysis of samples. For help and support: E-mail: carl-erik.dempfle@med.ma.uni-heidelberg.de

**Kunihiko Nakahara** from Iatron Inc. presented data on a new soluble fibrin assay. The assay is based on MAb IF-43. IF-43 does not react with fibrinogen, but reacts with FM and fragments E of fibrin in immunoblot. In native material, the epitope alpha 52-78 is exposed only when FM binds to fibrinogen (or fragment D), resulting in a trimolecular complex with an apparent MW of 1 000 kD, equaling trimer of 2 x fibrinogen and 1 x FM. Using IF-43, a latex enhanced photometric immunoassay (LPIA) was developed. The assay uses serial dilutions of desAABB-FM in normal plasma for calibration. Human plasma may be replaced by bovine plasma for preparation of the calibrators. There was no correlation with D-dimer assay (Iatron) in clinical plasma samples. The time course in clinical samples (before/after surgery) differs both from TAT and D-dimer, with highest levels observed on days 2 and 3 after surgery. Elevated levels of soluble fibrin were found in patients with disseminated intravascular coagulation.

**Gordon Lowe** gave an update on fibrinogen and cardiovascular disease risk from the Fibrinogen Studies Collaboration (FSC), a collaborative meta-analysis of prospective studies of fibrinogen and risk of CHD and stroke. Since the collaboration started in 1999, individual data has been collected from 38 cohorts (total subjects included 137,000). After an additional 20% of data, 10,000 coronary events and 2000 stroke events will be included.

At the next meeting of the ESC in 9/2002 in Berlin, preliminary analyses of CVD endpoints will be presented.
A sub-analysis on type of fibrinogen assay used and standardization issues will be discussed. Input from the Fibrinogen Subcommittee was invited. Discussions included recalibration with the high fibrinogen standard.

Next steps of Fibrinogen Subcommittee:

- Gordon Lowe: fibrinogen as a risk factor; standardization and re-evaluation of epidemiological studies.
- Nicodemo Weinstock: Establishment of generally accepted reference values and risk percentiles by re-measuring available samples of risk factor studies at a central laboratory using the newly established high fibrinogen standard. Correlation of high fibrinogen values from different fibrinogen assays.
- Carl-Erik Dempfle: Initiation of study on standardization of fibrinogen measurement in animal models, in collaboration with Susan Lord.
- Colin Longstaff: Stability studies on thrombin standards.

N. Weinstock
C.E. Dempfle
G.D.O. Lowe
Fibrinolysis

Chair: P. Declerck, Belgium
Co-chairs: N. Booth, UK; C. Dempfle, Germany; Dirk Hendriks, Belgium; O. Matsuo, Japan; M. Nesheim, Canada

D-dimer

_Dr. Dempfle_ commented on the issue of novel assay technologies for measurement of D-dimer antigen, including latex-enhanced photometric immunoassays (LPIA) and quantitative point-of-care (POC) assays. Rapid quantitative D-dimer assay results are needed in clinical practice for exclusion of venous thromboembolic disease, diagnosis and monitoring of DIC, and diagnosis or exclusion of heparin-induced thrombocytopenia type 2. This cannot be achieved with microtiter plate ELISA systems, nor with qualitative D-dimer assays. Clinical studies have shown equivalence of LPIA to ELISA and a high analytical standard of the novel whole blood POC assay technology. The majority of assays currently available are LPIA. LPIA display variable reactivity with different size crosslinked fibrin derivatives, as well as variable influence of fibrinogen degradation products on assay results. In whole blood assays, there may be additional influence of RBC content, hemolysis, and platelets. Apart from the analytical optimization of assays, common standardization is required, especially when integrating POC assays into the diagnostic array of a hospital. For preparation of a D-dimer standard, reproducible procedures for preparation and standardization are needed. The standard should contain a 'physiological' variety of fibrin derivatives rather than simply purified fibrin fragment D-dimer. Specific antigen reactivity profiles of the assays should be taken into consideration when interpreting results. Due to differences in monoclonal antibody reactivity and influences of assay technology on assay response, clinical studies are needed for validation of each individual assay and assay-instrument combination.

_Dr. Walker_ discussed the heterogeneity of non-covalently associated products derived from the double-stranded fibrin protofibrils subsequent to plasmin-mediated fibrin degradation. Using a perfused clot system employing purified components, he characterized these FDPs with respect to their weight-averaged molar mass, chain composition, mass distribution and tPA / DSPAal cofactor activity. A comparison of two different paired monoclonal-based D-dimer assays showed marked differences in sensitivity toward D-dimer and the heterogeneous FDPs, underscoring the need for defined D-dimer standards. The methods employed for FDP production and analysis may prove useful in defining heterogeneous FDPs as standards for D-dimer assays.

_Dr. Meijer_ presented the results of the external quality assessment programmes of INSTAND and ECAT Foundation, the so-called D-Dimer Comparison Trial, performed in 2001. The aim of
this trial was to investigate the performance of quantitative D-Dimer assays in daily laboratory practice. A set of 7 lyophilised plasma samples was distributed to about 500 laboratories. Samples were prepared by the addition of different amounts of a patient pool with a high D-Dimer level to normal pooled plasma. The results of 423 laboratories were included in the final evaluation.

Besides differences in the absolute D-Dimer concentration measured by the different methods in the plasma samples, differences in the analytical performance (within- and between-laboratory variation) were also observed. This is the first study which shows the daily performance of D-Dimer assays in clinical laboratories on a large scale. The results were used to set-up a harmonisation model based on linear regression between the mean value of the different samples and the amount of patient plasma added to the sample. This was performed for each method individually. Linear regression is possible due to the number of samples used in this trial. A consensus line was constructed by correlation of the mean value of all included methods for the different samples to the amount of patient plasma added to the sample. The difference between a method related regression line and the consensus regression line has been estimated. This was used to recalculate the values for each method of the different plasma samples. With this procedure the between-laboratory variation for the methods was reduced from 70 – 80% to about 20%. The model presented seems to be a feasible method to harmonise the different D-Dimer methods currently used in the clinical laboratories.

**Dr. Dempfle** proposed the generation of an inventory of D-dimer assay reactivity in animal models. D-dimer assays are needed for animal models of thrombotic disease, as well as intravascular coagulation and related conditions. Studies observing the lysis of clots prepared from human plasma in animal models require D-dimer assays reactive with human D-dimer antigen, but without cross-reactivity with animal fibrin and fibrinogen.

No valid information is currently available on the reactivity of available D-dimer assays with animal fibrin. The proposal involves the preparation of individual pools from mouse, rat, rabbit, pig, and dog plasma and the preparation of standardized crosslinked fibrin preparations similar to those used in the FACT trial. These fibrin preparations will be diluted in plasma from the same species. In addition, similar fibrin preparations from human plasma will be diluted in the same animal plasmas for comparison of reactivity profiles with the D-dimer assays. Comparison of different sub-species and strains, using the D-dimer assays reactive with the specific species samples, will be performed at a later stage of the trial. Audience and readers are encouraged to contribute animal plasma for preparation of the samples.

**Standardization and Methodology**

**Dr. Longstaff** discussed the significance of methodology in the establishment and use of WHO
international standards based upon the experiences with tPA and molecular variants thereof. International Standards (IS) prepared by NIBSC and established by WHO have traditionally been calibrated in International Units (IU), determined in collaborative studies using a range of methods. Thus IU may be arbitrary and many have obscure origins. An alternative approach argues that primary reference materials should be calibrated as far as possible in SI units and using only clearly defined Reference Methods. The current status of Fibrinolysis IS was discussed, including some history of the origin of the IU for different IS. The theoretical and practical merits and problems of the different approaches to calibrating primary reference materials were also discussed. It was concluded that, obviously, appropriate standards are useful but need to be considered with sufficient flexibility. In view of the increasing number of recombinant, molecular, variants of particular factors (e.g. thrombolytic agents) it should be evaluated how useful the currently available standards are for these other variants.

**Procarboxypeptidase U / TAFI**

*Dr. Nesheim* discussed the development and characteristics of a functional assay for activated TAFI (plasma carboxypeptidase B, CPU). It is based on the TAFI-mediated reduction of the bat saliva plasminogen activator (bPA) cofactor activity of high molecular weight purified fibrin degradation products (FDPs). The plasma sample with TAFIa in it is incubated in a microtitre plate with the FDPs for three hours in the presence of hirudin and aprotinin. A recombinant variant of plasminogen (S741C), labelled covalently at C741 with fluorescein is then added along with bPA. The conversion of the fluorescent plasminogen to inactive plasin is accompanied by a 50 percent decrease in fluorescence intensity. The time course of cleavage of the fluorescent plasminogen is determined in a fluorescence plate reader. Initial rates are calibrated with TAFIa at known concentrations in TAFI deficient plasma. The assay is sensitive to TAFIa over the concentration range of 20 to 200 picomolar. No interference from the constitutive plasma carboxypeptidase, CPN, is evident in the assay. The assay has been developed for the analysis of research and clinical specimens in order to obtain a better understanding of the biochemistry, physiology and pathophysiology of the TAFI pathway.

*Dr. Declerck* gave an overview on the problems associated with the measurement of proCPU/TAFI activity and antigen. A procedure for the start of a collaborative study (coordination by Dr. Hendriks and Dr. Declerck) was proposed and discussed. In an initial phase 150 samples will be analysed by two or three different laboratories using all available methods. Also pooled (and depleted and supplemented) plasma will be included. Particular attention will be focused on the differential recognition of various polymorphic forms in the different assays. After presentation of the data at the 2003 meeting, a larger study, involving a smaller number of samples (but more defined) and a larger number of participating laboratories will be started.

*Dr. Booth* presented her group's recent work on the effect of physiological inhibitors of
fibrinolysis on lysis of thrombi. This included the use of model thrombi to assess the contributions of leukocytes and analysis of activity in situ. The effect of TAFIa has been assessed in these systems and the inhibition of lysis (induced by tPA, uPA or scuPA) by TAFI, present in the plasma in which model thrombi were bathed, was highlighted.

**Topics for 2003**

Upon request of the SSC Working Group on Coagulation Standards, the Fibrinolysis Subcommittee agreed upon evaluation of the "SSC-ISTH Secondary Coagulation Standard, lot 2" for t-PA and PAI-1 antigen and activity levels. Dr. Kluft raised the problem of large variations between the different methods. Dr. Declerck and Dr. Longstaf will work out a procedure (including previously evaluated NIBSC preparations and involving various laboratories). The data should be presented at the 2003 meeting. The meeting also agreed to include a topic on assays for measurement of global fibrinolytic activity in blood in various disease states. It was also suggested to consider discussions on assays for fibrinolytic therapeutics exerting their effect through a direct (i.e. non-plasmin mediated) degradation of fibrin.

Progress on proCPU/TAFI measurements and D-dimer standardization should be included.

The meeting was attended by 35-45 people including all but one co-chair. The meeting was adjourned at 12.10 pm.
Haemostasis and Malignancy

Chairman: A. K. Kakkar, UK
Co-chairs: A. Falanga, Italy; M. Prins, The Netherlands; L. Zacharski, USA

The subcommittee convened at 2 pm

Part I

Work in Progress
Trials of prophylaxis in cancer patients undergoing surgery

1. M. Rasmussen presented the Danish surgical Prolonged Thromboprophylaxis study. Ongoing
2. G. Agnelli presented the Pegassus study on in-hospital prophylaxis with Fondaparinux in surgical cancer patients. Ongoing
3. A. Kakkar presented the PRODIGY study of prophylaxis in post-surgical glioma patients receiving fragmin, on behalf of M. Levine. To start

Trials of prophylaxis in cancer patients receiving medical oncological therapy

1. S. Haas presented the TOPIC I and II studies on behalf of the investigators. These protocols are evaluating Certoparin against placebo in the prevention of VTE in breast or lung cancer patients receiving chemotherapy. Ongoing

Prophylaxis of central catheter related thrombosis. Ongoing

1. A. Young gave an update on the WARP study of warfarin prophylaxis. Ongoing
2. A. Kakkar gave an update on behalf of the investigators of the Pharmacia study evaluating Dalteparin for prevention of catheter related thrombosis. Complete
3. G. Agnelli presented the Ethics study evaluating Enoxaparin for prevention of line thrombosis. Ongoing
4. A. Falanga presented a registry of catheter related thrombosis, CATHEM, in hematological malignancy in Italy. Ongoing
5. G. Agnelli presented the ARITOS registry of surgical cancer prophylaxis.

Treatment of VTE in cancer

1. A. Lee updated the CLOT trial of prolonged Dalteparin for treatment of acute DVT. Complete
2. S. Deitcher updated the ONCENOX trial of Enoxaparin for treatment of acute DVT. Discontinued

Antithrombotics and Survival
1. A. Kakkar presented the FAMOUS study evaluating Dalteparin for the prolongation of survival in patients with advanced solid tumor malignancy. Complete
2. H. Buller presented the MALT study evaluating Nadroparin for prolongation of survival in advanced cancer. Ongoing

**A. Kakkar presented a summary of the 1st registry of clinical trials of anti-thrombotic therapy in cancer patients. Its style and content were approved.**

Part II.

**Basic Science**

Prof. J. Fareed presented an update on ongoing studies of assessment of hemostatic markers in cancer patients receiving novel anti-angiogenic therapeutic agents; *in vitro* studies on endothelial cell function; and markers in cancer patients receiving low molecular weight heparin therapy.

Prof. A. Falanga presented a summary of principal presentations from the First Bergamo Cancer and Thrombosis Issues Conference held last November focusing on areas of potential translation research.

Part III.

**Guidelines**

M. Prins presented an outline of the process the subcommittee might adopt in preparation of any future guidelines. A discussion followed during which it was decided the subcommittee should concentrate future activities on promoting/facilitating clinical trials in cancer on thrombosis, continue to raise awareness of the special problems in these area, assist in trial design issues, and promote dissemination of educational activities.

The meeting adjourned at 4:30 pm
Lupus Anticoagulants/Phospholipid-Dependent-Antibodies

Chairman: J. Arnout, Belgium
Co-chairs: M. Galli, Italy; Ph. G. De Groot, The Netherlands; S. Machin, UK; R. Roubey, USA; P. Sie, France

Number of attendees: 125-175

I. Diagnosis of APS: classification and nomenclature?

Drs. Arnout, Machin, Roubey, Harris, de Groot and Pengo reviewed how APS is currently diagnosed in their institutions and countries. Most of the investigators test for antiphospholipid antibodies in patients with clinical features suggestive of APS. In most institutions, the diagnosis is made based on the so-called Sapporo criteria (Wilson et al. Arthritis & Rheumatism, 42; 1999: 1309-1311). From these presentations some general conclusions could be made: A large variety of assays is on the market and the interlaboratory agreement is relatively poor. We need to understand better the reasons for the disagreement between assays. There is a lack of good guidelines both for the clinical aspects and the laboratory aspects of the syndrome. There is also a lack of good reference materials.

Dr. Pengo sent a questionnaire to 54 centers in Italy regarding the diagnosis of APS and obtained responses of 23. Twelve (12) centers only use the LA and the aCL test for the diagnosis of antiphospholipid antibodies. Eleven (11) centers use in addition the direct anti-b2GP1 immunoassay. Dr. Pengo himself has a longstanding experience with a homemade anti-b2GP1 immunoassay. He reported on the results that he obtained in more than 600 consecutive patients with antiphospholipid antibodies. According to his experience, patients testing positive for LA, aCL and anti-b2GP1 have the highest risk for thrombosis. In his proposal for a new classification of the laboratory criteria for APS, he considers this as the most important group (Type I). He also proposed to do a retrospective multicenter analysis of data to give more power to this observation.

Dr. de Groot critically reviewed the so-called Sapporo criteria. Several meta-analysis studies have now shown that a positive LA test is much more strongly associated with thrombosis than a positive aCL. In addition, a recent meta-analysis reported by Dr. Galli also showed that a positive anti-b2GP1 is more strongly associated with thrombosis than a positive aCL. The Sapporo criteria include a positive b2GP1-dependent aCL as a criterion for APS; however, it is unclear how to prove the b2GP1-dependency. Therefore it would be better to replace the aCL test by a direct anti-b2GP1 test. The SSC should make efforts via multicenter studies to obtain sufficient data to validate the following proposed classification.

- Type I anti-b2GP1 and LA positive
- Type II only LA positive
- Type III only anti-b2GP1 positive
• Type IV       all the rest (anti-PE, anti-prothrombin etc.)

Measured on two occasions, at least 6 weeks apart.
Well defined normal range; IgG, IgM and IgA included

II. Antiphospholipid antibodies: standardization issues

Dr. Ian Jennings reported on a recent UK NEQAS proficiency testing exercises in which the performance of potential LA reference materials was evaluated. Five (5) plasmas were sent to 231 centres for LA screening. Three samples comprised normal pooled plasma to which monoclonal antibodies against b2glycoprotein1 (anti-b2GP1), prothrombin (anti-II), and a mixture of both had been added. One further sample was from a patient with a previously identified strong LA and another from a pool of normal plasma. Although the majority of the centers gave a correct interpretation for all samples, variation between methods and reagents was observed. At identical antibody concentrations, strength of response was greater with anti-II than with anti-b2GP1 and responsiveness of APTT reagents showed greatest agreement between patient and anti-II samples. A high proportion of negative interpretations, however, (4/8 users) were reported with one DRVVT kit with the anti-II sample, and this was associated with incomplete correction with the concentrated phospholipid reagent. Further study of phospholipid content in DRVVT reagents indicated significant correlation (r>0.85, P<0.03) between normalized test/confirm ratios and the phospholipid concentration in confirm reagents for 2 out of 3 spiked samples and the LA positive sample, with higher ratios obtained using reagents with higher phospholipid concentrations. Monoclonal antibodies will be a useful tool in improving standardization between LA screening methods; it is important, however, to recognize that these artificial plasmas may not always behave in the same way as plasma from all patients with LA.

Dr. Philip de Groot reported on a new method to differentiate prothrombin from b2glycoprotein1-dependent lupus anticoagulants. Monoclonal antibodies, affinity purified patient antibodies, and selected patient samples were used to show that in an aPTT based clotting assay (PTT-LA, Diagnostica Stago), the use of cardiolipin-vesicles in the neutralization procedure discriminates between b2GP1- or prothrombin-dependent LA activities. Addition of cardiolipin-vesicles shortened the prolonged clotting time caused by anti-b2-glycoprotein I-antibodies with LA activity, whereas this procedure further prolonged clotting times caused by anti-prothrombin-antibodies with LA activity. In contrast, addition of PS/PC-vesicles, corrected prolonged clotting times caused by both anti-b2GP1 and anti-prothrombin antibodies with LA activity. The effects of CL on b2GP1-induced LA activity were specific for contact activation mediated clotting assays. Possible explanations for these findings are the relatively high affinity of b2-GP1 for cardiolipin, as determined by surface plasmon resonance analysis, and inhibition by anti-b2GP1 antibodies of the CL-induced prolongation of the PTT-LA.

Dr. Jef Arnout reviewed the current practices of reporting the LA test results. Current ways of
expressing results include the Rosner index, the delta value, a percent value, the Rosove index and several forms of ratios. The relatively complex criteria for LA diagnosis cannot, however, be translated into one simple figure. These so-called quantitative measures lack inter-laboratory and inter-method standardization and can at most be useful to help differentiating LA from other clotting defects, thereby increasing the probability of a positive LA test. No precise recommendations on these are available in the current guidelines. Dr. Arnout proposed that the SSC would recommend qualitative estimations of the results using a probability scale rather than a potency scale. He also proposed a large multicenter study involving the SSC, ECAT, UK-NEQAS and CAPS to validate the possibility of using calibrator plasmas consisting of normal plasma spiked with LA-positive Mabs at different potency levels in order to quantify the LA potency in clinical samples.

Drs. Nigel Harris and Silvia Pierangeli reviewed the status of standardization of the anticardiolipin assay. Many efforts to standardize the aCL ELISA have been performed. Despite this, the interlaboratory agreement is still poor, mainly due to laboratories using procedures not conforming to proposed guidelines. The European forum on antiphospholipid antibodies has recently published guidelines for the aCL test in *Thrombosis and Haemostasis*. Although homemade bench methods can provide reliable results if the test is performed according to accepted guidelines, Dr. Pierangeli made a plead for the use of well-validated commercial kits. In selecting an aCL ELISA kit, she suggested selection of a kit that utilizes a calibration curve and reports antibody levels in GPL, MPL or APL units. Utilization of a separate positive control having a defined value and error range are also helpful. The use of kits in which there is no calibration curve should be discouraged since other means of determining antibody levels, such as that based on the ratio of the O.D. of an unknown sample to the level, may not be reliable. Dr. Pierangeli also made a plead for the use of assays that are more specific for the syndrome than the original aCL assay. One of the major drawbacks of the original aCL ELISA test has been false positive results. Binding of sera from patients with a variety of diseases, other than APS is frequent to cardiolipin coated plates. Recently, new assays that either utilize phosphatidylserine, a mixture of negatively charged phospholipids (APhL® ELISA Kit) or β2glycoprotein1 have been proposed for more specific measurements of antibodies in APS.

Dr. Peter Schur reviewed the methodological aspects of the antiprothrombin immunoassay. From his review of the literature it is clear that the area is fraught with lack of standardization in that different investigator groups used different ELISA plates, different prothrombin preparations and concentrations, different buffers, different blockers, etc. In any case most authors agree that these antibodies are found in variable frequencies in patients with SLE and APS, but more frequently in those with both. The associations were strongest with thromboembolic events rather than miscarriages, however, Dr. Schur presented his own experience using two different buffer systems, with and without calcium. The frequency of positivity in his experience is much less than reported by others; and calcium indeed does seem to make a difference with some
specimens.

**Dr. Ian Mackie** reported that a manuscript is being drafted on the multicenter study to standardize the aPT ELISA. This manuscript will be forwarded via e-mail to the participating laboratories and will then be submitted to the *Journal of Thrombosis and Haemostasis*.

**Dr. Roubey** presented a new initiative to register patients with APS: the Antiphospholipid Syndrome Collaborative Registry (APSCORE). The goal of this NIH funded registry is to enroll 1500 patients with APS and 500 individuals with antiphospholipid antibodies but without clinical manifestations of the syndrome. APSCORE will utilize a web-based data entry/data management system. Serum, plasma, and genomic DNA will be stored at UNC. Enrollment started in April of 2002 and yielded approximately 50 patients so far. Investigators wishing to obtain additional information can take contact with Robert A. S. Roubey, M.D. Principal Investigator, APSCORE via the following E-mail address: apscore@med.unc.edu

**III. Concluding remarks.**

From several questions raised from the participants it became clear that people expect some more precise guidelines on how to diagnose APS and which assays are needed. There is also need for an update of the SSC guidelines for LA testing. A position paper will be drafted and circulated to the active members of the Subcommittee via e-mail.

A number of active members also agreed to exchange samples and patient data in order to obtain sufficient data needed to propose a new classification of APS.

Those who want to join this effort can take contact with Dr. de Groot Ph.G.deGroot@lab.azu.nl, Dr. Pengo pengo@ux1.unipd.it, or the chairman, Dr. Arnout jef.arnout@med.kuleuven.ac.be.
The chair and co-chairs and approximately 55 Subcommittee members were present. Issues discussed were as follows:

1. Platelets and Thrombin generation:

   Thrombocytopenia: J. Bussel summarized again the available data on intracranial hemorrhage (ICH) occurring in acquired childhood ITP in the US and other countries. Causative factors, predictors and outcome were discussed as a basis for an understanding of the disease. No controlled data are available to date on whether treatment can prevent ICH from occurring during childhood ITP. Thus, multicenter controlled therapeutic trials are recommended (study end-point: ICH).

   V. Bardet et al. reported on inherited thrombocytopenias with respect to new diagnostic techniques (flow cytometry, genetic analysis, ultrastructural studies) and therapeutic approaches. A consensus was discussed.

   Thrombin generation in neonatal plasma: W. Muntean et al. reported on the thrombin generation capacity in neonatal plasma compared with adult plasma. The authors focused on the role of antithrombin and tissue factor pathway inhibitor (TFPI). They clearly demonstrated that, under physiological conditions (low plasma dilution and low amounts of TF), cord plasma clots earlier than adult plasma, and FXa- and FIIa-generation starts earlier, due mainly to low levels of TFPI and AT. The authors concluded that, despite low levels of procoagulatory factors, sufficient hemostasis is achieved in neonatal plasma.

2. Venous thrombosis in children:

   Testing of prothrombotic risk factors and pediatric controlled studies: Based on the presentations given (P. Mathwey, M. Peters & F. Rosendaal, E. Grabowski & M. Manco-Johnson) and the literature available, important questions concerning pediatric population-based studies were discussed. One of the main questions was whether every child with symptomatic venous thrombosis should be tested for prothrombotic risk factors or whether a distinction should be made between low/intermediate- and high-risk patients. It was furthermore discussed whether meta-analysis of literature data on the incidence of genetic risk factors will help to classify children at risk, and whether meta-analyses have (dis)advantages compared with controlled multicenter studies in children and vice versa. Another question was whether pediatric studies should always be controlled by using age-matched healthy children. The last issue raised at this
session was whether the testing of asymptomatic family members is indicated, and if so in what cases.

3. Coagulation factor concentrates:

Protein C-deficiency: C. Escuriola et al. reported on indications, dosages, and laboratory monitoring during the use of human protein C concentrate in children with congenital and acquired protein C deficiency. Data obtained from case series suggest that human protein C is effective in treating congenital protein C deficiency. Six out of eight children with acquired protein C deficiency and with severe disturbance of the microcirculation due to meningococcal septicemia survived after the administration of human protein C concentrate. The authors conclude that controlled studies should be carried out in children to obtain evidence-based data on the cost/benefit ratio of human protein C concentrate. Furthermore, comparative studies between human protein C concentrate and activated protein C concentrate are recommended with respect to clinical benefits as well as to potential side effects.

Recombinant factor VIIa (rFVIIa): E. Grabowski discussed the in vitro hypothesis of inactivated r-FVIIa being used in preclinical studies to interrupt the TF pathway of coagulation. This hypothesis is based on experimental data showing the expression of enzymatically active TF-Factor VIIa complex by HGECs exposed to various combinations of TNF-alpha and Shiga toxin-1.

Von Willebrand disease: B. Zieger reported on indications, dosages, and duration of application of von Willebrand factor concentrate or Desmopressin in children with different types of von Willebrand disease. Unclarified issues, e.g., for what ages Desmopressin is acceptable, adequate postoperative management in view of the possibility of volume overload and hyponatremia, and the possible intranasal application of Desmopressin should be clarified in controlled prospective studies in children.

4. Pediatric Stroke

Population-based data on ischemic stroke in pediatric patients were summarized by leading stroke experts from ten countries (M. Bonduel, Argentina; W. Muntean et al. Austria; G. deVeber et al., Canada; I. Husson et al., France; R. Straeter et al., Germany; G. Kenet et al., Israel; S. de Vries et al., The Netherlands; A. Kemahli, Turkey; V. Ganesan & F. Kirkham, United Kingdom; J.K. Lynch et al., US). Underlying diseases, imaging methods, presence of prothrombotic risk factors, and therapeutic options were discussed. A consensus paper on uniform stroke classifications, imaging methods, and laboratory screening as a basis for international multicenter therapeutic trials was proposed.
Plasma Coagulation Inhibitors

Chair- F. Church, USA
Co-chairs: M. Aiach, France; F. Bernardi, Italy; H. Kato, Japan; D. Lane, UK;
K. Suzuki, Japan

Business:
The meeting was chaired by Subcommittee CoChairs, Frank Church and Koji Suzuki (the other co-chairs were unable to attend due to conflicts). It was attended by approximately 50-60 persons. There were four sessions organized this year, R2 Factor V, Antithrombin reference standard, quality assessment for screening thrombophilia, and a review on serpin nomenclature and evolution.

Scientific Program:
Dr. Castoldi presented two talks, the first an overview of the FV R2 haplotype and gave the historical perspective and the evidence that the risk between venous and arterial thrombosis with this is not conclusive. She did suggest that heterozygotes with FV R2 and Factor V Leiden may be predisposed to venous thrombosis, but again, this is not totally conclusive. Her second talk centered around the ability of Factor V to serve as a cofactor for APC-protein S inactivation of Factor VIII and APC inactivation of Factor Va, both of which could contribute to the action of this interesting FV variant. She found that FV R2 was associated with altered cofactor function with APC inactivating factor Va.

Dr. Castaman described a metanalysis of existing data comparing the risk factor for factor V R2 with thrombosis. While there is no conclusive evidence that this allele predisposes strongly to venous thrombosis, there appears to be some small risk, and that other studies should be performed with a larger population base and a better defined/detailed clinical description of venous thromboembolic events.

Drs. Jackson and Esnouf spent the next period of time offering advice and guidelines about the newly proposed recombinant standard of antithrombin, and they divided their time to an overview of the question and the reference method to analyze the antithrombin standard by Jackson and the guidelines for the reference material described by Esnouf. There was a lot of conversation generated by their plan, and it is not clear where we stand in testing the reference standard, yet we heard today that a company has offered 2 grams of purified protein.

The third session was occupied with testing analyses and evaluation of different laboratories and their ability to test protein C, protein S, and antithrombin. These were given by Drs. Meijer, Biguzzi, and Jennings. Dr. Meijer summarized his studies of a wide-ranged testing comparing antithrombin, protein C and protein S. He described the use of a simple linear regression model just published that supports his comparison within the laboratories. The goal was assessment of
long-term analytical performance of field methods. There was some widespread variation of CV within the various lab for antithrombin, protein C and protein S. His story was summarized by saying that we need to improve assay variability between the various labs that perform such tests, and there is much research going on to sort out where the large variability occurs. His second talk centered around 11 different labs and their ability to assay only for protein S, pointing to different methods, different calculations, different reagent standards and assays. It was clear that "handling" of samples was a key issue that caused the variance within the assays. Dr. Biguzzi summarized the work on PROSIT, and she provided the first set of preliminary data studies from this group of interesting protein S patients/families in Italy. Fourteen (14) of the probands out of 52 studied had no mutations. The others had one or more mutations (frameshift, nonsense, or missense), and as this work continues they are studying the in vitro biochemistry and activity properties of these variants, secretion and activity. This promises to provide new and interesting details about protein S mutations. The final talk was from Dr. Jennings, who summarized a large set of interesting data gathered in the UK (but not from labs all located in the UK). His study focused on protein S activity, free and total protein assays. There was general agreement that the free protein S assay was less precise but more complex, and that direct free protein S levels are more precise to testing. There was also data included that summarized PEG-based versus direct-free protein S methods, and surprisingly, the data set suggested that the PEG-based assay had a better CV and might be a better assay, however, evaluations are still ongoing. Finally, he talked about pre-dilution of a sample being a problem from testing.

The final session was an overview of nomenclature for the serpin superfamily, given by Dr. Silverman. He provided the rationale for the new nomenclature, basing it primarily on need: too many names that are confusing, too many other names being signified for other serpins. Thus, a committee (not from the SSC) was convened and the nomenclature discussed and adopted. A paper was published in the J. Biol. Chem. in 2001 giving the details to this naming system(Silverman G.A., P.I. Bird, R.W. Carrell, F.C. Church, etc. 2001. The serpins are an expanding superfamily .... revised nomenclature. J. Biol. Chem. 276: 33293-33296.). We are now approaching over 700 different serpins from many different species, not just mammals, and the naming system will be useful as more genomes are sequenced. In no way is he suggesting that the nomenclature will displace the existing names of serpins; antithrombin will remain as is, yet it now how a genetic description based on this nomenclature that will identify it to all.

Drs. Suzuki and Church thanked all of those in attendance and closed the meeting.
Platelet Immunology

Chairman: BH Chong, Australia
Co-Chairs: RH Aster, USA; JB Bussel, USA; M Ertem, Turkey; S Santoso, Germany; T Warkentin, Canada

This meeting was dedicated to Dr. Albert von dem Borne (Amsterdam, The Netherlands) for his substantial and significant contributions to the field of Platelet Immunology. Of his 353 publications from 1969 to 2001, 76 dealt with platelet immunology. His leadership and contributions to the field are poignantly illustrated by the observation that 8 platelet alloantigens were discovered by Albert and his coworkers. The meeting was divided into three sections: (1) autoimmune thrombocytopenia; (2) alloimmune thrombocytopenia; and (3) drug-induced immune thrombocytopenia. Specific tributes were made to Dr. von dem Borne’s contributions before each of these three segments.

Autoimmune thrombocytopenia

Dr. BH Chong. Critical review of laboratory testing for ITP.
In this presentation, the useful contribution of platelet antibody testing for diagnosis of ITP was summarized, but with the key caveat that positive laboratory testing is not essential for diagnosis given the moderate sensitivity of the tests for detecting platelet-reactive autoantibodies, even using the newest assays. The history of antibody testing was reviewed, including the recognition that the "classic" platelet-associated IgG assays are not specific for ITP. Brief descriptions of various platelet glycoprotein-specific assays were provided, particularly the glycoprotein immobilization assays (microtiter well assay, immunobead assay, MACE, MAIPA & PAICA). Technical problems of these assays were described. Prospective studies suggest that the sensitivity and specificity of the MAIPA for ITP (using anti-GPIIb/IIIa and anti-GPIb/IX monoclonal antibodies) are about 50-66% and about 90%, respectively. A concluding comment was that other laboratory information (eg, reticulated platelets, TPO levels, plasma glyocalcicin) may also be helpful in defining ITP.

Dr. JB Bussel. H Pylori and ITP.
This presentation summarized published and non-published data indicating that about 42% of patients with ITP can be shown to have H pylori infection. Of the 66 patients in whom infection was successfully eradicated, 48% of patients showed platelet count improvement. Unfortunately, the validity of the treatment effect remains uncertain, as the five studies reviewed show considerable variability in response (0/13=0%, 6/14=43%, 6/12=50%, 12/19=63%, 8/8=100%).

Dr. D. Beardsley. Animal model for study of ITP.
Although dogs can spontaneously develop ITP, this does not offer a practical animal model for ITP. A strain of murine ITP-prone mice may have some value. Other models were described, including human Fcg RIIA transgenic mice, and comparisons of the effects of murine antiplatelet antibodies in murine FcgRIII knock-out vs human FcgRIIIA transgenic mice.

A new animal model was described: NOD/SCID immunodeficient mice were reconstituted with human stem cells, allowing for subsequent experiments examining the effects of anti-HPA-1a antibodies (in animals with significant numbers of circulating human platelets) or following
transfusion with human platelets. Human macrophages and megakaryocytes are demonstrable in these "Harrington mice." Addition of high concentrations of IgG can inhibit alloantibody-mediated platelet clearance. A proposal was made that members of the Platelet Immunology Subcommittee interact with other groups having animal models and aim by 2003 to have a joint agreement with the Subcommittee on Animal Models.

**Alloimmune Thrombocytopenia**

*Drs. EA Kolb, JB Bussel. A prospective randomized trial evaluating the safety and efficacy of risk-based therapy for neonatal alloimmune thrombocytopenia (NAIT).*

The US group reviewed their experience performing randomized trials of prenatal therapy in various at-risk groups for NAIT, as defined by severity of thrombocytopenia at first fetal blood sampling (FBS) at 20-24 wk and history of intracranial hemorrhage (ICH) in previous siblings. Standard-risk fetuses (platelets >20x10⁹/L, no siblings with ICH) were randomized to IVIG (1 g/kg/wk) or prednisone 0.5 mg/kg/wk, with salvage therapies (prednisone 1 mg/kg/d or IVIG 1 g/kg/wk, respectively) in non-responding fetuses (assessed at follow-up FBS every 3-6 wk). No significant differences in outcomes were seen. In high-risk fetuses (platelets <20x10⁹/L or sibling with ICH), randomization to IVIG 1g/kg/wk plus prednisone (1 mg/kg/d) was compared with IVIG alone (salvage: add IVIG 1 g/kg/wk to total 2 g/kg/wk or add prednisone, respectively). Significantly better outcomes were observed in the group receiving IVIG plus prednisone.

Non-randomized studies were performed in very high-risk fetuses (sibling with ICH between 28-36 wk): IVIG (1 g/kg/wk) beginning at 12 wk, FBS beginning at 20-24 wk, with salvage prednisone (1 mg/kg/d). In this group, 8 out of 12 needed salvage therapy. Finally, an extremely high-risk group (sibling with ICH <28 wk), randomization to IVIG 1g/kg/wk plus prednisone (1 mg/kg/d) was compared with IVIG alone (salvage: add IVIG 1 g/kg/wk to total 2 g/kg/wk or add prednisone, respectively). Significantly better outcomes were observed in the group receiving IVIG plus prednisone.

Overall, in these studies, 6 patients had ICH (74 pregnancies); 211 FBS procedures in 91 patients were complicated by 3 fetal deaths, and 14 deliveries precipitated by FBS, among other complications. Taking these complications into account, the new protocols under current study emphasize early treatment with stratification according to risk and with initial FBS deferred until 30-33 wk. The standard-risk protocol (no sibling with ICH) now compares IVIG 2 g/kg/wk with IVIG 1 g/kg/wk plus prednisone (0.5 mg/kg/d). Therapy now starts at 20-24 wk and the first FBS is performed at 30-33 wk. Protocols for the high-risk (previously, very high-risk) and very high-risk fetuses were similarly modified.

*Drs. MF Murphy, J Birchall, C Kaplan & H Kroll, on behalf of the European FMAIT Study Group. European collaborative study on the management of fetomaternal alloimmune thrombocytopenia (FMAIT).*

Results of an observational study was reported. Fetuses with FMAIT due to HPA-1a incompatibility and a previously affected sibling, underwent initial FBS (generally, 20-24 wk) followed either by: (1) maternal therapy with IVIG (1 g/kg/wk) and/or prednisolone; (2) serial intrauterine platelet transfusions; or (3) fetal platelet monitoring by FBS with or without pre-delivery intrauterine platelet transfusion. There were 56 fetuses from 55 pregnancies in 50 women. Comparison of ICH in previous and present (treated) fetuses was: 15 vs 3 (suggesting a
treatment effect). Other conclusions included: (1) there was a good correlation between the severity of FMAIT in siblings and severity of thrombocytopenia in fetuses; (2) FBS can have major complications (4 premature delivery, 2 deaths); (3) fetal platelet count rose to >50 in the 67% who received maternal therapy. In future studies, antenatal care will be based on sibling history. Antenatal therapy will commence prior to first FBS. Therapy with IVIG will be given with higher-dose IVIG or prednisolone or serial intrauterine transfusions added as salvage treatment if required.

Drs. JB Bussel, C Kaplan, M Murphy & H Kroll (presented by H Kroll). Guidelines for diagnosis and management of fetal/neonatal alloimmune thrombocytopenia (FNAIT). The proposed new guidelines for diagnosis and management of FNAIT were presented (the last was developed in 1991 by Neonatal Hemostasis Committee of SSC/ISTH). This work is being developed as a joint ISTH/ISBT initiative. The topics to be discussed are when to suspect alloimmune thrombocytopenia in the fetus and neonate, lab testing, antenatal and postnatal management. The topic of screening for FNAIT will also be discussed. Specific technical aspects of testing, as well as test interpretation, will be reviewed. Emphasis on minimizing fetal blood sampling and using maternal therapy for "standard risk" fetuses will be made. For postnatal management, therapy with HPA-1a and -5b negative platelets is recommended, as >95% of neonates will respond, and there are logistical problems with providing washed and irradiated maternal platelets. In urgent situations, random donor platelets with IVIG can be given although response may be poor. Studies of antenatal screening are underway. The intention is to have the draft of this manuscript completed by September 2002, to receive comments by November 2002 with revisions made in December 2002, and to publish the report in Journal of Thrombosis and Haemostasis.

Drs. S Santoso, C Kaplan. Human platelet alloantigens: nomenclature and guidelines for new inclusions.
The history and terminology of the human platelet alloantigen (HPA) system for designating platelet alloantigens were reviewed and compared with "classical" and gene-based naming. Twenty-two platelet alloantigens were listed, with 4 not having been given HPA designations (Oea, Gova, Govb, Duva). The Platelet Nomenclature Committee was described, consisting of representative membership of scientists and physicians active in the field of platelet immunology (including members both from ISBT and ISTH), and officers, Chairman, Co-Chairman, and Secretary, elected from membership of committee, maximum two 3-year terms. Discussions would take place at ISBT and ISTH meetings, with acceptance of recommendations at ISBT meetings. Current representatives include C. Kaplan, Chairman, (representing ISBT), S. Santoso, Co-Chairman, (representing ISTH), and W. Ouwehand (Secretary). Other committee members are A. von dem Borne, R.H. Aster, V. Kiefel, Y. Shibata, J. Smith, and R. Kekomaki. Submissions should be made to the Nomenclature committee 3 months before ISTH and ISBT meetings. Procedures for acceptance of new alloantigen(s) were described in detail. Six reference labs were designated. Repository laboratories (Amsterdam & Giessen) would act as trustees for reference materials (sera, DNA, platelets).

Plasma was obtained from two patients with well-characterized anti-HPA-5b alloantibodies. Stability studies showed minimal loss of reactivity when stored at -20°C or 4°C. Extensive collaborative studies were performed with multiple laboratories from several countries employing a wide variety of laboratory techniques: when plasma from the two patients was pooled (designated "99/666"), anti-HPA-5b reactivity of 1/64 and 1/16 in MAIPA and platelet immunofluorescence (flow cytometry) was shown, respectively. With few exceptions, laboratories readily detected anti-HPA-5b reactivity with 99/666, without detecting other HPA or HLA alloantibodies.

Dr Chong asked the members to vote on whether the Platelet Immunology subcommittee should recommend that 99/666 be adopted as an international reference reagent for human antibody against HPA-5b. The vote was 9 for, none against, remainder abstaining. This recommendation will be presented to the SSC Voting members at this year's Business Meeting.

### Drug-induced Immune Thrombocytopenia (DIT)

**Dr. BH Chong. Drug-induced thrombocytopenia. Mapping of the epitope of the quinine-induced platelet antibody.**

The history of research into the pathogenesis of quinine-induced immune thrombocytopenia (first described in 1865) was presented. Immunoprecipitation of GP Ib by quinidine-dependent antibodies helped to support the view that this syndrome was not caused by an "innocent bystander" mechanism involving platelet Fc receptors. In general, GP Ib/IX is more frequently invoked as the target GP in DIT (particularly the GP IX component) compared with GP IIb/IIIa. A series of GP IX mouse-human chimeras were constructed to investigate the target region on GP IX more precisely. The epitope of the quinine-induced antibody was mapped to the C-terminus extra-cellular region of GP IX. Interestingly the epitope of SZ 1, an anti-GP IX monoclonal antibody which is known to block the binding of quinidine-, rifampicin- and ranitidine-dependent antibodies to GP IX, was also mapped to this region of the protein suggesting that this is a common binding site of several drug-dependent antibodies.

**Dr. TE Warkentin. Clinical and laboratory diagnosis of HIT: refining the scoring system.**

The concept of immune HIT as a clinicopathologic syndrome was emphasized. Thus, diagnosis requires both clinical (particularly, thrombocytopenia) and laboratory (detection of HIT antibodies) criteria. The availability of sensitive assays for clinically significant HIT antibodies and the fairly common occurrence of subclinical seroconversion in heparin-treated patients means that it is far easier to refute than to confirm a diagnosis of HIT. Indeed, a positive test result simply increases the pre-test probability of HIT to a higher post-test probability. A simple scoring system was proposed wherein a maximum of two points each (0, 1, 2) could be given for three criteria that help distinguish HIT from non-HIT disorders (timing of onset of thrombocytopenia; occurrence of new thrombosis; lack of another explanation for thrombocytopenia; maximum score, 6/6). Generation of sensitivity-specificity trade-off curves was shown for two assays: PF4/heparin-EIA and washed platelet activation assay (serotonin release test). Likelihood ratios for various test results were interpreted in relation to the estimated
pre-test probability. It was concluded that a scoring system might help physicians arrive at appropriate estimations of pre-test probability for HIT, however, wide variation in the available laboratory assays and their performance characteristics means that it will be difficult to generate scoring systems that take into account both clinical and laboratory criteria applicable to all clinical settings. It was also suggested that quantitative results of laboratory testing can provide diagnostically useful information.

Dr. JM Walenga. Platelet-leukocyte activation in heparin-induced thrombocytopenia. Evidence for a pro-inflammatory response in HIT was reviewed. Evidence was shown that neutrophils and monocytes (but not lymphocytes) can bind to platelets in the presence of platelet activation by HIT antibodies. Furthermore, this activation can be prevented by antibodies against P-selectin. Leukocyte activation leads to various pro-inflammatory and prothrombotic effects.
Platelet Physiology

Chairman: A. Koneti Rao, USA
Co-chairs: M. Berndt, Australia; C. Cerletti, Italy; C. Hayward, Canada; M. Hoffman, USA; A. Michelson, USA;
P. Newman, USA; P. Nurden, France; S. Watson, UK

There were two main themes addressed in this meeting of the Platelet Physiology Subcommittee. The first one was platelet-leukocyte interactions, an area where tremendous new information has become available over the last few years. The second theme was platelet function disorders with a focus on a specific methodology (Platelet Function Analyzer, PFA-100).

Platelet-Leukocyte Interactions

Several speakers reviewed recent information on different aspects of platelet-leukocyte interactions. Dr. Bruce Furie reviewed interactions between P-selectin and PSGL-1. Dr. Jose Lopez reviewed the interactions between GPIb and MAC-1. Dr. Chiara Cerletti focused on the signaling interactions between platelets and leucocytes with a focus on Srk kinases. Dr. Michael Berndt reviewed the regulation of P-selectin binding to PSGL-1 by elastase and cathepsin G. Lastly, Dr. Alan Michelson reviewed recent information on circulating monocyte-platelet aggregates as a sensitive marker of in vivo platelet activation in patients.

Working Group on Platelet Function Disorders

The second half of the session focused on presentations by the Working Group on Platelet Function Disorders. Despite major advances in our understanding of platelet physiology and available newer methods, our understanding of the mechanisms in patients with platelet function disorders remains low. In the vast majority of patients with inherited abnormalities in platelet responses, the underlying mechanisms leading to the platelet dysfunction are unknown. The focus of this session was on the role of PFA-100 in the diagnosis of platelet function disorders, excluding von Willebrand disease. PFA-100 has become widely available and is being used in the evaluation and management of patients with vWD and platelet function disorders.

Dr. Catherine Hayward presented information on developing evidence-based approaches to the diagnosis of platelet disorders. She presented information from recent ongoing studies in her laboratory, including the Clinical History Assessment Tool (CHAT). Several speakers addressed various aspects of PFA-100 in the diagnosis of platelet function disorders (excluding von Willebrand disease): Drs. Diane Nugent, Marco Cattaneo, Thomas Ortel, Berndt Jilma and Paul Harrison. The information presented included studies with the PFA-100 in the general group of patients referred for the evaluation of bleeding disorders, patients with menorrhagia, and those
on platelet inhibitory drugs. Following these presentations, there was a discussion regarding the advantages and limitations of PFA-100. It was also felt that the working group on platelet function disorders should continue to explore the need for setting up collaborations between various laboratories with relevant expertise to define the underlying mechanisms in patients with inherited disorders of platelet function.

Dr. James Bussell presented information on the ongoing studies and on the registry of patients with non-immune thrombocytopenias.
Predictive Haemostatic Variables In Cardiovascular Disease

Chairman: L. Iacoviello, Italy
Co-Chairs: M. Cushman, USA; P. Grant, UK; R. Hull, Canada; G. Lowe, UK

The number of attendees of this subcommittee meeting was approximately 100.

Dr. Licia Iacoviello gave an update of the activities of the Subcommittee during the past year. A WEB site of this SubCommittee has been developed at http://www.negrisud.it/ssc and linked with the official ISTH website.

The Registry of on going studies on the association between genetic and biochemical haemostatic variables and cardiovascular disease has been started and the forms to apply are now available at the above website. The forms can be completed and sent to Dr. Iacoviello directly through the website.

The Subcommittee's website is also linked to the CANVAS website (http://genecanvas.idf.inserm.fr/) which was presented by Dr. Cambien at last year's SSC meeting in Paris. CANVAS has the objective to facilitate the study of the impact of candidate gene polymorphisms on common cardiovascular disorders by accelerating the communication of information on candidate genes and DNA resources. It is also a single nucleotide polymorphism (SNP) resource that may be useful to those exploring the genomic regions where the candidate genes are located. In the next 5 years, 300 candidates genes are foreseen to be included in the catalogue. At the moment information on 566 polymorphisms, 114 genes and 29 studies are available.

Dr. Iacoviello also reported on the ETRO Working Party meeting on Population Genetics of Haemostatic Risk Factors for Arterial Vascular Disease that was held in Rome, on October 26-27, 2001. The aim of this Working Party is to bring together European investigators in genetics, environment and thrombosis with different expertise (molecular and cell biology, epidemiology, biochemistry) to evaluate the contribution of genetic and environmental components to the risk of arterial thrombosis, with a special emphasis on haemostatic factors. The hot topics of this year's meeting were pharmacogenetics and innovative epidemiological approaches, with particular attention to large cohort studies.

Finally it has been proposed to write guidelines for population association studies in genetics. Potential topics are: selection of controls, sample size, multiple comparisons, statistical analysis (permutation test), admixture bias, gene-environment interactions, selection of polymorphisms. Are D-Dimer levels a suitable marker of cardiovascular disease? Methodological and epidemiological aspects. Dr. Gordon Lowe presented an overview of fibrin D-dimer in prediction of cardiovascular disease. While there are a large number of available commercial assays which give very different values for plasma D-dimer, its association with cardiovascular risk appeared consistent regardless of assay type. (Standardization of D-dimer assay is being performed by the Subcommittee on Fibrinolysis). Variation on sample storage, and on repeated measurement after 4 years, appeared minimal. Epidemiological association of D-dimer included
age, female sex, country, oral estrogen use, obesity, lack of leisure activity, varicose vein, and prevalent arterial disease or venous thromboembolism. A meta-analysis reported the relative risk of coronary heart disease as 1.7 (top third of D-dimer compared to bottom third). There were fewer studies of the predictive value of D-dimer for stroke or venous thromboembolism, but these showed consistent association with risk. These associations were not explained by acute phase reaction (e.g. C-reactive protein). D-dimer levels are normalised by oral anticoagulant therapy and merit evaluation in selection of patients (and in monitoring) of such therapy.

**Homocysteine Evaluation: Problems and Predictive Value.** Dr. Marco Cattaneo reported on the association of homocysteine levels and risk of cardiovascular disease (CVD). While the association between homocysteine and venous thromboembolism is well established, only retrospective case-control studies or prospective studies in patients who already had an ischaemic event demonstrated a consistent association between homocysteine and CVD. In contrast, prospective studies in healthy subjects gave negative results. As a consequence a huge debate is going on whether high homocysteine levels are cause or consequence of atherosclerosis. A study in patients with stroke demonstrated that, following the acute phase, there is a decrease in homocysteine levels, concomitant with the increase in C-RP levels; at longer term, homocysteine levels got back to normal levels, never increased. Although a final conclusion on this debate can only be derived from interventional studies, lowering homocysteine levels (whose results are expected in the next few years), there are still some arguments for a role of homocysteine in the risk of cardiovascular disease. Probably to express its risk potential, homocysteine requires to synergise with other risk factors. Moreover, in prospective studies, the power of the association between homocysteine and CVD decreases with the length of follow-up, suggesting problems related to a longer sample storage. Homocysteine levels can be determined by genetic (MTHFR polymorphism) and environmental factors (vitamins). While there is no consistent evidence of an association between the MTHRF polymorphism and CVD risk, there is evidence for an independent association with vitamin B6.

Dr. Cattaneo also reported on the standardization of pre-analytical conditions for homocysteine evaluation. Homocysteine levels are stable over 6 hours in samples collected in both ACD and EDTA when stored on ice. After storage at room temperature, homocysteine levels increase about 13% if collected in EDTA and only 3%, if collected in ACD. Therefore, when room temperature storage cannot be avoided, ACD must be used as an anticoagulant.

**The ARIC Project: Haemostatic Variables, their genetic control and Prediction of ischaemic arterial disease.** Dr. Kenneth Wu presented an overview of the results of the ARIC study, a prospective investigation of a USA cohort of 15,792 healthy subjects aged 45-64 years who were followed for 10 years. A nested case-control study was performed by comparing 365 incident cases and 734 non-cases. Fibrinogen, factor VII, factor VIII, von Willebrand factor, protein C, APC resistance, antithrombin III, t-PA, PAI-1, plasminogen, activated factor VII, and XII, Protrhombin fragment F1+2, D-dimer, beta-thromboglobulin and soluble thrombomodulin levels were evaluated. Fibrinogen, plasminogen and D-dimer levels were significantly and independently associated with the risk of ischaemic events. Unexpectedly, sTM showed a negative association with the risk of CVD. No associations were found for the other haemostatic factors evaluated. Combined analysis of pro- and antithrombotic factors can provide a more precise estimation of the association: indeed, increased levels of ICAM-1, fibrinogen, F VIII, and
vWF interact with low levels of sTM in increasing the risk of CVD, while in the presence of high levels of sTM they did not affect the risk.

Polymorphisms in several haemostasis factor genes (fibrinogen, F VII, F II, F V, F XIII, TM, platelet glycoprotein IIIa and Ib) were also evaluated in a larger population of 800 cases and 900 controls. Only TM and GP Ib polymorphisms were associated with the risk of CVD; however, the association between TM polymorphisms and CVD risk was present in African American subjects but not in Caucasians. This suggests that different ethnic populations should always be analysed separately.

*Ethnic differences in genotype distribution and risk of cardiovascular disease: the case of the Japanese*

**Dr. M. Murata** further discussed ethnic differences in genotype distribution by presenting the case of the Japanese population. In Japan the incidence of venous thrombosis after surgery and myocardial infarction is lower in both males and females, despite the high prevalence of smoking. Some polymorphisms related to such disease (F V Leiden, prothrombin, F XIII, GP Ib/IIIa) are absent in the Japanese population, while many others have a different frequency as compared to the Caucasian population. Some of them are increased, while some others are decreased, without any apparent relation with the potential risk effect. While the reduced incidence of venous thrombosis can at least in part be related to the absence of related polymorphisms, the difference in AMI is related more to different environmental habits, especially diet and to a different pathogenesis. Indeed, 52% of AMI in Japan can be attributed to coronary spasm, compared to 11% in Europe. Independent predictors of spasm in Japan are smoking and NO synthase polymorphisms.

Dr Murata also showed some results concerning PAF acetylhydrolase gene in a Japanese population. This enzyme inactivates PAF. A F/V polymorphism is associated with plasma activity of the enzyme, showing the VV genotype higher activity, FF no activity and VF intermediate activity. Carriers of VV genotype also showed reduced platelet activation after PAF, but not after ADP or collagen stimulation. The F genotype was associated with an increased risk of stroke, particularly at younger age.

*Activation markers of coagulation and fibrinolysis in twins: heritability of the prethrombotic state. Dr. Robert Ariens* reported on the heritability of the prothrombotic state. Heritability is the proportion of variance in a phenotype due to additive genes. Twin studies are a useful tool to evaluate the heritability of a phenotype by comparing monozygotic twins (100% genetics) with dizygotic twins (50% genetics). The levels of F VII, FXII, F XIII, Fibrinogen, vWF and PAI-1 showed a high inheritability. This result was consistent with those obtained in family studies. However, new polymorphisms in genes related to these phenotypes only account for a small part of their heritability (e.g., PAI-1 4G/5G polymorphism accounts for 2% of PAI-1 level heritability, Fibrinogen beta chain polymorphism for 1% of fibrinogen heritability and F VII Arg353Gly polymorphism for 16% of F VII levels heritability). In a sample of 115 twin pairs (59 MZ and 56 DZ), it has also been demonstrated that the levels of coagulation activation markers are also inherited. In particular, prothrombin fragment F1+2, TAT and D-dimer levels showed a degree of heritability of respectively 45, 40 and 65%. Also fibrin structure shows a 39% heritability, although the effect of environmental factors is also important. Among others,
glycosilation can be an important determinant of fibrin structure in diabetic patients.

**Genetic regulation of Factor VII and Factor XII: insights from the GAIT Study. Dr. John. Blangero** provided an update of the results of the GAIT study, a project aimed at identification of QTL in large pedigrees of Spanish families. He suggested that, before studying the genetics of discrete traits (diseases) that require very powerful methods, a useful approach is to identify genetic loci that regulate continuous traits (factor levels), which are called QTL (quantitative trait loci). The study of large pedigrees is one of the more powerful tools for this purpose and allows both the localization and the identification of a QTL.

The GAIT study includes 398 subjects from 12 thrombophilic and 9 randomly selected families, with a total of 57 cases of thrombosis. F VII and F XII liability has been studied. In a first phase, QTL F XII and F VII have been identified by using a wide genome scan with 363 genetic markers. High lod score (LD) has been found in chromosomes 5, 10 and 2 for F XII levels. Chromosome 10 contains F XII gene, however, in chromosome 5 there should be another gene strongly influencing F XII gene. Since a 46C/T polymorphism in F XII gene has been associated with the levels of F XII, if whether such a polymorphism could be responsible for the large linkage signal found has been tested. After conditional analysis, the LD for chromosome 10, although decreased, remained still significant, suggesting that other polymorphisms can be relevant.

For F VII levels a significant QTL has been found in chromosome 13, that actually contains F VII gene. F VII gene was resequenced: all its polymorphisms (49) were identified with an allele frequency between 0.01 and 0.37. Posterior probability of functionality was analysed and 7 polymorphisms were identified as functional. Conditioned analysis including these polymorphisms showed that the LS for F VII levels was reduced to a non-significant level, suggesting that F VII gene was completely dissected.

Finally, Dr. Blangero commented on the limitation of the case-control approach for genetic studies, based on linkage disequilibrium (LD) mapping. Indeed, LD is too unpredictable and, therefore, negative studies give no information.

**Genetic polymorphisms of haemostatic factors: a word of caution. Dr. Pier Mannuccio Mannucci** discussed the need to use large samples in case-control studies to have enough power to detect association for polymorphisms in haemostatic genes. He presented the results of a case-control study performed in collaboration with the ANMCO (Italian Association of Hospital Cardiologist), including more than 1000 patients with AMI at young age (under 45 years) and a corresponding number of healthy controls matched for age and sex. Cases differed from controls for all common environmental risk factors, such as smoking, hypertension, diabetes, dyslipidemia, etc. Cases also had a higher prevalence of family history of AMI in respect to controls; however, this difference was not explained by any of the polymorphisms of haemostatic genes studied. Indeed, no associations were found between F VII, FV, F II, F XIII, GP IIIa, etc., polymorphisms and the risk of AMI at young age. The same results were obtained when only females were taken into consideration.

**Conclusion:**
The subcommittee meeting highlighted the need to continue to study the role of haemostatic variables in the prediction of cardiovascular risk. Activation markers such as D-dimer levels could be particularly relevant, and their evaluation, also in relation to other well established risk factors, should be introduced in future epidemiological studies. On the other hand, new haemostatic markers are emerging as possibly predictive, such as plasminogen and soluble thrombomodulin and need to be tested in future studies.

A possible powerful approach emerges for genetic studies:

1. QTL identification in family studies
2. identification of all possible polymorphisms of the genes related to the QTL
3. test for their functionality
4. dissect residual inference
5. assess the absolute or relative risk in population studies.
6. design the population study with a correct calculation of the sample size, selection of cases and controls and respecting the homogeneity of populations.
The VWF Subcommittee was attended by about 125 attendees at the first session and 100 participants for the second session. They actively participated in the discussion of the various talks provided by the program. The following represents a full summary of the program.

**Cooperative Studies (J. Eikenboom, Chair)**

**Dr. Goodeve** reported on the European study "Molecular and Clinical Markers for Diagnosis and Management of type 1 von Willebrand Disease" funded by the European Union. Twelve centers from 9 European countries cooperate in this study. The aim of this study is to determine the value of clinical, phenotypic and molecular markers for the diagnosis of type 1 VWD; to examine the contribution of polymorphism and mutation in the VWF gene to type 1 VWD; and to determine the proportion of type 1 VWD not associated with mutation in VWF gene. Two hundred families with VWD type 1 and 1200 healthy controls will be included. So far 142 families have been recruited. In each family data is collected on bleeding history. Basic laboratory tests for VWF parameters and advanced phenotypic tests (VWF:CB, VWF:FVIIIB, platelet VWF) will be performed. Linkage analysis will be performed to establish or reject co-segregation between the phenotype and VWF genotype and to establish the contribution of the VWF gene to the variability in VWF level. The influence of haplotype (5 SNPs) on plasma VWF level will be studied. Finally, mutation analysis will be performed in all families and mutations will be expressed in vitro. To date, mutations have been identified in 24 index cases and no mutation was identified in 2 cases.

**Dr. Lillicrap** reported on the Canadian study "Molecular Genetic Basis of Type 1 von Willebrand Disease" funded by the Canadian Institutes of Health Research. Seven clinics have recruited 172 individuals from 52 families. To date 29 families were analyzed. For genotype analysis they were all screened for seven polymorphic sites and two type 1 dominant negative mutations (C1130F and C1149R). Phenotypic analysis showed a slight excess of women among the affected individuals. Blood group O was over-represented among index cases. Overall phenotypic data from the source laboratories agree with the core laboratory, although there were some discrepancies.

**Dr. Rodeghiero** reported the final results of the "Validation of the Diagnostic Criteria of Type 1 von Willebrand Disease: an International Multicenter Study" that was initiated by the SSC in 1997. The aim of this study was to investigate the contribution of bleeding history to the diagnosis in a sample of obligatory carriers of type 1 (n=42) and type 3 VWD (n=70). Bleeding
history was compared with that of affected members and age and sex-matched controls (n=224). A standardized questionnaire was used to evaluate each hemorrhagic symptom at presentation, using a score system (0: no symptoms, 1: mild symptoms; 2: symptoms requiring medical attention only; 3: symptoms requiring hospitalization, replacement therapy or blood transfusion). The severity of epistaxis, menorrhagia and bleeding after tooth extraction is similar but not equal in obligatory carriers of type 3 VWD and controls; affected subjects in type 1 and 3 families show a clear prevalence of score 1, 2 and 3. For type 1 VWD obligatory carriers vs. controls, menorrhagia and epistaxis have a low sensitivity, whereas cutaneous bleeding is the most sensitive symptom. For type 3 VWD obligatory carriers vs. controls, menorrhagia and bleeding after tooth extraction have a low sensitivity, whereas bleeding after surgery is the most sensitive symptom. Laboratory screening in subjects with at least two hemorrhagic symptoms seems a reasonable option.

Database and Classification (A. Goodeve, Chair)

New Molecular Database. Anne Goodeve summarized the new ISTH VWF website, on behalf of Stuart Croft (Sheffield) and Ross MacLachlan (Kingston), who have designed the site. It is intended that this site will replace the previous VWF site, hosted at the University of Michigan. The web address is http://www.shef.ac.uk/vwf/.

The aim of the web site is to provide a convenient, user-friendly first-point of reference for the scientific community with an interest in von Willebrand factor and von Willebrand disease. The site contains searchable mutation and polymorphism data, the full human VWF genomic sequence plus links to several other VWF sequences, diagrams of VWF and links to other sites of interest.

Linkage analysis in the EU study. Jeroen Eikenboom described that three short tandem repeat polymorphisms in the VWF gene will be used to examine linkage to the gene in families with type 1 VWD. Analysis will be performed using software such as LINKAGE and MLINK. In addition to highlighting families with VWD not linked to the VWF gene, the contribution of the gene to the variability in VWF level will be determined.

VWD Classification Issues (J.E. Sadler, Chair)

Strengths and weaknesses of the current VWD classification system were discussed. Dr. Sadler suggested that a classification should be designed with a primary emphasis on clinical utility. Thus, disease categories should correlate with bleeding risk and response to specific therapies and should be assignable using common laboratory tests. Although the current classification satisfies these guidelines, some patients are difficult to classify. In particular, decreased VWF antigen and proportionally decreased VWF activity may be consistent with VWD type 1, but
circulating VWF may contain subunits with mutations that do not affect binding functions. These patients could be accommodated under VWD type 1 by including variants in which circulating VWF contained mutant subunits but otherwise had a normal proportion of large multimers with apparently normal function.

Dr Castaman presented data on VWD Vicenza, another difficult to classify variant. The consensus opinion was that it was premature to define this as a distinct variant. Some data is being generated on what features are related to specific mutations within the VWF gene in these patients.

Dr. Eikenboom presented the results of an extensive comparison between patients with a diagnosis of VWD type 1 and obligate carriers of VWD type 3 alleles.

Dr. Montgomery discussed the heterogeneity of mutations and mechanisms underlying the categories of VWD types 1, 2A and 2M.

Dr. Budde proposed an extended classification in which VWD type 2A would be split into subtypes based mainly on multimer structure and secondarily on mutation location. After discussion, the consensus opinion was that all such mutations converge on two classes of mechanism: impaired multimer assembly and increased catabolism of large multimers. All such patients lack circulating large multimers, which impairs hemostasis, and they generally have a poor response to DDAVP. Phenotypic variation within any of the proposed subcategories appears to exceed the variation observed between them. Splitting VWD type 2A therefore was not felt to have added clinical utility.

Dr. Budde presented the results of studies using multimer analysis to distinguish VWD type 1 from VWD type 2 variants.

Dr. Eikenboom presented results on mutations that affect collagen binding specifically. These currently would be included under VWD type 2M because they impair platelet adhesion.

While a number of issues were brought up, it was decided to continue the present classification system and to extend the 2A classification to incorporate the specific genetic mutation and perhaps a multimer description as a parenthetic remark. No working party was developed at this time.

**Nomenclature (G. Castaman, Chair)**

Dr. Mazurier summarized the current recommended abbreviations for VWF activities as published in *Thrombosis and Haemostasis* in 1991. All authors, reviewers, and Editors were
Dr. Mazurier proposed an abbreviation for VWF multimers as VWF:Mult or VWF:Mers. Since these were not quantitative measurements, it was decided to refer to these as VWF multimers rather than any further abbreviation. A group will be appointed to make recommendations by next year on methods to quantitate the relative concentration of the various size multimers.

Dr. Montgomery made two proposals. The first was to cease using the term VW AgII to refer to the VWF propeptide. Although VWFpp or VWF:pp were proposed as abbreviations, there was no consensus for a change. The first recommendation to stop using VW AgII was accepted. The second recommendation to use abbreviations for the propeptide was not accepted at this time. In publications the propeptide should be referred to as the VWF propeptide.

**Standardization Issues (A. Federici, Chair)**

Dr. Federici presented the interim report of the Working Party to study the standardization of VWD diagnosis. A working party and time-lines were presented, and it is expected to have an interim report by the next meeting in Birmingham in 2003. Thirty centers will be involved in the study and at least 1/3 of them will be in developing countries.

Anthony Hubbard presented data on the collagen binding of the VWF concentrate standard. It was recommended that there needs to be further work on this matter and that a small working group will study the use of different collagen binding assays in several assays using several techniques in each participating laboratory and will make a report at the next SSC Meeting in 2003.

The issue of standardization of the collagen-binding assay was discussed by Drs. Mazurier, Sietz, and Lillicrap. There continue to be difficulties with agreeing on a standard approach to this assay. Dr. Federici agreed to head a working party to address this problem and to report on this at the next SSC meeting.

Dr. Mazurier will head a working party to recommend standards reporting of quantitative assessment of multimer size. Dr. Budde presented data on the graphical approach and this needs to be extended to other laboratories and countries.

**Treatment Issues (P. Kouides and R. Montgomery, Co-Chairs)**

A working group headed by Dr. Federici will report at the next SSC meeting on treatment and monitoring of patients using DDAVP. Dr. Thompson summarized the recovery and half-lives of various treatment concentrates. Dr. Mannucci presented the results of his survey on thrombosis
in patients treated with VWF concentrate. Seven additional patients were identified through this survey. This brought up a problem with our network for the VWF committee since we previously had no method of disseminating information about a potential problem (thrombosis) with VWF treatment concentrates. We are attempting to develop an e-mail list for the future. Further information was provided on FVIII and VWF levels in patients and treaters are encouraged to monitor FVIII levels.

**VWF-cleaving protease (ADAMTS-13) (J. Rand, Chair)**

Dr. Pier Mannucio Mannucci reported on the results of the assay when applied to patients with diseases other than TTP. He reviewed data that were published in BLOOD last year (Sept 2002) that showed decreases of the VWF-protease levels, generally mild-moderate, in a variety of clinical conditions.

Dr Han Mou Tsai reviewed the recent identification of the VWF-cleaving protease as ADAMTS13 and reported on his assay methodology. The protease has been purified from plasma and the gene has been mapped to chromosome 9q34. Other studies report that some patients without TTP have low (<30%) levels of the VWF-cleaving protease and suggest that the decrease may have pathophysiological implications. A review of his data reveals that mildly decreased ADAMTS13 levels are detected in some patients with various pathological conditions, however, the decreases are generally not as profound as described by others. The discrepancy most likely results from difference in the assay methods, some of which generate broad ranges among normal individuals. Since the decrease does not correlate with the severity of the underlying diseases and is not associated with an increased VWF size, the significance of the observed decrease is unclear and should be interpreted with caution. He also compared the various current assay methodologies.

Dr. Martina Bohm presented her and Dr. Inge Scharrer’s data on a new assay method for the protease that utilizes measurement of VWF:RCo. This test appears to have benefits in its simplicity and nonreliance upon gel-based analysis of multimers or protein fragments.

There was general agreement that laboratories currently performing these assays be encouraged to evaluate comparability by testing shared specimens. Dr. Bohm informed the group that such an effort, sponsored by Dr. Laemle, was already underway.

**Final recommendations**

1. The use of VW AgII should no longer be used for the propeptide of VWF. No abbreviation should be used - use "VWF propeptide."
2. No abbreviation for VWF multimers should be formalized – use "VWF multimers."
3. A working party was formed for assessing distribution of VWF multimer size with Dr. Mazurier as head.
4. A working party on the collagen-binding assay was formed with Dr. Federici as head.
5. A working party on treatment and monitoring of patients after DDAVP was established under the direction of Dr. Federici.
6. An informal group to exchange plasmas for ADAMTS 13 was agreed to by the major labs and was encouraged to determine if the SSC plasma standard has normal ADAMTS 13 level and if it has potential for being a future formal or informal standard.

Robert R. Montgomery, Chair
Working Group on Women’s Health Issues

Chair: M. J. Manco-Johnson, USA

This was the first open working party meeting on coagulation issues of women. There were 40-50 persons attending and participation was active.

I. Organization of the Working Party, scope of activities, composition of the working group, method of approach and anticipated products.

Time was devoted to discussing and planning the above issues. This working party will be focused on coagulation values and clinical syndromes that are influenced by hormones. The working party will evaluate existing data, determine issues for which data is missing or deficient, identify areas of controversy and pressing clinical need and discuss methodologic approaches to answer questions raised. The working group may suggest or organize future collaborative studies as a result of these activities. The working group will incorporate laboratory scientists, epidemiologists, hematologists, high-risk obstetricians and gynecologists. All interested parties are encouraged to join an e-mail tree through marilyn.manco-johnson@uchsc.edu. The e-mail tree will be used to further discussions and continue active collaborations throughout the year.

Bleeding Disorders in women:
Anne Dilley, CDC, Atlanta, USA, discussed screening for von Willebrand’s disease and other genetic bleeding disorders in women with menorrhagia. The use and limits of existing screening tools for menorrhagia were reviewed. Difficulties in the diagnosis of vWD were discussed including cost, timing and number of evaluations, use of reference laboratories, and high prevalence of the trait. Current challenges include validation of a screening questionnaire for menorrhagia, exploration of screening tests, including whole blood and thrombin generation assays, for vWD, platelet dysfunction and other bleeding disorders.

Peter Kouides, Rochester, USA, addressed efficacy of various therapies (OCPs, DDAVP, tranexemetic acid, etc.) for menorrhagia. He raised the issue of safety of intrapartum DDAVP, endometrial ablation, Mirena pretestational ring, use of DDAVP for sports, and safety of hormonal therapies in menopausal women. Limitations of efficacy and adherence with various vWD therapies were reviewed. Dr. Kouides suggested an ISTH survey on DDAVP use, guidelines for screening vWD, guidelines for management, and a survey on the diagnosis of vWD while using OCPs.

James Bussel, New York, USA, reviewed current updating of the Platelet Immunology Subcommittee recommendations for the evaluation and management of neonatal alloimmune thrombocytopenia. Dr. Bussel discussed advances in platelet antigen typing, serologic testing, and antenatal treatment. Revisions to recommendations for fetal testing were discussed. Current in-process studies of combined use of IVIG and steroids prenatally were presented. New recommendations stratified by fetal risk were discussed.

II. Influence of hormones on coagulation values in women.
Dr. Margareta Blombäck presented data for coagulation proteins relative to the menstrual cycle, trimesters of pregnancy and hormonal therapies, including oral and topical administration of HRT during menopause. Professor Blombäck advised that data are lacking primarily for hormonal effects on fibrinolysis overall, and that data are weak for the three generations of contraceptive agents. The recommendation was made to explore global assays of thrombin generation, coagulation potential and fibrinolytic potential and apply these to the various hormonally influenced states noted above.

III. Risk factors for thrombosis and adverse outcome in pregnant women.

Dr. Ian Greer, Glasgow, UK, discussed risk factors for thrombosis during pregnancy. He stressed the gap between knowledge of risk factors (obesity, age, caesarean section, prior venous thrombosis, personal and family history of thrombosis) and appropriate application of diagnosis and interventions in pregnancy. Prof. Greer reviewed evidence for thrombosis and thrombosis recurrence during pregnancy, safety of prophylaxis with LMWH and cost of screening and complications. Suggestions were made to collaborate the study cost/efficacy of targeted screening as well as the use of aspirin and compression stockings for prevention.

Dr. Jacqueline Conard gave an elegant presentation of venous thromboembolism in pregnancy complicated by genetic antithrombin deficiency. Thrombosis risk was delineated by propositus vs relative for antithrombin presentation, history of previous thrombosis, history of previous pregnancy-associated thrombosis, anticoagulation prophylaxis and antithrombin concentrate prophylaxis. Recommendations were made to proceed with similar data construction for all known thrombophilic traits.

Dr. Marilyn Manco-Johnson presented evidence for the role of thrombophilic genes in adverse pregnancy outcomes including preeclampsia, abruption, intrauterine growth retardation, recurrent miscarriage and fetal demise. General trends support the role of thrombophilic genes to increase the risk of adverse pregnancy outcome and to worsen existent disorders. There is a wide variation in presence and strength of these effects, diluting the power of metanalyses secondary to methodologic differences in case definition, sample size, referral patterns, eligibility criteria, and other genetic and environmental variations in the study populations. Examples include divergent results regarding the role of antiphospholipid antibodies in adverse pregnancy outcome. Two large studies of more than 1000 cases failed to support a relationship of adverse pregnancy outcome to factor V Leiden. Suggestions were made for prospective, multi-center studies with large numbers, standardized case definitions and outcome determinations.

IV. New Business

Future efforts will address HRT effects on coagulation and thrombosis risk, oral contraceptives and HELLP syndrome. The e-mail tree will be used to continue these discussions, identify new members and prepare for an open meeting next year.
Meeting of the SSC Working Group on Coagulation Secondary Standards

Chairman: Jane Lenahan, US

The annual meeting of this committee was held on 18 July, 2002 with 24 attendees.

The major topic of the meeting was the need to start arrangements to produce lot 3. Lot 2 does not expire until 30 June, 2006; however, the use of this product has increased changing the estimated need for a new lot to 2 – 3 years. Drs. Hubbard and Barrowcliffe will work with the chairman to find potential suppliers, hopefully within a few months.

Dr. Hubbard reported on the status (amount and stability) of Lot 2 and Dr. Kitchen reported on a NEQAS survey that included the SSC plasma, lot 2. Both reports are available by contacting Drs. Hubbard or Kitchen or the chairman.


Dr. Gray reported that there would be a WHO standard for Factor XI in 2003.

At the Fibrinolysis Subcommittee Meeting on 19, July, Dr. Declerck will discuss the possibility of the fibrinolysis committee carrying out the calibration of tPA and PAI-1.

VWF:CB will be discussed at our next meeting when there should be more information available.

The decision of the committee was that there was not sufficient use of assays for Factors V and XII to warrant having an action item as there is no WHO standard for either of these analytes.
Report on ISO TC 212 WG 1 meeting in Berlin 04 16-18, 2002

The main issue of the agenda was to check and decide about the comments obtained with regard to the prEN/ISO DIS 15189.2 i.e. the document now called Medical laboratories-Particular requirements for quality and competence. This standard was supposed to become a ‘stand alone’ accreditation standard for medical laboratories.

The project started in 1995 and has been much discussed during the years but had been principally finalized in the autumn 2000. During the last two years the document has been discussed outside the TC 212 and become aligned with ISO 9001, which thus is a normative standard. The standard for accreditation of general purpose laboratories and calibration laboratories is also a normative standard.

TC 212 decided to push the work on a standard for Quality management of POCT (point of care testing) and also to create a Guidance document for EN/ISO 15189. This document will give the background for the special requirement as needed for laboratory medicine (such as for diabetes and monitoring oral anticoagulant therapy etc.) and will thereby be of importance for the use of the standards. A preliminary version was presented at a meeting in Uppsala in June 2002. Now only a final revision needs to be done.

EN/ISO 15190 "Safety in medical laboratories" is ready for final voting.

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Report on CEN TC 140 meeting in Berlin 04 19, 2002 (as of July 2002)

The main subject for the meeting was to clarify some confusing items about half-ready and ready documents. Most of the time was spent on the standard External Quality Assessment (EQA) in relation to the mandating requirements on the vigilance in the post-market stage. It is intended that the EQA systems should be used to detect errors or deviations in the performance of different instruments and reagents. This is a sensitive issue as it is required by the standard and the EU IVD Directive that the organizers use certified reference materials.

Several work items are now finished, acknowledged and published. There is a long standing debate as to which requirements have to be fulfilled with regard to the labeling of the vacuum tubes-the industry refuses to accept the requirements for a single and commonly accepted color coding system for the stoppers.

Re the EN/ISO 15189 CEN was of the opinion that the new voting must be made as soon as possible and put in use without translation to French. In this way the voting within CEN could be made at the same time as that of ISO 212 and finished by now. The most important achievement during the last few years is the accreditation standard that will be accepted and used worldwide. Thus, all accredited laboratories will follow the same standard and their quality systems will be comparable for benefit of patients. This will increase the possibility to provide transferable results i.e. results that will be the same wherever the
samples are measured. Thus major industrial countries including USA, Canada, South American States, Australia, New Zealand, Japan, and the leading South East Asian and European countries have voted in favour of this standard becoming the accreditation standard for laboratory medicine.

Anders Kallner, Liaison Officer IUPAC
Margareta Blomback, ISTH Liaison to CEN TC 140
20 July 2002
World Heart Federation
Scientific and Standardization Committee
International Society on Thrombosis and Haemostasis

Council on Thrombosis

Minutes of the Meeting of the Council, Paris, 10 July 2001

Present: Mannucci (Chairman), Booth (Secretary), Bachmann, Badimon, Conard, Francis, Lassila, White
Apologies: Gulba, Ikeda, Kyrle, Lüscher, Ostojic, Verheugt

1. Welcome
The chair was taken initially by Booth and later by Mannucci. Igor Bokarew, President of the Russian Haemostasis Society, who was attending in order to gather information relevant to running a postgraduate training course, was welcomed.

2. Minutes
The minutes of the meeting in Washington, August 1999, were approved.

3. Report of Chairman
The current position of the council was summarised, most points being covered by up-coming items on the agenda, in particular the election of new members (item 3a), the activities of the training centres (item 4) and the planning of postgraduate courses (item 6). Booth explained the background to item 5 (Restructuring of WHF Councils), which was on the agenda because of its deferral from the last meeting. Papers to provide information on the proposals to the Council had been circulated with the agenda. In fact, the idea of restructuring WHF Councils had apparently been shelved, and there was no need for further discussion. Bachmann confirmed that the current funding was 20,000 Swiss francs per council per year, which was essential for our training work (see item 4).

3a. Election of new members
Badimon, a retiring member of Council, was thanked for her excellent contribution to the work of the council, and the other retiring members, not present, were also commended. It was proposed that the election of new members, delayed from 2000, should take place as soon as possible. Nominations were requested and further nominations would be solicited by White, as chair of the nominating committee, after which Booth would circulate all members and gather their votes. The new members would be considered as the class of 2001.

4. TVTC programme
Fedor Bachmann reported on the TVTC scheme, tabling a detailed report and details of current
finances (attached to the minutes). He emphasised that our remit was not to compete with American Heart and other national programs. Rather, our aims are in line with those of the WHF, to give assistance to developing countries where lack of money was a major constraint, and to focus on low-cost initiatives, for instance courses on rehabilitation after MI.

The TVTC program is providing grants for 3 months direct training in recognised centres. This has been very successful, participants often returning to set up laboratories and other facilities in their country of origin. It was noted that there was a pressing need for equipment and anyone with aggregometers, centrifuges etc should let Bachmann know.

Finances continue to be limiting, and our current monies stand at $43226.07. Bachmann made a strong case for annual support from ISTH, as one of our sponsoring bodies, of $15,000. He appealed for this case to be made to ISTH by all relevant members of council. Mannucci offered to write to Seligsohn and to Chapel Hill to try to get increased funding from ISTH; the stipend for trainees has not been increased for several years.

The second part of the training program concerns postgraduate courses in Haemostasis and Thrombosis for cardiologists and scientists carrying out cardiovascular research. These courses should be delivered by leading figures in the underlying science and in drug treatment. Thus a mixture of haemostasis and cardiological aspects should be covered.

5. Restructuring of WHF Councils
This is no longer planned; see item 3 above.

6. Postgraduate Courses
Mannucci reported that the meeting in Naples in May 2001 had been a success and thanks to Dr Giovanni Di Minno were recorded. The financial success meant that there were funds available for the use of the TVTC program. There are constraints on transfer of this money from Italy, but tickets can be purchased with it for support of travel (perhaps not exclusively to/from Italy) or to pay for travel of TVCT trainers in Italian Centers.

The possible content of further courses was discussed and it was agreed that there should be a good balance of haemostasis of relevance to clinical practice of cardiology, cardiac surgery and neurology. Bokarew expressed enthusiasm for organising the next course, in Moscow, St Petersburg or another venue, with perhaps 50 participants. Suggestions included having a geographical balance of speakers and having a format of lectures morning and evening, with free afternoons to allow good social interaction between faculty and students. Money for external speakers would be needed (the Naples surplus might be used towards this) and companies should be approached. Conard offered to help with the program.
7. Other business
Bachmann highlighted the fact that we should report to SSC, as in article III of our statutes. Francis welcomed this and it was agreed that Booth/Mannucci should arrange for this for the SSC meeting in Boston in July 2002.

8. Date and place of next meeting
This will be at the SSC Meeting in Boston, in July 2002.

Note added to minutes on 10 May 2002: There will not now be a meeting of the Council in Boston.

Nuala Booth, Secretary
The Thrombosis Vascular Training Centers (TVTC) provide training in the diagnosis, prevention and treatment of diseases related to arterial and venous thrombosis and primary (arteriosclerosis) or secondary diseases of the vascular system. TVTCs have been established throughout the world, some larger countries have several such centers. There is a TVTC directory primarily intended as a help for thrombosis/vascular traineeship candidates. Applications for a TVTC traineeship must include a curriculum vitae, a bibliography and a notarized copy of a valid professional license. Recommendation letters of three referees who are familiar with the work of the candidates are sent directly to the director of the TVTCs programs. Successful candidates receive an APEX fare air ticket and a monthly stipend of US$ 1400 to 1600. At present traineeships are limited to a maximum duration of three months and are offered only to candidates from developing countries who are cardiologists, neurologists or scientists working in close collaboration with cardiology or neurology services.

In 2000 five trainees who had been awarded a fellowship in 1999 spent a period in one of the recognized TVTC. Three of these are physicians interested in cardiovascular disease, the other two are PhD’s performing basic research in, or are collaborating with a cardiology center. Their countries of origin were Georgia, Hungary, India, Iran and Russia. The chosen TVTCs were the Thrombosis Research Centres of the University of Milano (2) and of the Mayo Clinic in Rochester, the Dept. of Cardiology of the Karolinska Hospital, Stockholm and the Centre for Thrombosis and Vascular Research, Randwick, Australia. In 2000, four traineeships have been awarded to candidates from Colombia, India, and Russia (2); one application is pending. Training will take place during the year 2001.

The following three organizations are the main sponsors of this program which costs about U.S.$ 5,500 to 6,000 per candidate: World Heart Federation, International Society of Thrombosis and Haemostasis and the Sanofi Association for Thrombosis Research. Most of the trainees are junior faculty members in academic institutions and very likely future leaders in their field. With a few exceptions, the reports of the trainees on their learning experience have been enthusiastic. One trainee wrote: "the TVTC fellowship was the most outstanding event in my life".

As of Dec. 31, we had $ 57,656 in the account, administered by at the ISTH headquarters. The 4 candidates who have been awarded a TVTC fellowship in 2000 will start their training in 2001. In 2001 two traineeships have been awarded so far. Presumed expenses approx. $ 22,000. Excellent applications continue to be received and we project to fund another 2 to 4 new candidates in 2001.

E-mail messages, faxes or letters were sent to all directors of TVTCs in 1999/2000 requesting
updating of the 1997 directory listing. Of the 25 Centers 18 have responded so far. It is intended to publish on the website of WHF and of ISTH the updated directory in Summer 2001.

F. Bachmann, July 4, 2001