44th Annual Scientific and Standardization Committee Meeting

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The Subcommittee meeting was chaired by G. Johnson. Approximately 50 persons attended the meeting. Active discussion of the presentations and resolution occurred.

PROGRAM

The meeting was devoted to presentations on the following three topics:

1. Animal models useful for the study of fibrinolytic mechanisms.

   Dr. Jordi Felez described methods to study expression of plasminogen receptor binding sites on endothelial cells.

   Dr. Peter Carmeliet, Leuven, Belgium, described the utility of gene knockout mice in the evaluation of the role of fibrinolytic parameters in the development of atherosclerotic vessels and myocardial response to injury.

   Dr. Jose Paramo, Navarra, Spain, described studies of restenosis in atherosclerotic pig arteries and his observations of increased PAI-1 expression.

   Dr. Paul Holvoet, Leuven, Belgium, described the important differences between the atherosclerotic lesions seen in rabbits, pigs, and humans, and he presented data on the increased expression of MDA modified LDL in acute coronary syndromes.

2. Animal models useful for the study of venous thrombosis.

   Dr. Marcel Levi, Amsterdam, Netherlands, reported on an extensive review of the status of animal models of venous thrombosis. He emphasized the limitations of these models in predicting drug doses and efficacy in human venous thrombosis.

3. Animal models of restenosis.

   Dr. Gerhard Johnson, Minneapolis, USA, presented a position paper on the use of animal models in the study of arterial restenosis.

ACTION The position paper on restenosis, "The Utility of Animal Models in the Preclinical Study of Interventions to Reduce Human Coronary Artery Restenosis: Analysis and Recommendations," was approved by the Subcommittee for submission to the SSC and Thrombosis and Haemostasis.

FUTURE PLANS
1. To develop a position paper on animal models of venous thrombosis for presentation to the Subcommittee in 1999.
2. To explore the feasibility of having a program on \textit{in vitro} models at the 1999 meeting.
Professor Y. Ikeda gave a final report on methods and main conclusions on the effects of flow on endothelial cell function. Methods that were studied were (i) morphology, (ii) immunoassay of platelet and EC released products and (iii) immunoassay of EC-platelet reaction products, such as thrombin: ATIII:heparin, and this is done with and without presentation by immunoregulatory molecules such as cytokines. Of specific interest was the expression of tissue factor, and the combined influence of TNF-a and shear on this process. Both cone-and-plate and parallel-plate devices were useful.

Prof. J. Hubbell gave an intermediate report on the transport and biorheological issues related to the delivery of drugs to the artery wall and to thrombus adherent to the arterial wall. These methods included (i) various catheter designs with or without an imposed pressure gradient or electrical field, (ii) with permanent implants, such as graft sleeves, and (iii) with polymer depots, either within the artery lumen, within the media, or surrounding the adventitia.

Prof. M. Frojmovic presented a report on various issues in the study of platelet-platelet and platelet-leucocyte interactions under flow. There are biological issues, including presentation of new receptors (e.g., P-selectins), exposure to new agonists, and activation of intracellular signal transduction. There are biophysical issues, including flow-dependent collision, adhesion, and de-adhesion. These both relate to device issues, e.g., platelet-platelet collision in a Couette flow vs. platelet-thrombus collision in a parallel-platelet flow.

Prof. M. Frojmovic presented a brief overview of biorheological issues in the design of flow devices for clinical monitoring of thrombosis and coagulation. This served as an introduction for discussion of clinical devices.

Prof. D. Gabriel presented a brief overview of the clinical issues in monitoring of the thrombotic state, also as an introduction for discussion of clinical devices.

Prof. Marc Hoylaerts presented experience in the use of rectangular capillaries for the study of platelet deposition. Protein is deposited, the free surface is blocked, platelets are perfused, fixative is perfused, and then staining is performed. Quantification is performed by microscopy.

Prof. Y. Ikeda presented experience in the use of the quartz crystal microbalance in the study of platelet deposition on protein-coated surfaces. It was observed that the standard calibration equations that are employed for molecular overlayers are not appropriate for cellular overlayers, due to the viscoelasticity of the cellular overlayer. Nevertheless, the results could be quantitatively analyzed to provide real-time measurements of platelet deposition under various pathological conditions.
Dr. E. Heilman, from Dade-Behring, gave an overview on the PFA 100 instrument, which is based on the closure of a capillary with a collagen coating. The instrument is now used clinically to diagnose Glanzmann's thrombosthemia, von Willebrand's disease, and aspirin-induced bleeding.

Dr. C. Li, from Xylum, presented results with the Clot Signature Analyser. This orifice instrument has a flow through an orifice and a flow through a channel with a collagen fiber within. The instrument operates with native whole blood.

Dr. D. Varon, from the University of Tel Aviv, presented results on the use of cone-and-plate methodology for assay of platelet function. Both adhesion and aggregation are measured. Platelet deposition is examined both on extracellular matrix (produced by cell culture) and on polystyrene.

Dr. R. Hillman, from Accumetrics, was scheduled but was unable to attend.

Dr. J. Mitchell, from Hemodyne, presented methods for study of the forms involved in clot retraction. Over time after introduction of thrombin, the mechanical properties of the fibrin network and of the contractile form generated by the platelet are measured. The method has also been used to monitor fibrinolysis.

Each of the methods developed and described above has been used clinically and has been calibrated in standard coagulopathies.

Extensive discussion followed, with general agreement to hold a major symposium on the above topic of "Rheological Bedside Devices for Monitoring and Managing Vascular Diseases: Current Status and Future Needs" at the Washington meeting in 1999.

New topics were suggested, including "Rheological Models for Bleeding/Thrombotic Disorders", and (2) "Flow Effects on Blood Cell Function".

Attendance was excellent with 45 participants.
Control of Anticoagulation Subcommittee

Chair: F. E. Preston, UK
Co-Chairs: G. Agnelli, Italy; T. W. Barrowcliffe, UK;
A.M.H.P. van den Besselaar, The Netherlands; B. Boneu, France; A. Giles, Canada;
L. Poller, UK; A. Tripodi, Italy

Current Tasks:

The Chairman welcomed the participants and outlined the Programme. On this occasion the Subcommittee has been allocated two complete sessions. A report was received from the Working Party on the Preparation and Use of Calibration Plasmas for INR Determination (T. Barrowcliffe, Chair) and recommendations from the Working Party on LMW Heparin Monitoring (A. Giles, Chair). Reports were also received from E. Gray and A. Tripodi on Unfractionated Heparin Replacement of WHO International Standard and WHO/oral anticoagulation respectively.

Report from Working Party on Preparation and Use of Calibration Plasmas for INR Determination (T. Barrowcliffe, Chair)

Dr. van den Besselaar presented results of a collaborative study on INR calibrated plasmas. The purpose of the study was to evaluate different types of lyophilized plasmas for local calibration. Significant INR differences were demonstrated for the lyophilized plasmas between the prothrombin time systems. The differences were relatively small for the deep-frozen coumarin plasmas and three lyophilized coumarin plasmas from one manufacturer.

Professor Poller summarized 1998 results from the European Concerted Action on Anticoagulation (ECAA). He stressed the following important points:

- In thromboplastin calibration, artificially depleted and fresh coumarin plasmas give reasonable differences from conventional plasmas but these are acceptable in clinical terms.
- Although a minimum of 20 abnormal plasmas are ideally required for ISI calibration it may be possible to achieve this with ten.
- Fresh plasmas give reliable ISIs when calibrating human and rabbit reference reagents across species, but freeze-dried plasmas needed to be certified in terms of a similar species reagent.

INR correction by local ISI assignment appears successful with the ECAA human reagent but not with the rabbit reagent.

Dr. Houbouyan reported that substantial improvement of interlaboratory variability of INR determination could be achieved by the use of plasma calibrants. She demonstrated that at least three calibrants are required to cover the therapeutic range and that in respect of unproved interlaboratory variability AVK calibrants were superior to ART (artificially depleted) calibrants.

Dr. Taberner reported on the preparation and practical use of calibrated plasmas. He particularly highlighted the practical difficulties of preparing calibrants and discussed different methods of analysis.
Dr. Johnston reported the results of a local calibration exercise using Immuno reagents and Thromborel S. She reported apparent lot to lot variability of the calibrants and she expressed the view that international validated reference plasmas were required for calibration.

Dr. Yetsuka presented results from Japan of a comparison of manual and instrument-derived INR results using NIBSC control plasma. The study confirmed recognized problems associated with instrument use.

Dr. Fischer (Organon Teknika) presented the company strategy in respect of calibration plasmas for INR determinations.

Dr. Jaklitsch and Dr. Lang (Immuno) presented the results of a study in which target values of AK calibrants were compared with those of European Reference Plasmas.

Dr. Barrowcliffe summarized the good progress made in this area and concluded by indicating that the group will continue to work together toward developing draft guidelines in respect of the preparation and use of calibrated plasmas for consideration by the Subcommittee.

**Does the Lupus Anticoagulant influence INR Determination?**

Dr. Mackie reported that lupus anticoagulants have no effect on INR determination if low instrument-specific ISI thromboplastins are used. A minority of patients may have potent antiprothrombin antibodies and prolonged prothrombin times.

Dr. Moll presented data to support his view that lupus anticoagulants can influence the PT and lead to INR values in warfarinized patients that do not reflect the true level of anticoagulation.

Dr. Houbouyan presented results of a French study designed to address the influence of the lupus anticoagulant in INR determination. She concluded that the use of calibrants reduces and abolishes the LA effect on INR.

Prof. Tripodi presented details of a current collaborative study designed to investigate the influence of LA on INR determination.

Prof. Machin (Co-Chair, Lupus Anticoagulant Subcommittee) suggested a joint exercise involving this Subcommittee and the Lupus Anticoagulant Subcommittee to explore further the clinical impact of LA on INR determinations.

Dr. Gray reported on the Collaborative study to establish the 5th International Standard for Unfractionated Heparin. Twenty-four laboratories from 14 countries participated. Materials included two candidates, the 4th IS, the USP reference standard and the EP reference standard. After WHO consultation on June 17-18, 1998, it was agreed that sample A, 97/578 will be proposed to the SSC of the ISTH and the ECBS of WHO as the 5th International Standard for UFH.

Prof. Tripodi provided a detailed account of the long standing collaboration between the SSC of the ISTH and WHO. He reviewed the situation of currently available WHO International Standards for thromboplastin and summarized the main issues discussed in the WHO Guidelines.

Dr. van den Besselaar described the effect of sodium citrate concentration and collection systems on prothrombin time and INR determinations. He reiterated the WHO recommendation for 109 mmol sodium citrate concentration.

From the ensuing discussion it became clear that this recommendation is not always adopted and Prof. Tripodi recommended that the Subcommittee prepare a recommendation in respect of this.
Prof. Fareed described the pathophysiology of heparin induced thrombocytopenia and reviewed the methods of detection. He gave a detailed account of thrombin inhibitor monitoring.

**Recommendations from the Working Party on LMW Heparin Monitoring (Dr. A. Giles, Chair)**

Dr. Massicotte presented a report on behalf of Dr. Andrews on LMW Heparin monitoring in Illdsen. She stressed critical pediatric issues which included weight, age, dependency of pharmacokinetics and the necessity of monitoring during long term therapy. Detailed information is available for two LMWHs. Monitoring should be undertaken with anti Xa assays 4-6 hours after a subcutaneous dose. If unexplained thrombocytopenia occurs HIT should be considered. Two relevant international clinical studies are underway and should help clarify the issue of LMW heparin monitoring.

Prof. Samama considered LMW heparin monitoring in adults. He discussed the clinical indication for treatment with LMWH and stressed that dose responses were unreliable in obese patients, in the elderly, and in those with impaired renal function. He indicated that monitoring with anti Xa could be of value in these categories of individual and possibly in those at high risk of bleeding or postoperative thrombosis. Additional monitoring should be considered in pregnant women receiving long-term LMW heparin. Platelet count monitoring is strongly recommended.

Prof. Giles concluded by indicating the despite limited available information the Group will continue to work together towards developing draft guidelines in respect of recommendations relating to LMW heparin monitoring in children and adults.

S. Kitchen reported clinically important differences in anti Xa results obtained with different assay systems on samples from patients receiving LMW heparin.

L. Mitchell reported results of an in vitro study which showed that conventional chromogenic heparin assays are influenced by the patient's plasma AT level.

Dr. Kovacs described a series of studies of LMW heparin monitoring. He concluded that present methods of monitoring are inadequate in that there is poor comparability between different assay systems.

Prof. Harenberg discussed therapeutic ranges for new anticoagulants. He considered the use of heptest for LMW heparin monitoring and the ecarin clotting time for hirudin/PEG-hirudin.

Prof. Jespersen reported an ECAA multicentre randomized study on computerized anticoagulant dosage. Results using the DAWN system were presented. The computer dosage programme gave significantly better INR control than that obtained by clinical staff.

Dr. Palareti discussed factors which influence the risk of bleeding in oral anticoagulated patients. He emphasized differences in published definitions of bleeding classifications and suggested that the Subcommittee should provide recommendations in respect of this. He further indicated a need for guidelines in reversal of oral anticoagulant therapy.

The meeting closed at 17:55 hrs.
**DIC**

Chair: F. Taylor, USA  
Co-Chairs: M. Blombäck, Sweden; M. Kazama, Japan;  
M. Levi, The Netherlands; T. Matsuda, Japan; I. Bokarew, Russia;  
J. W. Ten Cate, The Netherlands; N. Sakuragawa, Japan

**Purpose:** Define overt-DIC with and without organ failure by 2001 using clinical criteria and laboratory assays currently available. This definition will be based on the Japanese Ministry of Health criteria. It will include the addition of soluble fibrin as a marker and additional major and minor criteria and degrees of severity based on the duration and rates of change as well as cut-off values of clinical and laboratory parameters.

**Goals:** These criteria should serve as a basis for monitoring and management as well as diagnosis of this disorder in conventional medical settings. It also should serve as a standard to be used in publishing research data in this area.

**Rationale:** The hemostatic system is an organ consisting of endothelium and monocyte/macrophage/platelet elements as well as the mediators and regulatory factors (e.g., thrombin, protein C) found in plasma. These components act in a coordinated fashion to regulate clot formation (coag., anticoag.) and clot removal (fib. and antifib.). Acquired DIC (overt and non-overt) arises when the regulatory functions of the endothelium are either overridden or attenuated by inflammatory (sepsis) or by ischemia reperfusion (trauma) events. Though the thrombosis and hemorrhage that often accompanies DIC may not always be a link in the lethal chain of events, the hemostatic organ system is the first to be perturbed and its malfunction is closely linked to and amplifies the inflammatory response. Sepsis, for example, could be described initially as an acute disorder of the vascular reticulo-endothelial system.

**Execution:** The Japanese, English, and Dutch have been engaged in collaborative studies on primates with our laboratories over the last year. The results are provocative and have engaged their active interest. New, young participants have been added to the committee (Levi, Toh and Wada from Holland, England, and Japan). The most active, experienced current members also are actively engaged, (Blombäck, Sakuragawa, Bokarew, and Frances). Together all have agreed on seven specific questions to be addressed this year. A draft then will be submitted to a group of external advisors (V. Marder, A. Giles, K. Bauer, C. Esmon, B. Dalbach, etc.) for review.

**Non-overt DIC:** The same approach as described above will be taken. An important difference will be that the newer laboratory procedures including soluble fibrin, TAT, APC/PCI, etc., will come into play in discriminating between compensated and decompensated hemeostatic stress, assuming that eventually they can be used on-line.
Factor VIII and Factor IX Subcommittee

Chair: J. Ingerslev, Denmark
Co-Chairs: D. DiMichele, USA; K. Mertens, The Netherlands; I.R. Peake, UK; C. Prowse (secretary), UK; C. Negrier, France; R. Schwaab, Germany; A. Yoshioka, Japan

Apologies have been received from Dr. D. DiMichele and Dr. R. Schwaab for their absence.

Final Reports

The Nijmegen-Kingston modification of the Bethesda assay has been published as a SSC Communication for *Thrombosis and Haemostasis* (*Thromb. Haem.*, 1998; 79, 872).

A manuscript on the North American Immune Tolerance Registry is soon to be expected (Dr. D. DiMichele).

The final report on the Registry of Factor VIII and IX Concentrates was presented by Dr. C. Kasper. The Subcommittee approved the report and it will be forwarded to members of the F VIII/IX SSC followed by submission to ISTH for publication. The FVIII/IX SSC recommended an ongoing update of the registry be made available on the ISTH homepage on the Internet.

Ongoing Activities

No activities were reported from the working group formed last year on unexpected bleeding in haemophiliacs treated with protease inhibitors. In discussion, the SSC suggested that case-controlled studies should be performed and links established to the North American Haemophilia Society Study Group in this area, and that this effort be coordinated by Dr. Thyn Yee of the Royal Free Hospital.

Dr. G. White stressed the advantage of using PTPs for study of viral safety and immunogenicity of novel or modified products, and was asked to draft a letter to the Editor of *Thrombosis and Haemostasis* on the matter, passing the letter through the Chair.

Dr. Berntorp had reported to the Chair that inclusion of patients was slow for the safety study of non-infected patients studied by PCR technology, and there was no new data to present this year. On behalf of Drs. P. Mannucci, C.A. Ludlam and himself, Dr. F. Hill presented the latest version of the amended protocol for safety studies in PUPs. Following discussion, it was recommended that this be the final version passed to the members of the F VIII/IX SSC for final comments prior to submission to ISTH for publication as a recommendation.

Standardization Issues

Dr. A. Hubbard reported on the collaborative study for the replacement of the existing WHO Standard for Plasma F VIII/vWF.

Dr. T. Barrowcliffe reported on the Collaborative Study for the replacement of the existing WHO Standard for Concentrates (new Standard: 6th International Standard). There was a significant increase of around 20% by the chromogenic method as compared to the one-stage technique. Dr. Barrowcliffe will contact participating laboratories and return to the F VIII/IX SSC with more information.
Dr. T. Barrowcliffe reported on the SSC/5 "Field Type" Collaborative Study on Concentrates, one involving F VIII, the other involving F IX, and proposed a further exercise by October 1998.

Dr. M. Weinstein reported on the status of the MEGA-II and hoped to have product vialled by the autumn.

Dr. A. Hubbard reported on the proposed International Standard on F VII Concentrate. 

Lastly, Dr. E. Gray summarized data on her study of FIXa standard material, and suggested a future proposal of an International Standard.

**FVIII Assay Discrepancies**

Dr. M. Mikaelsson summarized three studies on the characterization of recombinant factor VIII with and without B-domain, and high purity plasma-derived factor VIII, demonstrating equivalence of activity measured by chromogenic assay and antigen content. *In vitro* studies showed that excess phospholipid reduces chromogenic activity, particularly for the B-domainless recombinant FVIII. Pharmacokinetic comparisons showed that the use of a consistent assay yielded expected results for both B-domainless recombinant and high purity plasma-derived products.

Dr. D. Owens presented a follow-on study from that presented by Dr. C. Lee at last year's meeting showing that the use of a concentrate standard for assay of both product and post-transfusion samples yielded recoveries close to those expected for both recombinant and plasma-derived FVIII.

In discussion it was agreed that Dr. Barrowcliffe would write a letter of recommendation on this approach to pharmacokinetic studies for forwarding to the FVIII/IX SSC chairman. It was noted that this approach only addresses pharmacokinetic assessments, and not the link between these and patient dosing required for pharmacodynamic considerations.

Dr. J. Ingerslev summarized results from his centre demonstrating an excess of one-stage over chromogenic activity when assessing 68 patients with mild haemophilia, such that 12% of patients would be reclassified as moderate. Similar data had been published by Dr. Mazurier and shown to be associated with mutations at around residue 530 of FVIII.

Dr. Oldenburg presented six cases from four mild haemophilic families in whom chromogenic activity was normal despite one-stage coagulant activities being reduced and showed an association with mutations at residues 720 or 1689.

**Inhibitors**

Dr. Lusher summarized the results on transient inhibitors from the three recombinant FVIII PUP studies, suggesting that these might be characterized as (1) low level inhibitors present in serial samples but then disappearing, but associated with low recoveries while present, (2) low level in serial samples which then disappear but reappear later (3) inhibitors only ever described in single samples (probably false positives). In discussion the need for a consistent definition of inhibitor type, the need to define tolerance therapy regimes and the option of linking inhibitor type with specific FVIII gene defects were raised.

On behalf of Dr. F. Rosendaal, Dr. J. Ingerslev made a formal proposal to form a Working Party on the Nomenclature of Inhibitors.

Dr. S. Kitchen presented data from recent NEQAS studies among 67 UK haemophilia laboratories, showing a wide variation in inhibitor potency assessment for three (two
haemophiliac and one acquired) inhibitor plasma. He proposed that a similar exercise be undertaken internationally, led by Prof. E Preston, under SSC auspices, and invited interested laboratories to participate in this.

Dr. C. Hay presented the outcome of a recent meeting in Bonn on the planned "International Controlled Comprehensive Cohort Study of Immune Tolerance Induction." The plan is to recruit 150 patients over two to three years and compare a low and a high dose regime in this area. After some discussion the meeting agreed that the proposal merited support of the FVIII/IX subcommittee and that it would be circulated to invite their support.

Dr. J. Ingerslev reported that Dr. G. Mariani was inviting participation in a new registry of any patients entered into tolerance regimes, with a view to linking this to data on their gene changes.

Dr. Oldenburg provided a summary of recent progress on the FVIII gene mutations and HLA types associated with the formation of FVIII inhibitors. Together with Dr. Schwaab he proposed the formation of a "Working Group on the Surveillance of Genetics and the Development of Inhibitors."

Miscellaneous
Dr. W. Schramm gave a presentation on a multicentre European study comparing the costs of prophylactic and on-demand therapy of haemophilia, and providing some data on the improved quality of life afforded by the more expensive prophylactic option.

SUMMARY OF NEW ACTIVITIES

Formation of new Working Groups on:

- Proficiency Study on FVIII Inhibitor Assay
- Terminology and Definition of Inhibitors
- Genetics and Development of Inhibitors
- Protease Inhibitor Use and Bleeding (in HIV infected individuals)
- Support for International Controlled Comprehensive Cohort Study of Immune Tolerance induction
- Support for use of FVIII concentrate standard for FVIII recovery studies

Next meeting in Washington in 1999.
Factor XIII
Chair: L. Muszbek, Hungary
Co-Chairs: P. Board, Australia, C.S. Greenberg, USA,
A. Ichinose, Japan

The Subcommittee Meeting was chaired by A. Ichinose and L. Muszbek, C.S. Greenberg and P. Board could not attend. Attendance was approximately 50 throughout the meeting. Many valuable remarks, comments and questions came from the audience, and after each presentation there was a lively discussion. The Subcommittee concentrated on four different issues:

1. Role of FXIII-A subunit polymorphism in vascular diseases.
2. Methodology and the need for standardization of FXIII assays.
3. Animal models for inflammatory bowel disease (IBD) in which the effectiveness of FXIII supplementation could be tested.
4. FXIII B-subunit deficiencies.

1. Dr. P. Grant gave an overview of the most recent findings on FXIII-A polymorphism in different vascular diseases. Val34Leu was the only mutation among the several they tested which as a protective mutation showed (inverse) correlation with arterial diseases. It was also demonstrated that the cardioprotective effect was lost if PAI-1 level was high or if the patients had insulin resistance. The wild type was more common in DVT as well. There was an interesting discussion about the possible mechanism of the protective effect with special reference to the fact that the mutation is three amino acid residues up-stream of the thrombin cleavage-site.

2. Dr. Muszbek gave a critical review of FXIII methodology with a proposal for the requirements of reliable FXIII assays which are aimed to be used widely in clinical laboratories. Then, he presented two new assays, sample kits are about to be distributed among interested expert laboratories for evaluation. The functional assay was a modification of the UV spectrophotometric assay based on monitoring ammonia released during the transglutaminase reaction. The antigen assay was a one-step sandwich ELISA which detected only plasma FXIII, i.e., the tetrameric complex of the two subunits, and showed remarkable sensitivity.
Dr. Jennings from UK NEQAS presented the first external quality assessment survey on FXIII determination. A relatively high number of laboratories participated and the performance of classic clot solubility test was surveyed. There was a surprising variation in the set-up of the test and accordingly the results also varied considerably. Samples from patients with severe inherited and acquired deficiencies were not recognized as abnormal by 60% and 30% of the laboratories, respectively. There was a long discussion on the use of the clot solubility test, with the general conclusion that, although it still has a place in the diagnostics of FXIII deficiencies, the general practice of using it as a "screening test" should be abandoned. UK NEQUAS was encouraged to continue the survey and to include FXIII determination in its coagulation survey profile.
Dr. Longstaff from NIBSC presented a talk on the possible developments of FXIII reference plasma. The audience highly supported the idea and the need for such a reference material was emphasised and supported. A list of participating expert laboratories is to be provided to NIBSC by the chairman of the Subcommittee.

3. Dr. Bishop presented pieces of evidence demonstrating that dextrane sulphate-induced inflammatory bowel disease in mice could be a useful model for investigating the mechanism of beneficial effect of FXIII supplementation in IBD.

4. Dr. Ichinose reviewed the cases of FXIII-B deficiencies and the underlying molecular genetic defects, and the classification of FXIII deficiencies was discussed.

In the general discussion it was recommended that promotion of the development of a reference plasma should be number one priority of the Subcommittee during the coming year.
Fibrinogen

Chair: M. W. Mosesson, USA
Co-Chairs: F. Brosstad, Norway; M. Matsuda;
W. Nieuwenhuizen, The Netherlands

The Fibrinogen Subcommittee met to discuss a single subject, "Criteria for Characterization of Fibrin Sealants." Fibrin sealants are complex, multi-component, biological preparations that are used in a variety of clinical situations to promote hemostasis and wound healing.

There are few established criteria or standards for characterizing the content and functional behavior of these preparations. We had seven presentations on the subject, and a lengthy and lively discussion afterward concerned with criteria, methods for measurement, and possible standards. We agreed that certain characteristics such as fibrinogen content, thrombin concentration, and factor XIII concentration were of importance and furthermore, that standards for fibrinogen and thrombin be sought. We intend to pursue this goal by an interactive correspondence during the coming year. We will also use this means to define other characteristics of fibrin sealant content and extend the dialogue to include certain functional parameters.
Fibrinolysis

Chair: Nuala Booth, UK
Co-chairs: P. Declerck, Belgium; C. Kluft, The Netherlands; Osamu Matsuo, Japan

The meeting was attended by 50-90 members.

Carboxypeptidases, including TAFI

This was a new topic for the subcommittee. Dr. Dirk Hendriks summarized the current state of knowledge on these enzymes, including specificity and physiological location. He discussed assay methods and the inhibitors that were available to identify which enzymes are active in a particular situation. He recommended that the standard nomenclature be adopted, including the recently agreed EC number. Dr. Lazslo Bajzar then addressed the specific topic of TAFI (also known as carboxypeptidase U) and gave information on a functional assay and on a novel ELISA. He presented data on a small group of normal individuals. It was agreed that this topic was of growing interest and that it should be on the program for 1999.

Measurements of proteins of the fibrinolytic system in animal models

Dr. Paul Declerck presented an overview of methods suitable for non-human samples, both functional and immunological. Additional information on assays and availability of antibodies was presented from the floor (Dr. HR Lijnen). It was agreed that a written summary of available antibodies should be made available and Paul Declerck offered to prepare this with a view to publication in *Thromb. Haemost*.

Standards for proteins of the fibrinolytic system

Dr. Patrick Gaffney made the case for a new standard for plasmin. He presented data on assignment of a value of 5.3 IU/ml to the new standard. It was agreed that this material should be recommended to the WHO Expert Committee on Biological Standardization. He and Dr. Booth, as Chairman, would discuss its presentation to the Committee with the SSC's presentative to the WHO.

Dr. Gaffney's second topic was on single-chain uPA. A collaborative group had assayed two preparations of this material, one produced in *E coli* and one in CHO cells. The agreement between all five centres was excellent, but there was a discrepancy in the activity of the CHO product in chromogenic versus clot lysis assays. It was agreed that, in the light of this, it was too early to propose either of these materials as a standard. It was agreed that Dr. Gaffney should offer these two preparations as NIBSC reagents, giving the full data on site of production and activities in both assays. The average data from the collaborative group would be included in the information given.

Blood collection for assays of fibrinolysis

Dr. I Walker was unable to be present so Dr. C Kluft summarized a document she had prepared. Since this was of relevance to other subcommittees, he undertook to consult with other interested parties and to bring together a final version of the report to be made available to any member of the subcommittee for eventual publication.

Fluctuations in fibrinolysis throughout the menstrual cycle
Dr. C Kluft presented the results of a literature review of this topic. There were strong indications of changes in some components of the system over the menstrual cycle in some studies but no clear changes emerged. Dr. Kluft intends to pursue this topic in further studies but there was little support for making this a subcommittee initiative at this stage, in view of the difficulty of carrying out a sufficiently wide-scale study to achieve a clear outcome.

**Reports for PGM working group**

Dr. C Kluft chaired this session, reporting on the activities of the Project Group on Methods, consisting of Drs. J Gram, J Jespersen, TW Barrowcliffe, PJ Declerck and CW Francis, with Dr. Kluft as chair. He explained that this group was part of general SSC activities that had initially concentrated on fibrinolysis. It aims to examine the methods used to measure particular analytes and to produce reports on the criteria necessary to achieve reproducibility across laboratories. Two such reports, on tPA antigen and plasmin inhibitor, are in preparation.

Dr. B Binder presented a review of methods to measure scuPA. His conclusion was that there was no single analyte measurement of which there was good experience at this stage. It was therefore decided to put the issue of standardization aside until this became available.

Dr. J Sidelaman presented data on plasminogen measurements, showing the wide fluctuations in data achieved in a WHO EQAS initiative. It was decided that several collaborators were required to assay a number of samples and to report back to the subcommittee.

**The introduction of the IFCC/SSC working party**

Dr. C Jackson explained the background to the formation of The Joint Committee on Standardization of Coagulation Effects, comprising Drs. J Jespersen, J Rosing, P Esnouf, G White, TW Barrowcliffe, C Kluft and J Lenahan with Dr. Jackson as Chair. This initiative will impact on the Fibrinolysis Subcommittee in that it will cover some of the issues covered up to now by the PGM.

**Plans for 1999**

It was agreed that topics for the SSC meeting in Washington should include

- an update on carboxypeptidases
- local fibrinolysis on the surface of platelets and other cells
- D-dimer

Further suggestions should be sent to Dr. Booth (n.a.booth@aberdeen.ac.uk), Dr. Declerck (Paul.Deleclerck@farm.kuleuven.ac.be), Dr. Kluft (Kluft@euronet.nl) or Dr. Matsuo (kr9omto@asani-net.or.jp).

The meeting finished at 5:15.
Approximately 20 persons attended the subcommittee meeting. All participated actively in the discussions.

The first half of the meeting was devoted to hypercoagulability and thrombosis in the obstetrical patient. Drs. Margreta Hellgren and Jacquie Conard presented a comprehensive summary addressing epidemiology, diagnosis, therapy and prophylaxis of thrombosis during pregnancy and the puerperium. The management of women with thrombophilia received special attention. An enormous effort was evident in the breadth and depth of the data and was appreciated by all present. A proposal was made to develop this report into Guidelines to be presented to this committee at the Washington meeting with publication recommendations at that time.

Dr. James Bussel gave an update on Neonatal Alloimmune Thrombocytopenia. It was proposed that an updated Guidelines paper on this topic (last done in 1991) should be prepared and presented at the Washington meeting in anticipation of publication. We would like to see these guidelines published as a joint recommendation of the Platelet Immunology and Perinatal/Pediatric Subcommittees.

Drs. James Bussel and Anton Sutor reported on their assigned task to determine the scope and correlates of intracranial hemorrhage in children with ITP. A questionnaire has been prepared, IRB consent has been drafted and the joint project should commence in North America and Europe by September. Preliminary results will be reported in Washington.

Dr. Sutor reported on the Subcommitteeâs position paper on Neonatal Vitamin K deficiency. This paper is recommended for publication. Dr. Sutor will add the requested statement on breastfeeding while taking oral anticoagulants and will share this recommendation with Dr. Hellgren to ensure that the obstetrical recommendations are identical.

Dr. Patti Massacotte reported on use and monitoring of 2 LMW heparin preparations in infants and children, Enoxaparin and Reviparin. It was proposed that recommendations for use of unfractionated heparin, LMW heparin and thrombolytic agents would be combined and prepared for publication from this Subcommittee. That manuscript should be completed this year.

The discussion on coagulation testing in children with thrombosis had to be abridged due to the absence of Dr. Nowak-Göttl and the dearth of time. These recommendations will be drafted as a Guidelines paper and circulated to committee members for comment. A final report will be presented in Washington.

**New Business:** After discussing a number of potential topics, the participants in the meeting expressed interest in future exploration of the following issues:
**Obstetrical:** evaluation and management of bleeding disorders in pregnant women.

**Neonatal:** DIC (to be done in collaboration with the DIC subcommittee with Dr. Keith Hoots as a liaison).

**Pediatric:** venous occlusive disease in transplantation and chemotherapy: epidemiology, pathophysiology and implications for therapeutic trials.
Platelet Immunology

Chair: D. Beardsley, USA
Co-Chairs: R. Aster, USA; P. Berchtold, Switzerland; B. Chong, Australia; C. Kaplan, France; T. Kunicki, USA

The Platelet Immunology Subcommittee addressed topics in two broad areas: autoimmune thrombocytopenia and alloimmune thrombocytopenia. New subcommittee activities were initiated in both areas this year.

The pathogenic mechanism of autoimmune thrombocytopenic purpura (ITP) involves immunoglobulin opsonization of platelets by autoantibodies and clearance of the platelets in the reticuloendothelial system. Triggers for production of the autoantibodies include immunodeficiency states, viral stimulation, and possible immunoglobulin germline genetic states. Mechanisms for the emergence of B cells that produce autoantibodies are under investigation. This topic will be included in the agenda for the next meeting. It was also suggested that platelet immunology laboratories and ITP treaters be polled as to whether alternative markers can be used to eliminate the need to do a bone marrow examination on the patient suspected to have ITP. In all cases, it was emphasized that the peripheral blood film must be examined to rule out any evidence for microangiopathy, abnormal leukocytes, giant platelets, or myelophthisis (tear drop forms or nucleated erythrocytes.)

Alloimmune thrombocytopenia (AIT) can affect both the neonate and fetus. Management of the first affected infant in a family may include transfusion with alloantigen negative or random donor platelets, treatment with IV IgG and/or steroids, or possibly exchange transfusion. Management of subsequent pregnancies affected by AIT varies. In the United States, weekly immunoglobulin is usual, while in Europe, practices vary in different countries. A multicenter study is in the planning phase in Europe. The subcommittee decided to undertake preparation of a review and analysis of the results of antenatal IV IgG treatment for pregnancies affected by AIT. This should be valuable to the clinician who has to make decisions in individual cases. In addition, it was suggested that the subcommittee publish a letter detailing considerations and recommendations for the preparation of antigen negative platelets for transfusion in utero at the time of percutaneous umbilical blood sampling. Features to be considered include filtration, irradiation, HLA compatibility, plasma depletion, and testing for transfusion transmitted diseases.

Standardization of nomenclature for the platelet alloantigens is an objective of this subcommittee. It was decided to cooperate with the International Society for Blood Transfusion (ISBT) platelet serology group in an attempt to come to a consensus regarding nomenclature for the platelet alloantigens.
Platelet Physiology

Chair: M. Berndt, Australia;
Co-Chairs: C. Cerletti, Italy; H. Deckmyn, Belgium;
M. Hoffman, USA; J. L. McGregor, France; P. Newman, USA;
A. K. Rao, USA; G. C. White, USA

The Committee met under the chairmanship of Dr. M.C. Berndt (Australia) with co-chairmen, Dr. G.C. White (USA), Dr. K. Rao (USA), and Dr. H. Deckmyn (Belgium) also attending. Approximately 40 delegates attended the session. The subcommittee presented a final programme primarily covering recent basic and clinical insights into agonist-dependent signal transduction in platelets.

Dr. Koneti Rao spoke on the topic "Disorders of platelet activation" within a context of a proposed classification of congenital disorders of platelet function. He described several patients with abnormalities of platelet aggregation and emphasized the need for a detailed classification for those patients with disorders of platelet secretion and signal transduction. Analysis of these patients, one with low levels of Gaq and the other with defective pleckstrin phosphorylation, have provided important insights into signal transduction pathways induced by ADP and thrombin.

Dr. Michael Barnes described how studies from his laboratory with synthetic collagen sequence peptides have helped clarify the mechanisms by which platelets adhere to collagen and become activated. His studies have indicated that platelets primarily adhere to collagen via the integrin a2b1, but are activated via GPVI. Different sequences in collagen are involved in both these events.

Dr. Steve Watson further developed this theme in an elegant presentation describing research from his laboratory on collagen-dependent signaling pathways. These studies have made collagen-induced platelet activation one of the best understood signaling pathways and illustrated the value of employing multiple approaches in studies of signal transduction.

Dr. Michael Berndt presented a brief overview of genetic abnormalities in Bernard-Soulier syndrome and presented some recent data on structure-function of the GP Ib-IX-V complex using canine-human chimeras. A tabulation of the clinical reports and genotype of all reported Bernard-Soulier syndrome patients was presented as a framework for establishment of a Bernard-Soulier register and web site. There was discussion as to how new information/patients could be added. There was agreement by the subcommittee that the register and web site should proceed and that it would compliment the Glansmann's thrombosthenia register and web site.

The meeting closed with discussion of potential programme topics for the SSC meeting in Washington. There was general agreement that a session on evaluation of GP IIb-IIIa receptor function in the light of oral antagonists would represent an important topic. This and other potential areas for subcommittee evaluation will be further considered in the coming months. There was a general view that earlier announcement of the programme would be valuable for attendees.
Predictive Variables and Cardiovascular Disease

Chair: K. Bauer, USA
Co-Chairs: R. Hull, Canada; G. Lowe, UK; F. R. Rosendaal, The Netherlands

The number of people attending this subcommittee meeting was estimated at 75.

Hyperhomocysteinemia.

Dr. M. Cattaneo discussed the topic of hyperhomocysteinemia with special emphasis on methodologic issues. He first presented data regarding comparing the two- and four-hour methionine loading tests for the diagnosis of hyperhomocysteinemia. Based on results in 371 patients, he concluded that there was good concordance between the two tests. However they were not equivalent in that the four-hour test was more sensitive to methionine intolerance. It was therefore suggested that the two hour protocol should not replace the four hour protocol. He next presented data from a study of 628 healthy controls which determined that the methionine loading test does not only explore the trans-sulfuration pathway in homocysteine metabolism. Finally, he discussed results in 200 healthy controls and 52 patients with previous thrombotic episodes comparing an ELISA assay to standard HPLC methodology for plasma total homocysteine measurements. A good correlation was found for total homocysteine concentrations less than 30-35 micromolar; thus the diagnostic accuracy of the ELISA assay was considered excellent for the diagnosis of fasting hyperhomocysteinemia and good for methionine loading tests.

Genetic polymorphisms.

Dr. L. Iacoviello presented an update from the recent ETRO working party meeting on "Population Genetics of Hemostatic Risk Factors for Arterial Disease." She discussed meta-analyses of studies examining the role of the 4G/5G polymorphism in the PAI-1 promoter, the Bcl 1 polymorphism in the beta-fibrinogen gene, the repeat polymorphism in the tissue plasminogen activator gene, and the PLAIII polymorphism in the platelet glycoprotein IIIA as risk factors for myocardial infarction. Plans will be made to formalize interactions between the ETRO working party and the activities of this subcommittee.

Overview of Predictive Variables.

Dr. G. Lowe reviewed data from a recently published metaanalysis of fibrinogen as a risk factor for ischemic heart disease (Danesh et al., JAMA May 1998) indicating a two-fold increased risk with a tighter confidence interval in patients with fibrinogen levels in the top tertile. He reviewed the various methodologies for fibrinogen assays and emphasized the need for attention to this issue in the interpretation of the metaanalyses. He next presented results of a study in 1,661 healthy subjects (aged 25-74) comparing results of fibrinogen assays performed by Clauss assay, a prothrombin time-derived method, and immunonephelometry. Correlations between the three assays ranged between 0.69 and 0.85. Dr. Lowe is preparing an overview article on the topic of predictive variables and cardiovascular disease for submission by this subcommittee for submission to Thrombosis and Haemostasis.

Factor VII Polymorphisms.

Dr. L. Iacoviello presented her results on factor VII polymorphisms as a risk factor for ischemic heart disease in patients with a positive family history and young patients. Potential reasons for
different results (i.e., patient selection, ethnic differences in polymorphism frequency, as well as environmental factors) were discussed. Further discussion of this topic will be presented at the SSC meeting in Washington next year along with discussion of other genetic risk factors including factor V Leiden, prothrombin 20210A, and factor XIII-Val34Leu polymorphisms.
von Willebrand Factor

Chair: F. Rodeghiero, Italy
Co-Chairs: A. Federici, Italy; C. Mazurier, France; R. Montgomery, USA; J. E. Sadler, USA

The Subcommittee met Sunday, June 21, at the E1 room Cankarjev Dom, Ljubljana, Slovenia, from 1:00 to 5:40. Presiding chair was Dr. Francesco Rodeghiero. Dr. J.E. Sadler could not attend.

Attendance was approximately 80.

Dr. A.B. Federici chaired the presentations of existing national registries of VWD. Some countries appear to have well-structured registries including Italy (Dr. A.B. Federici with 1,314 patients from 16 centers), U.K. (J. Pasi with 5,100 patients). Dr. Pasi, on behalf of Dr. Lillicrap, presented data on VWD in Canada (720 patients). Dr. M. Nishino reported data from the 1991 survey in Japan (735 patients); a national survey of 1998 is being analyzed. No registry or surveys are available in Germany, as reported by Dr. U. Budde. Dr. A.J. Cohen (reported by A.B. Federici) analyzed the current practice in the management of VWD in North America, based on a questionnaire mailed to 194 physicians. Dr. A. Srivrastava (India) is trying to set up a registry of VWD and hemophilia in his country, including the most severe cases (personal communication).

Dr. A.B. Federici presented a progress report on the International Registry on acquired von Willebrand syndrome, co-chaired with Dr. J. H. Rand. So far, 50 Centers have provided information on 209 cases.

The issue of molecular diagnosis of VWD was discussed in a "round table" chaired by Dr. I. Peake. Dr. Peake described a strategy including the need for full gene analysis in type 1 and 3 and for the distinction of mutations from polymorphisms. Each new mutation should be proved by linkage within the family, by analyzing mRNA and possibly by expressing the mutated gene. Dr. R. Schneppenheim added an important comment on this issue by emphasizing the need of detailed phenotypic study to guide the subsequent molecular strategies and provided data on a cytosine deletion in exon 18 in type 3 VWD in Europe. Dr. D. Meyer updated the results of the French Network on molecular abnormalities in type 2 VWD: 182 unrelated cases characterized either in Bicetre (D. Meyer) or Lille (C. Mazurier). Dr. J. Eikenboom addressed the problems in tracing the mutated allele in VWD with intragenic polymorphisms. This approach led to unequivocal results in type 2A, 2B, 2M (subtype "Vicenza") and type 3 VWD, but proved difficult in type 1.

Dr. P. Jones suggested to rename "von Willebrand disease" with "von Willebrand disorder" to diminish the impact of diagnosis, especially in mild cases, in patients and in the community.

Dr. Rodeghiero presented the final version of the project for a multicenter retrospective study on VWD type 1 diagnosis. The study implies direct reinvestigation of carriers of type 1 and 3 and their relatives. Interested centers are invited to participate.
Dr. B. Montgomery chaired a session on the standardization of laboratory diagnosis of VWD and presented data on 12 centers assaying VWF, RiCof, VIII:C and multimers and making a diagnosis in established type 1 and 2 VWD samples. This set of plasma samples was proposed for a multinational performance evaluation. Dr. E. Preston reported the results of the UK NEQAS on the performance of a new "functional" antigenic assay, using MoAbs that recognize a specific epitope on VWF interacting with GpIb. This method does not appear to correlate well with VWF:RiCof and might misdiagnose type 2A patients. Dr. A. R. Hubbard presented the results of the calibration studies involving 25 laboratories from 10 countries of the proposed 4th IS Factor VIII/VWF plasma (now intended also for VWF: collagen binding) in replacement of the current 3rd IS plasma.

Dr. C. Mazurier chaired a session on the measurement of VWF in concentrates for therapy of VWD. Dr. Mazurier stressed the importance that manufacturers clearly state the type and characteristics of the assay used to label the concentrate VWF content. The need for a reproducible assay, as well for a standard reference material and appropriate clinical trials, was further addressed by Dr. M.J. Weinstein. Accordingly, products can be licensed for the VWD indications based on pharmacokinetic measurements, clinical trials and demonstration of product consistency. Dr. T.W. Barrowcliffe presented data indicating that a plasma standard may not be entirely adequate for measuring VWF content in concentrates and that a concentrate standard may be useful to improve parallelism of bioassays. Furthermore, the wide difference in the ratios of VWF:Ag to FVIII:C in available concentrates hampers any comparison of their therapeutic effectiveness.

Dr. P.M. Mannucci emphasized the importance of basing substitutive therapy in VWD on FVIII:C. In rare instances, mucosal bleeding could require normalization of bleeding time.

**SUMMARY OF SUBCOMMITTEE ACTIVITIES**

- **Issues voted:**
  The information provided by available national registries or surveys has been considered sufficient. Uniform entering criteria are needed before fostering additional national registries
  Creation of a WP to prospectively evaluate diagnostic criteria for AVWS
  Creation of a WP for molecular diagnosis of type 1 VWD
  Further studies are needed before the "functional" ELISA can be proposed as a substitute of VWF:RC of activity
- **Ongoing projects:**
  Updating of the International Registry on AVWS.
  The WP on AVWS will prepare a protocol for prospective evaluation of AVWS diagnosis.
  Activation of the multicenter, retrospective study for the diagnostic criteria for type 1 and type 3 VWD.
  Creation of a WP to test the ability to diagnose VWD subtypes through a kit of VWF plasma and concentrates standards, and plasma samples from VWD subtypes and controls.
The proposal that the 4th IS be assigned the mean of the estimates vs. the 3rd IS and the local plasma pools for VWF:Ag and VWF:Rcof will be submitted to the members of the Subcommittee.

The WP for VWF assay in concentrates will cooperate with FDA and NIBSC to establish a new concentrate standard to be calibrated against normal plasma.