47th Annual Scientific and Standardization Committee Meeting

July 6 – 7, 2001

Paris, France
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Animal, Cellular and Molecular Models of Thrombosis

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

The meeting was chaired by G. Johnson at the request of Chairman, P. Carmeliet

Attendance: ~100 persons

Scientific Presentations:

P. Jagadeeswaran, San Antonio, reported on innovative studies of hemostatic parameters and thrombosis in zebrafish. Using techniques developed in his laboratory, Dr. Jagadeeswaran has been able to perform clotting assays on extremely small volumes of blood, evaluate platelet aggregation and induce thrombosis. Anticoagulant effects on hemostasis and thrombosis and platelet inhibitory drugs have been evaluated. Genes for all the human coagulation factors, except Factors VIII and IX, have been identified in the Zebrafish. An additional Factor VII-like gene has also been identified. Combination of the functional assays with the enormous potential of the zebrafish for rapid genetic analysis make this model a very promising one for analysis of the complex interactions of multiple proteins in hemostasis and thrombosis.

G. Johnson, Minneapolis, presented an overview of the signaling defect that exists in thromboxane-insensitive dog platelets. This defect in activation of PLC-beta results in impaired secretion stimulated by thromboxane A2 or thromboxane analogs. The cause of this signaling defect appears to be an elevated level of basal thromboxane receptor phosphorylation that is reversible by epinephrine. Thromboxane-insensitive dog platelets are a useful model for the study of the control of G protein-coupled receptors by phosphorylation.

L. Drouet, Paris, reported progress on the development of a pig model of unstable atherosclerotic plaque. Homozygous, LDL mutant, hypercholesterolemic pigs with spontaneously developing atherosclerosis had carotid stenosis lesions induced by application of an externally constricting collar combined with a ligature. These lesions develop flow reduction and down-stream thrombosis. The effects of angioplasty and antithrombotic agents on these lesions has been evaluated. Some spontaneous plaque rupture develops in this model when these animals are fed an atherogenic diet. Additional studies are in progress to further refine this very promising model.

Berend Isermann, Milwaukee, reported on a series of studies performed in mice with targeted disruption of genes for components of the thrombomodulin\protein C pathway. These studies have highlighted the contributions of components of this pathway to normal in-utero development. Thrombomodulin expression is required in the placenta during mid gestation, before development of a functional cardiovascular system. During the second half of pregnancy and at birth, embryos with disrupted hemostatic systems often succumb to hemorrhage or thrombosis. Several successful strategies have been developed to circumvent the intrauterine lethality of mice with defects in the thrombomodulin\protein C pathway. Animal models with defects in the thrombomodulin\protein C pathway will be very useful in the identification of
Old Business: The manuscript, The Utility and Limitations of Animal Venous Thrombosis Models, approved for publication by the SSC at the 2000 meeting in Maastricht, has been revised to meet the editorial criteria of Thrombosis and Haemostasis. It has been reviewed and approved by the co-authors and the Chairman. It will be resubmitted to Thrombosis and Haemostasis as an official SSC publication.

New Business: Discussion of future activities included critical evaluation of the important characteristics of animal models that must be considered in the selection of appropriate models for the study of research topics, such as arterial thrombosis in large and small animals. Characteristics, such as levels of hemostatic parameters, availability of antisera, functional characteristics of platelets and endothelium, and other important parameters would be compiled and made available via a registry. A particular need exists for this type of information for mouse models. Subcommittee members will submit proposals for this type of analysis to G. Johnson who will coordinate this activity for the Subcommittee. A goal will be to have a draft developed for submission to the Subcommittee at the 2002 meeting.
Biorheology

Chairman: S. Diamond--USA
Co-chairmen: M.M. Frojmovic--Canada; D. Gabriel--USA; M. Hoylaerts--Belgium;
C. Li--USA; K. Preissner--Germany; M. Hoylaerts--Belgium

Invited Talks: T. Diacovo (USA), M.B. Lawrence (USA)

Professor Scott Diamond opened the session with introductory remarks noting the emergence of
tools to investigate single receptor-ligand bonding kinetics under applied mechanical loads such
as hemodynamic shear forces. Quantitative kinetic and mechanical descriptions of bonding and
debonding of individual receptor-ligand chemistries during homotypic and heterotypic
interactions provide a starting point for analysis of cell mediated coagulation under
hemodynamic forces. Studies of individual chemistries is required before multiple receptor-
ligand pair interactions in the contact area can be defined from an adhesion strength perspective.

Symposium:

Professor K. Preissner presented an overview of neutrophil-endothelium and neutrophil-matrix
interactions with a focus on CD11b/CD18 (Mac-1). Using a thioglycollat injection to the
peritoneum as a inflammation/extravasation model in uPAR(-/-) mouse followed by peritoneal
lavage at 1-4 hr, a 30 to 50 % decrease in neutrophils in the KO mouse was noted relative to wt
mouse. Also, large decreases in eosinophil and monocyte levels were noted in the KO mouse.
This suggested an important role of uPAR interactions with Mac-1 where uPAR may modulate
the avidity of Mac-1. uPAR may exert a "preactivating" effect on beta2-integrins without effect
on beta2-integrin antigenic levels on the cell surface. In static adhesion assays, the interaction of
uPAR with vitronectin was enhanced by zinc ion and uPA. It was noted that free zinc ion exists
in platelet granules and can be released at sites of thrombosis. Only 1 to 2 % of the 50 uM total
zinc in plasma is considered free. The role of domain 5 of kininogen especially H475-486 binds
uPAR and interferes with uPAR preactivation of Mac-1. The effect of flow on local zinc ion at
the site of local platelet adhesion remains to be determined.

Professor M. Frojmovic presented an overview of platelet surface receptors for vWF, fibrinogen
and thrombospondin (TSP-1). Importantly the alpha granule contains fibrinogen, TSP-1,
fibronectin, vWF, 2b/3a (20,000 copies), and P-selectin. Release of granules by washed platelets
in buffer is sufficient to provide fibrinogen and vWF for aggregation in flow conditions.
Measurement of initial singlet consumption in a sheared suspension of platelets in a couette
viscometer allows determination of the collision efficiency which typically ranges from 0.01 to
0.3. Using beads for presentation of individual ligand to the platelet allows probing of specific
receptor-ligand interactions. It was convincingly shown that resting platelets have no binding
interaction to fibrinogen immobilized to the bead surface. Activated platelets are captured by
fibrinogen presented by immobilized 2b/3a beads with an efficiency of about 0.1. Interestingly,
beads with immobilized active 2b/3a can bind fibrinogen which in turn binds TSP, allowing the
creation of a remarkable 169 nm linkage between beads.
Professor T. Diacovo demonstrated using recombinant A1 domain of vWF that the platelet GPIb bond with A1 domain lasts for under a second when subjected to hemodynamic forces. Values of the dissociation rate of about 5 s⁻¹ indicate that the bond is short lived. Additionally, work with the 2B mutant A1 domain showed that the GPIb-2B A1 domain interaction was about 10 times longer lived. Furthermore, a critical level of shear stress was needed (about 1 dyne/cm²) to maintain platelet rolling on A1 domain. Platelets, at shear stresses above 1 dyne/cm², maintained stable rolling which was rapidly destabilized when the shear stress was dropped below 0.3 dyne/cm². This rapid reversal of adhesion with a decrease in flow was mediated by a monomeric A1 domain that was physisorbed or biologically linked to the surface. This finding suggests that flow does not effect the A1 domain. Placement of the A1 domain on beads suggested that the dissociation process of GPIb-A1 is very similar to that of P-selectin-PSGL-1.

Professor M. B. Lawrence presented work on the membrane deformation of neutrophil microvilli during rolling on selectin coated surfaces. Beads coated with PSGL1 and fixed neutrophils had very similar bond breakage kinetics as described by the Bell Model, however, unfixed neutrophils had different kinetics for the breakup of the bonds. This was shown to be due to the extension of membrane tethers which were pulled at longer lengths at higher shear forces. These tethers were clearly detected by SEM as well as by DIC light microscopy. The tethers are pulled as viscoelastic elements for short periods of time and then undergo viscous deformation. Extension of the tether enhances the lever arm and thus reduces the forces experienced by the bond. Membrane tethers thus shield the bond from loading and enhance the bond life. The Bell model remains the standard for analysis of force induced debonding (koff = koff(0) exp (r Fb/Kb T) where koff(0) is the zero stress off-rate, r is the reactive compliance, Fb is the force on the bond, Kb is the Boltzman constant, and T is the temperature. Membrane deformation alters lever arms which in turn changes Fb, the force on the bond.

Professor S. L. Diamond explored the role of neutrophils in fibrin formation. Neutrophils adherent to fibrinogen coated surfaces led to massive fibrin deposition after 15 min of perfusion of recalcified PFP. This deposition was substantially reduced with anti-b2 antibody and by corn trypsin inhibitor to block XIIa pathways triggered by contact activation. Almost no fibrin was detected after 15 min perfusion of CTI-treated recalcified plasma over neutrophils adherent to fibrinogen. Similarly, no fibrin was detected after 15 min perfusion of CTI-treated recalcified plasma over platelets adherent to fibrinogen. In contrast, large amounts formed under these conditions when CTI-treated plasma was perfused over p/n mixtures adherent to fibrinogen. This fibrin formation was reduced by inhibitors against elastase or cathepsin G. This suggested that neutrophils and release proteases that activate platelets that are spread on fibrinogen. It was noted that Dacron graft models where fibrin deposition is reduced by anti-p selectin may be due to the fact that the Dacron promotes fibrinogen deposition in the absence of collagen and neutrophils may help cause greater levels of platelet activation after their spreading on fibrinogen as indicated by PS, calcium, and prothrombinase activity.

Professor M. Hoylaerts gave an overview of monocyte-platelet interactions as well as the role of monocyte-tissue factor in stirred blood coagulation. Through measurements of thrombin activity it was determined that WBC-platelet interactions were important for thrombin generation in a rotated plate assay.
SUMMARY

The Subcommittee will help to standardize receptor-ligand assays that seek to define single bond mechanics in cellular and cell-mimic systems under flow conditions that are physiologically relevant. With definition of individual bond dynamics, it may become possible to understand cell contact area dynamics where 3-5 different bonding mechanisms are holding the cells together. Further emphasis will be given to phenotypic performance of blood components in the context genotyping and phenotyping chip and microarray data.
Contact Activation

Chairman: I. Schousboe--Denmark
Co-chairmen: M. Berrettini--Italy; R. De La Cadena--USA; M.J. Gallimore--UK;
H. Saito--Japan; A.H. Schmaier--USA; A. Zivelin--Israel

The session on Contact Activation continued the discussion whether there is a correlation between decreased concentration of FXII and thromboembolism. In addition, since numerous new investigations have indicated that the kallikrein-kininogen system plays a major role in cellular as well as vascular development, we found it of great importance to focus also on these new observations which may be relevant to many unanswered clinical problems.

Venous thrombosis: Whether there is a correlation with venous thrombosis seems to depend upon the methodologic approach. A common denominator over the years is a frequency in normal individuals of 1-3 % having less then 50 % of FXII, while the frequency of FXII-deficiency among thrombotic patients is in the range of 7-10 %. An update from the Italian Registry of Congenital Coagulopathies indicates that the deficience of FXII measured by the classical APPT-test did not correlate convincingly with venous thromboembolism; however, this last study was prospective in nature and it seems to suggest that FXII-deficiency alone is not a risk factor for thrombosis.

A deficiency of FXII was reported to be caused by a genetic polymorphism (46C/T in exon I of the 5 prime untranslated region) located close to the AUG translation codon of the FXII gene.

Also a significant number of thrombotic patients, when compared to normals, were deficient in either HK or prekallikrein.

The importance of FXII as a participant of an alternative pathway for plasminogen activation supports a correlation between Factor XII deficiency and thromboembolism. However, recent investigations have identified HK and PK as anti-thrombotic and the FXII and the HK/PK system is interrelated by activation. Both FXII and HK have been shown to be inhibitors of platelet activation.

Myocardial infarction: Studies have reported elevated levels of activated FXII (FXIIa) in patients with myocardial infarction. Although the method used in this study measured FXIIa, questions from the audience were related to the nature of the compound being measured. Nevertheless, this observation involves variations in concentration of a form of FXIIa circulating in plasma and may represent a risk factor for myocardial infarction. The levels of this nature of FXIIa appeared to increase further upon thrombolysis in patients with MI.

Regulation of cellular activity: The severity of FXII deficiency caused by the presence of auto antibodies seems to be far more serious than just the genetically determined deficiency. In a significant number of patients with Lupus-anticoagulants (LA) and primary anti-phospholipid antibody-syndrome (APS) the auto-antibody has been identified as anti-FXII antibody. Some of the features of these syndromes are thrombosis and fetal loss. While no correlation is found between antibodies to cardiolipin and/or β2-Glycoprotein I and recurrent fetal loss, a strong
correlation was observed with the presence of anti-FXII antibodies. This suggests that FXII has a role in cellular development. Further studies are required to complement these observations, namely histology of the placentas from those individuals afflicted by recurrent fetal loss as well as the determination of cross-reactivity of these antibodies with plasminogen. It was noted that these antibodies do not cross-react with prothrombin.

In a controlled animal model of APS (NZW x BXSB F1 mice), auto-antibodies to HK were identified, providing an additional explanation for a thrombotic manifestation in APS. HK-knockout mice confirm that the kinin-kallikrein system is anti-thrombotic, pro-inflammatory and involved in angiogenesis. Anti-angiogenic properties of HK were Zn\(^{2+}\)-dependent and limited to HKα and not to its single chain form in cell culture experiments. The inhibition was associated with apoptosis. The anti-angiogenic effect was mapped to the C-terminal region of the cell-binding domain, domain 5. The mechanisms behind these regulations remain to be determined. They may be several.

Additionally, a product of proteolytic cleavage of bradykinin by angiotensin converting enzyme (ACE) has been found to have anti-thrombotic properties. This proteolytic product corresponds to a pentapeptide, RPPGF.

**Factor XI:** Structure-function studies using recombinant proteins and monoclonal antibodies confirm regions within the molecule important for interacting with platelets, heparin, HK and FIX. Unlike previous mapping studies using synthetic peptides derived from different apple-domains, recombinant proteins revealed that apple II, and not apple I, is important for HK interaction; however, these findings did not rule out the possibility for apple I and apple II to participate in the interaction with HK and are thus in agreement with the data obtained using synthetic peptides. An interesting finding was that apple III is capable of binding both to platelets and FIX, thereby providing a reason for the dimeric nature of the FXI molecule. Finally, the FXIIa substrate binding region has not been identified yet by this last study.

The functional aspect of FXI as reflected by natural mutations and acquired inhibitors was discussed. It was noted that the mutation could be connected to either synthesis, secretion or activation. Several mutations affect Cys- Cys bonding. The mutation Cys\(^ {35} \Rightarrow \text{Arg} \) in BHK transfected cells is associated with a lack of secretion. Another mutation not involving the Cys-Cys bonding, namely the Gly\(^ {555} \Rightarrow \text{Glu} \), which is located within the catalytic domain, is associated with the presence of the antigen but with loss of function. The loss of function of this last mutation was determined by a combination assay where ultimately FXa activity was measured by a chromogenic substrate.

It was emphasized that all but one FXI-deficient patient had developed antibody inhibitors against FXI after transfusion. These antibodies were long-lasting. The majority of the antibodies (61.5 %) were associated with type II mutations. Thus, future studies aiming at a characterization of new mutations should start by evaluating the presence of type II mutations.

It was proposed that the molecular basis of platelet FXI-deficiency should be further analysed in view of the knowledge of unpublished data contradicting a result from a single published report.
Conclusions: The presentation of several animal models in this session indicates that the significance of proper plasma concentrations of FXII, HK and PK has an impact on the vascular system. It has been proposed that in order to correlate FXII, HK and PK deficiencies with thromboembolism, the studies have to focus on individuals who have developed the disease as opposed to prospectively trying to identify these deficiencies as risk factors. The association with and activation of FXII and the HK/PK complex on the cell membranes, including the vascular wall, appear to have a hitherto unrecognised significance in cellular development, namely apoptosis, angiogenesis and possibly proliferation and differentiation. The newly observed properties of FXII and the kinin-kallikrein system indicates that these zymogens of proteolytic enzymes are involved in a variety of cardiovascular diseases and mutations in these proteins or activation disorders may result in serious pathological conditions related to the vascular system.

The Working Group to establish plasma standards for determination of FXII, HK, PK and FXI recommends that this work be considered a low priority task. This Working Group suggests high priority to establish a name other than "Contact Activation", which more precisely defines the function of FXII, XI, PK and HK in the vascular system.
Control of Anticoagulation

Chairman: M. Greaves--UK
Co-chairmen: T.W. Barrowcliffe--UK; H. Bounameaux--Switzerland; J. Harenberg--Germany; C. Kearon--Canada; F.E. Preston--UK; F. 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 THROMBOSIS
S. SCHULMAN and C. KEARON, Chairmen

L. PINEDA
Three months vs. 6 months for first DVT (DOTAVK Study)

A report was presented on a study published in the May issue of Circulation. This was an open label, randomized, controlled trial of duration of anticoagulation for objectively confirmed first VTE. Calf DVT subjects were randomised to 6 and 12 weeks of warfarin and proximal DVT/PE to 3 vs 6 months. Enrollment ceased after patient 736, 539 patients were included with proximal DVT/PE. In this group the recurrence rate was similar in the two arms at around 8%. Serious bleeding occurred in around 2% in each arm. Higher recurrence rates were noted in those with idiopathic events than temporary risk factors. It was concluded that for proximal DVT/PE there was no advantage of 6 months of OAC over 3 months.

G. RASKOB
Oklahoma Optional Duration Studies

These studies report that patients with a first episode of idiopathic DVT have an annual recurrence rate of around 12% per year for years 1-3 after stopping OAC at 6 months. Extended treatment is effective but the annual risk of major bleeding during years 1, 2 and 3 was around 6%, 2%, and 0%, respectively, offsetting at least some of the benefit. It was concluded that indefinite treatment may be of benefit for those patients at low risk of bleeding who prefer to continue therapy.

C. KEARON
Recurrence rate after 27 months of OAC therapy.

A follow-up to the study published in the New England Journal of Medicine was reported. 3 months of OAC was compared with 27 months after first episode of proximal DVT. Extended follow-up of those treated for 27 months (n=116) and untreated for 10 months was reported. Recurrence occurred in 13%, this being lower than in the cohort stopped after 3 months. (It is noted that the recurrence rate was unusually high in that group, however.) It is concluded that long-term treatment after a first idiopathic event is an option, but the significant risk of bleeding must be considered.

G. AGNELLI
WODIT DVT and WODIT PE studies.
In the DVT study, the initial advantage of 12 over 3 months of OAC treatment was lost eventually as the recurrence rate caught up in the longer treatment arm. In PE there was no difference in recurrence rates between those treated for 6 and 12 months (around 7% in each arm). A lower recurrence rate was again noted in those with temporary risk factors.

Dr. Kearon summed up the results of studies to date. Although OAC therapy is very effective at preventing recurrence, there is an average 10% per annum recurrence rate after discontinuation, regardless of the duration of therapy (3 months treatment in his own study giving an unusually high recurrence rate compared with 3 other studies). It was concluded that the minimum duration of therapy to achieve the lowest recurrence rate is 6 and possibly 3 months. This lowest achievable rate of recurrence may be as high as 10% per year.

During discussion the question of use of less intense anticoagulation for longer periods was raised. Dr Kearon reported that a study of target INR of 1.75 vs. 2.5 had just completed enrollment.

The Chairman congratulated the speakers on their excellent efforts to provide an evidence base for the management of venous thromboembolism.

WORKING PARTY ON NEAR-PATIENT TESTING AND SELF-MANAGEMENT OF ORAL ANTICOAGULANT CONTROL
F.E. PRESTON, Chairman

S. KITCHEN
Proficiency testing of near-patient devices.

It was reported that although there is a spread of INR results in QA exercises in near-patient testing, this spread is no broader than that in hospital laboratories. Some outlying results are due to inappropriately handled QC materials. It was demonstrated that improved performance could be achieved in centres by continuing enrollment in external QA exercises. Results of health care providers improved with time, as did those of patients using self-management.

It was concluded that external assessment of near-patient testing is achievable and that appropriate EQA materials equivalent to whole blood can be developed for use with near-patient devices.

J. ANSELL
Clinical Trials.

Results of 13 clinical trials of PST/PSM (Patient Self Testing/ Self Management) were reviewed. In each case outcome was at least equivalent to comparator (usually a hospital-based clinic). Time in therapeutic range averages 77% in PSM studies compared with 72% in the anticoagulant clinic and 50% in unstructured anticoagulant management.
It was hypothesized that the improved outcomes with PST/PSM are due to increased access to and more frequent testing as well as improved consistency (use of the same equipment for each test) and better understanding and empowerment of the patient in their own care.

Barriers to adoption of PST/PSM were considered to be lack of physician awareness, unfounded concerns regarding safety and efficacy, lack of training opportunities and funding difficulties.

M. SPANNAGL
Update on ISO TC212

The properties of ISOs were described and a report given of a meeting held in Dublin earlier this year. Common issues between self-testing for blood glucose and INR were identified, including performance, validation, QA and training issues.

H. JOIST
NCCLS Update

An update of the programme of the NCCLS (National Commission on Clinical Laboratory Standards) on both heparin and warfarin monitoring was presented.

WORKING PARTY ON CALIBRATED PLASMAS FOR INR DETERMINATION
T. BARROWCLIFFE, Chairman

It was reported that the guideline is close to completion. There was a continuing debate over use of linear or orthogonal regression for setting of calibration curves. The chairman reported that statistical expert advice had been solicited but no consensus had been achieved. A meeting of the working party will be scheduled during the ISTH 2001 meeting in order to resolve this issue. Progress is essential here.

WORKING PARTY ON LABORATORY MONITORING OF LOW MOLECULAR WEIGHT HEPARIN
M. GREAVES Chairman

H.C. HEMKER
The ETP Test as a global method for anticoagulant control.

The extensive laboratory validation of this approach was reviewed. It was emphasised that all antithrombotics tested influence the ETP. The relationships between APTT and ETP during heparin therapy and INR and ETP on warfarin were illustrated. Professor Hemker highlighted the technical simplicity of this approach as well as its sensitivity to all antithrombotics and to mixed treatments.

J. FAREED
Monitoring of high dose LMWH heparin and newer agents.
New data on monitoring of the anticoagulant effect of high doses of LMWH, such as those used in unstable coronary syndromes, were reported. It was concluded that there are clear limitations of anti-òXa assays and that the ACT correlates with anticoagulant efficacy and bleeding. It was also suggested that the global anticoagulant effect can be assessed using a standardised APTT method.

J. HARENBERG
Relationships between anticoagulant effect and Marder score.

It was reported that, in two studies, the anti-Xa level by chromogenic assay was higher in those subjects treated with LMWH in whom the Marder score improved, compared with the level in those with no improvement in Marder score. The D-Dimer level was also lower at day 12 in those with improved Marder score. Use of UFH was associated with comparable D-Dimer results but not anti-Xa results. These data are important as they are one of the few reports of a relationship between anticoagulant efficacy of LMWH and anti-Xa level.

P. MASSICOTTE
Status of paediatric guidelines

The special considerations in relation to anticoagulant therapy in infants and children were emphasised. The higher doses needed in infants of less than 3 months of age than older children were noted. The presenter favoured the use of anti-Xa assay for monitoring of LMWH dosage in paediatric practice but acknowledged the need for the performance of randomised trials to assess the safety and efficacy in relation to anti-Xa level.

In relation to UFH use, it was reported that over 70% of APTT values do not match with anti-Xa levels. A plea was made for clinical studies of standard doses of UFH compared with lower doses in paediatrics as there is a perception of an unacceptably high rate of bleeding using standard doses.

M. GREAVES
Status of adult guidelines.

It was confirmed that these had reached an advanced stage and the principal contributors had agreed on the content. The limitations of anti-Xa assay in the assessment of antithrombotic efficacy and bleeding risk in treatment with LMWH had been conceded. These limitations relate largely to the role of other mechanisms in the antithrombotic effect, variation between heparin preparations, the poor standardisation of assays and calibration issues. The chairman confirmed that the final version should be approved imminently. He also urged caution in relation to promoting the routine use of anti-Xa assays in any situation of monitoring of LMWH and suggested that the major limitations of this approach to monitoring be borne in mind.

ORAL ANTICOAGULANT MONITORING
7th July, 08:00-13:00
A.H.M.P. van den BESSELAAR & L. POLLER, Chairmen
A.H.M.P. van den BESSELAAR
A method for citrated plasma samples on CoaguChek and TAS whole blood PT monitors

Calibration of 3 near patient PT devices for whole blood according to WHO ISI procedures is not practicable because it would require parallel samples of blood and citrated plasma from a large number of subjects. A new procedure is required, therefore. Plasma would be preferable but it must be demonstrated that plasma recalcified with a suitable concentration of calcium chloride gives satisfactory results. A study was conducted to show this. Mean plasma PT was higher than mean whole blood PT for all calcium concentrations tested. Further work is needed in deriving a procedure which will allow ISI calibration of individual instruments and permit expression of results as INR.

A. TRIPODI
A method for determination of ISI of 2 whole blood PT monitors using citrated plasmas.

Calibrant plasmas have been developed by the ECAA, but it is important to validate these for PT monitors which use whole blood. In 3 centres whole blood and plasma were used to calibrate using three home PT monitors: CoaguChek Mini test strip, CoaguChek Low ISI test strip and TAS PT-NC test cartridge. CV of slopes and test lines were <5%. ISI on plasma were less than those on blood but there was reasonable comparability with two of the three systems. A further study is in progress to investigate the greater difference with the TAS system.

M. KEOWN
An assessment of PT and INR variability of CoaguChek Home and TAS Near-patient testing PT monitors.

A QA study of 28 instruments of two different manufacturers (Brands A and B) was performed. The same operator and samples were employed. The samples were 2 pooled coumarin plasmas, 3 lyophilised depleted plasmas and blood and plasma from 3 normal adults. The results demonstrated problems of instrument variability, largely due to duplication problems with both brands of monitor, giving cause for concern in terms of patient care and oral anticoagulant dosage.

L. POLLER
The European Concerted Action on Anticoagulation (ECAA) Computerized Dosage Clinical End Point Study

The ECAA performed a randomized prospective study of dosage using Dawn AC to compare the results of computer dosage with those of experienced medical staff at 5 centres. With all INR ranges over the whole period the time in range was 72% for computer and 59% for medical staff dosing.

Professor Poller reported on a clinical end-point study being conducted at over 40 European centres. Dawn AC and Parma 4 are to be employed. Target recruitment over the 4 year period after induction will be 16,000 patients.
J. HARENBERG  
Preliminary results on a quality of life questionnaire.

A non-randomised study of patients using self-testing and INR control who had been on OAC for 5 to 6 years at enrolment demonstrated that there was improved independence, organisation of vacation time, self-assurance and plans and prospects.

**0RAL ANTICOAGULANT REVERSAL**  
FE. PRESTON, Chairman

**M. MAKRIS**  
Use of factor concentrates and FFP

The relative merits of use of FFP and factor concentrate to reverse of the effect of OAC in subjects with life-threatening bleeding were discussed. In those with INR >5 the factor IX concentration is generally < 10%. In serious bleeding it is necessary to correct this to normal levels. This would require 3000 mls of FFP in a 70 kg individual. This dose is difficult to administer and cannot be delivered rapidly. It is concluded that factor concentrate (II, IX, X, {VII}) must be administered, along with vitamin K, for anticoagulant reversal in life-threatening bleeding. Centres responsible for the management of OAC therapy should have appropriate material available. Although there is a risk of thrombosis associated with use of these products, in the situation of life-threatening bleeding this is more than balanced by efficacy. Dr. Makris suggested that a reporting system for thrombotic complications should be established.

**H.G. WATSON & M. CROWTHER**  
Oral and intravenous vitamin K

Dr. Watson reported on a 3 centre non-randomised study of intravenous versus oral vitamin K for OAC reversal in non-life threatening situations. In 64 subjects with INR above the therapeutic range there was clear evidence for more rapid reversal in those given intravenous vitamin K. Factor levels and INR showed a significant response after 4 hours with IV but not oral vitamin K. There was also some evidence for differences in responses to various oral formulations. It was concluded that vitamin K should be administered intravenously when reliable and rapid response is required.

Dr. Crowther reported on a study in which 114 subjects with INR >6 were followed for 2 weeks without intervention. 11 sought attention for bleeding and there were 5 major bleeds with 2 fatalities (Arch. Int. Med. 160:1612, 2000). The OVVAL I study was also reported. In 92 subjects with INR 4.5-10 given 1mg Vitamin K or placebo orally, the INR was 1.8-3.2 in 56% and 20% on the following day, respectively. Finally, preliminary data on subcutaneous administration of vitamin K were presented. In 51 subjects, by the next day the proportions in therapeutic range were 58% versus 24% for oral. These data suggest that the subcutaneous route is less efficient.
WORKING PARTY ON STANDISATION OF METHODS TO DETERMINE DIRECT THROMBIN INHIBITORS.
J. HARENBERG, Chairman

E. GRAY
Analysis of data from the thrombin inhibitor study

A preliminary analysis was presented. Seven methods were examined on 4 sets of plasmas. Sensitivity and robustness of methods were examined. Using most methods clotting times differed between the same concentrations of hirudin and argatroban. A more detailed analysis will follow.

J. WALENGA
Developments in the monitoring of direct thrombin inhibitors

Results of detailed studies were presented. It was concluded that the APTT is not reliable for monitoring of all thrombin inhibitors due to variable sensitivity. New methods, such as ECT and celite ECT are being developed but individual calibration curves are required. Also, drug interactions between thrombin inhibitors and other antithrombotics occur and should be measurable by the assay used for monitoring.

REPORTS ON WHO WORKING GROUP ACTIVITIES ON UNFRACTIONATED HEPARIN AND LOW MOLECULAR WEIGHT HEPARIN.
T. BARROWCLIFFE, Chairman

E. GRAY
A pilot study of comparability of LMWHs

8 LMWH are now licensed, with anti Xa:anti IIa of 1.5-10. A report was given on the relationships between these LMWH and UFH and a search for candidates for a new LMWH standard. 10 coded samples, including the first IS for LMWH and the 5th IS for UFH, were distributed to 9 laboratories. Each laboratory performed 4 independent assays. The 2 standards generally assayed well against each other but there was wide inter-laboratory variability. Four of the 8 LMWH were identified as potential standards to replace the 1st LMWH standard. It was concluded that UFH should not be used as a calibrator for anti-IIa/Xa activities of LMWH due to non-parallelism and poor reproducibility.

A. PADILLA
Anti-IIa chromogenic assay protocol and assessment

An outline protocol was presented

HEPARIN-INDUCED THROMBOCYTOPENIA
H. BOUNAMEAUX, Chairman
S. AHMAD
Endogenous factors contributing to generation of heparin-PF4 antibodies

Some clinical observations were reported. It was noted that IgG antibodies, rather than other species, were associated with thrombosis. A high rate of anti-PF4 antibodies in knee surgery patients was noted, possibly due to activation by thrombin generation. The possible role of various inflammatory markers in HIT was reviewed.

B. CHONG
Report on a survey on testing for HIT & Essential criteria for diagnosis

Dr. Chong indicated the need for standardisation in view of the wide range of assays in use. He presented results of a survey of the tests used by 42 expert laboratories and 23 routine laboratories. The laboratories were asked to investigate 6 sera (2 neg, 2 weak and 2 strong positives) by their usual methods. Expert laboratories were correct in 86-90% of cases. Many routine laboratories failed to detect weak positive antibodies.

In expert laboratories both ELISA and functional assays were used. ELISA performed well, but 1 gave some false positives. Aggregation tests performed well when selected donors were used, but not random donors. Overall ELISA and functional assays had equal specificity, but the ELISA was a little more sensitive. In the routine laboratories ELISA results were good but aggregation tests were unreliable and insensitive.

Dr. Chong also discussed criteria for diagnosis of HIT and proposed a new scoring system which included both clinical and laboratory parameters. This provoked considerable discussion and should form the basis for standardization of diagnosis.
The meeting of the SSC DIC subcommittee consisted of two parts: 1) an overview on recent progress in animal models of DIC and 2) a discussion on the definition of DIC, scoring criteria for overt and non-overt DIC and establishment of a scoring system.

The first part of the meeting consisted of a mini-symposium on animal models of DIC. Dr. Asakura (Japan) discussed differences between endotoxin-induced and tissue factor-induced DIC in rats. Dr. Rijneveld (the Netherlands) presented data on the cross-talk between fibrinolytic proteins and inflammation and infection in transgene mice models. In the paper of Dörfller, Levi and Carmeliet (Switzerland, the Netherlands, Belgium) the hypothesis that subjects with thrombophilia are potentially more vulnerable to severe DIC was supported by observations in mice with a one allel targeted deficiency of protein C. Dr. Taylor (USA) discussed the dynamics of coagulation and inflammatory parameters in his model of sepsis in baboons, clinical and laboratory effects. Lastly, Dr. Jilma (Austria) gave a comprehensive overview of the effect of various anticogulant interventions in models of human endotoxemia.

M. Levi started the discussion in the second part of the meeting by providing an historical overview of the work of this subcommittee to establish a definition of DIC and a diagnostic scoring system. The activities of the special working group between 1999 and the present meeting were summarized. A working group, consisting of 10 active subcommittee members, convened a number of times to discuss a consensual definition of DIC and a practical (diagnostic) scoring system for the syndrome. A small writing committee summarized the results of the discussion in a draft report. This was sent to over 50 experts in Hemostasis/Thrombosis or Critical Care Medicine. More than 30 experts have sent extensive reviews of the draft proposal. The draft proposal consists of a background position statement (one concept and six considerations) followed by a definition of DIC. Along these lines a diagnostic scoring system for overt DIC is proposed. In addition, a template for a scoring system for non-overt DIC is added that can be further developed depending on the specific needs of the user. Dr. Levi demonstrated that, in an initial prospective evaluation and validation of the scoring system for overt DIC, the scoring system was not only feasible in clinical practice but had a 92-98% concordance with the clinical diagnosis of DIC.

Dr. Hoots (USA) summarized the objectives of the subcommitteeis proposal and outlined in detail the scoring system for overt DIC. The scoring system consist of a simple algorhythm, using widely available coagulation tests.

In the general discussion following these presentations some issues were raised by the audience, including the validity of the score for patients that have undergone cardiac surgery or patients with advanced liver disease. These concerns diminished when it was taken into account that patients need to have a classifying diagnosis known to be associated with DIC to enter the scoring system, although liver disease remains a problematic area. Another issue regarded the
inclusion of clinical criteria, such as organ failure and bleeding, into the score. At the urge of critical care specialists, these items were left out of the score, since the DIC score is likely to become part of organ failure scores, which is impossible if organ failure itself is part of the DIC score. In the prospective validation of the score, special attention will be given to this matter.

Overall, the subcommitteeis proposal was received with enthusiasm by the audience.

Dr. Levi formally proposed to submit the paper to the SSC, which was unanimously approved. If accepted by the SSC, the paper will be published on the ISTH website and a short version will appear in Thrombosis and Haemostasis.

Lastly, future activities of the subcommittee were discussed. The program of the subcommittee for the next year will consist of (1) a prospective validation of the score for overt DIC in a multicenter clinical trial, (2) a further refinement of the non-overt diagnostic scoring system, and (3) a critical appraisal of animal experimental models for DIC.

The attendance was about 225-250 people. There was adequate discussion opportunity in both parts of the meeting.
Exogenous Hemostatic Factors: Registry

Chairman: R. M. Kini--Singapore
Co-Chairmen: C. Bon--France; F. Markland--USA; N. Marsh--Australia

Eight members of the Registry were in attendance plus about 75 guests (Largest gathering for our subcommittee meeting).

1. Meeting was brought to order by the Chair Dr. Manjunatha Kini.
2. The new members of the Registry, Dr. Tur Fu Huang, Taiwan and Dr. Aura S. Kamiguti, UK were introduced.
3. The minutes of the last meeting were read and there were no questions. It was proposed to be accepted by Dr. Frank Markland and seconded by Dr. Mary Ann McLane.
4. Publications of subcommittee reports: Three subcommittee reports have been completed. So far two of these reports on exogenous factors affecting platelet aggregation have been published in Thrombosis and Haemostasis. The third report on the Classification and Nomenclature of the prothrombin activators from snake venoms will be published in August or September issue of Thrombosis and Haemostasis.
5. A short report was made on the organization of the International Conference on the Exogenous Factors affecting Thrombosis and Haemostasis at Pasteur Institute on July 12 and 13.
6. Work on a new inventory on Disintegrins by Dr. McLane is progressing well. This will cover the interaction of disintegrins with various integrins and cell types.
7. Dr. C. Bon presented the role of phospholipase A2 in various biologic activities. He described the classification of secretory phospholipase A2 enzymes and the importance of interface binding site and penetrability to their anticoagulant effects. He discussed the mechanism of inhibition of the prothrombinase complex by Naja nigricollis snake venom enzyme as well as human platelet enzyme. Both these enzymes bind to human coagulation factor Xa at the same site as factor Va and interfere in the formation of the prothrombinase complex.
8. Dr. R. M. Kini presented the four groups of prothrombin activators from snake venoms and the new classification. Group A and B prothrombin activators are metalloproteinases and their structure-function studies would contribute to segments that are important for substrate specificity and recognition of prothrombin by these activators. Group C and D prothrombin activators are structurally and functionally similar to mammalian coagulation factor Xa-Va complex and factor Xa, respectively. Structure-function relationships of these prothrombinase activators contribute directly to our understanding of the prothrombinase complex formation.
9. Dr. A. S. Kamiguti described antiplatelet effects of a metalloproteinase Jarrarhagin. This enzyme cleaves vWF and renders it inactive in platelet adhesion. It also hydrolyzes $\beta_1$ subunit of $\alpha_2\beta_1$ integrin and thus interferes in the collagen-induced platelet aggregation.
10. Dr. F. S. Markland described the clinical effects of fibrolase in removing clots in both ex vivo and in vivo models. This enzyme at high concentrations also induces transient hypotension. He discussed the target substrate specificity and kinetics of cleavage of various synthetic peptide substrates of fibrolase. These studies indicate the possibility that the transient hypotensive effect is most likely is due to its ability to cleave low molecular
weight kininogen with ultimate release of bradykinin and then cleavage of kallidin and bradykinin.

11. Dr. M. A. McLane described the functional diversity of disintegrins. In addition to their role in inhibiting platelet aggregation, various disintegrins and related proteins interact with many different normal and cancer cells. This is due to their ability to bind and distinguish closely related integrins. She also described both in vivo and in vitro studies and their role in angiogenesis, anti-cancer and bone resorption effects.

12. Dr. T. F. Huang described the effect of accutin on neovascularization in both in vitro and in vivo models. This disintegrin inhibited the bFGF induced but not VEGF-induced angiogenesis. It also slows the growth of melanoma in mice. It is a $\alpha_v\beta_3$ antagonist and inhibits HUVEC proliferation by affecting the cell cycle.

13. Dr. T. Morita discussed the structural and functional diversity of C-type lectin related proteins. He described the three dimensional structure of several proteins of this class and their importance in recognition of the Gla domain of vitamin K-dependent coagulation factors, vWF and other ligands. He also discussed the importance of Mg$^{2+}$ in blood coagulation and binding of Mg$^{2+}$ ions to Gla domain of factor IX.

14. Dr. N. A. Marsh described some new hemorrhagic proteinases and the possibility of putting them in an inventory. The last inventory is currently seven year old. He discussed textilinins, the proteinases inhibitors that specifically inhibit plasmin. They bind to plasmin and inhibit it with a $K_i$ of $10^{-9}$ M. It prevents blood loss using mouse tail vein bleeding model. It appears to be more effective then aprotonin.

15. In the new business, Dr. R. M. Kini proposed to institute a review on the importance of exogenous hemostatic factors as research tools, diagnostic agents and prototypes of therapeutic agents. Dr. C. Bon asked whether such a review should include both exogenous animal and plant sources. It was decided that the review should focus only on the animal factors for the time.

16. We also discussed the importance of proper taxonomical identification of the sources as there is a significant geographic variation among animals and plants. In addition, there are significant changes in taxonomy and our inventories should reflect most recent taxonomic classification of the sources.

17. The next meeting of our subcommittee will be held in Birmingham during XIX congress of the ISTH, 2003.

18. Meeting adjourned. The Registry will not meet formally in 2002.
Dr. Donna DiMichele welcomed the audience and outlined the program.

1. Completed/Submitted Reports and Recommendations (2000/01)
Papers submitted to Thrombosis and Haemostasis for peer-review:

*DM DiMichele, B. Kroner*

Subcommittee recommendations for ISTH web site publication:

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

Revision of the Protocol Recommended for Studies of Safety from Hepatitis and Other Blood Borne Virus Infections of Clotting Factor Products which are Plasma Derived or Contain Plasma Derived Component.  
*Frank G.H. Hill, Christopher A. Ludlam, Pier M. Mannucci.*

2. Notes on the new publications policy
Dr. J. Ingerslev informed on the newly adopted policy for reporting SSC Communications to Thrombosis and Haemostasis. Only original research will be published in full length. All other contributions, including subcommittee reports and recommendations, should be limited to manuscripts of 1200 words accompanied by full ISTH web site publication.

3. Registries and Studies in Progress

*International F VII deficiency registry*

On behalf of Prof G. Mariani and the Registry Steering Committee, Dr. J. Ingerslev presented an update of the International Registry of Congenital FVII Deficiency. This registry now contains 420 patients submitted from 54 centres around the world. Twenty two percent of the subjects have severe deficiency (< 2%) and 52% have FVII levels of 20% or below. The racial background in 85% of pts is Caucasian, with an unexpectedly large distribution of pts from Eastern Europe. A Letter to the Editor was submitted to Blood in an effort to recruit more extensively from the US in an effort to establish more diversity of racial background among registry subjects. Data being collected includes 1) Method of diagnosis including antigen/activity correlation; 2) Characterization of hemorrhagic and thrombotic symptoms; 3) Treatment complications; and 4) Gene mutation analysis. Of note, 23% of patients are a symptomatic. Mucocutaneous bleeding accounted for 59% of the bleeding symptoms. The registry has documented eight thrombotic episodes and no inhibitors. In 210 patients, an assumed causative
mutation has been established. Missense mutations represent the majority of these mutations. An analysis of genotype-phenotype correlation is planned. Enrollment continues with enrollment forms available through Dr. Mariani (contact by email).

*Registry on FIX Inhibitors and Anaphylaxis in Children*

Dr. I. Warrier presented an update of the registry that now contains 77 patients worldwide, 57% of whom have allergic symptoms associated with inhibitor development. In 15/24 patients with a genetic diagnosis, a complete gene deletion was found. The median age at inhibitor development was 19.5 months (9-156), the median exposure days to FIX was 11 (2-180) and the median peak titer was 30 (1-960). ITI had been successful in only 2/19 patients. Nephrotic syndrome was reported in 12 cases, all associated with ITI in patients with allergic manifestations. The renal complications seemed unresponsive to steroids and cyclophosphamide. Enrollment continues with forms available through Dr. Warrier. Prof. Mannucci’s protocol for the immunological characterization of these pts is still open, but no new patients have been documented since this study has been established. Family studies were suggested by Dr. Hill. There was a request from Dr. Lee to have this information on the ISTH web site for easy reference. Dr. Warrier agreed to this.

*International Registry of Clotting Factor Concentrates*

Dr. C. Kasper had written an update of her previous registry. All current manufacturers responded to her request for information on factor VIII and IX concentrates. A paper copy of her registry was distributed to attendees. Dr. DiMichele will speak to Dr. Kasper about the distribution of this information through the web site in the future.

*Gene Therapy Registry.*

Dr. K. High presented an overview of ongoing gene therapy trials. Currently, 4000 patients have been treated with gene transfer for genetic diseases with one death reported in a non-hemophilia patient. General information can be obtained from [www.wiley.co.uk](http://www.wiley.co.uk).

Dr. High summarized the results of two clinical safety trials with a B-domain deleted factor VIII gene product and one clinical safety trial with factor IX. In the first FVIII trial sponsored by TKI (Dr. David Roth, PI), an electroporation cell gene transfer model in skin fibroblasts, resulted in 1-2% FVIII expression for <12 months in 4/6 subjects. The reason for cessation of expression as well as the fate of transfected DNA and cells is not known. The planned enrollment of 6 more patients has already begun.

In the second FVIII trial sponsored by Chiron, 13 patients were intravenously infused with a FVIII gene retroviral vector. The liver was the targeted organ. 6/13 transiently acquired >1% but <2% FVIII activity levels. The trial has been stopped and the data is to be presented at this ISTH meeting.

In a single trial in hemophilia B (Drs High and M. Kay, PIs), AAV/F IX was delivered by multiple intramuscular injections into both quadriceps muscles. FIX levels above baseline with
decreased replacement product utilization were observed in 3/8 subjects. In all, 19 hemophilia A pts and 8 hemophilia B pts have been treated with no safety issues identified.

Upcoming trials were discussed. A gutted adenovirus/FVIII trial using a full-length DNA construct (Genstar, Dr. G. White, PI) has just enrolled its first subject. Based on data demonstrating improved expression with liver targeting, an intrahepatic AAV-FIX trial is set to begin later this month. Dr High indicated that the NIH Office of Biotechnology Activities is setting up a registry of all trials. She will explore the possibility of a coordinated gene therapy registry on the ISTH web site subsequent to a meeting with the NIH later this month.

*International IT Study.*

CRM Hay reported that, despite approved funding by all major manufacturers and ongoing ethical approval of a final protocol in all participating countries, the start of study has been postponed due to a global recombinant and plasma-derived FVIII shortage. A Steering Committee meeting is planned in conjunction with this meeting to further discuss study logistics and a potential start date based on manufacturers’ projections.

### 4. Standardization Issues

*Report from the NIBSC/ FDA standardization meeting (June 2001) - T. Barrowcliffe*

T. Barrowcliffe reported from the NIBSC/FDA standardization meeting in London, June 2001, with respect to the following issues:

**Concentrate working standards:**
A harmonized US/European working standard is still the goal. A report on the new Mega 2 std was presented by Dr Mark Weinstein of the FDA. Full discussion was deferred to Dr Weinsteinís later presentation; however, as discussed at the NIBSC, no decision on the adoption of Mega 2 as a harmonized working standard will be made until after the FDA/CBERís completed report is studied further and any concerns addressed. A decision is expected by next yearís meeting.

Data presented in London continue to demonstrate one stage clotting (OS) and chromogenic (Chrom) assay discrepancies among some concentrates and working standards. When this discrepancy exists, the SSC recommendation to use the chromogenic assay for potency labeling still stands, but is difficult to enforce among manufacturers. Currently, 60% of the manufacturers use the chromogenic assay for labeling.

**Concentrate reference standards**
The current WHO ref standard 6, the first recombinant, was established in 1999 as the 5th IS concentrate standard and the 4th IS plasma standard. Replacement in the near future will be necessary, but the Mega 2 standard is not the likely candidate. At the London meeting, discussion centered on whether the replacement should be recombinant, plasma derived (PD) or both. It is likely that a single standard will continue to be used. A possible candidate could be the FDA ampouled material (N). A decision will be made by the next SSC meeting. A survey of
manufacturers indicate that they are using internal standards calibrated against either Mega 1 or the WHO reference. Most regulatory agencies are using either Mega 1 or EP 2.

**Post Infusion Plasma Measurement**

The NIBSC /FDA meeting discussion focused on whether to use a plasma or concentrate standard to assay concentrate in plasma. The merits of the OS vs the Chromogenic assay was also discussed. Dr. Barrowcliffe recommends that further investigation into the reason for discrepancy be done, but that a concentrate standard identical to the infused material be currently used. The practicality of this recommendation was discussed. Dr Mertens noted that a survey of the FVIII concentrate field studies indicates that inter-lab variability is still 10-20% and has not improved since 1995. Lack of compliance with SSC recommendations for the assay is largely responsible. Since concentrate calibration and licensing is the most pressing problem, the proposal from Prof. Mertens was as follows:

1) Use of the infused concentrate as standard should be recommended for manufacturers' PK studies; however, manufacturers must still assay the product according to SSC recommendations which include: a) calibration of their internal standards against a concentrate working or reference standard; b) perform assay according to SSC recommendations; and c) use the chromogenic assay.

2) No recommendation should be made at this time on the standard to be used for the routine assay of concentrate in plasma. Although a suggestion was made to have manufacturers use both OS and chromogenic assays, the committee thought that the data would be too confusing. There was no dissenting opinion on Prof Mertens' proposal. Prof Mertens suggested that the meeting summary be placed on the ISTH web site for easy reference.

*The Mega 2 Standard-M. Weinstein*

Dr. M. Weinstein spoke to the development of the new Mega 2 concentrate standard. Dr Weinstein reiterated the goals of the new standard which is to develop a uniform international working and reference standard to replace WHO 6 that would demonstrate 1) parallel dose response curves; 2) no OS/Chromogenic assay discrepancy; and 3) long term stability.

Six candidate plasma and recombinant materials were screened by the FDA and narrowed to two high purity plasma products. They were sent to 18 reference laboratories for assay correlation studies. Candidate A demonstrated a consistent 5-10% increase in OS compared to chromogenic assay results in all labs. Candidate B showed variable OS/chrom assay ratios, but better correlation in aggregate, and was therefore chosen. Vials (100,000) and ampoules (5000) were prepared under different conditions and sent to 38 international labs for assay using local methodology and balanced assay design. The results are as follows: Mega 2: OS = 11.3 u/ml (10.2-12.6) Chrom = 8.6 (8.1-9.4) For Sample N : OS = 10.5 u/ml (9.5-11.8) and Chrom = 10.7 (10.1-11.3). Questions that remain to be answered include reasons for the Mega 2 assay discrepancy; how the working standard potency will be assigned as well as how the potency assignation will affect vial fill, factor supply and clinical outcomes. Further investigation of these issues will be done by the FDA/CBER and a final report submitted to the NIBSC for further
consideration. Dr Weinstein also discussed the pros and cons of US manufacturers reverting to the chromogenic assay for potency labeling.

**WHO Standard for Factors II, VII, IX and X- E. Gray**

E. Gray summarized data from a 19 lab collaborative study to evaluate the 3rd International Standard candidate (99/826). Inter-lab variability for all factors assayed against both the 2nd IS standard (CV <4%) and local plasma (CV 6-10%) was excellent. Stability was established by accelerated degradation assay. Potencies/ampoule were assigned as follows: FII 0.91; FVII 1.00, FIX 0.86 and FX 0.93. This proposal was circulated to subcommittee members before the meeting with the majority responding and approving. This standard was approved.

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

On behalf of Dr. E. Preston, Dr. S. Kitchen reported on the Inhibitor Working Party’s attempt to develop a working inhibitor standard. Six lyophilized candidate materials including 3 patient plasmas, 1 rabbit polyclonal antibody(ab) and 2 monoclonal abs were assayed in 15 labs by OS(8), chromogenic (6) or two stage (1) assays. Wide inter-lab variability was seen for all assays. Chromogenic determinations were 15% lower than OS. Within labs, there was good correlation between patient samples and the rabbit polyclonal (r=0.85) but not between patients and monoclonals (r= 0.2 and 0.02). The working group will pursue further studies to obtain reliable standards especially for both very low and high/low responder breakpoint inhibitor assays.

**Measurement of FVII following RFVIIA therapy in HA inhibitor patients: impact of the thromboplastins used. E. Preston**

Dr. E. Preston reported on a collaborative study performed in 27 European hemophilia centers. Lyophilized plasma samples obtained 15 min and 3 hrs in a single pt following a rFVIIa infusion of 108 micrograms/kg was assayed for FVII activity. The labs employed 23 different instrument/reagent combination methodologies. Inter-lab CVís for both samples were 35.6-40%, although good intra-lab assay correlation was noted (r=0.87). Dr Preston suggested that assay standardization should be pursued if clinicians thought monitoring to be useful. Clinicians suggested some utility in life and limb threatening bleeding if a reliable assay was available. Suggestions on the alternative use of a FVIIA or FVII antigen assay were discussed.

**6. FactorVIII Measurement: Is a New Paradigm Possible?**

Dr DiMichele opened with an explanation for a timely related discussion of optimal FVIII measurement and dosing. Optimal FVIII measurement was necessary because of 1) non-correlation of current assays; 2) the ultimate need to measure FVIII effect on blood clotting and not "drug levels" in plasma; and 3) emerging new paradigms of coagulation. Optimal FVII dosing was required because clotting factor was proving not to be an inexhaustible resource. Furthermore, an increasing worldwide need for this product necessitated a new cost-effective approach to resource management.
The Role of FVIII in a TF-based model: Relevance of the APTT- K. Mann

Dr Mann clarified the two distinct issues with respect to FVII measurement: 1) assay for potency labeling and 2) assay in the individual that takes into account all aspects of bleeding predictability. Using his in vitro blood coagulation modeling system, Dr Mann illustrated several principles of blood clotting: 1) physiologic reactions are subtle and do not go to completion; 2) initiation phase of clotting is TF- based and consumes fibrinogen; 3) the propagation phase generates almost all the thrombin secondary to tenase activation of FXa; 4) 99% of this reaction is invisible to a clot-based assay; and 5) reaction kinetics rather than endpoint is important to the understanding of hemophilic bleeding. His view of the ultimate assay integrates procoagulant and anticoagulant physiology in order to determine the amount of FVIII required for clotting in an individual. Ideally, this model should be adapted to point of care monitoring.

The role of FVIII in FX Activation- P. Fay

Discussing detailed data from his own study of FVIII activation kinetics in a purified system, Dr Fay added that the assay of choice needed to measure the prime role of FVIII in FX activation.

APTT Waveform Analysis: A possible Alternative?- A. Giles

Based on work performed with the waveform APTT on the MDA 180 by Organon Teknika and Drs Shima and Yoshioka, Dr Giles added that there was more to clotting than the endpoint clot. The waveform APTT documents the maximal acceleration of clotting kinetics in a plasma-based optical system. In this system, Drs Shima and Yoshioka noted 5 different patterns among 23 severe HA patients. Re-addition of purified FVIII resulted in a linear correlation between waveform normalization and FVIII activity (r= 0.98). The individual optical responses create the possibility for genotype-phenotype correlation studies.

The Chromogenic Assay- K. Mertens

In a discussion of this assay, Dr Mertens demonstrated that manufacturersí assay recommendations may need to be modified to create optimal activation times necessary for linear kinetics. For this reason, he recommended standardization of the methodology. He reiterated that concentrate PK measurements in plasma serve a different function than the impact of FVIII on clotting. A single assay for both purposes may not be possible.

Discussion- T. Barrowcliffe, P. Lollar, R. Montgomery

A round table discussion emphasized the dual reasons for measuring FVIII; the complexity of that measurement; the need for ongoing study of optimal measurement; and the importance of optimal measurement when using cost effective dosing for bleeding prevention. All participants were encouraged to design and participate in these investigations and to submit data to the next subcommittee meeting.

7. FactorVIII Dosing: Developing Future Strategies
H. Roberts reviewed the history of management of bleeding in hemophilia. He suggested that adequate dosing strategies depended on whether treatment or prevention of bleeding was the goal. Historical data suggested that doses of 17 u/kg FVIII could result in breakthrough bleeding and advanced arthropathy. Consequently, minimum dosing strategies have increased over time.

Alternative Dosing Strategies- A. Srivistava

A. Srivastava reported on his extensive experience in India with lower dosing protocols in severe HA patients undergoing primarily orthopedic surgery. His results were as follows: 1) excellent operative hemostasis with target FVIII levels of 60-80%; 2) 14% minor breakthrough bleeding post operatively with target FVIII levels of 17-20%; and 3) total FVIII usage of < 300 u/kg. Based on his experience and a review of the literature, he suggested that there may not necessarily be an inverse correlation between total dose used and bleeding morbidity. A discussion of the generalizability of these strategies ensued.

The Role of TAFI in Hemophilic Bleeding- M. Nesheim

M. Nesheim reviewed the role of TAFI in the attenuation of fibrinolysis through the elimination of the fibin positive feedback loop on conversion of plasminogen to plasmin. He presented data to show that low concentrations of TAFI are sufficient to correct early clot lysis in intrinsic factor-deficient plasma, including FVIII and FIX-deficient plasma. The subcommittee discussed the potential role of fibrinolysis in hemophilic bleeding. The subcommittee debated a potentially greater role for fibrinolytic inhibitors in the optimal treatment of hemorrhage, especially with respect to decreasing the FVIII requirement.

Minimum Effective Dosing: Study Design.- M. van den Berg

On behalf of her collaborator, Dr Gil White, Dr van den Berg presented a rationale and design for a multicenter randomized double-blinded crossover dosing study for hemarthrosis. In this study, 30 patients with severe HA who are >18 years of age and have non-target joint bleeding into knees, would be evaluated for clinical response of acute hemarthrosis to FVIII doses of 30, 20 and 10 u/kg. A salvage dosing schedule is included. Short-term response (24 hours) is to be evaluated by exam and VAS pain score. Long-term response (2 years) is to be measured using clinical and orthopedic joint scores. The subcommittee raised concerns about the delay in outpatient treatment due to double-blinding as well long-term joint morbidity due to potential breakthrough bleeding on low dose regimens. The study was encouraged to proceed upon consideration of the following options: 1) home treatment to prevent delay; 2) comparison of higher dose regimens; 3) potential addition of anti-fibrinolytic therapy to one study arm; 4) a statistical review of cohort size; and 5) a longer follow-up period.

Round Table Discussion: C. Kasper, J. Oldenburg

Dr. Oldenburg (sitting in for Prof Brackmann) discussed the perspective of government-mandated standard of care requirements that would preclude the possibility of a sub-optimal
outcome in hemophilia patients. Dr Kasper emphasized the need for reliable cost-effective data to guide the treatment of patients in the developing world. This project will continue in an effort to meet these divergent goals.

**Meeting adjourned at 17.30.**

**Attendance**

Most of the time 250-300 people attended the meeting.

Minutes prepared by Drs Ingerslev and DiMichele
Factor XIII

Chairman: A. Ichinose--Japan
Co-Chairmen: B. Bishop--USA; P.G. Board--Australia; C.S. Greenberg--USA;
L. Muszbek--Hungary

Fortunately, the factor XIII Subcommittee had more than 100 participants this year, probably because almost all of its members attended the ISTH congress, as usual. Thus, the chairman of this committee would like to suggest that the SSC meeting be associated certain closely related meetings, in order to discuss important issues in the factor XIII field with a large number of members.

1) Recent Progress in Factor XIII Science: Gene Targeting of Factor XIII.

CHARACTERISATION OF COAGULATION FACTOR XIII A DEFICIENT MICE by Gerhard Dickneite (Germany).
A German group have established a transgenic factor XIII A deficiency mouse model (FXIII A knock-out (KO) mice) with an exon 7 deletion of the XIII A gene by homologous recombination in embryonic stem cells. Transglutaminase activity in plasma was <5 % in homozygous XIII A KO mice, no gamma-dimerization of fibrin in the plasma of the XIII KO mice could be detected. Mortality rate was higher in the XIII KO mice compared to normal mice because of bleeding episodes (one-year survival: 70 % in FXIII KO mice vs. 100 % in normal mice). When examined for the bleeding disorder in more depth, XIII deficient mice were found to have an increased bleeding time. Thrombelastography experiments demonstrated impaired clot formation in the XIII KO mice, the maximal amplitude was decreased and premature clot destruction was observed. It was concluded that these KO mice represent a good model to study the impact of factor XIII deficiency.

GENE TARGETING OF FACTOR XIII IN MICE by Akitada Ichinose, Shiori Koseki, Masayoishi Souri, Naoki Takeda, Gerhard Dickneite (Japan, Germany)
Prof. Ichinose's group have identified a number of mutations in the XIII A and the XIII B genes in patients' genomic DNA and also analyzed the molecular mechanisms using in vitro procedures. However, one cannot understand completely the clinical pathological mechanisms of this disease in vivo. To generate its disease model and ascertain the role of XIII B in vivo, XIII B knock-out (XIII B KO) mice have been established. Both homozygous and heterozygous KO mice showed no marked difference from the wild-type mice in general appearance. Although XIII B KO mice had somewhat prolonged bleeding time of their tail tips than the wild-type mice, this result should be reproduced by more standardized bleeding test.

As to XIII A KO mice, three pairs of the homozygous XIII A KO male and female mice were mated. All female mice became pregnant, and one of these mice died after 2 weeks because of massive bleeding from its vagina. Another female mouse also bled from its vagina and had abortion. These observations remind us the spontaneous absorption in human female patients with factor XIII deficiency. Further analysis of these XIII KO mice would lead to understanding the physiological and pathological functions of XIII in vivo.
2) Clinical Research on Factor XIII Deficiency


A working group including all the French physicians in charge of FXIII severely deficient patients has been organized in 2000 and named GEFF XIII (Groupe d'Etudes Francophone du FXIII). This group of physicians from 16 centers conducted the first study to assess the tolerance and safety of the FXIII plasma concentrate Fibrogammin P (Centeon Aventis). Eight out of the 19 patients had previous histories of intra-cerebral hemorrhages (ICH). Seven out of 8 episodes of IHC occurred in non-treated children before the age of 11, indicating the need for a systematic prophylactic replacement therapy in these patients.

This group plans future studies on the retrospective collection of data on patients undergoing surgery, systematic characterization patients' genotype, prenatal diagnosis, standardization of the FXIII assays, etc.

3) Activation and Its Implication of "Variant A subunits" of Factor XIII.

CHARACTERIZATION OF ENHANCED CATALYTIC EFFICIENCY OF RABBIT FACTOR XIII IN COMPARISON WITH HUMAN ENZYME by Lee S.Y., Lee I.H., Oh J.T., Kim I.G. and Chung S.I. (Catholic Univ., Seoul National Univ., and Korea)

Dr. Chung purified rabbit and human enzyme and characterized their enzymatic, physicochemical and structural properties. Rabbit factor XIIIA (RXIIIA), like the human enzyme (HXIIIA), showed the same mechanism where the deamidation step (k3) during the acyl enzyme formation step was found to be rate-limiting. However, the kinetic efficiency measured by methylamine incorporation into acetylated oxidized B chain of insulin showed rabbit enzyme was significantly greater than human enzyme (V/K: 427.45 for RXIIIA; 62.12 for HXIIIA). Structure modeling of RXIIIA by a fit to the known HXIIIA 3D structure coordinates again showed a similar resemblance of active site pocket and Ca ion binding domains. In light of the report that Val34Leu variant retained greater catalytic activity, the N-terminal activation peptide domain of rabbit enzyme, which is quite heterogeneous from human, may contribute in the enhancement of catalytic efficiency in an unknown manner and correlates well with the previously reported rat enzyme catalytic efficiency and its amino acid sequence.

EFFECT OF VAL34LEU PENOTYPE ON THE ACTIVATION OF FACTOR XIII: HOW IMPORTANT IS IT? by Laszlo Muszbek (Hungary)

To address the problem regarding Val34Leu, Prof. Muszbek reported the following: As observed with purified proteins in the absence of plasma, the rate of FXIII-A cleavage and fibrin polymerization was higher with the homozygous Leu/Leu variant than with wild type (Val/Val) FXIII. However, when plasma or whole blood from patients of various genotypes were compared, Val34Leu polymorphism did not seem to be a major contributor to the speed of FXIII activation. The onset of fibrinogen clotting, i.e., the release of fibrinopeptide A and fibrin polymerization were of predominant importance in determining the time course of FXIII activation. The release of FXIII-A activation peptide always lagged behind the release of
fibrinopeptide A and activated FXIII-A never appeared in the fluid phase. Prof. Muszbek concluded that the appearance of polymerizing fibrin and its accelerating effect on FXIII activation are the dominant factors that regulate the process of FXIII activation in whole blood.

FACTOR XIII VAL34LEU: RELATION TO THROMBOTIC DISORDERS by Peter J. Grant (UK)
Prof. Grant reviewed existing reports including his own on the implication of Val34Leu polymorphism of the XIII A gene. The factor XIII genes are highly polymorphic. There are 5 common coding polymorphisms in the factor XIII A gene, of which a valine to leucine transition at residue 34 is of interest due to its vicinity to the thrombin cleavage site and its relation to thrombotic disorders. The relationship between Val34Leu and thrombosis has now been analyzed in approximately 18 epidemiological studies, of which 7 regard patients with cardiovascular disease, 6 venous thrombosis and 5 cerebrovascular disease. Several of these reports have shown that Val34Leu is protective against thrombosis in different vascular beds.

FACTOR XIII VAL34LEU: EFFECTS ON FIBRIN STRUCTURE AND FUNCTION by Robert A.S. Ariens (UK)
Factor XIIIA Val34Leu occurs three amino acids upstream of the thrombin cleavage site between arginine 37 and glycine 38. Dr. Ariens previously reported that the substitution of Val 34 with Leu accelerates the thrombin cleavage of the factor XIII activation peptide. He showed that early covalent cross-linking of the fibrin clot by factor XIII Leu34 reduced lateral aggregation of the fibrin fibers, leading to a reduction in fiber thickness from 121.0 +/- 23.9 nm to 75.7 +/- 11.3 nm and alteration in rates of fibrinolysis of the fibrin clot. The effect of Val34Leu on fibrin structure and function appeared to alter the interaction with platelets and was dependent on fibrinogen levels. Dr. Ariens concluded that Val34Leu is the first example of a mutation in the factor XIII activation peptide that alters transglutaminase and fibrin structure and function.

4) Improvement of Screening Tests for Factor XIII.

SCREENING FOR FXIII ACTIVITY IN PLASMA by Lewis K.B., Heffernan J., Khuu Kien, Bishop P.D. (USA)
Although a variety of assays exist for screening FXIII levels in plasma samples, in many cases the reagents are not readily available and the protocols are not easily transferred to different laboratories. Rather than develop a new assay, an American has chosen to evaluate the Berichrom assay (not commercially available in the US). Where necessary, they have made simple modifications using readily available reagents. The principal modification has been to increase the thrombin concentration in the assay. A second modification has been the use of an absolute rFXIII [A2] standard rather than a pooled human plasma standard. With these modifications, FXIII activity levels have been measured in plasma from humans and other animal species.

5) Search for Standard Materials for Factor XIII.

SEEKING STANDARDIZATION MATERIALS FOR FACTOR XIII by Paul Bishop (USA)
Dr. Bishop proposed the following objectives: 1) Comparing the accuracy of factor XIII assays
in common use for clinical evaluation. 2) Establishing an international standard for factor XIII activity. 3) Defining a specific activity for factor XIII. He also proposed to establish study participants and a work plan:

1) Solicit laboratories interested and willing to participate in such study.  
2) Identify and designate reference plasma of normal FXIII activity (a lot of pooled normal plasma) and a FXIII deficient reference plasma.  
3) Determine which assays will be employed by the various participating laboratories  
4) Establish a protocol for testing the accuracy and availability and uniformity of FXIII assays in common use.  
5) Discuss the possibility of providing FXIII (A2B2) plasma concentrate and preparation of pure recombinant FXIII (A2) as an external reference.

FACTOR XIII AS A COMPONENT OF THE FIBRIN SEALANT, "BOLHEAL(R)," AND AN ASSAY FOR FACTOR XIII ACTIVITY by Hiroshi Kaetsu (Japan)  
Factor XIII, one of the active ingredients of the fibrin sealant, "Bolheal(R)," is included in the fibrinogen component. Fibrinogen and factor XIII are purified individually in the production process of the fibrinogen component of Bolheal(R). Dr. Kaetsu uses the amine incorporation method as a routine assay for factor XIII activity. This method gave the dilution linearity in samples of "standard human plasma," "purified factor XIII," and "the fibrinogen component of Bolheal(R)." The linear range of the assay system was 0.05-2 units/ml for both factor XIII concentrate and fibrinogen concentrate including factor XIII. Attention needs to be paid as to whether or not the values of factor XIII activity obtained with different assay systems are comparable.

Dr. Kaetsu proposed a process to establish the reference preparations of factor XIII in which "standard plasma" is established as the first step, and thereafter "purified factor XIII standards" are evaluated, based on the value of activity in "standard plasma."

PROGRESS REPORT ON STANDARD FACTOR XIII MATERIAL by Trevor W Barrowcliffe (UK)  
Last year, Dr. Barrowcliffe reported that he developed two XIII concentrates which are considerably stable at both 4 and 20 degrees when measured by a chromogenic assay. He also extended this study at higher temperatures, such as 37 and 45 degrees, where the two concentrates showed much less stability of 40-60%. Since these concentrates showed more than 90% stability, these should be used as one of candidates of XIII concentrates in the standardization study.

Dr. MacIntosh, co-chairman of the Fibrinogen Subcommittee, raised several issues regarding factor XIII materials and assay method; some fibrinogen preparations and clot solubility tests need to be?included in the future study.

6) General Discussion and Topics for 2002  
Since the meeting was behind the schedule, and passed 10 min over 12:00, it was strongly suggested by a congress personnel to conclude the discussion. Accordingly, the chairman of this
subcommittee just announced that factor XIII assay and material standardization activities would be continued during the year with reports at next year's meeting in Boston. Laboratories, institutes, and companies which are interested in this collaborative task force were requested to send an E-mail to Akitada Ichinose, <aichinos@med.id.yamagata-u.ac.jp>.
Fibrinogen

Chairman: S.T. Lord--USA
Co-chairmen: P. Feldman--UK; R. McIntosh--UK; N. Weinstock--Germany

Dysfibrinogens

Dr. Hanss (France) summarized the current content of the on-line database of dysfibrinogens, which he compiled, and that is now also available on the ISTH web site. He described the specific information associated with each case in this database. Dr. Hanss proposed developing a form for on-line submission of further entries to the database. It was agreed that Dr. Hanss should moderate and update this data base. Investigators should submit new dysfibrinogens online to Dr. Hanss. As this database may provide the basis to associate specific structural changes with clinical symptoms, the subcommittee encouraged Dr. Hanss to include a description of the clinical data available for each patient and family member.

Fibrinogen in mice

Dr. Lord (USA) presented data on the potentially significant variations in "normal" plasma fibrinogen levels in laboratory mice, which appear to vary with diet, age and mouse strain. The mouse model is commonly used in experimental studies that relate fibrinogen levels to a variety of disease states. There appeared to be a consensus that standardization of the measurement of plasma fibrinogen in mice would be desirable. Dr. Lord undertook to return to the Subcommittee with proposals for a standardization exercise. The proposals will include a method to draw blood, specifying an anticoagulant, and the assay method itself (e.g, ELISA). A reference preparation for mouse plasma would be a desirable aspect of the standardization. The mouse standard would be related to previous functional measurements of either human or mouse fibrinogen.

Fibrinogen plasma standards

Dr. Weinstock (Germany) summarized the critical features of his subcommittee report that described the proposed high fibrinogen reference plasma. Dr. Weinstock also showed further results on the potential use of such a preparation in clinical laboratory measurements. Dr. Lord confirmed that Dr. Weinstock’s report has been received and she indicated that this report had been reviewed by expert members of the Subcommittee. (Note added after the meeting: the statement made by the chair that the review process was complete was incorrect, as in fact, the review process is ongoing. This position will be clarified with Dr. Weinstock and Dr. Padilla, WHO, who was present at the meeting.)

Standardization of the measurement of components in fibrin sealants

Dr. McIntosh (UK) reported that it was his understanding that the revision of the fibrin sealant monograph is still under consideration by the European Pharmacopia.
Dr. McIntosh (UK) summarized the steps taken to approve the report from Dr. Barrowcliffe (UK) on the collaborative study (initiated by the SSC Fibrinogen subcommittee in 1999) to establish an international standard for fibrinogen concentrate. The WHO has adopted the recommended preparation from this report as the First International Fibrinogen Concentrate Standard. Dr. Lord will report on this procedure to this year’s SSC Annual Business Meeting.

Dr. Longstaff (UK) and Dr. Chang (USA) presented a proposal to characterize and calibrate a new reference preparation for thrombin. Thrombin is a key component of Fibrin Sealant kits and the subcommittee had previously decided to provide continuity by considering the standardization of other Fibrin Sealant components in addition to fibrinogen. The proposal from Drs. Longstaff and Chang was generally accepted with specific agreement that 100IU/vial was sufficient for a reference preparation; an intermediate purity preparation formulated in human albumin would be acceptable; results from a functional assay (thrombin/fibrinogen clotting time) would be preferred in assaying the potency and the calibration should be carried out against both existing NIH and WHO reference materials. The calibration of thrombin activity using clotting time should use the First International Fibrinogen Concentrate Standard, or material calibrated against that standard. The remaining stocks of the NIH and WHO thrombin standards are low, so there is an urgent need to establish a new reference material. Thus, Drs. Longstaff and Chang will report on their progress to next year’s SSC meeting in Boston.

On behalf of Dr. Barrowcliffe (UK), who was detained at another subcommittee meeting, Dr. McIntosh presented summary slides on the progress of proposals to establish reference materials for the measurement of FXIII. These proposals will also be discussed at the FXIII subcommittee meeting. The purpose of presenting them here was to determine whether or not there would be value in including a candidate preparation that could be used for the measurement of FXIII in Fibrin Sealent kits. It was agreed that the Fibrinogen Subcommittee would welcome the opportunity to work with the FXIII Subcommittee on this study. Furthermore, it was suggested that the inclusion of a preparation to determine if FXIII can be accurately measured in a concentrated solution of fibrinogen would be particularly useful. In addition to the assay methods proposed by Dr. Barrowcliffe, data on measuring FXIII using the solubility of fibrin in a suitable solvent, e.g., urea or chloroacetic acid (the latter is reference method in the Fibrin Sealant Monograph), would also be useful.
Procarboxypeptidase U / TAFI
Dr. D. Hendriks provided an overview on the nomenclature, on the current assays and on the function of this enzyme in the fibrinolytic system. This was followed by the presentation of a newly developed chromogenic substrate based activity assay that, in contrast to existing functional assays, might allow to analyse large numbers of samples. Dr. Hendriks briefly mentioned that at a recent wet workshop in Leiden, three commercial assays (one for activity, two for antigen) had been evaluated. Since only a limited number of people (often unexperienced) participated and only a limited number of samples were included, no firm conclusion could be drawn. Dr. Mosnier reported the results of their study on the measurement of TAFI levels in 25 normal plasma samples using 5 different assays (3 antigen and 2 activity assays). As presented at the 2000 Maastricht SSC meeting, the TAFI levels as measured by the 5 assays, showed good correlation. However, additional analysis of the results indicated that there might be a systematic variation in the TAFI levels (related to the use of the commercially available sheep anti-TAFI polyclonal antibody) that needs further investigation. The meeting agreed with the organisation of a collaborative interlaboratory study on available proCPU/TAFI assays. Laboratories interested in participating were requested to sign up at the end of the session.

Standards for proteins of the fibrinolytic system
Dr. Longstaff reported that at the WHO/ECBS meeting in October 2000 it was agreed that the recommendations of the SSC be adopted to change the name of the new IS for tPA (98/714) FROM: Alteplase (recombinant tissue plasminogen activator), 1st IS, TO: Tissue plasminogen activator, human, recombinant, 3rd IS.

Dr. Longstaff presented the results of a collaborative study that was organised in 2000/2001, involving 16 labs, to replace the 2nd IS for Streptokinase. The study included 4 coded Streptokinase samples and participants were asked to perform one or more of the 2 methods provided, or their own in-house method. At the end of the study there was universal agreement by participants with the two proposals arising from the study, 1) on potency and identity of the new standard; and 2) on methodology. 1) It was agreed that preparation 00/464 should be recommended to the WHO as the 3rd IS for Streptokinase with a potency of 1030 IU/ampoule. 2) It was agreed that a chromogenic assay without fibrin (one of the methods provided in the study) would make a suitable reference method for Streptokinase activity determinations. It is likely that this method will be adopted as the reference method of the European Pharmacopoeia. Prior to the SSC meeting the report of this study had also been sent to various members of the subcommittee for comments. Eleven responded and all approved the report. The report was subsequently approved by the meeting.

D-dimer
Dr. Nesheim briefly discussed the structure of fibrinogen, the polymerization of fibrin
momomers, as well as the subsequent cleavage of fibrin by plasmin to form a family of fibrin degradation products. This was followed by a brief presentation of results wherein the products released from a Factor XIIIa cross-linked clot perfused with dilute plasmin were characterized with respect to their average molecular weight, their molecular weight distribution, and their chain composition. Because the fragments are soluble and well characterized with respect to absolute concentration, chain composition, molecular weight and D-dimer content, they would be excellent candidates for D-dimer standards by which to calibrate and compare various assays.

Dr. C.-E. Dempfle presented the results of the FACT-3 trial (Fibrin Assay Comparison Trial) in which 23 samples (including normal samples as well as samples from different pathologies) were subjected to analysis by 22 commercially available assays. A wide range of values was observed when comparing the results obtained with the various kits; however, a good correlation between the methods was obtained. The latter allowed the generation of conversion factors by which the data from each respective assay could be normalized, resulting in a harmonization of the data obtained from all DVT and most of the DIC samples. For some particular assays this procedure appeared not to be applicable for the data obtained with normal samples. Larger studies will be needed to confirm the general applicability of these conversion factors. The study also demonstrated that a harmonization of the data obtained with the different assays could also be achieved by using a common calibrator consisting of a high-molecular weight, partially plasmin degraded, cross-linked fibrin preparation.

Dr. P. Meijer presented data of the "D-dimer comparison trial," a joint project of the ECAT Foundation and INSTAND performed within the framework of their external quality assessment programmes. The scope of this project was to evaluate the analytical performance of quantitative D-Dimer assays in daily laboratory routine and was focussed at the inter-laboratory variability per assay and the difference in outcome between different methods. A set of 7 samples generated by mixing pooled normal plasma with pooled patient plasma containing elevated D-dimer levels, at different ratios to about 450 laboratories. Preliminary data analysis on the results of about 200 participants from the ECAT Foundation was discussed. The mean inter-laboratory CV ranged from 5 ó 80%. The ratio between the lowest and highest mean value of different methods on different D-Dimer levels ranged from 3 ó 14. Dr. P. Meijer concluded that, with respect to harmonisation of D-Dimer methods, not only the standardisation of the calibration but also the difference in sensitivity should be taken into account.

Dr. Kitchen presented data of recent studies (September 1999 and November 2000) in which two test samples were distributed to more than 300 centres. Latex agglutination was used by 165-215 centres. During this period the use of quantitative (automated and ELISA) methods increased from 40 to 110. Each sample was a lyophilised pool of plasmas from hospitalised patients with elevated D-dimer. Results were grouped by technique and the median D-dimer with different latex agglutination methods varied from 400 to 2000 ng/ml. This was similar for both samples. For one automated assay the median result was 359 ng/ml (range 250-430) with a CV of 15%, for another method the median was 3030 ng/ml (range 1800-3200). For one automated method the CV of results was 54%. For one method the median test result was only 20% above the upper limit of normality. For others the median test result was more than 6-fold higher than the upper limit of normal. It was concluded that D-dimer assays in routine use vary widely in the results.
obtained, in the discrimination between normal and abnormal, and in their precision. Improved standardization is required.

**Matrix Metalloproteinases**
The matrix metalloproteinases (MMPs) form a family of over twenty closely related zinc dependent proteases. MMPs are involved in the degradation of many components of the extracellular matrix. MMPs are believed to be involved in various (disease) processes involving matrix remodelling like rheumatoid arthritis, scar formation and invasion and metastasis of tumor cells. Increasing evidence for a role of MMPs in fibrinolysis and cardiovascular disease is accumulating, i.e. MMPs can be activated by plasmin as well as by thrombin and activated MMPs may induce activation of platelets. Dr. J. Verheijen gave an overview on assays for quantitation of MMP activity and antigen. Quantitation methods include: zymography, degradation of matrix components, immunological methods like ELISA and fluorogenic or chromogenic methods involving synthetic peptide or protein substrates. The specificity of the methods is often poorly documented. In biological samples MMPs occur in multiple forms, i.e., free active enzyme, inactive pro-enzyme, active or inactive complexes with protein inhibitors and membrane- or matrix-bound forms. The extensive structural and functional similarity between the various MMPs, the occurrence of multiple forms and the lack of standard preparations and reference methods complicate a reliable quantitation of these enzymes and make interlaboratory comparison of results very difficult. In vitro and in vivo methods for the investigation of MMPs were subsequently discussed by Dr. Z. Galis. Her studies, involving a variety of experimental approaches (in vitro, in vivo, including particular knock-out animal models), clearly demonstrated the role of upregulation of gene expression of various MMPs in atherosclerotic disease, vascular remodeling and angiogenesis. It could also be concluded that MMPs play an important role in the degradation of fibrin matrices during angiogenesis.

**Topics for 2002**
Attendees were encouraged to communicate with the co-chairpersons. At the 2002 meeting progress on proCPU/TAFI measurements and D-dimer standardization should be included as well as the topic "Standards".

The meeting was attended by 130-140 people including all the co-chairs. The meeting was closed at 4.55 pm.
Hemostasis and Malignancy

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

1. The subcommittee convened at 8 AM
2. The chairman opened the meeting and authorized the morning’s program by explaining that the session would be divided into 2 parts. Work in progress: clinical trials and coagulation markers in cancer. The objectives of the session were the following: to learn more about ongoing clinical trials and interface laboratory research in the field with a view to compiling an International registry of studies of antithrombotic therapy in cancer and preparation for the eventual development of International guidelines on the management of thromboembolic disease in the cancer patient.
3. Part I: Work in Progress
   a. Trials of thromboprophylaxis in cancer patients undergoing operation.
      1. Professor David Bergquist presented the Eroxacan II Study of the low molecular weight heparin (LMWH). Enoxaparin for prolonged thromboprophylaxis in patients undergoing major abdominal/pelvic surgery for malignancy ó The study is complete and demonstrates an advantage in prolonging thromboprophylaxis in this high risk population.
      2. Dr. Morten Rasmussen presented a study evaluating the LMWH dalteparin for prolonged thromboprophylaxis in patients with malignancy undergoing major abdominal/Pelvic operations. The study is ongoing and will complete this year.
   b. Trials of thromboprophylaxis in non-surgical cancer patients.
      3. Mr. A. Kakkar presented outlines of the TOPIC I and II studies which are designed to evaluate the efficacy and safety of the LMWH Certoparin in the prevention of venous thromboembolism in patients with advanced breast (Topic I) or non-small cell lung (Topic II) cancers on behalf of the principal investigators, Professor M Freund and Professor U. Gatzmeier. Both studies are currently actively recruiting and will undergo planned interim analysis in the autumn.
      4. Dr. S. Deitcher presented the INVEST study that will evaluate the LMWH Tinzaparin for the prevention of venous thromboembolism in patients with advanced cancer and its effect on prolonging survival. The protocol will open for recruitment in September 2001.
      5. Professor M. Levine presented a study proposal for the evaluation of the LMWH dalteparin in the prevention of venous thromboembolic disease in patients with advanced glioblastoma. The study may commence early in 2002.
   c. Trials of thromboprophylaxis in patients with indwelling central venous catheters.
1. Dr. A. Reichart presented a large study evaluating the LMWH dalteparin in the prevention of central catheter associated thrombosis. The study is closed and data will be available in the autumn.

2. Ms. Annie Young presented the WARP study which is evaluating low fixed dose with dose adjusted wafarin for the prevention of central catheter related thrombosis. The study continues to recruit.

3. Dr. A. Lee presented the results of a survey on the management of catheter related thrombosis. This demonstrated considerable variations in practice and uncertainty about management of this clinical problem. A study in this area, although difficult, would be valuable.

4. Professor G. Agnelli presented the ETHICS study which is evaluating the LMWH enoxaparin in the prevention of central catheter related thrombosis. The study continues to recruit.

The subcommittee felt pooled analysis of the data from these studies would be valuable in the future.

d. Trials of DVT Treatment in cancer patients.

1. Professor M. Levine presented the CLOT in cancer study, which is evaluating the LMWH dalteparin against the oral anticoagulant wafarin/coumadin in the secondary prevention of venous thromboembolism after initial treatment with dalteparin. The study will complete recruitment in September 2001.

2. Dr. S. Deitcher presented the ONCENOX study which is evaluating the LMWH enoxaparin in secondary prevention of VTE. This pilot dosing/feasibility study has just opened recruitment.

3. Dr. R. Hull presented the outline of the LITE/HOME LITE studies evaluating the LMWH Tinzeparin in the treatment of DVT. Cancer subgroup analysis is not currently available.

4. Dr. A. Lee presented a proposal for a study on the duration of anticoagulation after initial treatment of DVT in cancer patients.

e. Trials of Low Molecular Weight Heparin to prolong survival in cancer patients.

5. Mr. A. Kakkar presented the FAMOUS trial, designed to determine whether the LMWH dalteparin given for 1 year can prolong survival in patients with advanced cancer. The study is now closed to recruitment and will report early next year.

6. Professor H. Büller presented the MALT study designed to evaluate whether the LMWH Nardoparin given for 6 weeks can prolong survival in patients with advanced cancer. The study should complete recruitment by the end of this year.

f. Surveys and registries
1. Mr. A. Kakkar presented the FRONTLINE survey, which is the first comprehensive global survey designed to assess current perceptions and patterns of practice towards the risk, prevention and management of venous thromboembolism in cancer. It is hoped to collect information from 5,000-10,000 clinicians. The survey can be accessed online at <www.frontlinesurvey.com>.

2. Dr. S. Schulman made a proposal for joint registries on cancer outcomes in patients receiving long term anticoagulation on behalf of the control of anticoagulation subcommittee. This was accepted.

Part II: Coagulation markers in cancer.

3. Dr. A. Falanga presented an overview of the state of the art with regard to markers.

4. Dr. D. Walsh presented a study on markers of Haemostasis in Brain Tumor patients.

5. Dr. J. Fareed and Dr. B. Kuenen presented data on Haemostatic abnormalities and clinical thrombosis in patients receiving anti-angiogenic therapies for treatment of cancer.

The subcommittee will proceed with loading its registry of clinical trials on the ISTH website.

The meeting closed at 12:15.
Lupus Anticoagulants/Phospholipid-Dependent Antibodies

Chairman: J. Arnout--Belgium
Co-chairmen: J.T. Brandt--USA; M. Galli--Italy; S. Machin--UK; R. Roubey--USA; I. Scharrer--Germany; P. Sie--France

Number of attendees: 250-350

I. Diagnosis of APS: which assays should we recommend?

Dr. Galli presented data on the thrombotic risk associated with different antiphospholipid antibodies currently measured such as Lupus anticoagulants (LAs), anticoagulolipin (aCL), anti-ß2-glycoprotein I (aß2-GPI), and anti-prothrombin (aPT) antibodies. Her data were based on a systematic review of the relevant literature, published from 1988 to 2000. For the risk analysis of LA and aCL a large number of studies were analyzed giving information on more than 4000 patients. The association of LAs with thrombosis was significant in all studies, irrespective of the site and type of thrombosis. The association between aCL and thrombosis was only significant for a small minority of studies. LA appeared more strongly associated with thrombosis than aCL. From this analysis, it may be concluded that the detection of LA helps to identify patients at risk of thrombosis whereas measurement of aCL antibodies is of little value. Analysis of the thrombotic risk associated with aß2-GPI and aPT was mainly based on retrospective data. aß2-GPI appeared to be a risk factor for DVT but not for arterial thrombosis. aß2-GPI showed to be more strongly associated with thrombosis than aCL. Also aPT seem to predict DVT. The clinical relevance of aß2-GPI and aPT needs to be confirmed by prospective studies based on better standardized assays.

Dr. F. de Groot and Dr V. Pengo reviewed how APS is currently defined and came up with a proposal for a new classification. They prepared this discussion together via e-mail conversation. Dr de Groot who presented the first part, critically reviewed the currently accepted diagnostic criteria (the so-called Sapporo criteria. Wilson et al. Arthritis & Rheumatism, 42; 1999: 1309-1311) and showed how internationally recognized experts continuously violate these. Dr. Pengo added a series of arguments why the Sapporo criteria are not optimal and proposed alternative criteria that separate APS into different types based on the antigen that is measured in the test. This presentation did as hoped initiate discussion and was concluded by the initiation of working party that would further discuss this important issue via e-mail. The table with the proposed criteria for APS is as follows:

<table>
<thead>
<tr>
<th>Proposed Laboratory Criteria for APS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I anti-ß2-Glycoprotein I and LA positive</td>
</tr>
<tr>
<td>Type II only LA positive</td>
</tr>
<tr>
<td>Type III only anti-ß2-Glycoprotein I positive</td>
</tr>
</tbody>
</table>
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.

**Dr. Finn Wisloff** compared LA results obtained with an aPTT, a PT and a RVVT using identical phospholipid composition, instrumentation and calculation of the results. He found that about 20% of LA positive plasmas give discordant results. The majority of these discordant samples were low positive. These data are in support of the SSC criteria on LA testing that at least 2 different assay systems should be used. In addition, a uniform way of expressing the results should be recommended.

II. **Clinical registries**

**Dr. Guido Finazzi** gave an update on the WAPS trial which was initiated by prof. Barbui under the auspices of our subcommittee. The purpose of this prospective study is two-fold: 1) in a randomized arm, to assess the risk/benefit ratio of high-dose oral anticoagulation vs. conventional antithrombotic prophylaxis; 2) in an observational arm, to obtain information on the natural history of non-randomized patients. 454 patients have been enrolled so far: 112 in the randomized and 342 in the observational arm. Median INR values of patients in the "high-dose" group were continuously around 3.2 as compared to around 2.3 in the "conventional treatment" group. Baseline samples of plasma, serum and DNA have been collected from all patients and are available for biological studies. Study projects are welcome and should be submitted for approval to the Chairmen and Steering Committee of the study. There was some discussion whether the difference in INR between the high intensity patients and the low intensity patients would be sufficiently large to come to a meaningful conclusion.

**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 will begin in the summer of 2001. Investigators wishing to obtain additional information can take contact with Robert A. S. Roubey, M.D. Principal Investigator, APSCORE via the following E-mailaddress: apscore@med.unc.edu

III. **Antiphospholipid antibodies: standardization issues**

**Dr Ian Mackie** discussed the importance of the use locally derived reference ranges as well as a standardized calculation of the test results. He presented data generated from the
UK NEQAS surveys where various commercial DRVVT kits as well as in-house methods are used. Laboratories showed extreme variability in reporting their normal reference ranges. The need for local, analyzer and reagent, specific normal reference ranges for DRVVT is clear. The current SSC guidelines make no particular recommendations on the limits of normality. Arbitrary and generic reference ranges should not be used. Test/normal clotting time ratios should be performed for quality control purposes and to allow comparisons between laboratories and methods. The use of different algorithms for interpretation of the data has led to confusion between laboratories and also requires standardization. More precise recommendations by the subcommittee on locally derived reference ranges and a standardized way of expressing the results is needed.

Dr. Ian Jennings reported on two recent UK NEQAS proficiency testing exercises in which the performance of potential LA controls or reference materials were evaluated. In the first exercise, three plasmas were distributed to 43 centers in the UK. These plasmas comprised the National Institute for Biological Standards and Control (NIBSC) 1ST British Reference Plasma Panel for Lupus Anticoagulant, a sample prepared using plasma from positive LA donors and one LA negative plasma pool. The results from this survey made it clear that LA screening performance with this reference panel is sub-optimal. The second set of data came from a recently completed UK NEQAS exercise, in which a series of plasmas spiked with monoclonal antibodies to β2GP1, prothrombin and a mixture of each antibody, were distributed together with a LA negative plasma and a LA positive patient plasma. The majority of the 277 centers agreed on the diagnosis for each sample but complete consensus was not achieved. The results were agreement with earlier studies by dr Arnout who provided the monoclonal antibodies for this survey. Multicentre studies with such materials may be useful to assess assay sensitivity over a range of antibody concentrations and may allow further standardization, particularly in the accurate detection of weak LA.

Dr. Guido Reber reported on a multicenter evaluation of the ELISA for β2-GPI antibodies. This study was initiated by the European forum on APL and involved 21 centers who received 2 normal samples and 28 patient samples selected by 4 centers. The participants also received 6 prediluted calibrators which enabled them to report their results into "Forum Units". Home made techniques as well as commercial kits were used. Marked differences in positivity rates were observed, ranging from 50 to 93% for IgG and 13 to 70% for IgM. Agreement was only found with 37% of the samples for IgG and for 27% of the samples for IgM. This study demonstrates that both home made assays and commercial kits for aβ2-GPI are poorly standardized. Two points have to be addressed: First, an uniform way to determine the cut-off of positivity should be tentatively adopted. Second, the influence of β2-GPI purification method and batch-to-batch consistency on assay results has to be evaluated.

Dr. Siobhan Donohoe reported on a multicenter study initiated by our subcommittee to standardize the aPT ELISA. Protocols for a calcium free aPT method employed at UCH and a calcium containing method used at Utrecht were distributed to 15 centers. These methods were to be run in parallel with the in-house method. Human prothrombin, kindly provided by Diagnostica Stago was supplied to all participants along with microtitre plates. A series of 17 samples were distributed (April-May 2001). By lack of a suitable standard all assays were performed against the in-house standard. A high degree
of variability was observed between the % activity of in-house standards making it impossible to compare results directly. However, by expressing the results in SD above Normal (< geo mean +2SD) it was possible to observe some consensus. More than 90% agreement on positivity or negativity was obtained with respectively 11 and 13 of the samples for aPT IgG and aPT IgM as determined with the calcium free protocol. Significantly less agreement was found with the other methods. A better standardization of aPT assays may thus be achieved but a international reference preparation is needed.

IV. Antiphospholipid antibodies and prethrombotic state: new developments.

Dr Françoise Dignat-George developed a method to measure endothelial microparticles (EMP) in plasma and showed previously that such microparticles display procoagulant properties. EMP may be measured by flow cytometry after immunolabelling with a monoclonal antibody against αvβ3. Plasmas of APS patients had significantly higher levels of EMP than controls plasma. Elevated EMP levels were also found in other patient groups. She also showed that plasma from APS patients and other patients induces vesiculation of cultured endothelial cells. However, when the procoagulant activity of EMP was assessed using a clotting assay, only EMP released in response to APS plasma were highly procoagulant. Dr Dignat-George proposes that by disseminating procoagulant activities in the circulation, EMPs could contribute to the hypercoagulable state of APS patients.

V. Concluding remarks.

From several questions raised from the participants it became clear that people expect some more precise guidelines on how to diagnose aPS, which assays are needed, useful and ready for widespread distribution. There is also need for an update of the SSC guidelines for LA testing.

Questions or comments regarding LA diagnostic criteria as well as concerning the newly proposed criteria for APS can be addressed to Dr.de Groot, Dr. Pengo as well as to the chairman. A working party will come together during this conference in Paris and will further work on these requests via email conversations in order to prepare the program for the next meeting in Boston: Ph.G.deGroot@lab.azu.nl, pengo@ux1.unipd.it, jef.arnout@med.kuleuven.ac.be
Perinatal/Pediatric Hemostasis

Chairman: U. Nowak-Gottl--Germany
Co-chairmen: E.F. Grabowski--USA; M. Hellgren--Sweden; A.S. Kemahli--Turkey;
M.J. Manco-Johnson--USA; M.P. Massicotte--Canada; W. Muntean--Austria;
M. Peters--The Netherlands

The chair and co-chairs and more than 200 participants/subcommittee members were present. Issues discussed were as follows:

1. *Epidemiology, diagnosis, management and prophylaxis of venous thrombosis during pregnancy*: This topic was presented by M. Hellgren. She stated that hereditary or acquired thrombophilia increases the risk of VTE and that screening for thrombophilia should be performed following obstetric complications such as early-onset severe preeclampsia, abruptio placentae, severe IUGR, repeated fetal loss and IUFD. In addition, M. Hellgren pointed out that objective diagnosis verification by suitable imaging methods is mandatory. Treatment modalities of VTE during pregnancy, e.g. i.v. infusion of unfractionated heparin (UFH) followed by s.c. UFH or low molecular mass heparin (LMMH) until delivery were discussed as well as the use of prolonged anticoagulant treatment with oral anticoagulants or s.c. LMMH post partum. Thromboprophylaxis with s.c. LMMH is suggested during pregnancy for women with previous VTE and thrombophilia, recurrent VTE, hereditary antithrombin deficiency without previous VTE, and antiphospholipid antibody syndrome. In addition to M. Hellgren, B. Brenner presented data indicating that inherited thrombophilia is associated not only with gestational thromboembolism but also with fetal loss, intrauterine growth retardation, preeclampsia and placental abruption. The antithrombotic therapy in this setting was further discussed. In addition, the placenta as the target organ for future research was pointed out.

2. *Prothrombotic risk factors and imaging methods in ischemic stroke in children*: F. Kirkham (London) and R. Sträter (Münster) reported on ischaemic stroke in children. Both underlined the importance of prothrombotic risk factors at the onset of the disease and the possible role in recurrent stroke. In addition, they pointed out that stroke types may influence recurrent stroke and that an adequate imaging work-up is mandatory in classifying stroke children. The future of therapeutic multicentre studies was discussed.

3. *Deep venous thrombosis in children*: Dr. Mitchell reported the results from the PARKAA Trial (Prophylactic antithrombin replacement therapy in kids with acute lymphoblastic leukemia treated with asparaginase) in which imaging methods, i.e. venography and ultrasound, were compared. She concluded from the data obtained that venography was superior to ultrasound in detecting thrombosis in the central venous system, whereas ultrasound was more sensitive in detecting venous thrombosis in the jugular veins. M. Briones& T. Abshire discussed a management report on catheter-related thromboses in children older than 12 months of age. M. Peters presented an objective new but not yet validated scoring system in adolescent patients with deep venous thrombosis for the use of heparin prophylaxis. This scoring system should be validated in different ethnic groups before it can be used routinely. M. Manco-Johnson reviewed data on TPA thrombolysis in children and concluded that the value of TPA thrombolysis compared to standard
anticoagulant therapy in reducing morbidity should be studied in prospective clinical trials. P. Massicotte reported on bone mineral density in a pediatric cohort patients suffering from DVT receiving long-term warfarin therapy. This paper gave evidence that BMD remains significantly decreased, with bone formation more affected than bone resorption, resulting in a net result of increased bone resorption. The need for further studies in the field were discussed. M. Peters reported on postthrombotic syndrome in children after venous thrombosis. Data from their study suggested that approximately two-thirds of children with DVT of the limbs are at risk of developing postthrombotic syndrome, with a higher risk in children carrying prothrombotic defects, suffering from recurrent thrombosis, and with a first onset during adolescence. The need to study compression stockings in these children was discussed.

4. Normal values of hemostatic parameters in the pediatric population: H.G. Koch reported on fasting homocysteine concentrations in healthy children and pediatric patients with thromboembolism. He reported that children carrying Hcy concentrations > the 90th age-dependent percentile have an increased risk of developing thromboembolism. In addition, in the upper Hcy quartile the odds ratio for suffering thrombosis was significantly increased also in neonates, infants and children. V. Balasa reported on prothrombin and PAI-1 in healthy children. He stated that a significant relationship between the G20201A allele and elevated plasma prothrombin levels was not recorded among children. The PAI-1 4G/4G polymorphism was associated with elevated PAI-1 activity levels in children. The PAI-1 genotype was an independent, significant determinant of PAI-1 antigen levels in children as well. The 4G allele of the PAI-1 gene is more commonly present among Caucasians than among African-Americans variants and its role in pediatric thromboembolism has to be evaluated in future studies. S. Israels reported on the evaluation of platelet function in children using the PFA-100 platelet function analyzer. Mean closure times (CTs) are significantly shorter in neonates, and slightly, but not significantly, longer in healthy children than in adults, and similar normal ranges can be used for screening children with bleeding disorders. CTs can be used for simple and accurate evaluation of the response to desmopressin in children with vWD and platelet function abnormalities. Patients with type 1 vWD consistently showed correction of CTs, while type 2 patients showed variable results that correlated with changes in ristocetin cofactor activity (RCoF). Some patients with partial storage pool deficiency or secretion defects also showed CT correction following desmopressin. For patients with vWD not responsive to desmopressin, vWF factor concentrates did not correct the CT, although levels of vWAntigen and RCoF were increased. In comparison to older children and adults, mean CTs of cord blood from healthy neonates are significantly shorter. E.F. Grabowski reported on platelet adhesion/aggregation in flowing blood in pediatric hemostatic disorders. He concluded that platelet function under physiologic flow conditions is clearly more informative regarding hemostasis (or thrombosis) than platelet count or aggregation in platelet-rich plasma, and may very well correlate more significantly with clinical bleeding (or thrombosis). Nicole Schlegel introduced the results of a study on integrin expression and function in neonatal platelets. She reported that a significant defect in GPIIbIIIa expression and function and a specific GPIb behavior were registered in neonatal platelets. B. Kehrel introduced also normal age-dependent values for the distribution of platelet membrane glycoproteins in neonates, children and young adults. She demonstrated first results showing that, despite neonatal
platelets expressing normal amounts of glycoprotein (GP) Ia/IIa, GPVI and GPIb/V/IX, neither CD62-P expression nor CD63 expression nor fibrinogen binding was induced by collagen (up to 1.5 µg/ml), whereas 0.5 µg/ml induced maximal activation of adult (age 20-35 years, n=25) platelets. In contrast ristocetin-induced vWF-binding to neonatal platelets was increased significantly. Even 0.3 to 0.5 mg/ml ristocetin induced maximal vWF-binding. 0.8 to 1.0 mg/ml ristocetin is needed to induce maximal vWF-binding in normal adult platelets. She concluded that knowledge of normal platelet functions in children are essential for the reliable diagnosis of platelet function disorders.

5. **Influence of factor VIIa on thrombin generation:** A. Chan reported the influence of exogenous factor VIIa on thrombin generation in cord plasma of full-term and preterm infants. He found that FVIIa enhanced IIa generation in plasma from different age groups, with the effect being more pronounced in plasma from preterm newborns, possibly due to increased levels of plasma TF.

6. **Hemophilia:** M. Manco-Johnson and P. Petrini introduced the development of international standards for assessment of physical outcomes in children with hemophilia. The WFH and three new scales were compared in 43 hemophilic children. The three new scales all showed better correlation with the WFH pain instrument than the original WFH physical examination instrument (p< 0.01 for each of the new instruments vs. > 0.05 for the WFH instrument). In addition, results of the new Colorado child physical examination instrument best conformed with a normal distribution (p=0.35) and displayed better overall statistical performance. This instrument should be studied further in prospective, longitudinal clinical trials of young children.

7. **Thrombocytopenia:** J. Bussel reported on a series of intracranial hemorrhages in ITP. In addition, he introduced a newly developed registry of non-immune thrombocytopenia. The Non-Immune Thrombocytopenia registry will begin in the United States, Canada, and Europe. The intention is to identify cases of chronic thrombocytopenia that are not ITP, including amegakaryocytic thrombocytopenia (CAMT).
Plasma Coagulation Inhibitors

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

The meeting this year was sub-divided into 5 different categories: (1) Protein S genotype, phenotype and deficiency; (2) Factor V gene and mechanisms of APC resistance, protein C deficiency and prothrombin G20210A; (3) Serpins (serine protease inhibitors); (4) Thrombophilic risk factors; and (5) Assay performance results for testing for familial thrombophilia.

1. **Protein S genotype, phenotype and deficiency**- consisted of talks by Drs. Walker, Soria, Bates and Faioni. Dr. Walker described her work of the West Scotland Donor Study (~3800 people), and it was stated initially that identifying protein S deficiencies has always been a difficult problem. She found that men had more total protein S than women, and a trend for more free protein S than women. They also found in women that with increasing age there was more total protein S, and when adjusting for age, post-menopause women had the same amount of protein S. Dr. Soria described work on the GAIT Project focusing on protein S, and his work focused on identifying candidate genes for hemostatic defects, and his work found a linkage with the gene region D1S194 to the factor V gene. Genome-wide linkage analysis found that C4B-binding protein was near, and they concluded that 1q32 could influence protein S levels and that this may be related to expression of C4b-BP. Dr. Bates talked about levels of evidence and the link with hemostatic disorders, asking questions about: a) methodology, accuracy, verification and variability?; b) evidence that the abnormality is associated with venous thrombosis?; c) potential confounders?; d) magnitude of abnormal significance; and e) link between abnormality and venous thrombosis and does it make biologic sense? She used warfarin therapy, pregnancy and antiphospholipid antibodies to study their link with protein S deficiency and disease phenotype. Her studies found no clear evidence with these 3 different states (inherited or acquired) and venous thromboembolic disease linked to protein S deficiency. A primary problem seems to be a lack of statistical power in these analyses. Without a doubt her studies are suggesting that we need more powerful tools to appreciate protein S deficiency, and again it reinforces the notion that describing protein S deficiency is difficult and is problematic for clinicians and laboratory workers. Dr. Faioni presented data exploring the possibility that a dimorphism, A2148G (Pro 626) influence protein S levels - she studied the effect of phenotype, effect of risk, and effects on diagnosis. She used the PROSIT patient group, and she found that Pro 626 modulates free protein S in healthy women independent of a protein S alpha gene mutation; and that Pro 626 had little effect on total protein S levels in healthy or protein S-deficient individuals.

2. **Factor V gene and mechanisms of APC resistance, protein C deficiency and prothrombin G20210A** consisted of talks from Drs. Spek, Bovill, Bernardi, and Magdelaine. Dr. Spek reviewed data about protein C polymorphisms of the promoter region, and the 3 studies found an overall risk that ranged from 1.00-2.00; and the results
suggest that protein C promoter polymorphisms do not increase thrombotic risk in factor V Leiden patients, and that homozygous or compound heterozygous states of protein C deficiency have no association between clinical severity and polymorphisms of the promoter region. It is not clear how promoter polymorphisms in protein C affect protein C antigen levels. Additional studies found no clear candidate for different transcription factor binding sites, but there is a moderate thrombotic risk associated with these promoter polymorphisms, but there is no biological explanation yet for the differences in plasma levels. Dr. Bovill described a large pedigree (>700 live individuals) in Northern Vermont U.S.A., Quebec Canada and now some studies in collaboration with others in Europe. They are characterizing the founder effect of the 3363 C mutation gene insertion found in this family, and he has obtained funds to fully sequence the genome of 300 family members. Dr. Bernardi presented an overview of the R2 factor V, and reported that up to 10% of western-European persons are carriers of the 2 polymorphisms found in R2 factor V. The studies described to date are not in agreement in that some report decrease in factor V levels, some do not, and others report an increase in APC resistance, while some do not. The risk in some studies reaches 2.0 while in others it is <1.4. There is much evidence to further describe the molecular basis for this phenotype, correlation of biochemistry versus in vivo finding, and the basis for the discrepancies between clinical phenotype and laboratory values. Dr. Bernardi suggests the formation of a Working Group in this SSC to further define the clinical and biological significance of R2 factor V. The audience and the Chair/co-Chairís were in agreement with this suggestion. Dr. Magdelaine described an interesting single case of a double thrombomodulin mutation (Ala 25→ Thr and Ala 455→ Val) with homozygous factor V Leiden. Her results highlight that we should continue to study multi-genetic defects in thrombotic-related genes, since these single thrombomodulin mutations are not strongly associated to thromboembolic disease.

3. Serpins (Serine protease inhibitors) consisted of talks by Drs. Church, Hayashi, and Rezaie. Dr. Church reviewed a landmark paper of Dr. Kenneth Brinkhous (in memory about) from 1939, where he described the "heparin cofactor" responsible for the biologic activity of heparin. Dr. Church also mentioned a paper in press (Gary A. Silverman, Phillip I. Bird, Robin W. Carrell, Paul B. Coughlin, Peter G.W. Gettins, James I. Irving, David A. Lomas, Cliff J. Luke, Richard W. Moyer, Philip A. Pemberton, Eileen Remold-O'Donnell, Guy S. Salvesen, James Travis, and James C. Whisstock, 2001, "The serpins are an expanding superfamily of structurally similar but functionally diverse proteins: Evolution, mechanism of inhibition, novel functions, and a revised nomenclature", published in J.Biol. Chem., published July 2, 2001 as 10.1074/jbc.R100016200) where the suggestions for nomenclature of serpins, new and old, are summarized. Dr. Hayashi described his work with protein C inhibitor in renal cell carcinoma (RCC), and he reported that in RCC there was an absence of protein C inhibitor antigen, with no change in urokinase levels. Adding back protein C inhibitor to a RCC cell line resulted in the inhibition of invasion; thus, suggesting that protein C inhibitor might function to regulate tumor cell invasion. Dr. Rezaie summarized his work about vitronectin interacting with plasminogen activator inhibitor type-1, which then becomes a potent inhibitor of activated protein C. This work suggests that the potential pro-fibrinolytic effect of
activated protein C might be due to a process involving PAI-1/vitronectin inhibiting activated protein C.

4. **Thrombophilic risk factors included talks by Dr. Miyata and Dr. Moll.** Dr. Miyata described the prevalence of protein C, antithrombin and plasminogen deficiency in the general population of Japan. The study used blood donors/patients seeking normal check-ups from the ages of 30-90. This work identified that 0.2%, 0.18% and ~4% of the population had protein C, antithrombin and plasminogen deficiency, respectively. The odds ratio for patients with deep vein thrombosis, compared to Western man, was higher for protein C and antithrombin deficiency, yet the Japanese have an occurrence of 0% with factor V Leiden. Dr. Moll proposed an International Collaboration Group through this SSC to study "combined thrombophilia" focusing on three rare compound thrombophilias: homozygous Factor V Leiden/heterozygous Factor II G20210A; heterozygous Factor V Leiden/ homozygous Factor II G20210A; and homozygous Factor V Leiden/homozygous Factor II G20210A. The proposal is based on the occurrence of these defects ranging from 1/1,000,000 3/100,000,000 for these combined thrombophilias. It was proposed that an international collaboration be established, genetic labs contacted to inquire about their database; and patients could respond to a questionnaire posted on the Internet. Discussion from the audience was enthusiastic and the co-Chairs found the idea worthy of further discussion, except Dr. Aiach raised a point about should we include other combined thrombophilic defects along with these suggestions. Dr. Moll commented that he thought this was complicated enough to do as proposed.

5. **Assay performance results for testing for familial thrombophilia consisted of talks by Dr. Jennings and Dr. Siegert.** Dr. Jennings reviewed the laboratory pitfalls of their experience in the "UK NEQAS/EQUALS Experience", and he went on to describe the frequency of how often incorrect diagnosis was made for factor V Leiden, antithrombin, protein C, and protein S. He summarized most of the finding as differences in assay methods, assay kits used, reference plasmas, methodology details, and expression of the results using normal ranges. Dr. Siegert reported on the influence of low molecular weight heparin (LMWH) on various assays including protein S, RVVDT, and TFPI. In all cases, LMWH had a global effect on clotting assay measurements, which differs from unfractionated heparin, especially when LMWH is used at >1 U/mL. These results caution us that even when neutralizing LMWH with protamine sulfate, there may be differences in the properties of the plasma samples when analyzed.

In conclusion, Dr. Church thanked the co-chairs for help in organizing this SSC meeting and for help in chairing individual sessions, he thanked the speakers for keeping within the given time constraints of this many presentations, he thanked the attendants provided by the congress for their help with the speakers, he reiterated the call for formation of 2 Working Groups (from Drs. Bernardi and Moll), he thanked the audience for their participation in asking questions, and encouraged them to communicate with the subcommittee chairman. The meeting was then adjourned by Dr. Church—Au revoir ôtil next year!(It was estimated by those in attendance that ~300 people were in this session)
Platelet Immunology

Chairman: B.H. Chong--Australia
Co-chairmen: R.H. Aster--USA; D. Beardsley--USA; J.B. Bussel--USA;
M. Ertem--Turkey; S. Santoso--Germany

This year the Platelet Immunology scientific session was divided into 4 parts: idiopathic thrombocytopenic purpura (ITP), alloimmune/isoimmune thrombocytopenia, heparin-induced thrombocytopenia and thrombocytopenia associated with GPIIb/IIIa inhibitors.

i. **Idiopathic Thrombocytopenic Purpura (ITP):**

For the past 10 or more years, there has been no new treatment for ITP, and in particular there is a lack of prospective randomized studies. The current treatment options for ITP are far from satisfactory, particularly for treatment of refractory ITP. Dr. J Bussel (USA) gave a brief overview on ITP clinical trials. Most are small and non-comparative studies. Among those completed and published recently, two are interesting. One showed that rhuIL-11 is not effective in refractory ITP, and another revealed that H pylori eradication led unexpectedly to improvement in the co-existing ITP. Ongoing studies which may provide clinically useful data are trials investigating the use of anti-CD 40L, rituxin, thrombopoietin, CBP1011, anti-FcR1, ATG and mycophenalate mofetil in ITP and autologous bone marrow transplant in refractory ITP.

The pathophysiology of ITP remains to be fully elucidated. Animal models may be helpful in this regard. Dr. D Bearsley described an animal model for immune thrombocytopenia using NOD SCID mice grafted with human bone marrow. She found that platelet destruction by the anti-HPA-1a antibody could be prevented by I.V. IgG.

ii. **Alloimmune and Iso-immune thrombocytopenia:**

Management of neonatal alloimmune thrombocytopenia remains a very difficult problem despite many years of clinical studies in Europe and USA. Dr M. Murphy presented the results of a recent European collaborative observational study on the antenatal management of neonatal alloimmune thrombocytopenia (NAIT). The patients received (i) maternal therapy (MT); (ii) intrauterine platelet transfusion (IUT); or (iii) serial fetal blood sampling (FBS) only. 56 fetuses from 55 pregnancies were studied. 18 patients received MT consisting of IVIG + prednisolone. This treatment was successful in 12 (67%). 33 received IUT and it was successful in 19. 24 cases had only FBS initially but 17 subsequently received other treatments. Only in 1 patient, the fetal platelet count dropped below 20x10^{9}/L warranting early delivery. In all treatment groups, no intracranial hemorrhage (IH) occurred after the treatment had commenced but IH occurred in fetuses before commencement of treatment. One case of IH was associated with infection following cordocentesis. In conclusion, all three treatment strategies appear effective, but IUT should perhaps be reserved for 'high risk' patients as it was associated with significant mortality: three deaths in this series. Dr. J Bussel presented the results of a US multi-center study on NAIT with stratification into 4 risk groups prior to randomization.
and treatment with IV IgG ± prednisone. The higher risk groups were given higher doses of IV IgG and at an earlier time. Like the previous US studies, IV IgG ± prednisone were found to be effective.

Every few years a new platelet alloantigen is discovered. There is an urgent need for a nomenclature of human platelet alloantigens that meets the approved of most investigators in the field. A proposal was made at this meeting for the formation of a Platelet Nomenclature Committee consisting of both members of ISBT Working Party on Platelet Serology and the representatives of the SSC Platelet Immunology subcommittee. This nomenclature committee will decide on a system of naming alloantigens, probably based on the current HPA system. The committee will also decide on the membership of reference and repository laboratories.

Iso antibody associated with pregnancy poses a difficult management problem. Dr. C Kaplan presented a review of the literature and 3 pregnant women with Glanzmann's Thrombasthenia and anti-GP IIb/IIIa isoantibody. Despite regular fetal cranial ultrasound monitoring, intra-uterine death due to intracranial haemorrhage occurred in two fetuses. One mother delivered a healthy infant without thrombocytopenia. Dr. Kaplan suggested the establishment of a registry to record cases and improve clinical management.

Recently there have been many reports describing an impact of platelet alloantigen genotypes on the occurrence of coronary artery disease (CAD). Some reported an impact but others showed no impact. Dr. S. Santoso presented his data as well as others and he concluded that platelet alloantigen genotypes have only a minor influence on the occurrence of CAD.

iii. **Heparin-induced thrombocytopenia (HIT).** Although the pathogenesis of HIT has been extensively studied, some issues remained unclear. Dr. P. Visentin (USA) presented convincing data that confirmed the role of T cells in the pathogenesis of this disorder. Dr. J. Walenga presented interesting results suggesting the role of leukocytes and cytokines in HIT. Dr. M Poncz and his colleagues, using a transgenic mouse model, confirmed for the first time the pathogenetic roles of platelet factor 4(PF4) and FcγRIIa in vivo.

There is a growing concern that laboratory testing for HIT lacks standardization. Dr. B Chong conducted a HIT serology survey under the auspice of the SSC Platelet Immunology subcommittee. The study showed that the hospital clinical laboratories performed the functional tests, e.g., platelet aggregation test, badly although their performance of the ELISA was excellent. In contrast, the referral or expert laboratories performed both the functional tests and ELISA very well. These findings suggest that functional tests are technically difficult. Unless there are properly trained staff to do the functional tests, clinical laboratories should use the ELISA or refer the HIT test to an expert laboratory.

Management of HIT patients with 'isolated thrombocytopenia' remains controversial. Drs T. Warkentin and A. Greinacher presented data from their studies to show that these
patients are at very high risk of developing thrombosis subsequently. Anticoagulant therapy is strongly recommended in these patients.

iv. **Thrombocytopenia associated with GP IIb/IIIa inhibitors:** With the increasing use of GP IIb/IIIa inhibitors in cardiac patients, more and more cases of thrombocytopenia associated with the use of these drugs have been reported; however, there is little data on its pathogenesis, diagnosis and management. Drs. R. Aster, A. Greinacher and H. Kroll presented their preliminary data on this "new disorder." The thrombocytopenia usually occurs abruptly, even on the first exposure to the drug, suggesting the presence of a natural occurring antibody. > 90% of patients had antibodies that would bind to platelets coated by the drug, but 70% of normal individuals also have the antibody, though a weaker form. The antibody can be detected by flow cytometry and the M.A.I.P.A., but treatment options are limited.
Platelet Physiology

Chairman: A.K. Rao--USA
Co-chairmen: M.C. Berndt--Australia; C. Cerletti--Italy; M. Hoffman--USA; A.D. Michelson--USA; P.J. Newman--USA; P. Nurden--France; S. Watson--UK

The main theme of this yearís session was platelet signaling and signal transduction defects. This was a continuation of the discussions initiated at the last SSC meeting in Maastricht. Drs. Deborah French and Dermott Kenny presented the reports on the existing databases on Glanzmann thrombasthenia and Bernard Soulier Syndrome (BSS), respectively. Both of the databases are running effectively. Dr. French indicated that all of the submissions were patients published in the literature. Dr. Kenny reported that the BSS web site had 1386 visitors so far. Dr. French agreed to update and incorporate a classification of thrombasthenia on the web site. Dr. Steve Watson discussed the ongoing efforts for establishing a database that covers platelet signaling and signal transduction defects. The main stumbling block is the need for financial support for setting up the database and this is still unresolved.

Several speakers reviewed the current information available with respect to different aspects of platelet signaling and information from specific knockout models and human platelet defects. Dr. John Hartwig reviewed aspects related to signaling and shape change. Dr. Guy Reed reviewed signaling mechanisms governing platelet secretion. At the receptor level, Dr. Athan Kuliopulos provided information on platelet thrombin receptors. Dr. Lawrence Brass described studies in mouse knock out models of Gᵦi family, including GᵦZ. Dr. Jean-Max Pasquet reviewed the signaling pathways involving tyrosine kinase/phosphatase pathways. Dr. Hidehiko Saito presented recently described mutations in non-muscle myosin heavy chain A in patients with May-Hegglin anomaly. Lastly, Dr. A. Koneti Rao summarized recent studies in patients with phospholipase C-β2 and Gαq deficiency with respect to thrombin-induced responses.

At the end of the meeting there was a discussion regarding the potential topics for the next meeting of the SSC in Boston in 2002. In addition, there were discussions regarding the working group on platelet signal transduction defects and about the areas that it should address. These were extensive discussions on the need for developing guidelines on specific methods widely used to study platelets. The specific areas that were included: 1) preparation of human platelets for studies, 2) preparation of mouse platelets, 3) platelet aggregation, 4) shape change, 5) flow cytometry, and 6) adhesion. In addition it was felt that a working party on platelet signal transduction defects should address the issues regarding the laboratory evaluation of patients with platelet function defects. Dr. James Bussell announced to the Platelet Subcommittee regarding a registry being established on patients with non-immune thrombocytopenia to facilitate studies on the molecular basis of the platelet abnormality.
Predictive Haemostatic Variables in Cardiovascular Disease

Chairman: L. Iacoviello--Italy
Co-chairmen: K.A. Bauer--USA; M. Cushman--USA; P.J. Grant--UK; R. Hull--Canada; G.D.O. Lowe--UK

The number of attendees of this subcommittee meeting was approximately 200.

Dr. L. Iacoviello presented the planned activities of the SubCommittee for the next year. Major issues were:

- Preparation of Guidelines for planning studies of genetic epidemiology
- Information on genetic polymorphisms available in "candidate" genes for haemostasis and thrombosis, on their functionality, on the protocols for their determination and on the validation of genotyping
- Standardisation of genetic polymorphism nomenclature
- Registry of on-going studies on the association between genetic and biochemical haemostatic variables and cardiovascular disease
- Development of a WEB page on our Subcommittee, in connection with the ISTH Web-page (http://www.negrisud.it/ssc)
- Systematic meta-analysis of the studies on predictive genetic and biochemical haemostatic variables in cardiovascular disease
- Preparation of Guidelines for blood management for homocysteine evaluation.

The first session of the meeting was focused on different methodological approaches to study the association between genetic haemostatic variables and ischaemic vascular disease.

Ongoing meta-analysis on biochemical predictive Haemostatic Variables in Cardiovascular Disease.

Dr. G Lowe underlined the necessity for meta-analysis to include a minimum of 1000 CHD cases in prospective studies, to consider the effect of confounders and the nature of samples to be analysed for antigen levels of haemostatic variables. In the framework of the British Regional Heart Study, they found a strong correlation between D-dimer, von Willebrand factor (vWF), and t-PA antigen levels measured in paired citrated plasma and serum samples. However, carefullness is still necessary in the interpretation of these results, until validation by further studies will be available. In contrast, their self-association over 5 years was lower and needs evaluation in further studies. Results of meta-analyses for fibrin D-dimer, vWF, and t-PA as markers of coronary risk were presented. They all included more than 1,000 CHD cases and showed a significant association with the risk of CHD. Adjustment for confounders did not modify the association for D-Dimer and vWF, while the association with t-PA decreased. A Fibrinogen Trialist Collaboration has been started to perform meta-analyses including more than 10,000 cases.

Methodological approaches for association population-based studies in genetics. Dr. A. Di Castelnuovo presented the use of association population-based studies in genetics. Association
studies are based on linkage disequilibrium (LD), a population-based concept identified by noting than certain alleles of two genes occur together in the population more often than by chance.

A major limitation of the approach is confounding by population stratification that can occur in ethnically mixed populations. A way to measure and correct for stratification has been recently proposed, by genotyping a moderate number of genetic markers unlinked with the disease and using the average of association across the unlinked markers as a direct measure of stratification.

Correct sample size estimation is of fundamental importance, especially in testing for gene-environment interactions. Methods to calculate sample size in the presence of interactions have been reviewed. Interaction must be supported by statistical tests, and not solely suggested by data. Synergy index (S) is a useful measure of interaction in case-control studies.

Relevant references to the topic are:

1. MJ Khoury et al. Fundamentals of Genetic Epidemiology, Oxford University Press, 1993
2. KJ Rothman et al. Modern Epidemiology, Lippincot-Raven, 1998
5. ER Reich et al. Genetic Epidemiology 20:4-16,2001
10. A Di Castelnuovo et al. Statistica 2001, in press
11. J Hoh et al. PNAS; 97:9615-9617,2000


The Utility of Family Studies for Understanding the Genetics of Thrombosis. Dr. J. Blangero provided an overview of a method for linkage analysis in extended pedigrees known as Variance Component Method (VCM). VCM is a powerful strategy to map genes related with quantitative trait loci (QTL) involved in pathogenesis of complex disease. VCM used data from large pedigrees containing more than 20 individuals in more than 3 generations. The method is based on a decomposition of the variability of the QTL in genetic and environmental causes. He showed that for a disease with large prevalence (35%), the VCM method is more powerful than other linkage-based strategies.

The author presented also some results of the GAIT project, a project aimed at identification of QTL involved in thrombosis, that made use of the VCM strategy in analysing data from Spanish families.

Twin studies to evaluate the genetics of haemostatic variables. Dr. P Grant presented examples on how to define the genetic components of single factors related to a syndrome, such as the insulin resistance syndrome. The inheritability of these factors has been suggested by
studies on offsprings of probands with diabetes, which showed increased levels of factors related to the metabolic syndrome (including haemostatic factors) as compared with those of the normal population. He presented results on 501 twin pairs that showed a high inheritability of F VII, F XII, F VIII, fibrinogen, vWF, PAI-1, F XIII levels. Moreover, also levels of activation markers of coagulation and fibrinolysis, as such as D-Dimer, prothrombin fragments, thrombin-antithrombin complex, showed a significant inheritability. These results were confirmed by the Leeds Family Study on 537 subjects from 89 families. However, when the most studied polymorphisms in haemostatic genes were tested in these models, they accounted only for a very small percentage of the total inheritability.

The CANVAS project and the candidate gene approach. Dr. F Cambien presented the "candidate gene approach" to understand the genetic component of ischaemic cardiovascular disease. The strategy consists in identifying all the possible candidate genes and all the possible relevant polymorphisms in functional parts of the genes. 300-600,000 single nucleotide polymorphisms in regulatory portions of the genes could be expected. It is necessary to genotype all the polymorphisms of a certain gene, since the functional one could be not in linkage disequilibrium with the others (the example of Apo A1 has been given). Dr Cambien then presented the GENE-CANVAS project. The project started in 1998 with the aim of screening for polymorphisms in candidate genes for ischaemic cardiovascular disease, providing assay conditions, allele and genotype frequencies and linkage disequilibrium of identified polymorphisms, and testing associations with ischaemic cardiovascular disease. At 2001, 29 studies, 102 gene and 454 polymorphisms have been analysed and are available at the Web site http://genecanvas.idf.inserm.fr.

The second session of the meeting was focused on differences in the association between levels of haemostatic and inflammatory variables and the risk of ischaemic vascular disease.

Homocysteine evaluation: problems and predictive value. Dr. M Cattaneo could not be present at the meeting.

Inflammation variables in the prediction of cardiovascular disease. Dr. Kluft reported on the ‘Leiden Meeting on Inflammation and Cardiovascular Disease’. Information is available at the web site www.haemost.nl and published in the Italian Heart Journal 2001;2:155-199. Three inflammatory markers have been discussed: CRP, SAA and SFLA2. CRP seems to perform better, since it presents very limited preanalytical problems, has a reliable assay, is widely available and can be considered as both a marker and a functional parameter. It is a systemic marker, but is also localised at the site of atherosclerotic plaques.

The intraindividual variation of CRP levels is about 30%. An upper quartile of PCR level >3mg/L has been established as a cut-off for high CAD risk.

Finally, treatment options have been discussed. The DALI study showed that atorvastatin treatment is able to strongly reduce CRP levels at the dose of 80 mg/day. The dose response for effects on CRP levels was not parallel with those of cholesterol lowering, suggesting that the reduction of CRP was not dependent on the effect of atorvastatin on cholesterol. Evaluation of
cost/effect benefit of statins in primary prevention showed an acceptable cost/benefit for people over 45 years.

**Inflammatory markers and sexual hormones.** Dr. Cushman presented an overview of the studies on the effect of hormone replacement therapy (HRT) on CRP levels. HRT doubles the levels of CRP and this effect could explain the results of the two trials that showed an increased risk of CAD after HRT. On the contrary, tamoxifen therapy reduced CRP levels. In the HERS trial, the increased risk of CAD in the first months of follow-up was associated to an increase in the levels of CRP.

**The VITA project: haemostatic variables and prediction of deep vein thrombosis and arteriosclerosis progression.** Dr. Tosetto presented the VITA study (1993 to 1997), a cross-sectional randomised study of 15,109 subjects in the area of Vicenza, Italy. The principal aim of the study was to define the prevalence and the impact of haemostatic variables on the thrombotic risk. For each subject recruited into the study information on family structure, history of thrombosis and DNA samples are available. The main results of the study confirmed the association of APC resistance with the risk of VTE and did not support an association of prothrombin polymorphism with VTE.

Secondary aims were the prospective evaluation of the risk and a cross-sectional evaluation of the impact of haemostatic variables on arteriosclerosis (evaluated as IMT of the common carotid artery) in a subsample of 2,000 subjects. The results showed an association between high fibrinogen levels and the risk of both high IMT and incident myocardial infarction. The VITA study is also part of a European collaborative study on the genetics of thrombosis, the GENERALE project.

The final discussion pointed out that the different methodological approaches to genetic studies are complementary; that genetic studies may be relevant in the understanding of the physiopathology of haemostasis and in defining the genetic components of haemostatic variables levels, rather than the risk of CVD; and that it is important to determine genetic variables together with the circulating levels of the corresponding proteins.
von Willebrand Factor

Chairman: R.R. Montgomery--USA
Co-chairmen: J. Eikenboom--The Netherlands; A.B. Federici--Italy; A. Goodeve--UK; P.A. Kouides--USA; C. Mazurier--France; J. Rand--USA; F. Rodeghiero--Italy

Presiding chair was Robert R. Montgomery (Milwaukee, USA); all co-chairmen were present.

The attendance was approximately 425 with many individuals needing to stand.

The session was divided into five general topics ó Clinical Epidemiology, Molecular Classification, Laboratory Standardization, TTP and VWF, and Potential Future SSC Studies.

The first session on Clinical Epidemiology was introduced by Dr. S. Seremetis (New York, USA).

Dr. P. Kouides (USA) presented data on the effect of menstruation and birth control pills on VWF testing. He proposed and will carry out a study to review the approach to VWD diagnosis at participating centers with regard to women on oral contraceptives and phase of their menstrual cycle.

Dr. A. Srivastava (India) presented data on VWD testing in developing countries. He noted that these data were from specific centers in third world countries and did not necessarily reflect the availability of testing throughout these countries. Many countries did not respond and the reasons for this were discussed.

Dr. C. Miller (USA) presented data on the differences of VWF ranges in different ethnic groups and focused specifically on the African American population. Discussion included comments that absolute level, rather than normalcy range of VWF, probably predicts clinical bleeding. Dr. Sadler emphasized the misnomer of "disease" when discussing a continual variable.

Dr. C. Mazurier (France) discussed the variety of concentrates available in Europe and their differences in labeling. Most are labeled in VWF:RCo but one is labeled in VWF:CB and another in VWF:Ag. The concentrate standard was identified as important.

Dr. Federici (Italy) discussed his survey on the use of DDAVP and the lack of uniform use and availability on a world-wide basis. He proposed the development of guidelines for its use by the SSC.

The second session on Molecular Classification was introduced by Dr. J.E. Sadler (USA).

Dr. Goodeve presented the approved plans by the European Union on centralized molecular testing in Europe. The study on molecular and extragenic causes of VWD or low VWF was discussed.
Dr. D. Lillicrap (Canada) reviewed polymorphic haplotypes in type 1 VWD. These studies are going to be done collaboratively with the EU Study presented by Dr. Goodeve.

Dr. D. Ginsburg (USA) presented updates on the VWF Database. A number of deficiencies were discussed and most related to the cost and longevity of database management. Dr. Ginsburg will see if both numbering systems can be included for ease of converting. This database is voluntary and includes prepublication and non-published reports. The subcommittee will consider the need for financial support and whether this can be sought commercially.

Dr. Montgomery introduced the third session on Laboratory Standardization.

Dr. A. Hubbard (UK) discussed the recent results on the new proposed VWF concentrate standard. Although both VWF:Ag and VWF:RCo units varied when assayed against the 4th IS Plasma Standard, most of this variability was corrected when one concentrate was compared to another using the concentrate standard. Much of the discussion focused on the collagen binding assay. A small working group was appointed to review this matter but the Concentrate Standard was recommended for SSC approval with the VWF:Ag and VWF:RCo activities as determined from his study.

Dr. R. Seitz (Germany) discussed the collagen binding standardization of concentrates using various collagens. Equine collagen appears to measure HMW preferentially compared to other collagens. The proposed European Pharmacopoeia use of collagen binding was discussed at length and it was recommended for further discussion between the ISBC, USA FDA, and interested European members.

Dr. A. Federici (Italy) discussed the need for an international standardization program. He proposed a future standardization study with a steering committee to guide its implementation. Dr. E. Preston (UK) discussed a program he was charged with to deal with third world proficiency testing. Drs. Preston and Federici will discuss this further to develop a coordinated approach rather than duplication. Since racial differences exist, it will be important to reference studies to the 4th IS Plasma Standard.

Dr. E. Fressinaud (France) discussed the use of ratios of VWF:Ag, VWF:RCo, and FVIII in the diagnosis of type 2 VWD phenotypes. These were correlated with molecular mutations that they have discovered.

The final session on TTP and VWF Cleaving Protease, introduced by Dr. T. Raife (USA) was extensively review by Dr. H-M. Tsai (USA). This was followed by a brief discussion by Dr. S. Vesely (USA). There are considerable differences in the phenotype (HUS or TTP) relationship between some centers. Standardization of the assay and standard plasma is needed.

Dr. Montgomery concluded with a brief discussion of laboratory partnering with third world countries to improve availability of quality testing. Time limitations prevented a discussion of this in detail. Dr. Srivastava will identify countries in need and Dr. Montgomery will identify centers willing to partner.
SUMMARY OF COMMITTEE ACTIVITIES

Approval and recommendation to the full SSC that the VWF:Ag and VWF:RCo assay be accepted for 1st Concentrate Standard and the SSC should recommend its adoption by the WHO.

For the present, the Concentrate Standard should not be labeled in VWF:CB units because of the variability of the collagens used for assay.

The committee recommends the acceptance of the VWF:CB units of the 4th IS Standard but that this should not be used to calibrate concentrates at this time and should only be applied to plasma assays.

Ongoing projects:

Survey on diagnosis of VWD in women on oral contraceptives and varying times in their menstrual cycle.

Study of general availability of VWF testing in developing countries.

Improvement of the VWF Database through corporate sponsorship or assimilation by another database system.

Development of SSC Guidelines for the use of DDAVP in VWD.

Study of VWF in more diverse ethnic groups.

Determine if recommendations on the type of collagen used for VWF:CB should be made before wide-spread development of assays has occurred.

Continue standardization of the VWF-cleaving protease assay.

Laboratory partnering for greater availability of VWF testing worldwide.

Proposed standardization of assays VWD subtypes on a world wide basis.