45th Annual Scientific and Standardization Committee Meeting

August 14-15, 1999

Washington, DC
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Animal, Cellular, and Molecular Models of Thrombosis and Haemostasis

Chair: L. Badimon, Spain
Co-Chairs: P. Carmeliet, Belgium; L. Drouet, France; G. Johnson, USA

The program and invited speakers addressed the issue of how representative are animal models of thrombosis and vascular disease of the respective human pathologies. Speakers were selected to discuss spontaneous atherosclerosis, resterosis, venous thrombosis and angiogenesis.

The first speaker was Dr. John MacGregor. He reported that the mechanism implicated in the initiation and perpetuation of vascular lesions and their subsequent rupture leading to thrombus formation and total lumen occlusion remains poorly understood. In an effort to investigate such mechanisms his laboratory selected the use of ApoE deficient mice (generated in the laboratory of Dr. Maeda in Chapel Hill, NC), and compared them to C57BL6 wild type, fed a fat or a chow diet. The work focused on the quantitative expression of major adhesion molecules (ICAM-1, VCAM-1, PECAM-1, P-selectin) on the aortic arch of ApoE deficient mice compared to controls (C57BL6). Immunohistochemistry and Northern blots were used to assay the levels of these adhesion molecules at the endothelial cell level or by the whole vessel. Results show modulation of the level of expression of these adhesion molecules at different stages of vascular lesions in ApoE deficient mice. A similar type of result has been observed for human tissue samples. However, no plaque rupture and/or thrombotic occlusion have been observed for these ApoE animals. Great caution has to be taken in interpreting the data observed in the mouse ApoE model. Further work needs to be performed to humanize the ApoE deficient mouse model.

Dr. J.J. Badimon reported on experimental studies of cell cycle inhibition as a therapeutic strategy to decrease coronary restenosis following stenting. Rapamycin was found to inhibit smooth muscle cell proliferation in pig coronary arteries by a direct effect on p27, a negative regulator of cell proliferation, rather than by an apoptosis-related mechanism.

Direct comparisons with data in ApoE KO mice in terms of markers of proliferation/apoptosis were discussed. More research is needed because intra-stent restenosis remains a significant problem in clinical revascularization.

Dr. M. Levi presented the results of a comprehensive review of the literature on animal models of venous thrombosis. In general, experimental venous thrombosis studies have been useful to determine if experimental agents are useful for the inhibition of thrombus formation. However, they have been of lesser utility in dose-finding and comparative pharmacology studies. Discussion followed on species differences regarding pharmacokinetics and coagulation/fibrinolytic system. Research is needed to develop new methods that can provide a clear insight in the etiology of venous thrombosis.

Dr. L. Drouet described the characteristics and utility of a large animal model of chronic venous thrombosis. Thrombi with the same morphologic characteristics as chronic human venous thrombi developed in Gortex grafts implanted in pig external jugular veins, although the distal normal segments did not thrombose. Studies of anticoagulant therapy in this model indicated that
heparin was more effective than low molecular weight heparin and low-dose hirudin was ineffective.

Existing models of venous thrombosis, and namely the Wessler model (and derivatives) are:

- studying only the acute thrombotic reactivity for a few hours (while patients have clinical manifestations of thrombosis after days of evolution of the thrombotic process).
- developed in small rodents (rabbit, rats, etc.) whose flow condition (due to vessel size) are different from human pathology.

A model of chronic venous thrombosis in the pig seems of relevance because the coagulation system of this animal is fairly well known and quite similar to the human system and this animal is accepted as a good model of arterial pathology. Ultrastructural examination shows, as in human thrombosis, an onion skin like structure of the thrombus. It is formed with several layers of dense platelet deposits alternating with layers of fibrin network imprisoning various amounts of red blood cells. The rate of occlusion was 100%.

Dr. P. Carmielet described studies on embryonic and neonatal angiogenesis in mice. He showed data on the role of VEGF in angiogenesis. VEGF knockout mice were found to have defective vascular sprouting. VEGF 121 mutant mice were observed to have a significant decrease in the density of coronary blood vessels. These mice had decreased intramyocardial blood flow and subendocardial ischemia, and they died prematurely of heart failure. Placental growth factor (PLGF) was also found to interact with VEGF in the differentiation of large blood vessels. PLGF seems to play a role in pathological angiogenesis while VEGF seems to play a role both in physiological and pathological angiogenesis. VEGF was found to increase myocardial capillary formation in wild type mice but not in UPA knockout mice.

Dr. G. J. Johnson reported that the Subcommittee report titled "The Utility of Animal Models in the Preclinical Study of Interventions to Prevent Human Coronary Artery Restesosis: Analysis and Recommendations" was recently published in *Thrombosis and Haemostasis*.

Dr Badimon reported that because the content of the document was of interest to other scientific communities, the editors of *Circulation* were contacted. They showed interest and asked for a document that possibly could be published as an editorial.

Finally, Dr. Badimon, as the Chair of the Subcommittee, proposed new tasks for the next year. Due to the lack of models specifically addressing the pathophysiology of venous thrombosis, a consensus document on this issue will be prepared by Drs. Levi, Drovet, Johnson, and Badimon. The plan is to have a preliminary draft circulated by January 2000 and a final draft ready for presentation at the next SSC meeting. It will be submitted for approval at a later date.

This year we had a large attendance at our SSC meeting (over 100 participants).

Next year we plan to focus on models of pulmonary embolism and on coagulation factors in animal models.
Professor Yasuo Ikeda presented the final working party report on "Effects of Flow on Endothelial Cell (EC) Functional Activities" cochaired with Prof. Kjell Sakariassen. He described the various factors affecting platelet function that will be subject to modulation by flow effects: PGI2 and NO, both increasing with shear stress, associated with decreased platelet aggregation; tPA increase and PAI-1 decrease, with elevated fibrinolysis; tissue factor (TF) decrease, and thrombomodulin ™ increase, associated with decreased coagulation; all the changes generally associated with anti-thrombogenicity associated with increasing shear stresses. The synergistic effects of cytokines (IL-1 and TNF) with shear stresses on tPA and PAI-1 were described at shear stresses of 12-24 dynes/cm². In addition, shear stress decreased TNF expression in HUVECS. Flow device most useful was the cone-in-plate microcouette.

Prof. Jeff Hubbell gave his final working party report, cochaired with Prof. Steve Hanson, on "Local Drug Delivery and Flow." He discussed the issues of transport and biorheology associated with the delivery of drugs to the artery wall, and to thrombi adherent to the arterial wall. He outlined the methods best used to date, including the use of a) permanent implants such as graft sleeves, b) polymer depots, either within the surrounding adventitia, within the media, or within the artery lumen, and c) a variety of catheter designs, with and without imposed pressure or electric fields. He described some distinct chemical structures designed to promote drug deliveries, including bilayer depots where the drug-loading gel is covered by a polymer gel with low permeability not containing any drug, and the use of heparin binding of growth factors for delivery devices.

Prof. Mony Frojmovic gave a final working party report on "Blood Cell Aggregation in Flow."

Both biological and biophysical issues associated with methodological approaches and data interpretation were described. The biological issues included the presentation of new receptors (e.g., P-selectins and activated GPIIbIIIa), exposure to new agonists, differential importance of newly secreted ligands that remain platelet surface-bound versus soluble ligands, multiple cross-associations of receptors and ligands, the time between cell activation and actual cell collisions, as well as the ‘age’ of the activated cell in circulation, and activation of intracellular signal transduction. Biophysical issues include the effects of flow on collision frequency, adhesion and de-adhesion related directly to surface molecules present on platelets and other blood cells at the time of actual collisions. The choice of flow devices, and model surfaces or model spheres in suspension, is critical to proper and biologically meaningful evaluation of the molecular biorheology of platelet-platelet and heterotypic blood cell collisions, best done with particles in suspension in couette flow. Parallel plate flow devices, on the other hand, are more appropriate for studies of platelet-thrombus collisions. The build-up of local metabolites with time in ‘closed’ devices must be properly evaluated, quite possible with rapid measurements of shear-associated aggregation within seconds of cell activation versus longer incubation times prior to shear. Possible effects of hydrostatic pressure on cell function must also be examined.
Prof. Mony Frojmovic introduced the symposium on "Rheological Bedside Devices for Monitoring and Managing Vascular Diseases" with a brief overview of current status and future needs. He discussed biorheological issues in the design of flow devices for clinical monitoring of thrombosis and coagulation, and the relation between platelet biology, flow and the choices of anti-thrombotic drugs. He also described the need for relevant in vitro flow-dependent test for monitoring drug efficacy.

Prof. Don Gabriel presented a brief overview of the clinical issues in monitoring the thrombotic state, as a complementary introduction to the use of bedside clinical devices. He reviewed flow effects on rheopheresis of platelets concentrating near vessel walls; effects of flow on fibrin organization; and some of the molecules regulating platelet adhesion to collagen and fibrin.

Prof. Marc Hoylaerts discussed the use of rectangular microcapillaries for the study of platelet deposition onto well-defined surfaces, including seeded endothelial cells (EC); studies of leukocyte-EC interactions are also readily made with this approach. Quantification is performed with videomicroscopy and computer interface. He described the use of anticoagulated hamster blood evaluated between 300 and 2700/sec shear rates. Good platelet deposition was observed onto fibrin, vWF or collagen I—coated glass surfaces in the rectangular micro-capillaries. Anti GPIb ‘drugs’ inhibited hamster platelet deposition onto collagen I by 50% at 1300/sec, but anti-GPIIbIIIa drugs were without effect; whereas at 2700/sec, anti GPIIbIIIa drugs potentiated, platelet surface coverage, while anti-GPIb drugs were without effect.

Critical factors that need to be considered in choosing a flow system include: blood volume; shear stress; parameters measured such as closure times and cell rolling; mono versus re-perfusion; reproducibility and sensitivity of the system; ease of use; and evaluation of anti-platelet drugs. The rectangular capillaries are good for low volume, but not practical for bedside assays at this time, largely due to need for ‘unstable’ protein-coated surfaces. They are, however, very useful because of direct visualisation of platelet deposition via videomicroscopy. Dr. Sakariassen pointed out the importance of eventually testing fresh non-coagulated blood in platelet function testing.

Prof. Yasuo Ikeda described the use of a quartz crystal microbalance for the evaluation of platelet deposition onto protein-coated surfaces. In spite of problems in analysing the cellular overlayers with standard equations applicable to molecular reactions, due to viscoelasticity of the cells, quantitative analyses were feasible and provided real-time measurements of platelet deposition under various pathological conditions. The usefulness of this system for monitoring the time course of binding of liposomes to vWF-coated surfaces was well demonstrated with liposomes containing different densities of rGPIbalpha, as well as liposomes containing GPIa-IIa binding to collagen.

Dr. Eric Heilmann, from Dade-Behring, provided a detailed overview of the PFA-100 flow device, which is based on the closure of a capillary with a collagen or ADP-Adrenaline coating. The clinical usefulness of this device was described for a variety of situations, including von Willebrand’s disease, aspirin-induced bleeding, and Glantzmann’s thrombasthenia. A new, very important observation on the two patient populations with distinct response to treatment with the
anti-GPIIbIIIa drug, Reopro, observed 19 hours after administration, was described: about 1/3 of patients returned to normal, with 2/3 still showing abnormal closure times.

Dr. Bruce Lages, on behalf of Dr. Conan Li, from Xylum, gave a detailed outline of the Clot Signature Analyser. The instrument generates an orifice with flow through both the orifice and a channel containing a collagen fiber. Native blood is used, with both platelet function and clotting times readily monitored. The platelet-mediated hemostasis time (PHT) reflects mainly platelet adhesive function, with no effects of fibrinogen/fibrin, while the collagen-induced thrombin formation (CITF) time reflects fibrin formation actually visualised in the platelet fibrin-rich thrombi formed. Useful data from a number of clinical situations were reviewed. In particular, low molecular weight heparin had more effect on PHT, likely related to thrombin formation affecting platelet function and not fibrin formation. Two anti-thrombotic drugs showing total block of platelet aggregation in aggregometry showed some rebound effects in analyses of the blood with the CSA analyser, paralleled by measurement of platelet-bound fibrinogen by flow cytometry.

Dr. David Varon, from the University of Tel Aviv and Tel Hashomer Hospital, presented results on the novel use of a micro cone-in-plate device for the assay of platelet adhesion and aggregation with anticoagulated whole blood from patients. Bedside data output is provided by computer interface analysis of platelet surface coverage and aggregate size. The relative usefulness of native polystyrene versus ECM-coated plastic was described. A number of clinical situations were described which could be ‘characterised’ from changes in these platelet parameters.

Dr. Robert Hillman, from Accumetrics, described the use of fibrinogen-coated beads in a bedside device for monitoring blood drug concentrations for those directed at the GPIIbIIIa platelet receptors. Of the more than 300,000 patients per year in N.A receiving Reopro, about 60% are getting sub-optimal treatment, with high variability of reopro concentrations in patients’ blood leaving hospitals, with observation that less than 80% GPIIbIIIa blockade associated with much higher event rates in cardiovascular disease. Blood drug monitoring and receptor blockade measurements will become increasingly important with the generation of increasing numbers and types of antithrombotic drugs.

Dr. Marc Carr, from Hemodyne, described a bedside device for monitoring clot retraction and fibrinolysis. Following in vitro addition of thrombin to a patient’s blood, the mechanical properties of the fibrin network and the contractile forces generated by the platelets are measured. He described the very different sensitivities seen for effects of reopro on whole blood aggregation with 80% inhibition versus effects on clot retraction associated with maximal platelet activation due to use of thrombin for the observation. The high interdonor variabilities in responses for clot retraction were noted. Some impressive examples of clinical situations showing altered clot retraction and fibrinolytic capacities were described.

Each of the methods developed and described above has been used clinically and calibrated in standard platelet-defective states and coagulopathies.
Prof. Gordon Lowe, in collaboration with Prof. J Danesh, told the gathering to attend an afternoon subcommittee session as there was no time to give a brief final report on "predictive values of rheological variables for vascular events," focusing on measurable changes in fibrinogen concentrations as a likely marker for acute phase reaction associated with vascular events. A written report will however be submitted to our committee in the coming months.

The chairman solicited suggestions for new topics for future meetings, and specifically for the Maastricht meeting in June of 2000, which include: 1) a follow-up symposium on ‘bedside devices," possibly jointly with other subcommittees such as the platelet physiology group; 2) Rheological Models for Bleeding/Thrombotic Disorders; 3) flow effects on cell structure-function; 4) what makes arterial blood physiology so distinct from that of venal?

Attendance was excellent with 115 participants.

P.S. Any ICTH attendees wishing to become ‘consulting members’ of our Biorheology subcommittee are invited to e-mail the chairman at: mony@med.mcgill.ca.
Contact Activation

Chairman: I. Schousboe, Denmark
Co-Chairmen: M. Berrettini, Italy; R.A. De La Cadena, USA; M. Fischer, Austria; B. Lämmle, Switzerland; H. Saito, Japan; A. Schmaier, USA; P.N. Walsh, USA

Analyze the need for standardized methods to measure contact activation during extracorporeal circulation and hemofiltration.

The subcommittee has focused on this topic as extracorporeal circulation, hemofiltrations, and artificial valves and joints are all procoagulant surfaces for contact activation. The degree of activation of the system significantly influences the non-specific reactions that support systemic inflammatory responses and hemostatic perturbation. Precautions are being taken therefore to minimize contact activation by increasing the biocompatibility of the artificial device. However, the necessity to respect the integrity of the organism during the procedure precludes most systemic interventions and limits the control of blood activation on the area of the device.

The presentations by Dr. Olsson, et al. from Sweden and Dr. te Velthuis, et al. from Holland in combination with our knowledge from the literature suggest the need for proper assays to follow the degree of activation during and after extracorporeal circulation.

It was agreed among the committee that a working group (Dr. Berrettini, Dr. De La Cadena, Dr. Lämmle, Dr. Saito, Dr. Schmaier and Dr. Schousboe) be formed to begin standardization of contact activation proteins (Factor XII, Factor XI, prekallikrein and high molecular mass kininogen). Levels of each of the contact factors by activity and antigen will be measured. It is planned that each investigator uses his/her own pool of NHP for standard and test the SSC plasma against this standard. In this context, the subcommittee contacted Dr. Jane Lenahan and Dr. Anthony Hubbard (National Institute for Biological Standards and Control) and arranged for delivery of the standard. The committee also agreed to look further into assays for Factor XIIa.

International Patient Registries:

The efforts taken two years ago to determine retrospectively and prospectively the medical history of patients with contact protein deficiency was updated. Dr. Bylesjo from Sweden, Dr. Dalsgaard-Nielsen from Denmark, Drs. Berrettini and Castaman from Italy and Dr. Lammle et al. from Switzerland all agreed that there is no correlation between non-severe Factor XII deficiency (2-50% of normal Factor XII) and thromboembolic events. There seems to be an indication of a correlation between severe factor deficiency (<2%) and arterial, rather than venous thrombosis. The report from Dr. Dalsgaard-Nielsen indicated that there seems to be a higher risk of thrombosis among patients with LA and antiphospholipid antibodies directed against Factor XII compared to the risk connected with the presence of other antiphospholipid antibodies. The committee agreed to look further into this possibility.

The committee also agreed to analyze the statistical need for investigating whether severe Factor XII deficiency is connected with increased risk of thrombosis. Dr. Berrettini will be in charge of this investigation.
Control of Anticoagulation Subcommittee

Chair: F. E. Preston, UK
Co-Chairs: T. W. Barrowcliffe, UK; A.M.H.P. van den Besselaar, The Netherlands; L. Poller, UK; F. Rosendaal, The Netherlands; A. Tripodi, Italy

Current Tasks

The Chairman welcomed the participants and outlined the program. As on the previous occasion the Subcommittee had been allocated two complete sessions. Draft recommendations were received from the Working Party on the Preparation and Use of Calibration Plasmas for INR Determination (T. Barrowcliffe, Chair) and on Low Molecular Weight Heparin (LMWH) Monitoring in infants from the Working Party on LMWH Monitoring (A. Giles, Chair).

New Working Party on the Duration of Oral Anticoagulant Control for Venous Thromboembolism

The Chairman reported that the new Working Party had been established under the chairmanship of Dr. J. Hirsh. The membership comprises Drs. S. Shulman, C. Kearon, G. Agnelli and Dr. F.R. Rosendaal.

Dr. J. Hirsh:

The session addressed two unresolved issues in management of venous thromboembolism. These are: 1) the optimal duration of anticoagulant therapy for venous thromboembolism; and 2) the need for long-term anticoagulant prophylaxis in subjects with inherited thrombophilia.

The optimal duration of anticoagulation therapy in patients with venous thrombosis and pulmonary embolism is controversial. Three adequately designed studies have been performed, producing somewhat discrepant findings. Each of the senior authors of the studies (Drs. Kearon, Schulman and Agnelli) were invited to present the results of their studies and to present their views on the optimal duration of oral anticoagulant therapy in the following patient groups: proximal vein thrombosis without a provoking cause (idiopathic); proximal vein thrombosis after a reversible cause (secondary); idiopathic calf vein thrombosis; calf vein thrombosis after a provoking cause. The effect (if any) that the presence of associated inherited or acquired thrombophilia would have on this recommendation would also be considered.

For how long should the treatment with vitamin K antagonists be maintained?

(Dr. S. Schulman)

Recent prospective randomised studies on the optimum duration of oral anticoagulation with vitamin K antagonists after VTE have demonstrated that the probability of recurrence is reduced when the secondary prophylaxis is prolonged from four weeks to three months or from six weeks to six months or from six months to 27 months. The patient material and the duration of follow-up differed, however, between those trials. The reduced risk of recurrence is valid for almost all
subgroups of patients except for those with the combination of a calf vein thrombosis and a temporary triggering risk factor, such as surgery. For those patients, a prolongation beyond six weeks of secondary prophylaxis is not of any benefit. For proximal deep vein thrombosis (DVT) or symptomatic pulmonary embolism (SPE) and a temporary trigger, a prolongation from six weeks to six months yields a significantly lower incidence of recurrence, but after three - four years this difference is not significant, and the optimal duration may be between three and six months. For those with an idiopathic or permanent triggering factor and any DVT or SPE, a prolongation from six weeks to six months confers a significant advantage regarding incidence of recurrences which remains even after six years. Even longer durations should be considered in patients with cardiolipin antibodies, due to a significantly higher probability of recurrence and a higher mortality due to all kinds of thrombotic events after cessation of anticoagulation at six months. The risk of recurrence is of the same magnitude in patients with proximal deep vein thrombosis or with symptomatic pulmonary embolism.

Patients with hereditary thrombophilia do not seem to run an increased risk of recurrence in case of heterozygous Factor V Leiden or prothrombin mutation. For the heterozygous forms of protein C, protein S, or antithrombin deficiency the risk of recurrence is at least 10% annually, which is twice that in patients without hereditary thrombophilia, and a prolongation beyond six months is warranted. For patients with hyperhomocysteinemia there is an increased risk of recurrence, but substitution with folate and vitamin B12 may provide a better benefit/risk ratio than vitamin K antagonists.

After the second event of VTE, prolongation of oral anticoagulation from six months to 48 months markedly reduces the rate of recurrence but with a trend toward more major hemorrhages in the latter group. Arguments against very long durations of conventional treatment with vitamin K antagonists aiming at an INR of 2.0-3.0, include the necessity of monitoring the prothrombin time with direct and indirect costs and the constant fear of interactions with other drugs or food products. This has to be weighed against the reduction or elimination of a risk of recurrence of about 5% per year after cessation of anticoagulation.

If lower intensity of anticoagulation (INR of 1.5-2.0) turns out to be safer regarding hemorrhages and more convenient by allowing for less frequent monitoring of prothrombin time, the recommendations for duration of anticoagulation will require revision.

Optimal duration of anticoagulant therapy for symptomatic venous thromboembolism: One perspective

(Dr. C. Kearon)

Six large, well-designed trials have recently been completed that have compared different durations of oral anticoagulation for the treatment of various categories of patients with venous thromboembolism (VTE). Their findings, in conjunction with those of a number of other prospective studies that have helped to identify risk factors for recurrent VTE and anticoagulant-related bleeding, have led to a better understanding of the optimal duration of therapy for individual patients with VTE. Current evidence suggests that three months of therapy is adequate for patients with VTE that was provoked by a major reversible risk factor such as surgery.
Idiopathic VTE should be treated for a minimum of six months, and a longer duration of therapy (e.g., two years) is likely to be preferable. Patients with continuing (non-reversible) risk factors, such as cancer, should be treated until the risk factor resolves. Six months of therapy is reasonable for patients who do not fall clearly into one of these categories. The presence of an antiphospholipid antibody, deficiencies of protein C, protein S and antithrombin, homogeneity for Factor V Leiden, combined thrombophilic abnormalities; and a previous episode of idiopathic VTE, support longer durations of therapy within each category of patients. Conversely, a high risk of anticoagulant-related bleeding, isolated distal deep vein thrombosis, and patient preference support a shorter duration of anticoagulation, particularly in patients with idiopathic VTE (i.e., less than two years of therapy). Heterozygous Factor V Leiden appears to have little bearing on the optimal duration of anticoagulation.

Dr. G. Agnelli presented results from a study, yet to be published, that compared the rates of recurrence of venous thromboembolism following treatment with oral anticoagulants for three months or 12 months after a first event.

Dr. F.R. Rosendaal presented prospective data on thromboembolism from two studies. One, the European Prospective Cohort on Thrombophilia (EPCOT) Study includes patients from high risk families with familial thrombophilia (deficiencies of protein C, protein S, antithrombin, carriers of Factor V Leiden and of combined defects) from 11 European centers. The second study is the follow-up of a group of unselected consecutive patients with a first thrombosis, the Leiden Thrombophilia Study (LETS). In the EPCOT study 1,781 patients were followed for 4,838 patient-years. About half of them had not experienced thrombosis prior to entry in the cohort, while the other half had experienced on average 2.5 events prior to entry; again half of these patients received long-term anticoagulation. During follow-up 107 DVT/PE occurred. Annual risks were 0.9% for first events, and 3.8% for recurrences for those not receiving long-term anticoagulation, and 1.6% in those receiving long-term anticoagulation.

In the LETS 474 patients were followed for 2,784 patient-years and 81 recurrences occurred after the conclusion of three months of oral anticoagulation. The overall annual recurrence rate was 2.9%, varying from 1.9% in those without blood abnormalities [deficiencies of protein C, protein S, antithrombin, carriership of Factor V Leiden, Factor VIII levels 150IU/dl or hyperhomocysteinemia (>18.5 μM/l)] to 4% in those with such abnormalities. The recurrent events were evenly distributed over time, i.e., there was not a particularly high risk following discontinuation of oral anticoagulation; in fact, over half of the recurrences occurred more than three years after the first event.

Dr. Rosendaal concluded that even in patients with familial thrombophilia from high-risk families, long-term anticoagulation is not warranted in asymptomatic patients; although the thrombotic risk is reduced in patients who have suffered prior thrombosis, it is uncertain if the benefits of such treatment would outweigh the risks. For consecutive patients with DVT, even those with blood abnormalities, these data do not suggest a benefit of extending the duration of anticoagulation beyond three months.

The Working Party will attempt to provide guidelines on the optimal duration of oral anticoagulant therapy.
New Working Party on the Monitoring of Direct Thrombin Inhibitors

The Chairman reported that a new Working Party has been established under the chairmanship of Prof. J. Harenberg to address issues relating to the monitoring of direct thrombin inhibitors. These are:

1. Methods to determine direct thrombin inhibitors:
   - APTT, different reagents
2. Thrombin clotting time methods:
   - Ecarin clotting time
   - Whole blood ecarin clotting time -- bedside method
   - Chromogenic substrate methods
   - ELISA methods
   - Others
3. Antithrombin agents:
   - r-hirudins
   - PEG-hirudin
   - Argatroban
   - Melagatran
   - Others

Dr. J. Fareed discussed monitoring issues related to the development of Factor Xa inhibitors. Currently, there are no guidelines for the monitoring of these agents. Since these agents are expected to enter into a fast-track clinical development in both the therapeutic and interventional indications, some recommendations for their monitoring are warranted. This presentation also provided comparative data on DX 9065a and a synthetic pentasaccharide SR-90107 in various laboratory assays.

Report and Recommendations from the Working Party on LMWH Monitoring

(Chair, A. Giles)

Adult Monitoring
The Subcommittee Chairman reported that despite detailed discussions there remained important and unresolved differences of opinion between Working Party members and, unfortunately, it had not proved possible to present any recommendations in respect of adult monitoring.

The membership expressed regret at this outcome and supported the view of the Subcommittee Chairman that in view of the importance of this subject the Working Party should continue its activities and attempt to present appropriate therapeutic guidelines.

**Pediatric Monitoring**

Dr. P. Massicotte presented proposed guidelines on behalf of herself and Dr. Andrews. Critical issues for the use of LMWH in children include the age (or weight) dependancy of the pharmacokinetics of LMWHs with small infants having increased requirements per body weight. There is increased use of LMWH, rather than oral anticoagulants, for long-term therapy. This also has implications in respect of monitoring.

It is anticipated that in the very near future the guidelines will be forwarded to the Chairman of the Subcommittee for distribution to Subcommittee members.

Dr. A. Giles requested that he be allowed to stand down from the chairmanship of the Working Party on account of changed personal circumstances. He indicated that, if required, he would be willing to serve as a member of the Working Party. The Chairman accepted his request and thanked him on behalf of the Subcommittee for his active contribution.

**Progress Report on the Preparation of Heparin Reference Plasmas for Unfractionated Heparin**

Dr. Elaine Gray reported that two sets of heparinized plasmas, one set of ex-vivo patient plasma, and one set of in-vitro spiked plasma have been ampouled. Each set of plasma consists of a negative normal pooled plasma and three plasmas with varying concentrations of unfractionated heparin. A pilot study involving approximately ten laboratories will now be initiated and the results of the study will be reported at the next SSC meeting.


(Dr. Duncan Thomas)

One of the proposals resulting from the June 1998 WHO/ISTH consultation on biological standardization of unfractionated heparin was to set up a Working Group on Biological Standardization for Unfractionated Heparin. The first remit of the working group is to review the methodology currently used for measurement of heparin biological activity. The first meeting of the group will be held in Geneva in September of this year, with a view of promoting harmonization of the USP and WHO standards for unfractionated heparin.

**Reports and Recommendations from the Working Group on Calibrated Plasmas for Local INR Determination**
(Chair, T. Barrowcliffe)

D. Yetsuka presented results of a study on the influence of thromboplastins of various ISIs on INR. This confirmed the advantages of thromboplastins with an ISI of approximately 1.0.

Dr. M. Johnston reported the results of a study that confirmed the greater precision and accuracy of thromboplastins with an ISI < 1.5.

Prof. J. Jespersen reported the results of an ECAA study that addressed the effect of sample size on fresh and lyophilized plasma thromboplastin ISI determination.

Dr. T. Barrowcliffe presented highlights from the draft guidelines on the preparation, calibration, and use of calibrated plasmas for INR determination.

It is anticipated that the draft guidelines will be forwarded to the Subcommittee Chairman in the very near future.

New Working Group on Near-Patient and Self-Management of Oral Anticoagulant Control

The Chairman announced the establishment of the new Working Group (Chair, F.E. Preston; Members: J. Ansell, T. van den Besselaar, S. Kitchen, G. Muller-Berghaus, G. Palareti.)

The aims of the Working Group are to address the issues arising out of the increased use of point-of-care testing devices for oral anticoagulant control with a view to publishing guidelines and recommendations.

Working Group members made the following presentations:

Prof. Muller-Beghaus: Patient self-testing of oral anticoagulation -- A new approach in controlling oral anticoagulant therapy.

Dr. T. van den Besselaar: Instrument calibration issues.

Dr. J. Ansell: Patient selection for patient self-testing.

Prof. G. Palareti: Dosing issues.

Dr. S. Kitchen: Quality control issues.

Prof. L. Poller presented details of an ECAA research study: Standardization of whole blood testing in home prothrombin time monitors.

Dr. Ginette Michaud, Chairperson, Workshop Steering Committee, US Food and Drug Administration, presented a report arising out of an International Workshop on the Standardization of Whole Coagulation Devices, held in Washington on August 13th, 1999. The
Workshop was sponsored jointly by the U.S. Food and Drug Administration Center for Devices and Radiological Health and the College of American Pathologists.

Participants at the Workshop were asked to establish the requirements of a project focused on standardizing the calibration of whole blood clotting devices.

Discussions centered on the PT, aPTT and ACT. There was a general consensus that the calibration issue required further study and that the magnitude of the problem needs to be better understood. Any effort to standardize the calibration of these assays should focus on the clinical impact of these tests and should not be driven exclusively by the correlation of test results between methods. Manufacturers would carry the primary responsibility for reliable calibration of their test systems. Standards would be specific to each assay type and clinical application.

The meeting closed at noon August 15th, 1999.
DIC

Chair: F.B. Taylor, USA
Co-Chairs: M. Blombäck, Sweden; M. Kazama, Japan; M. Levi, The Netherlands; I. Bokarew, Russia; W.K. Hoots, USA; N. Sakuragawa, Japan

The DIC Subcommittee of the SSC of the ISTH met at the Washington Convention Center August 15th from 8:00 AM to 12:00 Noon. Dr. Taylor, in his opening remarks, outlined the goals and timetable for completing a draft document defining overt and non-overt DIC. He emphasized that 1) this was to be based on the data to be presented at this meeting, 2) that these definitions and diagnostic criteria for DIC had to meet the needs of all parts of the world, 3) that this would require starting with clinical and global coagulation test criteria commonly available and graduate to include more sophisticated tests when necessary, and, 4) that these should be completed by a working group in time for review by the full committee in the year 2000 at Maastricht.

The working group will meet in the fall and spring and consists of M. Blombäck, I. Bokarew, C. Dempfle, K. Hoots, M. Levi, S. LaRosa, A. Schorer, F. Taylor, C.H. Toh, and H. Wada. The work of this group will be submitted for review by critical care physicians, clinical trialists, and hemostasis experts not on this committee during the preparation and rewriting of this draft proposal.

The first topic covered was overt DIC chaired by Dr. K. Hoots. In his opening remarks he emphasized that DIC was one of the manifestations of a dysfunctional reticuloendothelial/microvascular organ and that DIC was to this organ as azotemia was to the kidney or hypoxia was to the lung. He emphasized that there were at least two categories of DIC. The first is characterized by a simple override of the normal endothelial anticoagulant factors by foreign procoagulant factors such as occurs in transfusion reactions or envenumation. The second is characterized by an override combined with a degradation of regulatory factors such as occurs in sepsis, SIRS following trauma, etc.

Drs. Hoots and Blombäck then reviewed studies by Drs. Gando, Bredbeck and Gramander in which trauma and leukemia patients were evaluated for DIC using global coagulation tests and clinical criteria combined with evaluation of the underlying disorder using criteria developed for assessing sepsis, SIRS, MOF, ARDS, and head trauma. The DIC and criteria for underlying disorder correlated almost exactly supporting the hypothesis that disorder of the microvasculature/hemostatic systems as reflected by DIC scores were closely linked to and possibly contributed to initiation or amplification of the underlying disorder of the RES/microvascular organ. The contribution of molecular markers of endothelial injury (sTM) and thrombin cleavage of fibrinogen (sFM) to the global coagulation test data also was reviewed.

The second topic covered was non-overt DIC chaired by Dr. Toh. In his opening remarks he emphasized that definitions of useful criteria for diagnosing impending DIC or non-overt DIC was critical for patient care because it would provide the opportunity to intervene under more favorable circumstances. He stressed that though global coagulation tests (that were useful in evaluation of overt-DIC) were not as useful in diagnosing non-overt DIC, there still could be a
place for them together with molecular markers. These comments were based on his analysis of prospective studies by Dr. Woda of 40 patients who progressed from non-overt to overt DIC. He also stressed the importance of keeping the laboratory aspects of the diagnosis of both overt and non-overt DIC as simple as possible because of cost and availability throughout the world. Finally, Dr. Toh introduced data concerning whole blood or plasma function in DIC as measured using thrombelastography, wave form analysis of whole blood and plasma, respectively. In contrast to the ELISA assay of molecular markers, the assessment of whole blood or plasma clotting in patients could be done almost immediately. The technology and understanding of abnormal wave forms or clotting signature of whole blood or plasma samples may advance to the point where they could be used.

Drs. Wada and Taylor reviewed primate models of E. coli sepsis and peritonitis. They observed a dose response of global coagulation markers and molecular markers. Special interest centered on soluble fibrin which in human and non-human primate studies exhibited a second very large peak 24-48 hours after sublethal E. coli or endotoxin respectively. This second peak coincided with a fall in Factor VIII and rise in tissue factor and was unexpected as these events occurred long after mediators had returned to normal and after complete clinical recovery. They emphasized that these late procoagulant events probably reflected an opening up or reperfusion of the microvascular beds 24 to 48 hours following the initial insult. sTM also remained elevated even though all clinical and global coagulation tests were normal. These observations were important, for they increased our understanding of the characteristics of non-overt DIC and the settings in which it occurs.

These findings led naturally to the observations of Drs. Bokarew and Dempfle on continuous low grade, non-overt DIC and soluble fibrin as a potential marker of these more subtle forms of DIC. Dr. Bokarew reviewed the history and offered data showing elevated concentrations of thrombin fibrin products in patients with hemophilia, arthritis, and coronary occlusion ischemia. Dr. Dempfle reviewed the chemistry of soluble fibrin and the challenge of validating and standardizing assays of soluble fibrin such that they could be used reliably.

The third and final topic covered was treatment of DIC chaired by Dr. Levi. In his opening remarks he emphasized how important an adequate, useable set of criteria for defining overt and especially non-overt DIC is to those conducting clinical trials. He reviewed studies done in primates using molecular markers which, if made available at the bedside, would aid in the selection of patients to be treated.

Drs. Kessler and LaRosa who have been conducting clinical trials of antithrombin and activated protein C (APC) reviewed their experiences. Dr. Kessler emphasized the need for infusion of antithrombin (AT) in concentrations sufficient to raise AT levels above normal. He spoke of AT anti-inflammatory properties. The response to AT therapy in studies remains to be fully evaluated. Dr. LaRosa emphasized the effect of APC on coagulant factor generation and activation in patients with sepsis. He spoke of treatment of patients with meningococcal sepsis. Again, the response to APC therapy in ongoing trials remains to be fully evaluated. Both emphasized the need for updated, more sophisticated criteria for diagnosis of non-overt DIC.
The attendance was in excess of 300 people. There was adequate discussion opportunity the first session. What was of interest was that in spite of the session lasting the full four hours allotted, the audience remained at about 300 throughout the session.
Exogenous Hemostatic Factors: Registry

Chair: N. Marsh, Australia
Co-Chairs: M. Kini, Singapore; F. Markland, USA

Four members of the Registry were in attendance plus some 16 guests.

Welcome: Neville Marsh, Chair

Francis S. Markland, R. Manjunatha Kini, Co-chairs

Introduction of new Registry member: Dr. Takashi Morita

Retirements: Hubert Pirkle, Che-Ming Teng, Fritz Markwardt. Letters of thanks will be sent to these retiring members.

Reports on inventories

Snake venom fibrinogenolytic enzymes (Francis Markland)

Dr. Markland reported on recent work on fibrolase including coupling with the cyclic RGD peptide, Diatide P734. This chimeric compound retained azocasein and fibrin plate activity and \( K_m \) and \( V_{max} \) were unaltered by coupling. The chimera inhibits platelet aggregation (IC\(_{50}\) of 105nM, compared with an IC\(_{50}\) of 76nM for P734 alone). The chimera binds via GPIIb/IIIa fibrinogen receptors, whereas fibrolase has no such binding activity. Studies to find the binding site of P734 on fibrolase revealed that there were seven lysine sites available. Digestion with endopeptidase LysC gave eight peptides and Lys\(_{184}\) was identified as the site of P734 attachment, distant from the active site. It is anticipated that the chimera will be targeted to fibrin by fibrolase binding activity and thus brings platelet aggregating inhibitory activity to the thrombus. In vivo tests are in progress.

Arthropod hemostatic factors (Carmen-Luisa Arocha-Pinango)

Dr. Pinango was unable to be present to give her report.

Hemorrhagic factors from snake venoms (Neville Marsh)

Dr. Marsh reported on new factors and recent work following publication of the first inventory in 1994. Previously identified hemorrhagins that have been further studied include rhodostoxin (Calloselasma rhodostoma), LHF-I and LHF-II (Lachesis muta muta) and HR2b (Trimeresurus flavoviridis). Rhodostoxin has been completely sequenced and is the first 4-disulphided hemorrhagin described. LHF-I and II have been studied in detail, in particular, their action on insulin bond cleavage. HR2b has also been completely sequenced. New hemorrhagins include the following: AaHIV (Agkistrodon acutus), a 44kD protein; ACL toxin 1 (Agkistrodon c. laticinctus) which has been completely sequenced and also appears in the previous inventory of fibrinogenolytic factors; BHRa and BHRb (Bitis arietans), large MW proteins (68 and 75kD);
catrocollastatin, a large 50kD protein of the MDC family binding to collagen and VRH-I (*Vipera russelli*) a small 22kD non-zinc dependent protein. It was agreed that the inventory be updated by Dr. Marsh and submitted for publication as an official communication of the Registry.

*Factors affecting platelet aggregation (Manjunatha Kini and Geraldine Chow)*

Dr. Kini reviewed the main classes of factors that inhibit and/or induce platelet aggregation. Inhibitors include enzymic factors (metalloproteinases, nucleotidases, phospholipases) and non-enzymic factors include antagonists of GPIIb/IIIa, vWF-GPIb, thrombin-thrombin receptor interaction. Metalloproteinases include fibrinogenases, kistomin which cleaves GPIb and jararrhagin which cleaves the β 1 subunit of α 2 β 1 integrin. Recent work on GPIIb/IIIa antagonists has been triggered by trigramin-echistatin publications and the possibility of developing therapeutic agents. They have a common function from a diverse structure including echistatin (49 residues, 4 disulphide bridges), kistrin (68 residues, 6 bridges), mambin and decorisin, all of which have RGD loops. Inducers of platelet aggregation include the serine proteinases, including the "thrombin-like enzymes, C-type lectins and factors from the sea anemone (equinatoxin) and sea cucumber. It was agreed that the inventory will be published as an official communication of the Registry and if possible as a review in *Thrombosis and Haemostasis*.

*New business*

*Textilinin*

Dr. Patrick Gaffney gave an update on textilinin, a plasmin inhibitor from the common brown snake (*Pseudonaja textilis*) venom. This is a loose, fast reversible inhibitor (Kᵢ, 10⁻⁷) which only inhibits plasmin and trypsin. Textilinin has a 47% homology with aprotinin and reduces bleeding in a mouse tail vein bleeding model.

*Bacterial fibrinolytic agents*

It was decided not to proceed with an inventory of bacterial agents, as the number of such compounds was small.

*C-type lectins (Takashi Morita)*

Dr. Morita reported on various classes of C-type lectins. These are mainly heterodimers and include habu IX/X-bp, botrocetin (vWF), echicetin (GPIb), rhodocytin (Ia/Ii) and alboaggregin-B (Ib). Dr. Morita described the 3D structure of flavocetin A. It was agreed that the inventory be published as an official communication of the Registry. Dr. Kini will liaise with Dr. Morita over omission of C-type lectins from the inventory of platelet factors.

*Plant factors*

Dr. Kini will contact Prof. Teng to determine if a manuscript has been prepared on plant factors.
Satellite meeting in association with ISTH Paris 2001

It was agreed that members of the Registry would be involved in a satellite on Animal Hemostatic Factors. Dr. Kini will approach Dr. Cassian Bon in Paris for his help. The meeting will be organized by Drs. Kini, Markland and Marsh.

Change of chair

This meeting concludes Dr. Marsh’s term of chairmanship and he formally handed over the chair to Dr. Manjunatha Kini. Co-chairs for the next period will be Drs. Francis Markland and Neville Marsh.

Next Registry meeting

The next meeting of the Registry will be held in Paris, 4-5 July 2001.

The meeting was adjourned at 3:50pm.
Factor VIII and Factor IX Subcommittee

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

PUBLISHED REPORTS


COMPLETED REPORTS

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 Components. FGH Hill, CA Ludlam, PM Mannucci.


Collaborative Study for the 3rd International Standard FII and FX Concentrate. E Gray et al.


REGISTRIES AND STUDIES

The chairman reminded the audience of the following registries and the contact persons to address for further details:

International Registry on Congenital FVII Deficiency: Prof. G Mariani, fax no.: +39-0-91655-4402, e-mail address: marianigu@tin.it

International Registry on Outcome of Immune-Tolerance and the Gene Defect of the Inhibitor Patient. Prof. G Mariani, as above.

Randomized Immune Tolerance Study Protocol. Dr. Charles Hay, fax no. +44-161-276-4814, e-mail: haemophilia@man.ac.uk

(Continued)

STANDARDIZATION ISSUES

Collaborative Study for the 3rd International Standard for Factors II and X Concentrates.

E Gray. Approved.

S Kitchen commented on planned calibration of the 2nd SSC Secondary Plasma Standard for FVIII:C, FIX: C, and vWF, and presented additional data on the usage of the 1st SSC Secondary Standard for FVIII:C, FIX:C and vWF, which was collected during the UK NEQAS assay trial.

M Weinstein reported on the progress of the MEGA II Standard with planned filling in the fourth quarter of 1999 and collaborative calibration thereafter. Launch is predicted within 12 months.

**Factor VIII Assay Discrepancies**

E Preston reported on circulation of a postinfusion sample from a recombinant factor VIII clinical study, as part of the NEQAS proficiency program. Less assay discrepancy was found when using the 6th International Concentrate Standard compared to the usage of a plasma standard. The importance of the individual APTT reagent used was less prominent than for the ReFacto data reported at the previous meeting.

M Mikaelsson summarized previous and new findings from her studies of the assay discrepancy phenomenon, emphasizing the impact of the phospholipid composition on the one-stage assay.

J Ingerslev attempted to explain the nature of assay discrepancy, including patients with certain variants of mild hemophilia A, from a clinician’s point of view.

**WORKING GROUP ACTIVITIES**

At a meeting in London in February 1999 the subcommittee established five working groups to discuss issues felt to be of import in relation to the subcommittee’s activities. Brief updates on each of these were presented:

**Working Group on Definitions and Terminology in Hemophilia**

*(G White et al.)*

Three activities were highlighted:

- the definition of severe, mild and moderate hemophilia, for which the discussion suggested that the definition should be based on biological factor FVIII levels (e.g., <1, 1 to 5 and >5 u/dl) rather than clinical severity,

- a cut off between low and high titre inhibitors, for which a level of five Bethesda units was suggested,
- and, possible experiments in hemophilic dogs or patients to define the minimal level of FVIII required for hemostasis. While the London meeting had been concerned about the ethics of carrying out such studies in humans, the consensus at this meeting was that such a study was feasible, possibly in countries where the alternative was no therapy, and would probably involve a dose de-escalation design.

Working Group on Quality Assessment in Measurement of Inhibitors

(S Kitchen for FE Preston et al.)

The wide range of results obtained in the UK NEQAS group for inhibitor samples was emphasized and a planned collaborative study to assess the impact of assay variables was proposed. Potential participants were invited to fill in a form.

Working Group on Genetic Aberrations and Inhibitors

(R Schwaab, J Oldenburg et al.)

The group proposed to collect data on FVIII gene mutations, MHC II type (and possibly other immune status markers) in a prospective manner for previously treated patients, both prospectively and retrospectively from previously treated patients and from mild/moderate patients (e.g., with the A2 domain R593C or the C2 domain W2229C mutation). There was some debate as to whether such data would be best collected on national or European/US databases. It was noted that a number of centers can offer FVIII gene typing and that there was an FDA meeting on the topic during the SSC meetings in Washington in which Dr. Gill proposed a study on sibling pairs in the US (but not limited to the US). A discussion concluded that in any concentrate assessment the gene type of the mutation should be determined but should not influence the treatment.

Working Group on Protease Inhibitors and Bleeding in Hemophilia

(C Lee et al.)

A summary of published data was presented, with an emphasis that this was a problem in groups other than hemophilia as well. Mechanisms of the bleeding effect remain unclear.

Working Group on Pharmacokinetics in Hemophilia Substitution

(M Lee et al.)

An update to the 1991 recommendations was prepared and a draft recommendation is anticipated imminently.

MISCELLANEOUS
E Santagostino summarized data from her own and published studies on the association of chronic hepatitis C with B cell non-Hodgkinis lymphoma and/or cryoglobulinemia and invited participation in an expanded ongoing study (fax no.: 39-021545-7074, email: hemophilia ctr@polic.cilea.it).

P Mannucci suggested a centralized study of immunological parameters in patients suffering from severe hemophilia B with inhibitors in whom anaphylactic reactions toward factor IX concentrate had occurred. Controls from non-reacting inhibitor patients were felt to be appropriate. Detailed study plans will be proposed.

D Brettler summarized the ethical and practical concerns that will emerge as gene therapies become increasingly available.

(End)
There was an acceptable attendance throughout the subcommittee meeting. The number of attendees varied between 50 to 70. A short additional presentation (Wartiowara and coworkers, University of Helsinki, "Different rate of thrombin activation of wild type and 34Leu mutant Factor XIII (FXIII) A") was added to the original program.

Dr. Yee (USA) provided the audience with a general state of the art overview on the three-dimensional structure of Factor XIII A subunit (FXIII-A) which was an introduction to some of the problems elaborated by other speakers.

Dr. Greenberg (USA) elaborated how site-directed mutagenesis could be utilized to study the biochemical mechanism of FXIII activation and inactivation. As an example he used mutations at the Ca$^{2+}$ binding site of FXIII-A to demonstrate the usefulness of such an approach in studying structural-functional relationship in the mechanism of the activation process.

Dr. Adany (Hungary) gave a detailed methodological overview on measuring the gene expression by quantitative PCR, in general, and measuring the expression of FXIII-A, in particular. She demonstrated how to overcome technical difficulties and how to complete the findings with morphological techniques by monitoring the expression of FXIII-A during the process of differentiation of monocytes into macrophages.

Dr. Bishop (USA) demonstrated the biochemistry of gelatin cross-linking by activated FXIII (FXIIla) and the techniques by which the altered physicochemical characteristics of non-fibrin gel can be studied.

Dr. Ichinose (Japan) gave an overview of FXIII deficiencies and proposed a new classification. He proposed to abandon the terms FXIII type I and type II deficiency and replace it with FXIII-A, FXIII-B and, if such a case is found, combined subunit deficiency. Subcommittee members agreed with the proposal and by a unanimous vote suggested supplementing the formerly accepted, but not yet published, paper on FXIII nomenclature with the new classification.

Dr. Muszbek (Hungary) demonstrated a new one-step sandwich ELISA method that made it possible to measure the mass concentration of tetrameric plasma Factor XIII without any interference from free FXIII subunits, fibrinogen and other plasma components. He used this method to establish a reference interval for plasma FXIII mass concentration for the first time.

Dr. Grant (UK) summarized the findings of his group on Val34Leu FXIII-A polymorphism as a risk factor for arterial and venous thrombosis and showed the importance of its relationship to the expression of PAI-I. Dr. Balogh (Hungary) presented a study in which no increased risk for venous thrombosis could be related to this polymorphism.
Dr. Ariens (UK) presented data showing that the thrombin-induced release of activation peptide from plasma FXIII containing the mutant A subunit proceeded significantly faster than from the wild type plasma factor. The Finnish group (Wartiowara et al.) reported similar results for cellular FXIII. In three very recent publications it was reported that FXIII containing 34Leu allele had higher activity than wild type (Val/Val) FXIII. Evidence was presented that showed that this is not the case. Although in the mutant thrombin activation proceeds faster, fully activated mutant and wild type FXIII had identical specific activities.

At the previous subcommittee meeting it was decided to explore the possibility of preparing a reference plasma for FXIII measurement. Dr. Longstaff (UK) looked into this matter; however, FXIII did not present the required stability in the plasma preparations he investigated. It was agreed that a reference plasma would be essential for calibrating present and further assays and further effort is to be made in this direction.
Dysfibrinogens:

The subcommittee undertook a discussion of dysfibrinogenemia including a general overview of dysfibrinogens from the standpoint of structural and genetic abnormalities and correlative dysfunction. Drs. Michio Matsuda and Susan Lord made formal presentations. Drs. Jose Martinez and Steve Brennan were unable to be at the meeting. The subcommittee expressed the view that it would be useful to compile and publish an updated and annotated summary of dysfibrinogens, including afibrinogenemia and hypofibrinogenemia, with emphasis on those with known structural defects. The abnormalities should be primarily identified by the molecular defect but the city name, when available, should be retained. The subcommittee also recommended that the list should serve as a repository for reporting newly characterized dysfibrinogens that are not intended for full publication.

Dr. Michio Matsuda agreed to coordinate the compilation effort with a working party consisting of Drs. Susan Lord, Steve Brennan, Jose Martinez, Michael Mosesson, and Jan McDonagh. Dr. McDonagh has compiled an extensive database on dysfibrinogens and will make this information available to the working party.

Fibrinogen Plasma Standard:

Following the results of an international collaborative study presented by Pattrick Gaffney, the Fibrinogen SSC agreed to recommend to the ECBS of the WHO that the UK NIBSC plasma preparation coded 98/612 should be adopted as the Second International Plasma Standard for Fibrinogen. This standard is defined to contain 2.2 mg of clottable protein per vial.

The subcommittee also discussed the requirement for a plasma standard (Dr. Nicodemo Weinstock presenting) containing high levels of fibrinogen. Dr. Ian Mackie also presented in relation to this topic. There was no consensus and the subcommittee agreed to discuss this question more fully at the next meeting.

Standardization and Fibrin Sealant:

Dr. Pattrick Gaffney presented the results of a preliminary study that had been initiated at the Fibrinogen Subcommittee meeting in Ljubljana concerning a fibrinogen concentrate standard. The subcommittee agreed to proceed to an International Collaborative Study using the materials prepared by the UK NIBSC with a defined assay protocol for participants.

Dr. Ronald McIntosh summarized proposals for the standardization of the measurement of other components in fibrin sealants in addition to its principal constituent, fibrinogen. It was agreed that future discussion on the standardized of measurement of these components (e.g., thrombin
and Factor XIII), should continue within the Fibrinogen Subcommittee, as this will provide continuity rather than have other constituents discussed as separate subjects in different subcommittees.

**Characterization of Fibrin Sealants:**

Following a presentation by Dr. Rainer Seitz, there was a strong view expressed in the meeting that the European Pharmacopoeia monograph "Fibrin Sealant" is not suitable in its present form for the regulatory characterization of new fibrin sealants. The subcommittee agreed to discuss the monograph at the next meeting with the intention of offering its recommendations to the European Pharmacopoeia and establishing its own view on constituents that should be measured in fibrin sealants.

Drs. Israel Nur and Gilbo Soe presented papers on characteristics of certain fibrin sealant preparations. The subcommittee will continue to hear papers on the characterization of fibrin sealant in the broader sense.
Fibrinolysis

Chair: N. Booth, UK
Co-chairs: P.J. Declerk, Belgium; C. Kluft, The Netherlands; O. Matsuo, Japan

The meeting was attended by 80-120 people.

Procarboxypeptidase U / TAFI

This topic was first discussed by the subcommittee in 1998, since then there has been considerable progress both in functional and antigen assays. The recent work of five groups was presented, allowing a reasonable consensus to emerge. Dr. Dirk Hendriks summarised a functional assay in which all the procarboxypeptidase U (PCU) is activated, after which it can be measured by the release of hippurate by HPLC. This correlated well with a measure of total PCU by ELISA. There was a strong correlation between plasma PCU and time for t-PA-mediated plasma clot lysis. Dr. Laurant Mosnier then presented his studies, using very similar approaches, this time with released hippurate being measured colorimetrically. Dr. Anthony Chan then made a presentation on behalf of Dr. Lazslo Bajzar, whose group has developed ELISA methods, based on either polyclonal or monoclonal antibodies. The assay is sensitive to native TAFI and sees it less well after activation. They too examined the relationship between plasma TAFI and endogenous fibrinolytic activity, this time measuring euglobulin lysis time, and found no correlation. Dr. Kees Kluft presented data on TAFI, measured by ELISA, in normal individuals. The most notable findings were the wide variation in normal concentrations and correlation with markers of inflammation. Dr. Irene Juhan-Vague presented ELISA data on normal individuals and in a group of patients. Correlations were observed between TAFI and some known risk factors for cardiovascular disease. General discussion of all these findings included suggestions that both activity and antigen needs to be measured, that care should be taken to make sure that there is no interference in the assays from other plasma factors, and that the wide normal variation might reflect genetic polymorphisms. It was suggested that the speakers and any others interested should form a working group to summarise assays that are readily available, and to compare data before the meeting in Maastricht in 2000. Exchange of samples in order to compare different assays may also be possible.

Standards for proteins of the fibrinolytic system

Dr. Patrick Gaffney, in his final presentation before he retires, summarised the standards and reference materials that are available and discussed their use in different studies. The t-PA standard was discussed in detail by Dr. Colin Longstaff. A new standard for t-PA is now required and a collaborative study was recently completed, in which the new putative standard was compared to the existing t-PA standard. The report on this study was sent last month to a panel of members. All responses to date supported the acceptance of the new standard and several commented favourably on the study. The potency is 10000 IU based on 12 laboratories using fibrin-based assays. A minority of contributors to the study felt that the value of 10500 IU should be adopted; this was based on 14 laboratories, the additional 2 using a different assay. It should be noted that previous standard was measured only in fibrin-based assays. Both these issues, the acceptance of the new standard and the actual value, were put to a vote. There were no votes
against the acceptance of the standard. The value of 10000 IU was accepted by 12 votes to 1. It was noted that there were a very large number of abstentions, especially in the second vote, most of those present not having a strong view. This t-PA preparation will now be recommended as a new standard, with a value of 10000 IU, to the WHO Expert Committee on Biological Standardisation.

**Physiological fibrinolysis by u-PA**

Dr. Victor Gurewich reviewed the data that suggest that u-PA has a role in fibrinolysis independent of the receptor, u-PAR. This included data from animal models, including knockout mice, and from studies on human blood. His own work shows that clots of platelet-rich plasma were lysed more readily by single-chain u-PA (also called proUK) than were platelet-free clots; the opposite is true of lysis by t-PA. He concluded that physiological fibrinolysis is critically dependent on the strong binding by platelets of u-PA and encouraged others to assess its role.

**D-Dimer**

This was a new topic for this subcommittee, formerly having been discussed by the fibrinogen committee. Dr. Dempfle reviewed different assays for D-Dimer and concluded that some of them are equally sensitive to soluble fibrin and D-Dimer, some of the assays being related to plasmin degradation and some not. Dr. Piet Meijer presented the ECAT experience of measurement of D-Dimer. It became clear that there was a problem of using different assays and different plasma samples in many studies, such that a solid basis for comparison is lacking. Dr. Willem Nieuwenhuizen summarised his work on measurement of D-Dimer. He had hoped to make available a set of plasma samples, suitable for comparisons between different laboratories; he now intends to provide these. This was warmly welcomed and it is intended that studies on these samples should be presented to future meetings of the subcommittee and to liaise with the fibrinogen committee.

**Secondary SSC standard**

Dr. Kees Kluft discussed the measurement of the SSC standard for proteins of the fibrinolytic system, which had been hampered by discrepancies between assays. The problems of assaying t-PA and PAI-1 have now been resolved. Dr. J Sidelaman, who last year presented data on the wide fluctuations in plasminogen measurements in a WHO EQAS initiative, reported on the conclusions of a small working party on measurement of plasminogen. It recommends the use of streptokinase activation and functional measurement, essentially as in the ECAT procedure.

**Plans for 2000**

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

- PCU / TAFI
- D-Dimer
Results of current work on standards

Further suggestions should be sent to Dr. Booth (n.a.booth@aberdeen.ac.uk), Dr. Declerck, (Paul.Declerck@farm.kuleuven.ac.be), Dr. Kluft (Kluft@euronet.nl) or Dr. Matsuo (kr9o-mto@asani-net.or.jp).

The meeting finished shortly before 5 p.m.
Tests:

1. M. Levine reviewed the goals of the Hemostasis and Malignancy Subcommittee. The goals are to stimulate clinical trials that will address unanswered clinically important questions in thrombosis and cancer; and to facilitate basic research that will advance knowledge in the area of the pathophysiology of hemostasis and malignancy.

2. A. Falanga reviewed the topic of blood tests of hypercoagulation in cancer patients. The focus was on the ability of such tests to predict thrombotic events in cancer patients. There has been no new information since the Subcommittee’s previous meetings. There is clearly a need for further large prospective studies in which blood tests are performed.

3. A. Falanga reviewed the progress of the SOMIT Study, which is ongoing in Italy. In this study, patients who present with idiopathic venous thrombosis are randomized to an extensive search for an occult malignancy versus a minimal search. Entry to the study has been closed and over 200 patients have been recruited.

4. A. Lee presented the results of a prospective study that evaluated the SimpliRED D-dimer Test in cancer patients with deep vein thrombosis. The study demonstrated that in cancer patients compared to non-cancer patients, the specificity of the SimpliRED drops substantially and, in addition, the negative predictive value of the D-dimer test was substantially lower in cancer patients. The false negative rate was 21%. Thus, this test used in isolation does not reliably exclude deep venous thrombosis in cancer patients.

Surgical Prophylaxis:

5. A. Kakkar reviewed data showing that the risk of postoperative pulmonary embolism was higher in cancer patients than non-cancer patients. He described preliminary data on the ability of pancreatic tumor cell lines to inhibit the anticoagulant effect of heparin. He described a new trial, the Prism Study, that examines the duration of postoperative prophylaxis in cancer patients undergoing major surgery. Cancer patients undergoing a laparotomy receive initial tinzaparin and are randomized to continue with low molecular weight heparin versus placebo. They receive an end of study venogram.

6. M. Monreal discussed prophylaxis in the medical cancer patient. He reviewed the epidemiology of cancer and thrombosis in his hospital in Barcelona. The most common cancers associated with thrombosis were lung, colon, prostate, and breast. He reviewed data on the use of low dose warfarin and low molecular weight heparin in cancer patients with central vein catheters.

7. G. von Templehoff discussed the TOPIC Study (Thrombosis Prophylaxis in Oncologic Patients with Certoparin). Patients with metastatic breast cancer are randomized to low molecular weight heparin or no treatment. The duration of therapy is 180 days.

8. G. Agnelli presented the results on the use of dermatan sulphate in patients undergoing cancer surgery. In this multicenter Italian study, 842 patients undergoing abdominal, thoracic, gynecologic, or urologic cancer surgery were randomized to dermatan sulphate
intramuscularly starting two days preoperatively versus unfractionated heparin 5,000 units three times per day. A venogram was performed on day 7. The rate of DVT/PE in the dermatan sulphate group was 15% versus 22% in the heparin group (P=0.003). The rate of proximal DVT/PE was 1.1% versus 3.5%, respectively (P=0.08). There was no difference in the rates of bleeding and mortality. Dr. Agnelli also outlined the design of a study in cancer patients with central vein catheters. They are randomized to lovenox or placebo.

9. M. Levine also described: i) the design of a trial of fragmin versus placebo in patients with pancreatic cancer—the primary outcome is mortality, ii) the design of a trial of fragmin prophylaxis in patients with glioblastoma which is being conducted by ECOG—the primary outcome is prevention of VTE, and iii) a trial of fragmin versus placebo in cancer patients with indwelling central vein catheters—the primary outcome is prevention of VTE.

10. During the discussion period on prophylaxis there were questions and discussion concerning the use of placebo controls in studies evaluating antithrombotics in patients with central vein catheters. Given that the standard of care in most centers is no prophylaxis, the general consensus was that it is reasonable and ethical to use a no-treatment control. The use of warfarin in a three-arm trial would substantially increase the sample size.

**Treatment of Venous Thromboembolism:**

11. M. Levine reviewed the issues related to home treatment with low molecular weight heparin in cancer patients and the duration of anticoagulant therapy for secondary prevention in cancer patients with venous thromboembolism.

12. P. Richardson discussed veno-occlusive disease of the liver in patients undergoing transplantation. This is a clinical syndrome that involves cell injury, inflammation, thrombosis, fibrosis, and necrosis. He discussed potential therapies that might modulate these pathways. One such therapy with promise is defibrotide.

13. L. Zacharski reviewed the data on the antineoplastic effect of antithrombotics. There is no new data since the Subcommittee’s last meeting.

14. A. Kakkar updated the group on the FAMOS trial in which patients with advanced cancer are randomized to placebo or fragmin low molecular weight heparin. Recruitment should go on for one more year.

15. M. Levine updated the group on the CLOT study in which patients with acute symptomatic venous thromboembolism receive initial fragmin and then are randomized to continue low molecular weight heparin or receive oral anticoagulant therapy. The duration of treatment is six months.

16. S. Sonenberg described the design of the MALT trial. Cancer patients are randomized to six weeks of low molecular weight or placebo.

**Basic Science:**

17. P. Nawroth discussed the contribution of the coagulation and fibrinolytic systems to tumor angiogenesis. In particular, he discussed the effect of tissue factor and vascular endothelial growth factor (VEGF) on angiogenesis and wound healing.
18. F. Rickles discussed translational research and the potential of molecular targets of therapy, e.g., inhibition of tissue factor which suppresses tumor growth.
Lupus Anticoagulant/Phospholipid-Dependent Antibodies

Chair: T. Barbui, Italy
Co-Chairs: J. Arnout, Belgium; J.T. Brandt, USA; S. Machin, UK;
R. Roubey, USA; I. Scharrer, Germany

The Subcommittee was divided into three sessions

First, an overview of pathophysiology of phospholipid-dependent antibodies was presented by Dr. DA Triplett. He pointed out the heterogeneity of these antibodies, which differ in terms of antigenic targets, methods of detection, and, possibly, clinical correlations. The various mechanisms potentially predisposing to thrombosis were also analyzed and discussed.

The second session dealt with the problems that still exist in the detection of the different phospholipid-dependent antibodies.

Dr. S. Machin updated the clinical validation of new tests.

Dr. M. Taylor reported on the final results of the ISLA-5, an international survey which involved 29 laboratories from 12 different countries. Seven plasmas were provided in limited aliquots and each laboratory was asked to identify the presence or absence of lupus anticoagulants using the coagulation tests commonly carried out in daily practice. A good degree of inter-laboratory concordance was observed, less in the two cases with "weak" lupus anticoagulants.

Dr. J. Arnout reported the functional characterization of murine monoclonal antibodies directed against human prothrombin which behave as lupus anticoagulants in "in vitro" coagulation tests. LA screening tests performed on plasmas spiked with these monoclonals, variably mixed with LA-positive monoclonal antibodies against human Beta-2-glycoprotein I, showed that both types of LA antibodies prolong clotting in an additive way.

Plasma spiked with these antibodies have the potential to serve as reference materials to compare the sensitivity and responsiveness of the different tests proposed for the detection of lupus anticoagulants.

The NCCLS document on anticardiolipin assay was presented by Dr. J.T. Brandt.

Dr. A. Tincani, on behalf of the European forum, reported the results of two surveys that analyzed the standardization of the ELISA methodology to detect anticardiolipin and the antiβ 2-glycoprotein I antibodies. Twenty-four and 19 centers participated in the two studies, respectively. A reasonable degree of concordance was observed for IgG but not IgM anticardiolipin antibody measurement. Regarding the antiβ 2-glycoprotein I antibodies measurement, the use of a common cut off point appeared to improve the agreement among centers.

The prevalence and clinical correlations with thrombosis of antibodies directed against Factor XII were presented by Dr. W. Jones.
The third session dealt with clinical trials. Dr. A. Tripodi reported the final results of an international collaborative study on the monitoring of oral anticoagulation of lupus anticoagulant-positive patients. The main conclusion was that the PT-INR measured with the majority of the thromboplastins is not affected by the presence of lupus anticoagulants, provided that the reagents were calibrated against the same international standard on the same instrument.

Dr. R. Brey first gave an overview of the treatment of neurologic complications of the antiphospholipid syndrome. Next, she updated the results of clinical trials on stroke.

Three different trials are currently being conducted to establish the best primary and secondary prophylaxis of arterial and venous thrombosis in patients with antiphospholipid antibodies. Dr. G. Finazzi reported the ad-interim analysis of the WAPS study, a prospective, randomized clinical trial aimed at assessing the risk/benefit ratio of high-dose oral anticoagulation in patients with antiphospholipid antibodies and thrombosis.

Dr. M. Crowther described the PAPRE trial, a double-blind study that randomizes patients with antiphospholipid antibodies and one (arterial or venous) thrombotic event to receive warfarin targeted at a PT INR either of 20-2.9 or 3.0-4.0.

Dr. L. Mitchell described the PRECLUDE study, a randomized, placebo-controlled trial, aimed at assessing the efficacy of warfarin in the primary prevention of thrombosis in SLE patients with antiphospholipid antibodies.

Finally, Dr. T. Barbui summarized the activities of the last three years, pointing out the role of the Subcommittee in the development of clinical trials in patients with the antiphospholipid syndrome.

A booklet with the short reports of the presentations is available upon request to Dr. Barbui.
Perinatal/Pediatric Hemostasis
Chair: M.J. Manco-Johnson, USA
Co-Chairs: M. Hellgren, Sweden; M.P. Massicotte, Canada; A. Sutor, Germany

The Perinatal/Pediatric Subcommittee meeting was attended by approximately 200 persons. Participation was active, discussion was lively and substantial interest was expressed in the presented topics and activities.

I. Standardization of laboratory testing for coagulation abnormalities in children who present with thrombosis.

In the first part of the program, the types and prevalence of coagulation abnormalities detected in children presenting with thrombosis in various settings were reviewed and recommendations made, as follows.

Catheters, Dr. Tom Abshire, USA: Because of recent reports supporting the presence of genetic and acquired prothrombotic traits in the majority of children at the time of presentation with thrombosis, this group of infants and children would warrant a complete study.

Cancer, Leukemia, Dr. Ulrike Nowak-Göttl, Germany: An excess risk of thrombosis has been reported in the treatment of several pediatric malignancies. All episodes of thrombosis occurred in children with indwelling central venous catheters. The cost/effectiveness of screening children at the outset of therapy, and the need for prospective intervention trials in children identified with thrombophilia and cancer were stressed. In addition, the additive effect of chemotherapy was discussed. The association of an increased risk of venous thromboembolic events with the specific L-asparaginase obtained from Kyowa, Japan explained discrepant results of studies examining the relationship of thrombosis to L-asparaginase.

Stroke, Drs. Vinod Balasa, Ralph Gruppo, USA: The conclusion of Drs. Balasa and Gruppo was that a complete laboratory evaluation should be performed in children with stroke.

Hormones, Dr. Marjolein Peters, The Netherlands: Dr. Peters recommended that girls treated with high dose estrogens be evaluated, especially for free protein S level, prior to commencing therapy.

Bone Marrow Transplantation (BMT)/Venous Occlusive Disease (VOD): Dr. Donna DiMichele, USA: National collaborative groups should be utilized to collect data needed to develop recommendations regarding coagulation screening in children undergoing BMT and/or diagnosed with VOD.

Neonatal, Dr. Wolfgang Muntean, Austria: The consensus of the subcommittee participants was that the in the premature infant thrombosis is almost always related to blood flow, vascular obstruction by catheters and consumptive coagulopathies whereas the well term infant with thrombosis is more likely to manifest a genetic thrombophilia. Routine coagulation testing was recommended for term infants with thrombosis.
Antiphospholipid antibodies, Manco-Johnson, USA: Although more data is needed, children presenting with venous thromboembolism should be evaluated for the lupus anticoagulant and children with stroke should be studied for anticardiolipin antibodies. In children with thrombosis associated with SLE or varicella, the prevalence of antiphospholipid antibodies is quite high (70-80%). The overall prevalence of APA in neonates with stroke is probably low.

The Subcommittee resolved to draft a position paper making recommendations regarding laboratory coagulation testing of children with thrombosis in the absence of adequate data, and proposing future studies to obtain information necessary for a definitive recommendation. This will be accomplished in the next nine months and submitted prior to next year’s meeting.

II. Monitoring of long-term anticoagulation was reviewed by Drs. Patti Massacote (Canada) and Paul Monagle (Australia).

III. Intracranial hemorrhage in ITP, Dr. James Bussel, USA:

Dr. Bussel and Dr. Sutor reviewed consensus papers from North America, Germany and the UK. A decision was made to develop a consensus paper from this committee specifically regarding current areas of consensus and lack of consensus around "need to treat," not addressing the treatment per se, for infants and children with ITP relative to platelet count. This paper will also be submitted prior to next year’s meeting. The second activity proposed in this section is continuation of an international registry of cases of intracranial hemorrhage in children with ITP. Because all treatment is designed to prevent this dreaded complication, factors associated with ICH determined by the registry will be useful to design prospective studies, intervention trials and future recommendations around treatment of ITP in children.

IV. Papers: Dr. J. Conard presented on behalf of Dr. Hellgren who was unable to attend. A summary of the recommendations for diagnosis and treatment of thromboembolism during pregnancy and the puerperium were presented. Input was received from participating committee members. The paper has undergone numerous drafts and will be submitted to the SSC Publications Review Committee within two to three months.

V. New Business:

Rheology, Eric Grabowski, USA: Dr. Grabowski presented an elegant paper demonstrating that neonatal endothelial cells support increased activation of Factor Xa and an increased ability to express tissue factor in response to increased shear stress in vitro. In his model the neonatal phenotype is controlled by shear stress. Transcription of tissue factor as well as levels of TFPI and TPA was comparable in the neonatal and adult models. There was consensus that this type of model is needed to address many remaining unknowns in neonatal hemostasis and the subcommittee should expand its interest in the neonatal endothelial cell. Future meetings will devote more time to neonatal rheology and the subcommittee requests that members involved in this area of research submit suggestions for next year’s program.
Plasma Coagulation Inhibitors

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

Dr. M. Aiach presented an update of the protein S database, 204 mutations (140 unique events). There are only 3 large deletions, 99 missense mutations, as well as frameshift mutations, etc. There are mainly type I/III deficiencies, very few type II, all of the latter mutations in N terminal region. It was decided to look into the possibility of publishing the update as a SSC communication in *Thrombosis and Haemostasis* and also to explore the possibility of preparing a website for the database.

Dr. I. Jennings discussed the practical issues of APCR/Factor V Leiden tests, under the UK laboratory quality control scheme, NEQAS. In this routine quality control exercise many laboratories could not reproducibly detect heterozygotes with functional assays or even with PCR. Forty-seven centers (mainly UK) were sent fresh whole blood. Different extraction techniques were used with different primer sets for Factor V Leiden and PT 20210A. Finally, different endpoints (SSCP, restriction) were used. In the first study of only the APCR clotting test, five out of 47 tests resulted in the incorrect diagnosis of Factor V Leiden. A later study included genetic testing for FV Leiden and PT 20210A. Once again, there were many incorrect diagnoses. A further survey underway includes polymorphisms of the MTHFR gene, and there are still reporting errors. The conclusion is that proficiency testing for these common polymorphisms is very important in the routine laboratory context. There was some discussion whether identifying these polymorphisms is clinically valuable.

Dr. J. Emmerich summarized results of a meta-analysis of eight case control studies of venous thrombosis concerned with interaction of Factor V Leiden and PT 20210A. There were 2310 cases in total and 3206 controls. An overall OR for thrombosis for Factor V Leiden was 5.01, while that for PT 20210A was 3.88. Both mutations combined gave an OR 24.1. Age of first onset was significantly younger, 36 compared to 40.5 years. The OR for PE was approximately 1.0 for both Factor V Leiden and PT 20210A. Synergism was demonstrated between oral contraceptives for both Factor V Leiden and PT 20210A. It is planned to submit this as a SSC communication for publication in *Thrombosis and Haemostasis*.

NEW RISK FACTORS

Dr. A.K. Ohlin presented her results on association of thrombomodulin gene mutations and venous thromboembolism. Seven hundred patients (Swedish/USA/France) have had their thrombomodulin gene screened. Thirteen cases were found to be heterozygous for a mutation. A start has been made characterizing gene mutations by in vitro transfection but further work is required. Dr. G. Kunz presented results of thrombomodulin mutations in arterial disease. In a case control study of 104 patients, six different mutations were identified. Three promoter polymorphisms have been evaluated by reporter-gene analysis and one, —33G to A, had reduced reporter activity. A coding sequence mutation Ala25Thr was found in two individuals and evaluated in a large case control study of myocardial infarction (MI). The results suggest Ala25Thr is a risk, ~2 fold, for MI. There is also evidence that an insertion/frameshift mutation
in the coding sequence results in reduced expression of thrombomodulin in vitro and in vivo. It was concluded that thrombomodulin gene mutations may be important in MI but more work is required to clarify this.

Dr. R. Simmonds summarized the polymorphic nature of the EPCR gene. This gene is a candidate risk factor for venous and arterial thrombosis. The sequence of the EPCR gene has been completed. There are four exons coding for the receptor sparing 6kbp. Four common polymorphisms have been identified in healthy normals and their population frequencies determined, as a prelude to clinical studies. Dr. E. Faioni also addressed this issue with two clinical studies. Two hundred and two survivors of MI/190 controls were used as well as 209 patients with DVT/402 controls. A 23bp insertion was identified and its consequences evaluated in the clinical studies. Adjusted OR of 2.5 for MI and 2.2 for DVT were obtained, but there were wide CIs. This is the first indication that mutation of the EPCR may have a role in disease.

Dr. F.C. Church discussed nomenclature issues in SERPINS. First, he reviewed the recent advances in SERPINS, basic and clinical. An international committee has been set up chaired by Gary Silverman to develop a logical classification system, to link websites, and to form a SERPINS society. There are approximately 400 known SERPINs. These will be grouped as plants, insects, nematodes, and certain types of factors, e.g., PAI-1. Dr. Church asked for people with an interest in this area to contact him during the meeting.

Dr. F. Bernardi discussed the HR2 Factor V gene allele. Approximately 10% of subjects are carriers and are widely distributed. Polymorphisms between exon 8-25 are linked and form the HR2 allele. This seems to be associated with increased risk of thrombosis, OR around two, but this has been inconsistent. A large study of Italian patients suggests the HR2 might be a risk factor for coronary artery disease. The functionally important change is that HR2 alters APCR. The isoforms of factor differing in glycosylation, Factor Va1 and Factor Va2, seem to be altered in their relative distribution in the HR2 haplotype and this could explain the effect on function.

Dr. M. Murata discussed the polymorphisms related to coagulation genes in the Japanese population. Factor V Leiden, PT20210A, and PLA2 are almost absent in the Japanese population, but there are other possibly important ones, particularly G1b α and Factor XII.
ALLOIMMUNE THROMBOCYTOPENIA

The subcommittee continues efforts toward providing a consensus statement on the management of alloimmune thrombocytopenia caused by maternal alloimmunization against fetal platelets. In previous meetings the laboratory evaluation of suspected cases has been discussed. The name for this syndrome has been proposed to be "alloimmune thrombocytopenia" ("AIT"), rather than "neonatal" alloimmune thrombocytopenia ("NATP"). The proposed nomenclature is appropriate because thrombocytopenia usually is present during gestation in affected pregnancies and because an affected fetus can suffer from serious hemorrhage antenatally as well as during the perinatal period.

This year’s meeting focussed on the clinical management of families affected with alloimmune thrombocytopenia. Presentations by Drs. Bussel and Kaplan were followed by a focussed discussion among the members present at the meeting. The first affected infant in a family is usually diagnosed based upon the clinical presentation of isolated thrombocytopenia in an otherwise healthy full term neonate. The suspected condition often requires therapy before serologic confirmation can be obtained. Transfusion with antigen negative platelets is appropriate if there is severe thrombocytopenia or serious hemorrhage. Diagnostic imaging (ultrasound or CT scan) should be performed to determine whether there is intracranial bleeding. The mother is usually the donor for the antigen negative platelets. However, alternative therapy may need to be started if there will be a delay in obtaining, processing, and testing maternal platelets. Maternal platelets must be 1) washed or plasma depleted to minimize the transfer of antiplatelet alloantibody, 2) irradiated to prevent graft versus host disease, and 3) filtered to reduce the risk of embolization of platelet aggregates. Alternative treatments (e.g., IV IgG, steroids, exchange transfusion, and transfusion with random donor platelets) may be initiated while preparing the antigen negative platelets.

After the diagnosis of AIT is confirmed, the family must be informed about the risk of this syndrome in subsequent pregnancies. Paternal genotyping for the platelet allotype implicated is appropriate. Subsequent pregnancies should be managed with the consultation of a high risk perinatal center. There is currently no antenatal intervention that can prevent thrombocytopenia and hemorrhage in all cases, but IV IgG infusions (with or without steroids) and fetal platelet transfusions during pregnancy have been reported to be efficacious in some cases. Several intervention protocols are under evaluation in North America and Europe. Affected families should be informed about these protocols and be offered referral to a specialty center. The subcommittee will prepare a manuscript to summarize the areas of consensus in this field.

AUTOIMMUNE THROMBOCYTOPENIA
Dr. Bussel presented a summary of current therapies used to treat autoimmune thrombocytopenia (ITP). First line treatments include steroids (oral or intravenous), IV IgG, and anti-D. Dr. Bussel cautioned that the time to achieve a platelet response with anti-D is longer than with IgG (72 hours, compared to 24-48 hours). However, preliminary results with a higher dose of anti-D (75mcg/kg, rather than 50mcg/kg) indicate a more rapid response.

Dr. Warrier presented results of her retrospective review of 16 pediatric patients with Evan's Syndrome. This group of patients was distinguished by the presence of multi-system aspects of the disease such as lymphoproliferation. These patients had an extremely poor outcome with 36% mortality from hemorrhage and post-splenectomy sepsis. Discussion indicated that the prognosis for adults with Evan's Syndrome tends to be better than for the pediatric patients reported.

AUTOIMMUNE THROMBOCYTOPENIA: WHICH PATIENTS ARE APPROPRIATE CANDIDATES FOR STEM CELL TRANSPLANTATION?

Stem cell transplantation (autologous and allogeneic) has been reported to be effective therapy for autoimmune diseases. Dr. Ertem reviewed the published reports and European Bone Marrow Transplant data base experience with this therapy. For serious autoimmune disease overall the long term results are promising; remissions resulted in 87% of cases after allogeneic stem cell transplantation and in 33% of cases after autologous transplantation. The European League Against Rheumatologic Diseases has recommended specific guidelines for severity of rheumatologic diseases that might warrant transplantation therapy. The Platelet Immunology Subcommittee set a goal to prepare analogous recommendations for patients with immune thrombocytopenia.

The clinical results of stem cell transplantation to date for patients with ITP and Evan's Syndrome are not encouraging. After a 1997 publication reporting complete response of two ITP patients after autologous stem cell transplantation, both of the reported patients relapsed (12 and 18 months post transplant). In only eight ITP patients have autologous stem cell transplantation results been reported. Results are as follows: three patients - complete responses (two relapsed at 12 and 18 months; third patient in remission but currently only eight months post transplant), one patient - partial response at eight months post transplant, and four patients - no response. One Evan's Syndrome patient is in complete remission eight months after autologous transplantation.

Allogeneic stem cell transplantation has been effective in correcting autoimmune thrombocytopenia in an animal model. The risk of standard allogeneic transplantation may be too high to warrant this therapy for most patients with immune cytopenias although non-myeloablative preparative regimens might provide better options by decreasing the procedure-related mortality rate.

Only those patients who are refractory to available therapies should currently be considered candidates for stem cell transplantation. Data are needed on long-term outcomes of subsets of ITP patients to allow assessment of current mortality rates for refractory patients. There presently is an ongoing international registry of pediatric ITP patients, but a registry of adult patients is also needed. It was recommended that at the present time stem cell transplantation for patients
with immune cytopenias only take place as part of a clinical investigation protocol. Dr. Ertem will lead the subcommittee in preparing a consensus statement in this area.

**DRUG RELATED IMMUNE THROMBOCYTOPENIA**

A proposal by Dr. Chong to organize an international wet workshop to assess results of laboratory testing for heparin-dependent antibodies was enthusiastically approved. Interested laboratory directors will communicate with Dr. Chong. He will also invite participation from laboratories active in this area that may not have been represented at this meeting. The goal will be to present the results at the 2000 meeting in the Netherlands.

**NOMENCLATURE**

The subcommittee chair will circulate proposals for platelet alloantigen nomenclature to interested members. Proposals will also be shared with the ISBT working party on platelet antigens. The goal is to achieve consensus with the ISBT by the time of the 2000 SSC meeting. The WHO liaison representatives will be encouraged to participate in this consensus activity.
On Saturday afternoon, August 14th, the Platelet Physiology Subcommittee met to discuss the issue of "Assessment of GP IIb-IIIa Receptor Occupancy and Function during Therapy with GP IIb-IIIa Antagonists." The session was chaired by P.J. Newman, L.K. Jennings, and M. Hoffman. More than 300 attendees participated in the program.

Dr. Lisa Jennings opened the program with an overview of the biology of GP IIb-IIIa, the mechanism of action of the different antagonists, and the different pharmacokinetics of the currently available, FDA approved GP IIb-IIIa antagonists. She also reviewed the various oral GP IIb-IIIa antagonists remaining in phase trial. An issue emphasized by Dr. Jennings was the importance of choice of anticoagulant in sample preparation since citrate by chelating calcium alters the apparent receptor occupancy of the antagonist relative to native blood.

Dr. Jennings’ presentation was followed by fine presentations describing approaches and instrumentation for assessment of GP IIb-IIIa occupancy and function. Dr. Alan Michelson outlined the use of whole blood flow cytometric approaches for assessment of occupancy and functional response. An advantage of flow cytometry is that only small volumes of blood need be analyzed. Potential disadvantages included the cost of instrumentation and the requirement for a highly trained and dedicated operator. Care also is required to minimize dilutional effects that would lead to dissociation of agonist from receptor leading to underestimation of receptor occupancy. Dr. David Varon described use of a prototype research cone and plate analyzer for rapid assessment of platelet function. The method involves image analysis to quantitate platelet adhesion to polystyrene from whole blood and is sensitive to plasma levels of von Willebrand factor, fibrinogen, and to GP IIb-IIIa occupancy by antagonists. The next three speakers described the use of commercially available instrumentation for assessment of GP IIb-IIIa occupancy. Dr. Robert Hillman from Accumetrics discussed the Ultergra™ RPFA system for bedside assessment of oral and intravenous GP IIb-IIIa antagonists. Dr. Doug Christie from Dade Behring discussed use of a high shear system, the PFA-100, for evaluation of platelet dysfunction in patients receiving GP IIb-IIIa antagonists. Finally, Dr. Bruce Lages of Xylum Corporation presented data using the Xylum Clot Signature Analyzer. All three instruments would appear to provide appropriate methodology for assessment of GP IIb-IIIa receptor occupancy and function that would be useful depending on the clinical and research context.

Dr. Koneti Rao discussed the impact of GP IIb-IIIa antagonists on thrombin generation. He provided data from in vivo assessment in primates for a potential anticoagulant effect of GP IIb-IIIa antagonists on top of that provided by heparin during coronary procedures. Dr. Dick Aster discussed the issue of thrombocytopenia associated with the use of GP IIb-IIIa inhibitors. The incidence of moderate to severe thrombocytopenia reported in the literature associated with the use of GP IIb-IIIa receptor antagonists varies between one and five percent. Dr. Aster presented preliminary data that the thrombocytopenia occurs by an immune mechanism involving circulating pre-existing antibody but that the epitopes recognized by the antibodies may be
different depending upon the receptor antagonist that binds to GPIIb-IIIa. Finally, Dr. Barry Coller gave an elegant presentation describing when, where, and why GP IIb-IIIa receptor antagonists need to be monitored in the context of either acute or chronic therapy. It is hoped that Drs. Jennings and Coller will work with the subcommittee to prepare a discussion document on this topic for publication as a subcommittee report.
Predictive Variables and Cardiovascular Disease

Chair: K. Bauer, USA
Co-Chairs: R. Hull, Canada; L. Iacoviello, Italy; G.D.O. Lowe, UK

The number of people attending this subcommittee meeting was estimated at 200.

Fibrinogen Assays. Dr. Ian Mackie, chair of the British Society of Haematology Working Party on Fibrinogen Assays, reported on a comprehensive evaluation of commercial fibrinogen standards, Clauss fibrinogen assays, and prothrombin-derived methods. These studies were performed on both photo-optical and mechanical coagulation analyzers at several sites in the U.K. In addition to lyophilised plasmas, a large number of plasma samples were analysed from patients with disseminated intravascular coagulation, liver disease, dysfibrinogenaemia, as well as those with elevated fibrinogen levels. The major findings included apparent errors of calibration in some commercial fibrinogen reference preparations, differences in results between Clauss kits, and a variety of discrepancies in prothrombin-derived assays, which were dependent on the thromboplastin and standard preparations used.

Overview of the Results of the Second Northwick Park Heart Study. Dr. George Miller presented an overview of the results of this prospective cardiovascular survey designed to prospectively look for associations between coagulation activation markers and a first episode of myocardial infarction in middle-aged males.

Meta-Analysis of Haemostatic Variables in Prediction of Cardiovascular Disease. Dr. John Danesh presented the results of meta-analyses for fibrinogen, C-reactive protein, albumin, and leucocyte count as markers of coronary risk. Using studies published before 1998 that included 4,000 cases of myocardial infarction, a 1 gram/L increase in fibrinogen was associated with a 1.8-fold increased risk. The analysis of C-reactive protein included 1,053 cases and conferred a 1.7-fold increased risk. Among 3,770 cases of coronary heart disease, the population in the bottom third of the population with respect to serum albumin level had an increased risk as compared to the top third. Among 8,054 cases, the population in the top third of the population for hematocrit had a 1.3-fold increased risk as compared to the bottom third. Data was also presented for plasma viscosity and erythrocyte sedimentation rate.

Overview of Committee Report on "Predictive Variables and Cardiovascular Disease." Dr. Gordon Lowe presented an overview of an article on this topic that is being prepared for submission to Thrombosis and Haemostasis. The variables to be included include Factor VII, tPA, PAI-1, coagulation inhibitors, and fibrinogen. The preparation of this paper has been delayed awaiting more prospective studies and comparative studies of different assays. It is anticipated that a draft manuscript will be circulated by the end of 1999 and hopefully ready for SSC approval at next year’s Annual Business Meeting in Maastricht.

The Genetics of Factor XIII, Fibrinogen, and Platelet Glycoproteins and Vascular Disorders. Drs. Peter Grant and Rashta Anwar presented data regarding the protective role of the Factor XIII-Val34Leu polymorphism for arterial as well as venous thrombosis. Data was presented indicating that the activation peptide of Factor XIII is actually released by a lower concentration...
of thrombin for the mutant Factor XIII molecule as contrasted with the wild type molecule. Dr. Angela Carter presented data regarding fibrinogen polymorphisms and the PlA2 polymorphism in the platelet glycoprotein IIIA gene as risk factors for myocardial infarction. Dr. Pascal-Goldschmidt also presented data regarding the role of the PlA2 polymorphism as a risk factor for myocardial infarction.

**Report of the ETRO Working Party on "Population Genetics of Hemostatic Risk Factors for Arterial Vascular Disease." Meta-Analysis of Genetic Polymorphisms and the Risk of Myocardial Infarction in the Young.** Dr. Licia Iacoviello presented an update from this group which included a meta-analysis of the PlA2 allele. Among 9,274 cases and 14,675 healthy controls, the polymorphism had a weak effect on the risk of coronary artery disease. The effect however was double in subjects younger than age 60 (odds ratio=1.22) and strongest in restenosis after angioplasty (odds ratio=1.31).

**Overview of Activated Protein C Resistance (APCR) and Factor V Leiden in Prediction of Thrombosis.** Drs. Gordon Lowe and M. McColl presented data on the role of APCR due to the Factor V-Arg506Gln mutation in venous thrombosis. Recommendations were presented regarding populations warranting screening and the implications of a positive diagnosis with respect to patient management. Dr. Lowe presented a meta-analysis of the role of APCR in deep venous thrombosis following total hip replacement. He concluded that APCR is probably associated with an increased risk of both asymptomatic (venographic) deep venous thrombosis and confirmed clinical thromboembolism after elective total hip arthroplasty despite routine antithrombotic prophylaxis.
von Willebrand Factor

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

Presiding chair was Dr. Francesco Rodeghiero (Vicenza, Italy); all co-chairmen were present.

Attendance was approximately 350.

Dr. A.B. Federici (Italy) reported the final data of the International Registry on Acquired VWD, set up with Dr. J.H. Rand. A document including guidelines for diagnosis and treatment will be submitted to the SSC for publication as an official report.

Dr. J.E. Sadler (U.S.A.) presented the recommendation of the joint ISTH/WHO meeting on VWD held in London, October 12, 1998. WHO and ISTH agreed on the development of joint strategies for epidemiological data collection on the prevalence of VWD in developing countries, for laboratory diagnosis, and for optimal treatment of VWD patients. The Subcommittee on VWF is already active in this field.

Dr. A. Srivastava (India) presented data on the prevalence of VWD in developing countries collected by mailed questionnaires. A new questionnaire for the collection of additional data on the prevalence of VWD in comparison to severe hemophilia A and on the severity of VWD was proposed and approved.

Dr. I. Peake (U.K.) chaired a session on the molecular diagnosis of VWD. Dr. A. Goodeve (U.K.) presented an updated molecular and genetic terminology of VWF and VWD. A consensus document will be prepared in cooperation with other members for approval in the next subcommittee meeting. Dr. J.E. Sadler, on behalf of Dr. D. Ginsburg (U.S.A.), reported on the current status of the electronic database on VWD mutations and polymorphisms. Dr. I. Peake reported on the difficulties of a molecular diagnosis of type 1 VWD. His proposal for a registry of studies on phenotypically and genotypically investigated type 1 VWD patients was approved.

Dr. C. Mazurier (France) chaired the session of the Working Party on the measurement of VWF content in therapeutic products. Dr. A. Hubbard (U.K.) and Dr A. Chang (U.S.A.) reported on the joint NIBSC and FDA study for the identification of a suitable concentrate preparation to be used as a future standard. Dr. T. Barrowcliffe (U.K.) presented data on potency assignment of the 4th International Standard of FVIII:VWF adopted by WHO. Dr. R. Seitz (Germany) presented two methods for VWF:CBA in concentrates.

Dr. R. Montgomery (U.S.A.) chaired the session of the Working Party on laboratory diagnosis of VWD. Dr. K. Friedman (U.S.A.) reported on an international standardized study of VWD variants. Dr. A.B. Federici presented data on VWF:Rcof, VWF:CBA and on a commercial VWF:Ag "functional" assay in different types of VWD. He also presented data on the use of the above tests for the diagnosis of type 2 VWD. Dr. E. Fressinaud (France) also presented data on the VWF:Rcof/VWF:Ag and FVIII:C/VWF:Ag ratio for the diagnosis of type 2 VWD. Dr. C.
Miller (U.S.A.) reported on disproportionately high Factor VIII levels using the 3rd International Plasma Standard and on the problems encountered in VWF:Rcof assay using commercial kits. Dr. E. Favaloro (Australia) reported on the role of VWF:CBA in VWD diagnosis in a multi-laboratory study in Australasia. Dr. Z. Ruggeri (U.S.A.) suggested the term "functional activity" for any VWF measurement be avoided.

Dr. M. Furlan (Switzerland) and Dr. J.P. Girma (France) presented two methods for VWF-cleaving protease assay for use in the clinical laboratory. A new Working Party on the measurement of VWF-cleaving protease was created, under the coordination of Dr. Furlan.

Dr. F. Rodeghiero closed the session presenting the current status of the multicenter study on the validation of diagnostic criteria of type 1 VWD.

SUMMARY OF SUBCOMMITTEE ACTIVITIES

Issues voted:

Submission to the SSC of an official report on diagnosis and treatment of AVWS.

Creation of a new Working Party on the measurement of VWF-cleaving protease.

Creation of a registry of studies on phenotypically and genotypically investigated type 1 VWD patients.

Ongoing projects:

Multicenter, retrospective study for the validation of the diagnostic criteria for type 1 and type 3 VWD.

The Working Party for VWF assay in concentrates will continue to cooperate with FDA and NIBSC to produce an international standard consisting of a stable freeze-dried concentrate calibrated against normal plasma.

Survey on VWD in developing countries.

Proposals for genetic and molecular terminology of VWF/VWD.
von Willebrand Factor
Annual Report

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

The Subcommittee met Sunday, August 15, in Room 40 in the Washington Convention Center, Washington, USA, from 08:00 to 12:00. Presiding chair was Dr. Francesco Rodeghiero. Attendance was approximately 350.

FINAL REPORT OF THE International Registry on Acquired von Willebrand Syndrome:
Guidelines FOR DIAGNOSIS AND TREATMENT (A.B. Federici, Chair)

Dr. A. Federici (Italy) presented the final report of the International Registry on Acquired von Willebrand Syndrome (AvWS). Information about 221 patients from 52 Centers worldwide was collected and analyzed. AvWS was associated with lymphoproliferative (LPD, 47%) or myeloproliferative (MPD, 19%) disorders, cardiovascular diseases (CVD, 13%), neoplasia (NEO, 7%) and others diseases (OTH, 14%). The results (as % or mean values) are as follows:

AvWS (total 221): LPD(98) MPD(40) CVD(27) NEO(14) OTH(32)

<table>
<thead>
<tr>
<th></th>
<th>LPD(98)</th>
<th>MPD(40)</th>
<th>CVD(27)</th>
<th>NEO(14)</th>
<th>OTH(32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex (% of male)</td>
<td>59</td>
<td>38</td>
<td>56</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>age at onset (yrs)</td>
<td>63</td>
<td>46</td>
<td>57</td>
<td>61</td>
<td>62</td>
</tr>
<tr>
<td>bleeders (%)</td>
<td>70</td>
<td>38</td>
<td>11</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>in follow-up (%)</td>
<td>58</td>
<td>18</td>
<td>59</td>
<td>50</td>
<td>67</td>
</tr>
<tr>
<td>VWF:Ag (U/dL, mean)</td>
<td>25</td>
<td>68</td>
<td>120</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>VWF:RCof(U/dL,mean)</td>
<td>8</td>
<td>22</td>
<td>68</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>FVIII:C (U/dL,mean)</td>
<td>21</td>
<td>33</td>
<td>131</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>pos anti-FVIII/VWF (%)</td>
<td>14</td>
<td>2</td>
<td>n.t.</td>
<td>14</td>
<td>12</td>
</tr>
</tbody>
</table>

Effective therapy with:

<table>
<thead>
<tr>
<th></th>
<th>LPD(98)</th>
<th>MPD(40)</th>
<th>CVD(27)</th>
<th>NEO(14)</th>
<th>OTH(32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddavp (%)</td>
<td>31</td>
<td>15</td>
<td>7</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>fVIII/VWF conc (%)</td>
<td>38</td>
<td>5</td>
<td>7</td>
<td>43</td>
<td>22</td>
</tr>
<tr>
<td>immunoglobulin (%)</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>3</td>
</tr>
</tbody>
</table>
The data from this International Registry suggest that AvWS is highly associated with certain disorders such as LPD, MPD, CVD. A practical flowchart for the diagnosis and the management of AvWS in these underlying disorders was discussed and approved during the meeting. An official document of the SSC-VWF will be prepared by Federici, Rand, Mannucci, Budde, Mohri, van Genderen, Bucciarelli and Rodeghiero. This report will be submitted to Thrombosis and Haemostasis.

REPORT FROM THE JOINT ISTH/WHO MEETING IN LONDON

(J.E. Sadler, Chair)

Under the sponsorship of WHO and ISTH, a joint committee meeting was held in at the Royal Free Hospital in London, October 12-14, 1998, to consider the impact, prevention and control of von Willebrand disease (VWD). The committee members were Erik Berntorp, Nikolai Bochkov, David Ginsburg, Dominique Meyer, Ian Peake, Francesco Rodeghiero, J. Evan Sadler, and Alok Srivastava. The meeting was chaired by Pier M. Mannucci, and the WHO Secretariat was Victor Boulyjenkov. Observers were Christine Lee, Vince Jenkins, Dale Owens, John Pasi, and Flora Peyvandi. The goal of the meeting was to develop recommendations for actions that ISTH and WHO could take to improve the diagnosis and treatment of VWD, particularly in developing countries.

Dr. J.E. Sadler (U.S.A.) presented a summary of the WHO/ISTH meeting proceedings, conclusions and recommendations. Dr. Srivastavaís survey data on VWD in the developing world was a focal point of the proceedings, and he presented these results separately to the Subcommittee. Several of the joint committeeís recommendations are congruent with the mandate of the Subcommittee, focusing on standards for classification, diagnosis, and treatment. One purpose of Dr. Sadlerís presentation was to consider the role the Subcommittee could reasonably perform to assist in the implementation of these recommendations, which are summarized below.

Assessment and Monitoring: National Patient Registries

Data on disease prevalence are important for making decisions about the allocation of health care resources. The number of persons at risk for VWD was estimated to be 1,480 to 3,580 persons per million. The number of persons with symptomatic VWD was estimated to be at least 100 per million. Among all symptomatic patients, intensive and frequent medical therapy is required mainly by patients with VWD type 3, who constitute 0.55 to 3.2 per million. These values were derived from data of both developed and developing countries, suggesting that the prevalence of VWD may be similar worldwide. However, additional data would be useful to substantiate these very rough prevalence estimates.

Based on a world population of approximately 5.8 billion, there are 8.6 to 21 million persons at risk for VWD and at least 580,000 persons with symptomatic VWD worldwide. Approximately 80% of these persons live in developing countries. Accurate knowledge of VWD prevalence, symptoms, treatment, and outcomes is required to evaluate the need for additional resources. Longitudinal data will be critical to evaluate the efficacy of interventions. During the meeting,
ISTH and WHO agreed on the development of strategies for establishing national VWD patient registries and data collection methods applicable to developing countries. Guidelines for this activity may be derived from previous successful efforts for other diseases in developing countries, and for VWD in certain developed countries such as France and Italy.

The Subcommittee on VWF has been engaged in studies of VWD prevalence for some time and these efforts might be extended to encompass regions of the developing world.

**Laboratory Methods and Quality Assurance**

Patients with VWD and their physicians need access to the laboratory tests required for proper diagnosis, either locally or by prompt referral to qualified central diagnostic laboratories. The quality and accessibility of laboratory resources in the developing world varies considerably. Optimal strategies for laboratory testing in VWD have not been defined, and may vary depending on the medical resources that are available and their distribution. ISTH and WHO agreed on that ISTH should promote an analysis of laboratory testing for the diagnosis of VWD and for the discrimination between type 1 and type 2 VWD. The existing Subcommittee on VWF of the Scientific and Standardization Committee of the ISTH may be a suitable working group to pursue this objective. ISTH and WHO agreed that WHO should evaluate its External Quality Assurance schemes for participating diagnostic laboratories, with particular reference to coagulation laboratory services, to determine whether modifications are necessary to address quality control measures for laboratory testing in VWD. ISTH and WHO together recommended that WHO survey and inventory the laboratory services relevant to blood coagulation testing among developing countries, to facilitate the identification and correction of deficiencies that may currently limit the diagnosis and therapy of VWD and other bleeding disorders.

The Subcommittee on VWF has a natural interest in these specific issues and will consider methods to obtain more data on VWD prevalence, criteria for diagnosis and strategies for treatment in the developing countries.

**VWD in the Developing Countries**

Dr. A. Srivastava (India) reported on the magnitude of the problem of VWD in developing countries, which is undefined due to the lack of awareness of this disorder, inadequate facilities for diagnosis and limited resources for treatment. In preparation for the joint WHO/ISTH meeting on this disorder in 1998, a questionnaire survey was conducted by Dr. Srivastava to record the available information on the prevalence, diagnosis and treatment of VWD in as many developing countries as possible. Responses were received from 22/56 countries (39%) that were approached. The results are shown in the table. The salient features are: 1.) Majority of patients are unidentified; 2.) There is a higher proportion of patients with severe disease, particularly in those countries with significant levels of consanguinity; 3.) At least one center capable of performing VWF:Ag and/or RiCof exists in most countries but facilities for multimer analysis are uncommon; 4.) Entire range of products from FFP to purified factor concentrates are used for replacement; 5.) Countries where treatment is supported by government/insurance, "on-demand" replacement is provided, while in others only major bleeds are treated with factor replacement; 6.) Surgeries, both minor and major, are performed in most countries.
While this survey provided a glimpse of VWD in developing countries, more detailed and precise data would convey better the impact of VWD in these countries. At the joint WHO/ISTH meeting in London, it was suggested that a system be established to collect data on VWD from more developing countries. In collaboration with Dr. F. Rodeghiero, the previous questionnaire was modified to include data on mortality and on severe hemophilia from the same referral area to assist in estimation of the true prevalence of VWD in that population. This survey will be conducted over the next 12-18 months. ISTH could help the cause of VWD in developing countries with a training program and by promoting joint collaborative projects of mutual interest between centers in developed and developing countries. (See Table, attached.)

MOLECULAR DIAGNOSIS OF VWD (I. Peake, Chair)

Dr. A. Goodeve (UK) reported on the consensus on genetic and molecular terminology for VWF and VWD. Nomenclature schemes proposed for VWF nucleotides and amino acids in 1994 are still not in use by all investigators. Now that examination of the entire VWF gene for mutations, particularly in type 1 VWD, is becoming more common this is of greater importance. A reminder of the nomenclature convention was presented (Beaudet AL, Tsui LC, Hum Mutat 1993;2:245-8). This has nucleotides in the cDNA starting from the mRNA cap site as +1, intronic sequence numbered after Mancuso et al (1989) and amino acid numbering having the initiator methionine as +1. Using (c) to denote propeptide numbers should no longer be practiced. Dr. Sadler reminded that the presence of a mutation in the VWF gene is required as part of the definition of VWD according to the 1994 ISTH criteria. However, this definition excludes possible locus heterogeneity and could be therefore undesirable. Proposals for a renewed consensus on genetic and molecular terminology of VWF/VWD would require the involvement of the major centers working on the field.

Dr. J.E. Sadler (U.S.A.) discussed on behalf of Dr. D. Ginsburg the current status of database of VWF mutations and polymorphisms. A database of mutations in VWD and polymorphisms in the VWF gene was published in two articles during 1993. This depended on the contributions of many members of the Subcommittee on VWF as part of the "Consortium on VWF Mutations and Polymorphisms". Since then, the database has been maintained on a server at the University of Michigan and is accessible at http://mmg2.im.med.umich.edu/VWF. New data can be submitted online using either Netscape or Internet Explorer browser software. Submissions are reviewed and confirmed before posting. The current database contains approximately 200 mutation entries and approximately 50 polymorphism entries. Updating of the database relies on voluntary submissions from scientists, and there is no mechanism for ongoing review of Medline or published abstracts for new mutations or polymorphisms. Consequently, some published information is not included in the database. There were 13 volunteer submissions in 1997, 33 in 1998, and 13 in 1999 as of July. The database continues to be used heavily and usage is increasing. The database was accessed by users an average of 84 times per week in 1997, 116 times per week in 1998, and 161 times per week in 1999. The current rate of access is approximately 8,400 hits per year.

Other Internet resources also contain information on VWD mutations. The Human Gene Mutation Database Cardiff at the location http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html lists approximately 115 VWD mutations.
The Genome Database (GDB) contains approximately 28 VWF polymorphism entries. Online Mendelian Inheritance in Man (OMIM) includes VWD as MIM #193400 and lists 28 mutations. These resources overlap to some extent but each contains some unique information.

The international genetics community is attempting to encourage collaboration among databases to develop standard nomenclature, procedures for quality control, and a common database format and software. These efforts so far have not proceeded to the point that they can influence the format or management of the current VWD databases. However, standards for nomenclature have been proposed and these probably should be adopted for general use. For example, the abbreviation for the von Willebrand factor protein would be "VWF" (all capitalized). The abbreviation for the human gene would be "VWF" (capitalized italics). The abbreviation for the mouse gene would be "Vwf" (initial capital, italicized). The abbreviation for von Willebrand disease would be "VWD" (all capitalized). Detailed recommendations for the description of mutations have been published in Antonarakis. Hum Mut 11:1-3,1998. Salient features include numbering of nucleotides beginning with the first nucleotide of the initiation codon, and numbering amino acid residues beginning with the initiation codon. These changes will be incorporated into the VWD databases as time permits.

Dr. I. Peake (UK) reported on the molecular diagnosis of type 1 VWD. Since about 70% of VWD is phenotypically diagnosed as type 1, understanding the molecular basis of this condition is important diagnostically. In the dominant form, only a few mutations have been described. In order to further understand this condition, full VWF gene analysis in index cases from type 1 VWD families combined with precise phenotypic assays, family linkage studies, ABO blood grouping, VWF gene/promoter haplotype analysis and expression of detected mutations in mammalian expression systems, are considered necessary. Results from this type of study would form the basis of proposed molecular diagnostic recommendations to be put before this Subcommittee.

WORKING PARTY ON MEASUREMENT OF VWF CONTENT IN THERAPEUTIC PRODUCTS (C. Mazurier, Chair)

Dr. C. Mazurier (France) introduced the session by emphasizing that the evaluation of replacement therapy for VWD is complicated by the heterogeneity of the different concentrates and also by the adoption of different treatment strategies. There is a clear need for standardisation in terms of VWF potency estimation, and hence labeling of these concentrates, in order to promote improved harmonisation of treatment.

Dr. T. Barrowcliffe (UK) described the final results of the collaborative study designed to establish the 4th International Standard (IS) for FVIII and VWF plasma. For VWF:Ag and VWF:RiCof, mean potency estimates against the previous IS exceeded those against fresh plasma pools by 14 and 20 % respectively, indicating a possible drift of the International Unit away from normal plasma. It was proposed to make a partial correction for this drift by assigning potencies as the means of the two estimates. This proposal was supported by members of the Subcommittee to whom the report had been circulated, and was accepted by the Expert Committee on Biological Standardisation of WHO, which accordingly established plasma 97/586 as the 4th IS for FVIII/VWF plasma.
Dr. R. Seitz (Germany) asserted that a reliable assay of VWF potency in concentrates is a prerequisite for their clinical use for treatment of VWD. The European Pharmacopeia Expert Group 6B currently evaluated two candidate VWF collagen binding methods: (1) commercial method (Baxter), using pepsin-treated soluble type III collagen of human placenta, and (2) in-house method (Paul Ehrlich Institut, modified after Thomas et al, 1993), using fibrillary type I collagen of equine tendon. Method (1) is robust and reproducible and is sensitive also to low and medium MW multimers, while method (2) gives lower values in concentrates containing predominantly low MW multimers. The question is which method would more adequately reflect the clinically relevant VWF activity. The Expert Group 6B will go on with their evaluation of the methods and would appreciate the advice of the SSC on this matter.

Dr. A.R. Hubbard (UK) and Dr. A. Chang (USA) reported on the collaborative study between NIBSC (T. Barrowcliffe), FDA (M. Weinstein) and SSC VWF subcommittee (C. Mazurier) to produce an International Standard consisting of a stable freeze-dried concentrate. Trial fills of five concentrates, currently used to treat VWD, have been prepared for evaluation. Preliminary characterisation, in three laboratories, focus on stability, multimer composition and the validity of VWF potency estimation relative to the WHO International plasma standard and other concentrates. This will be followed by a multi-centre collaborative study that will include a variety of current assay methodologies. Based on these results, one or two suitable materials will then be selected as candidate preparations for inclusion in an international collaborative study which will lead to the establishment of the 1st IS for VWF concentrates.

WORKING PARTY ON LABORATORY DIAGNOSIS OF VWD

(R. Montgomery, Chair)

Dr. K. Friedman (U.S.A.) reported the results of an international multicenter study on interlaboratory variability in VWD testing. Ten fresh-frozen samples including normal plasma and plasmas from type 1, 2 and 3 VWD were sent to Centers in Europe and US for measurement of FVIII:C, VWF:Ag and VWF:RiCof.

On the basis of the encouraging results of this study, Dr. R. Montgomery (U.S.A.) made specific recommendations on behalf of the Working Party on Diagnosis of VWD. A number of different international laboratories should study a group of molecularly defined VWD variants with ratios of VWF:RiCof/VWF:Ag, VWF:CBA/VWF:Ag, and FVIII/VWF:Ag to determine agreement of assays. These assays should be compared to internal standards within that laboratory and between the study laboratories. A cohort of type 1, heterozygous type 3, and type 1 patients with different ABO blood groups should be compared by a small number of multinational laboratories. Multiple methods for ristocetin cofactor and collagen binding should be compared using multiple participating laboratories. All of above should be directly compared to the 4th International Standard. Commercially available standards should be compared to the 4th International Standard in multiple laboratories. Using the 4th International Standard, several laboratories should determine VWF antigen levels in 50 unselected individuals to determine the mean and standard deviation of normal individuals with this standard.

Shipping costs should be borne equally by participating laboratories.
Dr. A.B. Federici (Italy) reported VWF:RCof in comparison to two commercially available assays: functional ELISA and collagen binding assay (VWF:CBA). Dr. Federici showed the results of the comparison of two commercially available proposed functional assays with the home-made Ristocetin cofactor (VWF:RCof) activity in 81 VWD cases, diagnosed accordingly to the recommendations of the ISTH-SSC on VWF. The ELISA kit commercially available (VWF "ACTIVITY") proposed by Goodhall et al [TEST A] was compared to the home-made VWF:RiCof [TEST B] and the kit commercially available (IMMUNOZYM VWF:CBA), a two-step-ELISA which is made with collagen type III of human placenta [TEST C]. All the three assays were compared by expressing their absolute values (U/dl) by the ratio with the VWF:Ag measured by ELISA. Mean values (SD) of the ratios between each functional assay and the VWF:Ag are shown. Paired data [A vs. B and C vs. B] were analyzed by the paired Student’s t test and significant differences reported as follows:

<table>
<thead>
<tr>
<th>Individuals(n):</th>
<th>[A] VWF:Act/Ag</th>
<th>[AvsB]</th>
<th>[B] VWF:RCof/Ag</th>
<th>[C] VWF: CBA/Ag</th>
<th>[CvsB]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals (25)</td>
<td>0.90 (016)</td>
<td>ns</td>
<td>0.97 (0.20)</td>
<td>1.01 (0.16)</td>
<td>ns</td>
</tr>
<tr>
<td>1 PN (22)</td>
<td>1.03 (0.24)</td>
<td>ns</td>
<td>0.99 (0.17)</td>
<td>0.99 (0.16)</td>
<td>ns</td>
</tr>
<tr>
<td>1 PL (13)</td>
<td>0.88 (065)</td>
<td>ns</td>
<td>0.67 (0.25)</td>
<td>0.78 (0.29)</td>
<td>ns</td>
</tr>
<tr>
<td>1 PD (7)</td>
<td>0.79 (0.40)</td>
<td>0.015</td>
<td>0.34 (0.17)</td>
<td>0.97 (0.17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 A (10)</td>
<td>0.89 (0.34)</td>
<td>&lt;0.001</td>
<td>0.31 (0.18)</td>
<td>0.47 (0.29)</td>
<td>0.07</td>
</tr>
<tr>
<td>2 B (16)</td>
<td>0.96 (0.31)</td>
<td>&lt;0.001</td>
<td>0.56 (0.20)</td>
<td>0.75 (0.22)</td>
<td>0.004</td>
</tr>
<tr>
<td>2 M (13)</td>
<td>1.11 (0.63)</td>
<td>ns</td>
<td>0.94 (0.50)</td>
<td>0.86 (0.44)</td>
<td>ns</td>
</tr>
</tbody>
</table>

(Vicenza)


Dr. A.B. Federici (Italy) reported VWF:(RCo/Ag) ratio in the different types of VWD. According to the study of different VWF functional assays previously shown, Dr. Federici pointed out that a presumptive diagnosis of type 2 VWD can be performed just by the VWF:RiCof/Ag ratio considering multimeric analysis a second-step test. In fact values of ratio > 0.7 are always present in normal subjects and in type 1 VWD with normal VWF while values < 0.65 correlate very well with the loss of higher molecular weight multimers as those shown in type 1 PD, 2 A, 2 B. This approach can be very useful in case of laboratories where the multimeric analysis is not available.

Dr. E. Fressinaud (France), also on behalf of Dr. C. Mazurier and Dr. D. Meyer presented data on the ratio of von Willebrand factor ristocetin cofactor activity to antigen in types 2A, 2B, 2M
von Willebrand disease and ratio of factor VIII to von Willebrand factor antigen in type 2N VWD. VWF:RiCof/VWF:Ag ratio was studied in 93 patients, included in the French INSERM Network on molecular abnormalities in VWD. In all patients, the gene defect was characterized. Ninety-six healthy volunteers served as control group and exhibited a mean ratio of 0.99 ± 0.24 (2SD). Values were 0.32 ± 0.18 (mean ± SD) in 38 patients with type 2A and were very similar (0.36 ± 0.13) in 20 patients with type 2M (that is with a gene defect in the A1 loop). In contrast, the ratio was much higher (0.6 to 1) in 4 patients with the Vicenza variant that is with a candidate mutation in exon 27 of the gene. In 31 patients with type 2B, the mean ratio was 0.54 with extreme values from 0.25 to 0.9. Thus, the VWF:RCo/VWF:Ag ratio may be normal in patients with type 2B or the Vicenza variant.

Factor VIII:C/VWF:Ag ratio was studied in 47 patients with type 2N VWD. In the control group the ratio was 0.56 ± 0.24 (mean ± 2 SD). In 20 patients with compound heterozygous mutations and in 20 homozygous patients with the Arg 91 Gln mutation, values were 0.30 ± 0.13 (mean ± SD) and 0.31 ± 0.08 respectively. In 7 homozygous patients with another mutation, the mean ratio was particularly low: 0.10 ± 0.09.

Dr. C. Miller (U.S.A.) presented data showing disproportionately high factor VIII levels using the 3rd international plasma standard under clinical conditions. A population of 115 normal women studied recently at the CDC differed dramatically in factor VIII activity from a previously studied population (mean 150 vs. mean 106). FVIII levels were also significantly higher than VWF levels in the recent study and in 40 patients studied over 2 years. The 3rd IS was used in the recent studies. The 2nd IS was used for the earlier one, in which FVIII and VWF levels were equivalent. Adjustment of FVIII levels to the 2nd IS (60 IU/ml) rather than the 3rd (0.80 IU/ml) eliminated the discrepancies. Dr. Miller hypothesized that the potency of the third IS may be overestimated or that the preparation may behave differently with some instrument/reagent systems in current clinical use. Overestimation of FVIII levels in a clinical setting may lead to undertreatment of hemophilia patients and can complicate the diagnosis of von Willebrand disease. This issue continues to be of importance because commercial suppliers of reference materials may continue to use the 3rd IS for a long period of time before adopting the 4th IS. An ancillary concern is that many companies no longer specify against which standard their materials are calibrated, while clinical labs need that information.

Dr. C. Miller (U.S.A.) reported on difficulties with ristocetin cofactor measurement using commercial kits. A number of laboratories in the U.S. have had difficulties with the ristocetin cofactor assay over the past 2-3 years when using commercial reagents. The problems consist of inability to produce an acceptable standard curve, erratic results for the same sample during a single assay, and poor reproducibility of results from day to day. Quality control was unacceptable for patient care, causing many labs to abandon the assay. Reagents from three companies gave similar results, in two different brands of aggregometer. Dr. Miller has tracked the problem and found the common factor to be ristocetin, all of which comes from a single source. The U.S. companies have been reconstituting and then relyophilizing the material to be packaged in their kits. The ristocetin as originally supplied performed well in our hands, with over 250 tests on study subjects all with excellent results. The problem apparently lies with the quality of the processed ristocetin. Recommendation was made that lots of ristocetin be
characterized for activity and stability prior to packaging to assure that the consumer gets a reliable product.

**Dr. E. Favaloro** (Australia) presented data on the role of CBA in the laboratory diagnosis of VWD produced by a multicenter evaluation. A multi-laboratory evaluation for VWD testing was undertaken in Australasia. 25 labs were sent 10 plasmas (normal plasma pool (PNP), PNP diluted to 50%, normal individual, and typed VWD samples: severe 1 \([x1]\), 2B \([x2]\), 3 \([x1]\) and 2A \([x1]\)), and were asked to perform all available tests used to diagnose VWD, and to comment on results. All laboratories tested for FVIII:C, VWF:Ag and functional VWF (VWF:RCoF and/or VWF:CBA); only three labs performed VWF:Multimers. Assay methodologies varied widely (e.g. VWF:Ag, ELISA, EID, LIA, VIDAS; VWF:RCoF: aggregometry vs. 'functional' ELISA). VWF:RCoF gave highest inter-laboratory variation, and poorest sensitivity to low levels of VWF; VWF:CBA was better able to detect Type 2 VWD. VWF:RiCof by aggregometry gave greater variation & poorer sensitivity to low VWF levels compared to 'functional' ELISA, but was better for identifying Type 2 VWD. Most diagnostic predictions were correct (i.e. normal vs. VWD samples correctly identified, VWD subtype correct). 'Misinterpretations' usually linked to test panels used (i.e. VWF:Ag & VWF:RiCof combination performed worse than VWF:Ag & VWF:CBA). Conclusion: VWF:CBA outperformed VWF:RCoF as a functional VWF assay, and should be included in future multi-laboratory evaluations. During the following discussion, **Dr. Z. Ruggeri** (U.S.A.) stated that the term "functional activity" for any VWF measurement be avoided.

**VWF-CLEAVING PROTEASE ASSAY**

**Dr. M. Furlan** (Switzerland) addressed the issue of the deficiency of VWF-cleaving protease and its measurement. VWF-cleaving protease was found deficient in patients with TTP, whereas patients with hemolytic-uremic syndrome (HUS) had normal activity. The laborious assays of VWF-cleaving protease activity described hitherto include sodium dodecyl sulfate (SDS)-electrophoresis of digested VWF and analysis of degraded VWF multimers by immunoblotting. A new simple functional assay, based on previous observations that binding of VWF to collagen depends on the multimeric size of VWF, has been recently developed in Dr. Furlanís laboratory. VWF present in a human plasma pool that had been depleted of protease activity was digested by the VWF-cleaving protease of added test plasmas. Proteolytic degradation led to low molecular weight forms of VWF showing an impaired binding to microtiter plates coated with human collagen type III. This assay can be performed in a non-specialized laboratory and may be accomplished within 6 hours.

**Dr. J.P. Girma** (France) reported on a new two-step method to quantify the VWF-cleaving protease activity in plasma. In the first step proteolysis is performed using a constant amount of wild type recombinant VWF as substrate and serial dilutions of tested plasma as protease provider. In the second step, the degradation of VWF-Ag is estimated by a two-site IRMA using as coated antibody a monoclonal antibody (MoAb) directed against the C-terminal side of the cleavage site, and as labeled antibody a pool of MoAbs specific for the N-terminal side. Because the proteolytic process leads to the progressive separation of the C- and N-terminal portions of the VWF subunit the IRMA also shows a progressive apparent loss of VWF:Ag. The validity of the method was confirmed by showing a normal protease activity in relapsing thrombotic
thrombocytopenic purpura as well as in normal plasma following addition of an anti-protease antibody raised in mice against partially purified VWF-cleaving protease.

CLOSING REMARKS

Dr. F. Rodeghiero (Italy) reported the current status of the International Multicenter Study for the Validation of the Diagnostic Criteria of Type 1 and Type 3 VWD. Twenty-one Centers had definitely agreed to participate and a sufficient number of families is expected to be enrolled and available for analysis. Preliminary data will be presented during the next SSC meeting and could provide the basis for formal criteria for VWD diagnosis. Hopefully, before the next SSC meeting most of the ongoing projects of the Subcommittee will be concluded and other issues, like clinical management of VWD and its impact in women, could be addressed.

SUMMARY OF SUBCOMMITTEE ACTIVITIES

Issues voted:

- Submission to the SSC of an official report on diagnosis and treatment of AVWS
- Creation of a new WP on the measurement of VWF-cleaving protease
- Creation of a Registry of studies on phenotypically and genotypically investigated type 1 VWD patients

Ongoing projects:

- International Multicenter Study for the validation of the diagnostic criteria for type 1 and type 3 VWD.
- The WP for VWF assay in concentrates will continue to cooperate with FDA and NIBSC to produce an IS consisting of a stable freeze-dried concentrate calibrated against normal plasma.
- Survey on VWD in developing countries.
- Proposals for genetic and molecular terminology of VWF/VWD.