46th Annual Scientific and Standardization Committee Meeting

June 15 – 16, 2000

Maastricht, The Netherlands
# Table of Contents

Animal, Cellular and Molecular Models of Thrombosis and Haemostasis .................................................. 2  
Biorheology .................................................................................................................................................. 4  
Contact Activation....................................................................................................................................... 7  
Control of Anticoagulation, Parts I & II ...................................................................................................... 8  
DIC .............................................................................................................................................................. 18  
Factor VIII and Factor IX, Parts I & II ....................................................................................................... 20  
Factor XIII ................................................................................................................................................ 25  
Fibrinogen .................................................................................................................................................. 28  
Fibrinolysis ................................................................................................................................................ 30  
Hemostasis and Malignancy ....................................................................................................................... 33  
Lupus Anticoagulants/Phospholipid-Dependent-Antibodies ..................................................................... 36  
Perinatal/Pediatric Hemostasis .................................................................................................................. 40  
Plasma Coagulation Inhibitors ................................................................................................................... 42  
Platelet Immunology ................................................................................................................................ 44  
Platelet Physiology ................................................................................................................................... 47  
Predictive Variables in Cardiovascular Disease ......................................................................................... 48  
von Willebrand Factor ............................................................................................................................... 50
Animal, Cellular and Molecular Models of Thrombosis and Haemostasis

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

The Subcommittee meeting was attended by 30-40 persons. The program was devoted to new animal and cellular models. G. Johnson, Co-Chair, presided, and Pudur Jagadeeswaran, Co-Chair, attended and presented.

PROGRAM

Luc Schoonjans (Leuven) presented new data on novel transgenic techniques using embryonic stem (ES) cells and cloning. The majority of gene targeting experiments have been performed using 129 ES cells. However, the 129 strain is not well characterized, and the knockout phenotype depends on the genetic background of the mice. Therefore, a program was developed to derive ES cells from the most commonly used mouse strains. This has resulted in the establishment of ES cells from 10 different mouse strains with germline transmission capability.

Virginie Mattot (Lille) presented the results of studies of transgenic mouse models expressing different VEGF isoforms. VEGF 120/120 animals manifested renal vascular abnormalities that resulted in sclerotic glomeruli and decreased tubule formation and impaired renal function. Thus, angiogenesis is normal in VEGF 120/120 animals, but vascular remodeling is abnormal.

Heike Beck (Erlangen) discussed rodent models of stroke. The advantages and disadvantages of single and extended thrombotic occlusion and embolic occlusion were described, and the roles of Ang-2, VEGF and apoptosis in post-stroke angiogenesis were discussed.

Vivian de Waard (Amsterdam) presented the results of studies of the role of PAI-1 and VN in an experimental arterial constriction model. Ligated carotid arteries demonstrated enhance smooth muscle proliferation in PAI-1 -/- and VN -/- knock-out mice compared to wild-type controls. These studies indicate that PAI-1 and VN protect against smooth muscle-rich neointimal formation following arterial injury.

Pudur Jagadeeswaran (San Antonio) reviewed his studies of hemostatic parameters and thrombosis in zebrafish. Due to the ease of saturation mutagenesis and the rapidly progressing zebrafish genome project, this model has great promise for rapidly identifying specific genotypes. To evaluate hemostatic factors, micro-assays for coagulation, platelet function and bleeding time were developed, the effects of anticoagulants were assessed and thrombosis was induced. These developments will enable further evaluation of the utility of this model in genetic research in hemostasis and thrombosis.

Anne Angellillo (Geneva) presented the results of studies of growth arrest-specific gene 6 (Gas6), a homolog of protein S, on platelet function. Contrary to expectations, Gas6/-/- mice were resistant to thrombosis. Protection from thrombosis was found to be attributable to impaired platelet aggregation in response to several agonists and to reduced endothelial tissue factor.
BUSINESS

The Subcommittee voted to approve submission of the manuscript, USEFULNESS AND LIMITATION OF ANIMAL MODELS OF VENOUS THROMBOSIS, written by M. Levi, J. Dorffler-Melly, G. Johnson, L. Drouet and L. Badimon on behalf of the Subcommittee, to the SSC for approval as an official SSC document.

The Subcommittee voted to authorize a writing committee to prepare a document on the assessment of bleeding risk in animal models. The committee will be appointed by the Chair.

The Subcommittee authorized the development of a program to be developed jointly with the Subcommittee on DIC for the 2001 meeting. Dr. M. Levi will coordinate organization of this symposium.
I. Professor Mony Frojmovic presented highlights of the Final Working Party Report on "Rheological bedside devices for monitoring and managing vascular diseases". Basic biorheological principles were presented, centered on the dependence of drug efficacy affecting platelet function on the actual shear rates being evaluated. The spectrum of flow devices currently available for both basic research and clinical evaluations were reviewed. The types of blood samples used (anticoagulated versus native), volumes, shear conditions, measured in vitro parameters and their relationship to platelet or clotting functions, as well as clinical settings (including bleeding disorders and management of drugs targeted against GPIIbIIIa receptors), were reviewed. A few central take-home messages included (1) the shear rate choice for in vitro evaluations in current flow devices affect drug action, seen for example on effects of anti-GPIb/αWF agents on platelet function with the Clot Signature Analyzer (CSA) of Xylum Corp. at ~1000 versus ~10,000 s\(^{-1}\) shear rates; (2) rebound effects of anti-GPIIbIIIa drugs ~ 20 hours post administration are appearing for some patients with devices like the CSA and the platelet function analyzer (PFA) of Dade-Behring, not seen by aggregometry; (3) differential effects of anti-GPIIbIIIa drugs on platelet aggregation and clot retraction seen with the Hemodyne bedside device, which could perhaps be extended to more dynamic, shear-dependent fibrin clot formation; (4) the wide normal ranges seen with PFA and Hemodyne device may reflect important biological variations in even normal human donors (e.g., α-granule vWF content and secretion); and (5) the old bleeding time method is largely being replaced by a number of devices (including the Cone-Plate(let) Analyzer by Varon/Savion).

II. Symposium on flow-dependent blood cell adhesion and thrombus formation

1. Professor Mony Frojmovic gave the background and new data supporting the notion that unactivated platelets cannot recognize fibrinogen (Fg) either surface-immobilized or bound to GPIIbIIIa receptor on activated platelets. Rather, controlled activation of platelets by ADP allows recognition of surface or receptor-immobilized Fg. Latex beads containing pure surface-adsorbed Fg at varying coating densities, or purified activated GPIIbIIIa receptors, were used in coaggregation studies with "resting" or ADP-activated platelets studies in a cylindrical cylinder couette device at shear rates varied from 100-2000 s\(^{-1}\). Observations were shown to be physiologically relevant, with minor inhibition by soluble Fg (up to 9 µM). Comparisons of aggregation efficiencies were made with ADP-activated platelets and model GPIIbIIIa spheres, at comparable Fg coating densities. It is suggested that previous studies done in parallel plate flow devices, which require red blood cells in whole blood for delivery of platelets to the study surfaces coated with Fg, had <1% of platelets present as activated cells (e.g., via ADP generated by red blood cells), which could suffice to show platelet deposition in typical 5 minute flow times.
2. Dr. Hoylaerts discussed the role of platelets in monocyte (THP-1) rolling on PMA-stimulated (4 hour) endothelium via platelet P-selecting mechanisms. Endothelial NO or NO-donors help reduce platelet interactions, and THP-1 rolling with PMA-EC. Platelets: THP-1 at ratios of 3:1 were maximal in promoting THP-1 rolling. The platelet P-selectin interactions with THP-1 PSGL-1 helps reinforce THP-1 P-selectins interactions with PMA-endothelium, by utilization of platelet P-selectin.

3. Dr. S. Diamond discussed his studies of computer simulations and high resolution, high speed imaging of platelet neutrophil interactions in flow. Platelet homoaggregation by Fg-GPIIbIIIa in flow was successfully modeled over the shear range of 50-2000s for modest and full activation of platelets. Similar calculations predicted neutrophil aggregation from 100 to 2000 s⁻¹ for L-selectin and B₂-integrin mediated aggregation. Neutrophils were predicted to accelerate platelet capture in heterotypic aggregation by a volumetric mechanism. Finally, neutrophil membrane tethers were discovered which mediate string formation. These tethers may be prothrombotic.

4. Dr. Don Gabriel stated that conformational changes of vWF by shear forces are poorly understood, potentially very important, and supported only by one literature study using atomic force microscopy (AFM). In analogy, fibrin formation under flow results in highly aligned polymers. A₁ domain of vWF and A₃ domain are reactive for GPIb and collagen, respectively. A₁ and A₃ are similar to I domain. Potentially A₃-collagen binding can reveal the A₁ site and may move 5-25 Å to reveal A₁ domain. Quasi-elastic light scattering (QELS) and electrophoresis of vWF polymers can reveal up to 20 million MW multimers. DTT breaks vWF to monomer or dimers. Use of hydrophobic surfaces allows detection of adherent vWF. Loading at 35 dyne/cm² shear stress resulted in alignment. QESL gives Diffusion coefficients (D) of vWF of 5-10⁻⁷ to 4X10⁻⁸ cm²/s (function of MW and concentration). As salt concentration approaches zero vWF goes into extended form, D=3.6x10⁻⁸ cm²/5; at 100 mM NaCl, ~D~10X10⁻⁸ cm²/s. At T > 40°C, vWF denatures. A change in hydrophobic character of solvent (methanol, ethanol, and propanol) causes D of vWF to increase. As vWF can undergo hydrophobic aggregation, it may be aggregated in typical lab preparations.

5. Dr. J.J. Zwagining discussed interactions of leukocytes with platelets under flow conditions. In the context of endothelial inflammation and coagulation (thrombin, fibrin), shear forces regulate leukocyte and platelet rolling interactions which can transition to firm adhesion. Relevant binding interactions of: Lsel-PSGL-1, Psel-PSGL-1 and platelet coverage of >10% of EC matrix results in PMN adhesion. PMN cannot adhere to ECM above 300 mPa. With platelets/ECM, PMN can adhere up to 700 mPa. At 400 s⁻¹, PMN adhere to platelets (50% block by antiCD62L, >90% block by anti CD62P). Firm adhesion of PMN to platelet is via β₂ integrins (mostly Mac1, cd11b/18), with consequent increase in rolling fraction. PMN firmly bind fibrin via CD18 (100%) and Lsel (50%) up to 300 mPa (no rolling), and to fibrin/P* (activated platelets) up to 700 mPa (less rolling). Fibrin contributes to firm arrest. PMN bind to aligned fibrin formed under flow
better than random fibrin. Strings of neutrophils formed under flow to fibrin may control deposition of PMN onto Lsel/PSGL-1 rich neutrophil membrane.

III. Ideas for future meetings included: (1) a multi-subcommittee working group to evaluate the in vivo relevance of biorheological studies with flow devices, which could include bedside devices and management of patients receiving anti-platelet and anti-hemostatic drugs; (2) effects of flow on vWF and other proteins, as well on endothelial cell (EC) shear-sensitive receptors; and (3) effects of flow on EC exocytosis.
The Subcommittee of Contact Activation has found that the increased knowledge of the mechanism of contact activation in vitro and its putative functions has suggested a need for standardized methods to measure concentrations of the activated factors and their zymogens. In this context the Subcommittee contacted the SSC Working Group on Coagulation Standards at the ISTH meeting in Washington and it was agreed that the Subcommittee would consider labeling the SSC standard for factor XII, HK, prekallikrein and factor XI. During the past six months, the Subcommittee has made arrangements with six laboratories to perform the labeling independent of each other. A protocol for measurements and storage conditions was suggested and accepted by the Subcommittee of Contact Activation. In December we asked for the SSC plasma from the SSC Working Group and it was promised for January. The SSC Working group then decided that they could not dispatch the SSC standard as no WHO standards existed for the contact activation proteins. During the meeting in Maastricht this problem will be discussed. We knew that no WHO standard existed for the contact activation proteins and this was why the Subcommittee had also offered to label this standard. We hope that the SSC Working Group on Coagulation Standards proceeds with the labeling and the Subcommittee of Contact Activation looks forward to the outcome.

Following this we will discuss the methods of choice and the information obtained using these methods for certain pathological conditions.

Inger Schousboe, Chairman
Control of Anticoagulation, Parts I & II

Chairman: F.E. Preston--UK
Co-chairmen: T.W. Barrowcliffe--UK; H. Bounnameaux--Switzerland; M. Greaves--UK; C. Kearon--Canada; L. Poller--UK; F. Rosendaal--The Netherlands; S. Schulman--Sweden; A. Tripodi--Italy; A.M.H.P. van den Besselaar

Current Tasks

The Chairman welcomed the participants and outlined the programme. As on the previous occasion, the Subcommittee had been allocated two complete sessions.

Working Party on the Duration of Anticoagulant Therapy in Venous Thrombosis
(Co-Chairs: Professor J Hirsh and Dr C Kearon)

The membership of the Working Party comprises Professor J Hirsh; Dr S Schulman, Dr C Kearon, Dr G Agnelli and Professor F R Rosendaal.

Presentations on current evidence and future clinical trials were delivered by Dr. S Schulman, Dr G Raskob, Dr. C Kearon and Prof. G Agnelli in relation to optimal duration of anticoagulant therapy post-hospital discharge. It is conventional to continue anticoagulant treatment of most patients with idiopathic venous thrombosis for six months or longer. Patients with recurrent venous thrombosis or those with an inherited or acquired thrombophilia were treated for longer. Two published studies, one by Schulman and the other by Kearon have reported rates of thrombosis of 9% and 27% after six months and three months respectively, are discontinued. Despite these data, there is a difference of opinion on the optimal duration of anticoagulant therapy in patients with idiopathic venous thrombosis. It might be possible to develop guidelines based on the following assumptions:-

1. Recurrences occur at a rate of 9% to 27% in the first year that anticoagulants are discontinued (the ranges reported in the studies by Schulman and Kearon).

2. About half of the recurrences are idiopathic and therefore not preventable by prophylaxis during high-risk periods.

3. The incidence of major bleeding is 3% to 4% during a year of anticoagulant therapy but is likely to be higher in elderly patients with multiple risk factors and lower in your low-risk patients.


5. Based on these assumptions, the risk of unexpected fatal PE in the first year after discontinuing anticoagulants is 0.25 to 0.7, whereas the risk of a fatal bleed during one year of anticoagulation is 0.2% to 0.8%. If these assumptions are correct, patients at very
high risk of bleeding should receive anticoagulation for a limited period of time, whereas patients with a low risk of bleeding and an estimated risk of recurrent VTE of at least 5% would benefit from indefinite anticoagulation.

Factors that indicate a shorter course of anticoagulants include high risk of bleeding, unstable anticoagulant response, inconvenience of anticoagulation, and patientís fear of bleeding. Factors that would indicate a longer course of treatment include presentation with massive PE, poor cardiopulmonary reserve, severe post-thrombotic syndrome, and inherited or acquired thrombophilia.

Since clinical studies relating to the duration of oral anticoagulant therapy are currently on-going and since further trials are under consideration it was concluded that it would be premature to produce guidelines at this stage. The topic will be kept under review.


The Chairman announced the membership of the Working Group as (Chair, Professor F E Preston); Dr J Ansell, Dr AMHP van den Besselaar, Dr S Kitchen, Professor G Mueller-Berghaus, Professor G Palareti and Dr G Michaud (FDA).

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.

Professor J Michael Hasenkam presented a report of his experience of self-management of oral anticoagulant therapy in Denmark.

Dr Ginette Michaud of the U.S. Food and Drug Administration presented two topics.

1. The quality control of point-of-care coagulation devices and
2. Novel designs with traditional names. When should the name of an assay be changed?

Dr S Kitchen presented a report on "Is Quality Control Necessary?"

Near patient test (NPT) instruments are increasingly used for determination of International Normalised Ratios (INR). Some are calibrated for routine (non-anticoagulated blood), others for citrated whole blood or plasma. Some units, such as CoaguChek (CUC), CUC-S and the Thrombolytic Assessment System (TAS) or Rapid Point Coag can analyse anticoagulated plasma. It is not known whether external quality assessment (EQA) is possible using lyophilised anticoagulated test plasmas and there is not yet a consensus whether or not it is necessary.

Dr Kitchen then compared the relationship between NPT and conventional INRís with patient samples and the relationship between NPT and conventional INRís with NEQAS samples.
He concluded that the data suggests that EQA of NPT is necessary for continuous monitoring of the relationships between NPT and conventional INRIs and because the range of results in different centres using NPT systems is similar to that observed in centres using conventional INR methods, and because discrepancies between conventional and NPT systems can be highlighted by EQA.

Dr AMHP van den Besselaar presented a report on "Instrument Calibration ó Who is responsible and how should it be achieved?"

Dr Michelle Keown presented details of the European Concerted Action on Anticoagulation (ECAA) home PT monitors quality control study. Project No. SMT4-CT98-2269

Quality control of home PT monitors is essential to (i) ensure conformity to the WHO standard, (ii) provide checks of inter-instrument variation, (iii) check inter-batch variation of test strips/cards and (iv) check performance.

This study was performed to establish the extent of inter-instrument variation at the three Centres in Leiden, Milan and ECAA Central Facility in Manchester on 2 types of home PT monitors with 3 test systems. The same sets of 20 ECAA certified depleted plasmas were tested in duplicate on the CoaguChek Low ISI test strip at all 3 Centres and at Leiden and Manchester with the CoaguChek Mini strip and the TAS PT-NC.

The PT of the 20 plasmas had been certified at the Central Facility using ECAA rabbit thromboplastin with the manual technique by at least 4 operators. Mean values were calculated.

Code chips or a correction formula were used with the home monitors to give "real" seconds.

Mean prothrombin times were calculated and prothrombin ratios (PR) were derived. Derived ISIs were compared with the ISI from a fresh plasma calibration.

It was possible to conclude that ECAA lyophilised depleted plasmas appeared to work successfully on the Home PT monitors giving good comparability between results at the 3 Centres.

**Working Group on Calibrated Plasmas for INR Determination**

The Chairman of this Group, Dr Trevor Barrowcliffe presented an update on the guidelines for the preparation, calibration and use of calibrated plasmas for oral anticoagulant control. The Working Group are approaching consensus.

Dr Nils Egberg and Dr. Lindahl jointly presented data from Sweden in respect of the calibration of PT performed with combined thromboplastin reagents.

**Working Party on Standardization of Methods to Determine Direct Thrombin Inhibitors**
The Chairman, Professor J Harenberg reported that a Working Party has been established under his Chairmanship to address issues relating to the monitoring of direct thrombin inhibitors. A presentation by Dr Elaine Gray of NIBSC covered a collaborative study for monitoring thrombin inhibitors.

This Working Party had met twice during the ISTH Congress in Washington and agreed the following procedure:

R-Hirudin and argatroban will be used as antithrombin agents to determine the inhibitory effect on the following reagents: APTT, local and central (actin FS, Dade Behring, AG), ecarin clotting time wet chemistry (Professor Nowak Erfurt, with ecarin reagent precoated tubes), dry chemistry, ecarin clotting time on TAS-analyser and wet chemistry ecarin clotting time (Dr Mize, Raleigh), S 2336 chromogenic substrate assay (W.Rabe, Chromogenix, Essen), hirudin ELISA (Dr Hart, American Diagnostics, only for hirudin samples). The reagents will be supplied from the producers.

The following participants will analyse blinded spiked samples: Professor J Fareed (together with Dr J Walenga, Dr S Lewis and Dr Jeske), Professor Harenberg (together with Dr Huhle), Professor Agnelli, Dr Spannagel (together with Dr Calatzis), Dr Eriksson, Dr Lindhoff-Last, Dr Gray, Dr Schinzel, Professor Nowak, Dr Becker, Professor Weitz, Dr Carlsson, Dr Russi, Professor Gulba will be our independent scientific advisor.

Professor Fareed supplied r-hirudin and argatroban. Freeze-dried thrombin inhibitor spiked plasma samples have been prepared at NIBSC. Reagents and blinded samples will be sent to the participants from NIBSC, probably at the end of June. Participants will be asked to analyse the samples soon after receiving the samples and send the original data (optical densities, seconds) to Dr E Gray and to Professor Harenberg. Statistical analysis will be performed at NIBSC. A report of the results for this International collaborative study should be available by November 2000 and will be presented at the next ISTH Congress.

Dr S Schulman presented a report on the antineoplastic effects of heparin, low molecular weight heparin and warfarin and proposed the setting up of a registry of new data.

Reports on WHO Working Group Activities on Unfractionated Heparin and Low Molecular Weight Heparin

Dr Anna Padilla, WHO, presented this progress report on the biological standardisation of unfractionated heparin.

The WHO Working Group on Biological Standardization of Unfractionated Heparin met in September 1999 and agreed to examine the feasibility of replacing current pharmacopoeial assays for unfractionated heparin with more up-to-date methods and to adopt a strategy that would lead to an agreed global assay, based on a single heparin unit, thus promoting global harmonisation. The Working Group includes members from the EP, USP, FDA, NIBSC and heparin manufacturers. A sub-group has been set up to draft out a protocol for anti-IIa chromogenic assay for determination of unfractionated heparin. A collaborative study with the
aims of testing the robustness of the proposed method and establishing a new USP reference standard will be initiated in the latter half of this year.

Dr Elaine Gray, NIBSC, presented the following proposal for the replacement of the 1st International Standard for Low Molecular Weight Heparin.

A pilot study, including 8 current clinical low molecular weight heparin (LMWH) preparations, the unfractionated heparin International Standard and the current LMWH International Standard, will be carried out to study the comparability of the LMWHis and to select suitable candidates for the main large scale calibration of the replacement International Standard for Low Molecular Weight Heparin.

Dr Coyne presented data on behalf of Dr Walenga on pentasaccharide monitoring.

Pentasaccharide is a synthetic heparin analogue with sole anti-factor Xa activity devoid of any direct antithrombin effects, yet it is capable of inhibiting thrombin generation. Clinically, this agent is used for prophylactic and therapeutic treatment of venous thrombosis. Pentasaccharide is also used in conjunction with thrombolytic agents and anti-platelet agents and is potentially useful in combination with antithrombin agents. At currently used clinical dosages, pentasaccharide does not release endogenous mediators such as TFPI; however, it is capable of binding to vascular sites when ATIII and TFPI are present. Endogenous ATIII levels have been shown to be rate limiting for the pharmacodynamic effects of pentasaccharide.

This presentation provided a review of the mechanisms of the antithrombotic effects of pentasaccharide and the relevance of these mechanisms to the development of methods to monitor the pharmacokinetics and pharmacodynamics of pentasaccharide. It must be stressed that the anti-factor Xa methods currently used may not truly reflect the overall endogenous effects of pentasaccharide, nor the total circulating drug levels. Thus a clear differentiation between the pharmacokinetics and pharmacodynamics of this agent must be considered.

**Working Party on Laboratory Monitoring of Low Molecular Weight Heparin**
Chairman: Professor M Greaves, UK.
The membership comprises: Dr P Massicotte, Dr M Andrews, Professor J Fareed, Professor M Samama, Professor F Ofusu and Professor H C Hemker.

**Pediatric Monitoring**

Dr P Massicotte presented proposed guidelines on behalf of herself and Dr M Andrews, together with a report on anti-Xa monitoring in children.

Critical issues for the use of LMWH in children include the age, or weight, dependency of the pharmacokinetics of LMWHis 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, guidelines will be forwarded to the Chairman of the Subcommittee for distribution to Subcommittee members.

Adult Monitoring

A report was presented by Dr. Bacher on behalf of Professor J. Fareed on new data on the monitoring of intravenously administered low molecular weight heparins.

Several low molecular weight heparins are currently evaluated as anticoagulants in various therapeutic, interventional and surgical indications. Unlike the unfractionated heparin, these drugs are relatively weaker anticoagulants as measured by the global anticoagulant (PT, APTT and thrombin time) and whole blood clotting time (ACT) tests. In addition, when administered intravenously at equivalent anti-Xa dosages the agents produce markedly different product dependent anticoagulant responses. Thus, each of the individual LMWHs produces characteristically distinct anticoagulant responses. This situation is further complicated due to the simultaneous administration of antiplatelet drugs such as the GP IIb/IIIa inhibitors and thrombolytic agents. Several other drugs also show different degrees of interaction with low molecular weight heparins. Despite the lower anticoagulant response, low molecular weight heparins have been successfully used as anticoagulants for therapeutic, interventional and surgical indications. Currently, enoxaparin and dalteparin are developed as anticoagulants in percutaneous interventions, despite relatively weaker anticoagulant effects (ACT<200 secs). Enoxaparin at 1.0mg/kg dosage (IV) is found to produce comparable antithrombotic response as the standard dosage of heparin for this indication. Reduced dosage of enoxaparin (including 0.75 mg/kg) have been used with antiplatelet drugs and have shown acceptable anticoagulant responses. Dalteparin has been reported to produce dose dependent anticoagulant responses at 40 IU/kg (0.30 mg/kg) and 60 IU (0.45 mg/kg). These studies have provided useful data on the threshold dosages.

Additional studies on Enoxaparin at 0.70 mg/kg (70U/kg) and 60 U/kg of Dalteparin (0.45 mg/kg) with standard antiplatelet therapy are in progress at this time. Major differences in the pharmacokinetic and pharmacodynamic behaviour of these drugs are observed in terms of their effects on the ACT, anti-Xa and anti-IIa actions. Besides the low molecular weight heparins, the chemically synthesized Pentasaccharide is also used in various trials as an adjunctive antithrombotic agent. Because of the polytherapeutic approaches the monitoring of the end stage products such as F1.2 and TAT may be useful clinically. While the monitoring of the anti-Xa action of these drugs may not be relevant, it does provide important information on the dosing and pharmacokinetic/pharmacodynamic behaviour of these drugs and its relevance to the overall antithrombotic effects. This presentation will provide data on the circulating levels of these drugs and their relevance to the clinical outcome in several clinical trials.

Professor M Samama presented a report on the case for monitoring low molecular weight heparin in special situations and discussed the difficulties related to the anti-Xa assay in this respect.

Professor H C Hemker presented a report on the case for novel methods for monitoring low molecular weight heparin.
Professor M Greaves, (Subcommittee Chairman) presented this report on heparin monitoring and summarized the proposals from the Working Party. Heparin is the most widely used anticoagulant for the prevention and initial treatment of venous thromboembolism for haemodialysis and cardiopulmonary bypass procedures and, with aspirin, for the management of acute coronary syndromes. In venous thrombosis and coronary disease, low molecular weight heparins (LMWHs) have been demonstrated to be at least as effective and safe as unfractionated heparin (UFH) and the use of LMWH is rapidly increasing.

Most of the available LMWHs are depolymerised porcine mucosal heparin preparations prepared by chemical or enzymatic digestion. This process results in a variety of lower molecular weight products (or molecular mass between 4 and 8 kDa) and enhances the anti-Xa activity in relation to anti-IIa activity. However, this relationship varies between LMWH preparations, as do other potentially important properties including interaction with platelet factor 4 and heparin cofactor II. Furthermore, the mechanism of the antithrombotic action of LMWH is not fully understood and probably does not only depend upon anti-Xa and anti-IIa activities. For example, UFH and LMWH release Tissue Factor Pathway Inhibitor (TFPI) from vascular sites and this could explain the prolonged antithrombotic effect of subcutaneously administered LMWH, which appears to persist when anti-Xa activity is no longer detectable.

The activated partial thromboplastin time is used to monitor therapeutic doses of UFH in venous thromboembolism. A target ratio versus control of 1.5 to 2.5 is typically employed. This is principally based on evidence that delay in the achievement of adequate anticoagulation is associated with an increased rate of thrombosis recurrence or progression. It is, however, clear that the sensitivity of the test to heparin is highly reagent dependent and ideally local calibration of the APTT should be employed. The inconvenience and limited precision of monitoring of UFH therapy have contributed to the increasing use of LMWH preparations, as several randomised studies have demonstrated their efficacy and safety when administered in fixed dosage and without laboratory monitoring. Despite this positive development, there is still debate over the need to monitor treatment with LMWH. This arises in part because most clinical trials have excluded subjects at increased risk of bleeding, as well as children, pregnant women, the very obese and others in whom the antithrombotic and prohaemorrhagic responses may be less predictable, such as patients with severe renal failure. Where monitoring is performed the anti-Xa assay is generally employed, but there are crucial considerations in relation to the interpretation of results:

- Anti-Xa (and anti-IIa) activity represents the amount of heparin present but not necessarily the antithrombotic function of LMWH, because the same concentration of heparin may have varying effects in different plasmas and patients (above).
- LMWH has been standardised ultimately against the 4th International Heparin Standard. This may have resulted in an overestimation of the anti-Xa and underestimation of the anti-IIa activity.
- Relative anti-Xa and anti-IIa activities vary between preparations and the antithrombin activity appears to be the more important action in kinetic studies.
- The comparability between commercially available anti-Xa chromogenic assays is poor. Assays should preferably be LMWH, method and equipment specific.
- Anti-Xa level is a poor predictor of bleeding risk and antithrombotic efficacy in thromboprophylaxis with LMWH.
- Anti-Xa level is a poor predictor of bleeding during treatment with LMWH. The clinical status (WHO stage) of the patient and dose administered are more informative.

Consideration of these factors, and of the good results obtained in clinical trials where LMWH has been administered without monitoring as venous thromboprophylaxis and treatment, leads to the conclusion that routine monitoring by anti-Xa assay is not currently indicated. Furthermore, whilst anti-Xa assays may provide some clue to LMWH pharmacokinetics in individual subjects, such as pregnant women and infants, only limited information on antithrombotic effect and bleeding risk can be deduced from this measurement. Although more global tests of antithrombotic potential, such as the Heptest and measurement of the endogenous thrombin potential (area under the thrombin generation curve) are interesting, they are not yet fully evaluated in the setting of studies of efficacy and safety of anticoagulation with heparins.

Professor A Tripodi and Dr AMHP van den Besselaar presented a comparison of 2 citrate concentrations in 2 evacuated blood collection systems for PT and ISI determinations.

The prothrombin time is usually measured in citrated plasma. The WHO recommended concentration of sodium citrate for blood collection for laboratory control of oral anticoagulant therapy is 0.109M. Some evacuated blood collection systems include 0.105M sodium citrate. The purpose of the present study was to establish the difference in ISI calibration between 0.109M and 0.105M citrate, using 7 types of thromboplastin and various types of instrumentation. The two citrate concentrations were provided in both evacuated siliconised glass tubes and in evacuated polyethylene terephthalate (PET) tubes. The ISI difference between the two citrate concentrations was 5.4% for one system but not greater than 3% for all other systems when blood samples were collected with either siliconised glass or PET tubes. Most of the ISI differences between the two citrate concentrations were not significant at the 5% level. It is concluded that the ISI differences between 0.105M and 0.109M citrate are not of practical importance. In contrast, ISI differences between siliconised glass and PET tubes, using either 0.105M or 0.109M citrate, were significant (p<0.05) for most thromboplastin systems and amounted to 7%. ISI interchange between these glass and PET tubes could induce INR differences amounting to 14%, which could affect clinical dosage of oral anticoagulants.

Drs. van den Besselaar and Tripodi proposed that, with Professor Preston, they should submit on behalf of the Subcommittee a letter to Thrombosis and Haemostasis recommending that blood samples for calibration and use of the INR for monitoring oral anticoagulant therapy should be collected using citrate concentrations in the range 0.105M to 0.109 M.

Professor Leon Poller, ECAA, presented a report on the ECAA assessment of benefit of computer-assisted anticoagulant dosage study.

The study is a sequel to the first ECAA randomized multicentre study on computer dosage.
There is, at present, a vast demand for increased provision of oral anticoagulant treatment (OAT) in EU countries but urgent steps are required to improve success of clinical dosage to make OAT safer and measures are needed to provide for the massively increased medical workload.

A wide-ranging study is to assess the value of computerised OAT dosage in reducing thrombotic and haemorrhagic complications by improving on the indifferent success of dose prediction by doctors. Patients will be randomized to dosage by computer or medical staff. Two EU-devised computer-dose programmes will be compared with medical staff dosage and the conformity to WHO laboratory standards checked by previous EC-funded developments in control procedures.

Bleeding and thrombotic complications are associated with time "out of control" and labile control, both shown to be reduced by computer dosage so clinical benefit may be even greater than dosage improvement. Cost and medical manpower saving will be assessed.

In our previous study, there was difficulty initially with reduced success in achieving target INR as a result of interference by medical staff with computer derived dosage. This was due to lack of confidence in the computer programme by Centres using the procedure for the first time. The resulting improvement in dosage was limited until the medical staff gained confidence. In the new project an initial induction phase study will be conducted at all Centres to achieve familiarization with the relevant computer programme.

Dr. Bacher presented a report on behalf of Professor J. Fareed on "The Diagnosis of Heparin-Induced Thrombocytopenia - Do We Need Guidelines?"

Clinical guidelines are needed for this devastating syndrome affecting up to 5% of all patients receiving heparin. Do we have enough solid scientific data to formulate guidelines that would improve beneficial patient care? We have some reliable data but we lack high quality studies in areas of diagnosis as well as in the management of this syndrome. Many consultant physicians are insecure in their recommendations which are based primarily on which review article they may have read most recently.

An approach to this problem would include convening a broad panel of experts in various clinical, laboratory and pharmacological aspects of HIT. Sub-specialty panels would be desirable to focus on diagnostic testing in the laboratory, clinical evaluations and therapeutics options. Basic scientists, pathologists, pharmacologists and clinicians of diverse opinions should be able to arrive at some common ground to provide guidance even in areas where data is of low quality. Though it may appear to be a daunting task, the authors feel it can be accomplished.

At the present time, the laboratory diagnosis of heparin-induced thrombocytopenia includes the following tests:

1. Platelet count
2. Quantitation of anti-heparin platelet factor 4 antibodies (anti-HPF4 abs).
3. Platelet aggregation/agglutination studies
4. $^{14}$C Serotonin release
Not all of these tests can be skillfully performed by routine laboratories. Only the platelet count can be readily evaluated and constitutes the first line of diagnosis for this syndrome.

Dr Bacher presented Dr. Fareedís conclusion that antithrombin drugs such as hirudin and argatroban are recently approved for the alternate anticoagulant management of HIT and while some guidelines are available for the optimal use of the agent in HIT, additional clinical data are needed to clarify several unresolved issues. The presentation provided an objective review of the diagnostic and treatment issues in the management of heparin induced thrombocytopenia.

The possibility of producing guidelines related to the diagnosis of HIT was discussed. The possibility of collaboration with the Platelet Immunology Subcommittee in this activity was raised following comments from Dr Chong (co-chair of Platelet Immunology).

Part II of the meeting closed at noon on Friday 16th June 2000.
DIC

Chairman: M. Levi--the Netherlands
Co-chairmen: M. Kazama--Japan; I. Bokarew--Russia; W.K. Hoots--USA;
N. Sakuragawa--Japan; F.B. Taylor--USA; C.-H. Toh--UK

The meeting of the SSC DIC Subcommittee consisted of two parts: 1) a discussion on the definition and diagnostic criteria for DIC and 2) a mini-symposium on the role of the fibrinolytic system in DIC.

M. Levi started the first part of the session by summarizing the activities of the Subcommittee between the 1999 Washington, DC, meeting and the present meeting. A working group, consisting of 10 active subcommittee members, convened in the fall of 1999 in Liverpool to discuss a consensual definition of DIC and a practical (diagnostic) scoring system for the syndrome. At a second meeting in January in Amsterdam, a small writing committee summarized the results of the discussion in a draft report. This was sent to over 50 experts in hemostasis/thrombosis or critical care medicine. As of now, more than 30 experts have sent extensive reviews of the draft proposal.

Dr. Hoots summarized the objectives of the Subcommittee's proposal and outlined the scoring system for overt DIC. The scoring system consists of a simple algorithm, using widely available coagulation tests.

Dr. Toh emphasized the need for a system to grade sub-clinical DIC (non-overt DIC) and discussed the potential tests that could contribute to that diagnosis. The Subcommittee proposes a template for a scoring system in non-overt DIC that can be further developed depending on the specific needs of the user.

In the general discussion following these presentations the major issues that were raised by the reviewers as well as the audience were discussed. These major issues included the need for inflammatory activation as an essential part of DIC, how to integrate the existing scoring systems for organ failure in the proposed DIC score, how to refine the scoring system for overt DIC using the proposed parameters, the use of the scoring system in patients with advanced liver disease and standardization of (normal) values. Overall, the Subcommittee's proposal was received with great enthusiasm by the audience.

Dr. Levi proposed to revise the draft proposal according to all the comments that were raised by the reviewers and in the discussion. At the Paris 2001 meeting of the Subcommittee a final proposal will be discussed and hopefully approved, after which it can be submitted to the SSC.

The second part of the meeting consisted of a mini-symposium on the role of the fibrinolytic system in DIC.
Drs. Hack and Taylor (USA) discussed the dynamics of fibrinolytic parameters in a model of sepsis in baboons, followed by a presentation of Dr. Biemond (the Netherlands) on the pro- and anti-fibrinolytic effect of endotoxemia in humans and chimpanzees. Dr. Montes (Spain) summarized a series of studies on the role of PAI-1 in models of endotoxemia, in which various anticoagulant and anti-inflammatory interventions could be studied. Dr. Rijken (Belgium) presented the results of measurements of thrombin-cleaved urokinase in patients with DIC and Dr. Hazelzet showed the role of PAI-1 polymorphisms on the clinical outcome in patients with DIC. Finally, Dr. Wada (Japan) presented the result of fibrinolytic studies in large series of patients with DIC from a Japanese collaborative group of investigators.

The attendance was about 100 people. There was adequate discussion opportunity in both parts of the meeting.
Factor VIII and Factor IX, Parts I & II

Chairman: J. Ingerslev--Denmark
Co-chairmen: D. DiMichele--USA; K. Mertens--The Netherlands; C.G. Negrier--France;
J. Oldenburg--Germany; C. Prowse--UK; A. Yoshioka--Japan

The Chairman welcomed the audience and outlined the agenda of the full-day meeting. Apologies had been received from Co-chair Dr. Christopher Prowse.

Since the previous meeting in Washington, this Subcommittee has produced a number of documents.

Reports submitted for publication

D.M. DiMichele, B. Kroner and Members of the Factor VIII/IX Subcommittee.

Recommendation submitted for publication
The Design and Analysis of Pharmacokinetic Studies of Coagulation Factors.

Finished paper

Ongoing work with registries and clinical studies in progress
Dr Guglielmo Mariani gave an overview on ongoing inclusion of patients into the International Registry of Congenital FVII deficiency (IRF7), reporting that the registry had been successful recently with submission of patients, now amounting to over 200. Forms for submission can be found on the ISTH web-site.

Dr. Donna DiMichele reported from the American French Multicenter Comparison of Inhibitor Development on Plasma-derived versus Recombinant Factor VIII in Severe Hemophilia A Patients. This registry allows for further inclusions of patients, and an extension has been set forth including additional countries.

In the absence of Dr. C. Hay, Dr. D. DiMichele presented the development of the Randomised Immune Tolerance Study Protocol. All of the logistics and funding issues have been clarified, and this randomised, controlled study will begin inclusion of patients within the next few months.
On behalf of Dr. I. Warrier and herself, Dr. J. Lusher presented an update on the Registry on Factor IX Inhibitors & Anaphylaxis in Children with Haemophilia B. In total, 39 cases have been included. Of these, 11 cases have suffered a nephrotic syndrome under attempts to induce tolerance, and 9 of these 11 patients had demonstrated anaphylaxis.

During the following discussion, Dr. P. Mannucci wished to underscore the importance of progress in understanding the underlying mechanisms of this serious clinical problem, reminding the audience of the ISTH approved study protocol on complement activation, for which samples should be collected before and after infusion of factor IX concentrate for subsequent assessment of complement factors in Milan. There was agreement to facilitate cooperation.

Dr. J. Lusher presented a proposal for a Registry on Gene Therapy Studies in Haemophilia. It was agreed that such a registry would be very useful, and Dr. Lusher was asked to proceed with this work together with a smaller committee.

**Standardization**

Dr. T. Barrowcliffe reported on the (October 1999) WHO Report. Ongoing work attempts to clarify the standardization issues around activated factor IX, assumingly using a molar basis.

Work continues on the 3rd International Standard for FII and X Concentrate to replace the 2nd WHO Std. 98/590. Development of a standard for von Willebrand Factor Concentrate is ongoing, and data is expected to be reported at the year 2001 ISTH Congress.

Other work will attempt to raise the level of factor VIII:C in plasma standards for Factor VII. Further studies have shown that the 6th IS on Factor VIII Concentrate works well with plasma-derived concentrates of varying purity.

On behalf of Dr. E. Gray and himself, Dr. T. Hubbard gave a brief account of the scheduled activities around the proposed 3rd Int. Standard on factor II, VII, IX and X, that was ampouled in April of this year. Assays were to be performed between July and September of this year.

On behalf of Dr. E. Preston and himself, Dr. S. Kitchen reported on the calibration of the SSC Secondary Plasma Standard Lot #f 2 for F VIII:C, F IX:C and vWF. The previous material that had been used by 24 industrial companies expires by May 2001.

Dr. M. Lee, on behalf of a larger group of investigators, spoke about his study on Calibration of New Monoclonal-Purified and Recombinant Factor VIII Standards. Standards utilized in the exercise were the MEGA-1, the WHO # 6th Standard for F VIII Concentrate, and two in-house calibration standards, recombinant and monoclonal purified. Authors found a 6-8% lower clotting activity of MEGA-1 as compared to the WHO 6th, whereas the chromogenic assays gave similar results.

Dr. M. Weinstein gave an update on the work on the MEGA-II Standard. Vials (100,000) of the standard factor VIII concentrate material have been produced, and the material gave comparable
results with one-stage and chromogenic assays. Extensive testing was planned for FDA approval, for Ph.Eur. adoption and material would also be available to the SSC.

Dr. B. Verbruggen presented data from continued work on the Nijmegen modification of the Bethesda assay for determination of inhibitors carried out by himself and Dr. A. Giles, focusing on the possible adoption of less costly reagents, in particular the nature and composition of the control sample and the substrate plasma material. Chemically factor VIII depleted plasma displayed a high content of activated factor V, and immune depleted plasma gave lower titers than natural deficiency plasma. Based on the experiments made, authors recommended that chemically depleted or congenital factor VIII deficiency plasma were advisable for inclusion in the test sample, whereas a 4% albumin solution could be adopted for control samples.

On behalf of Drs A. Hubbard, J. Lusher and A. Padilla, Dr. S. Kitchen presented the audience with the suggestion to produce a proposal for (WHO) guidelines for assays utilized in measurement of factor VIII:C and factor IX:C. This initiative was met with approval and support by the Subcommittee.

**Factor VIII assay discrepancies**

Dr. S. Raut on behalf of Dr. T. Barrowcliffe and himself, presented data on a study on rFVIII full-length and a BDD factor VIII under true field conditions. Two candidate factor VIII concentrates had been circulated to several laboratories, which had all used their routine methods for determination of factor VIII:C by one-stage and chromogenic assay. A strikingly high variance was found, some of which was probably ascribable to deviations from the ISTH recommendations. Further data analysis and publication was forecasted.

Dr. M. Mikaelsson presented data on her studies on assay discrepancies in pharmacokinetic studies of recombinant factor VIII concentrates, by outlining the well-known difference detected when the factor VIII:C of some recombinant factor VIII concentrates was determined by a one-stage assay compared to the chromogenic assay. Recent experiments elucidated that if the APTT reagent, Dapttin, was utilised in the one-stage factor VIII:C assay, the discrepancy seemed to abolish. Further studies are expected.

On behalf of a group of laboratories in the Nordic Countries, J. Ingerslev presented data from a field laboratory study on the use of the ReFacto concentrate standard. In an attempt to correctly determine factor VIII:C utilising the one-stage technique in postinfusion samples after administration of a recombinant B-domain deleted factor VIII concentrate, a field study in nine laboratories has been carried out. In this, the working standard has been a ReFacto concentrate standard in order to measure by the "like-versus-like" principle. The main finding was that this practice reduced the assay discrepancy phenomenon to a level of around 10%.

An extended field study on the utility of a BDD concentrate standard was proposed, and interested laboratories can contact the chair for participation.

On behalf of a larger study group, Dr. K. Mertens presented an extensive pharmacokinetic evaluation of plasma-derived factor VIII employing multiple factor VIII assays and standards. In
a bioequivalence study in which all determinants were highly controlled, the authors failed to
detect any assay discrepancy when recording pharmacokinetic patient samples by one-stage and
chromogenic assays. On the contrary, authors could demonstrate that the peak value of factor
VIII in plasma was dependent on the accuracy of the factor VIII concentrate unitage (label).

**Molecular genetics and biology of F VIII:C**

Drs J. Oldenburg and R. Schwaab presented their mutation al findings in a total of 413 German
haemophilia patients. Using an algorhythm involving southern blots, DGGE, chemical mismatch
and denaturing HPLC, and sequencing all patients, except for 10 cases, could be assigned a
molecular genetic diagnosis. Hot-spot areas in exons 8, 11, 14, and 23 could be pointed out.
Further, authors reported some distinctly interesting findings from this group of patients and
patients from other countries investigated in their laboratory. The aim of the study is to seek out
a presumed causative diagnosis in all severe cases of haemophilia A in Germany within a three
year time-frame.

Dr. D. Scandella presented data from her recent studies on functional areas in the factor VIII
molecule detected through inhibitor epitope mapping. This series of investigations employing
haemophilic inhibitory antibodies and synthetic peptides adopted in competitive assays for
functional areas, had disclosed a number of previously unrecognised binding regions of the
factor VIII molecule.

Dr. G. Kemball-Cook presented cumulative data on the molecular pathology in mild haemophilia
by summarising the functional effects of recently detected mutations in mild haemophilia. In
quite many patients, a lowered factor VIII:C has been found by the chromogenic method as
compared to the one-stage technique. Adopting molecular modelling it could be shown that these
mutations are predominantly located in the intersections between the A2 domain and
neighbouring A1 and A3 domains, making the A2 domain less likely to maintain its association
with the two other A domains. If so, a lower functional F VIII:C by the chromogenic method
could result from premature disruption of the A2 domain during the prolonged incubation with
thrombin in the chromogenic assay.

**Reports from Working Groups**

Dr. T. Barrowcliffe reported on the ongoing work aimed at establishing standards for inhibitor
assays. Three candidates are being considered, a polyclonal rabbit antibody and two human
hybridoma inhibitor antibodies. Based on early data from two laboratories only, a need for
standardisation in this area was highlighted.

On behalf of the Terminology and Definitions Group, Dr. C. Rotschild gave an introduction to a
series of questions that she intended to circulate amongst members of the Factor VIII/IX
Subcommittee as a postal interview. The aim of this investigation is to attempt to give a common
definition on the term of *transient inhibitors*. The approach selected was approved by the
audience.
On behalf of Dr. G. White II and herself, Dr. M. van den Berg presented a draft proposal for a clinical study on critical haemostatic levels of BDD recombinant factor VIII by one-stage and chromogenic assays. The aim of the double-blind cross-over study is to evaluate the efficacy of quite low doses of BDD recombinant factor VIII in knee-joint bleeds in adult patients, and various clinical end-points were proposed. The study proposal was well received. Attendees presented a number of helpful comments and general advice.

Other business

Dr. C. Ludlam introduced the audience to documents for possible collaborative review that had been detected in the Cochrane Library. Members wishing to participate or communicate with Dr. Ludlam on Cochrane review issues were invited to do so.
Unfortunately, factor XIII Subcommittee attracted only 20 participants this year, probably because a limited number of its members attended the associated meeting, the 1st North Sea Conference on Thrombosis and Hemostasis. Prof. Muszbek was the only co-chair who attended and presented. Six speakers discussed important issues in the factor XIII field as follows:

1) Control Mechanisms of the Gene Expression of the A Subunit for Human Factor XIII. A. Ichinose, M. Kida, and M. Souri (Japan)

To study the mechanism of gene regulation for coagulation factor XIII A subunit (XIIIA) Prof. Ichinose's group characterized its 5'-flanking region. Deletion analysis, DNase footprinting, electrophoretic mobility shift, and reporter gene assays demonstrated that promoter elements for a myeloid-enriched transcription factor (MZFR-1-like protein) and two ubiquitous transcription factors (NF-1 and SP-1) were important for the basal XIII expression. DNA sequences for binding of myeloid-enriched factors (GATA-1 and Ets-1) were recognized in an upstream region, and the GATA-1 element was found to be responsible for the enhancer activity. These transcription factors play a major role in the cell type-specific expression of XIIIA, which clearly differs from other transglutaminases.

Prof. Ichinose also reported that G/A polymorphism at -246 bp in the 5'-flanking region of the XIIIA gene is not responsible for variable plasma XIIIA levels among individuals. Since it is inconsistent with the result obtained by a British group of Dr. Anwar, this discrepancy must be discussed in the future session. Finally, he showed the Val34-Leu polymorphism was absent in both Japanese healthy individuals and cases with ischemic heart disease.

2) Sensitivity of Factor XIII Screening Tests: Data from UK NEQAS Surveys. I. Jennings, and F.E. Preston (UK)

Dr. Jennings reported different sensitivities of various screening methods for XIII activity from a survey involving 160 centers. XIII deficient plasma, XIII inhibitor plasma, deficient plasma with replacement therapy were examined for clot solubility by Ca + Urea, Ca + Acetic acid, Ca + MCA, Ca/Thr + Urea, Ca/Thr + Acetic acid, or Ca/Thr + MCA. In general, thrombin methods are more sensitive than Ca alone methods, and tend not to miss abnormal XIII levels. Factor XIII levels obtained by these clot solubility tests vary widely between laboratories at both very low levels and normal levels. These results clearly indicate the necessity of standard methods for routine assay for plasma XIII. Accordingly, this Subcommittee decided to seek for recommended tests for screening of abnormal factor XIII levels.

3) The Specific Activity of Variant A Subunits. L. Muszbek (Hungary)
As the site of Val34-Leu polymorphism is close to the Arg 37-Gly 38 activation cleavage site, Professor Muszbek investigated if this polymorphism influences the rate of thrombin-induced XIII activation. The initial rate of release of activation peptide (AP) by thrombin from Leu 34 homozygous XIII was more than double the rate measured with the Val 34 wild type. Heterozygous XIII showed intermediate rate of AP release. Similarly, the transformation of zymogen XIII into an active transglutaminase in the presence of thrombin and Ca ion occurred more with the Leu 34 variant than with Val34. Earlier activation of Leu34 XIII resulted in higher initial rate of fibrin cross-linking. Although the activation of leu34 XIII proceeded at a faster rate, there was no difference in the specific activities of fully activated XIII of different Val34-Leu genotype.

Accordingly, the completed activation of XIII A must be achieved in the functional assay for XIII activity employing enough amounts of thrombin and proper incubation time.

4) Molecular Modeling of the Interaction of Thrombin with XIII-A Variants. I. Komaromi and L. Muszbek (Hungary)

Dr. Komaromi performed molecular modeling to understand how XIII reaches the active conformation and to get semiquantitative estimation on the reaction path of its catalytic reaction. The geometry corresponding to the active conformation has been determined. The active conformation has an open active site arrangement which is now accessible for substrates. Simulations predict four possible substrate orientations on the XIII A surface. Comparing the results of pure quantum chemical and mixed quantum chemical/molecular mechanical calculations suggests the crucial role of electrostatic field at the reaction center in catalytic activity. Dr. Komaromi claimed that on the basis of their results, a model for the activation mechanism, active conformation and enzymatic reaction of XIII A can be constructed at atomic and subatomic level.

5) Effect of Activation Peptide on the Expression and Activity of rXIII A in Eukaryotic Cells. S.K. Woo, I. Kim, and S.I. Chung (Korea)

In Dr. Kimís effort to understand the influence of AP on the expression, the cellular stability of rXIII A, and insight into generation of enzyme activity, deletion mutants: -11A, -37A, -37A/Ala314 were constructed and expressed in S.cerevisiae and CHO cells. The expression of various XIII A gene was analyzed by RT-PCR, western blot analysis, and immunoprecipitation. The full-length gene expressed a thrombin-dependent XIII activity in the cytosol. The ó11A gene expressed a comparable level of proenzyme with a partial activity independent of thrombin but still maintained the fibrin substrate specificity of a native enzyme. The ó37A gene transfected cells failed to show either enzyme activities or protein in spite of presence of comparable mRNA level whereas ó37A/Ala314 gene transfected cells expressed comparable levels of inactive protein. These results suggest that the full-length amino-terminal sequences of XIII A AP are required for a stable expression of XIII A AP are required for a stable expression of XIII A. First 11 residue is required for complete masking of active site and the 12-37 residue of AP was able to prevent cellular clearance of an active form of XIII A.

Dr. Barrowcliffe reported that he developed two XIII concentrates which are considerably stable at both 4 and 20 degrees when measured by a chromogenic assay. Although these concentrates look more promising than the lyophilized plasma presented last year, there is still uncertainty because of the difference in XIII values between two labs currently involved. Accordingly, this Subcommittee decided to call volunteers to help to solve this issue.

7) General Discussion

Three topics were chosen to be discussed in the next session in Paris; proper screening methods for factor XIII with high sensitivity not to miss its deficiency; reassessment of the assay method for XIII activity regarding thrombin concentration; and collaboration to seek for a standard material for XIII.
Dr. R McIntosh was the presiding chairman at this meeting.

**Dysfibrinogens**

On behalf of Dr. Matsuda, Dr. McIntosh presented a summary table of newly identified structural alterations with city names as work-in-progress from the dysfibrinogens working party. Dr. Michel Hanss (France) presented a paper on the database of published fibrinogen variants that he has constructed and made available on the Internet.

With the agreement of Dr. Hanss the meeting recommended that together with the work-in-progress and the database of Dr. McDonagh (referred to in the 1999 meeting) the working party should include Dr. Hanss in their efforts to compile and publish an annotated summary of dysfibrinogens.

**Fibrinogen Plasma Standards**

Dr. Nico Weinstock (FRG) presented the case for a reference material prepared by adding fibrinogen concentrate to normal plasma to give a significantly higher than normal level of fibrinogen. Dr. Ian Mackie (UK) presented the case against a high fibrinogen plasma standard, maintaining that existing standards allow the determination of when levels are higher than normal.

The meeting agreed that there is a need to know when fibrinogen levels were above normal but it may also be desirable to quantify the higher than normal level. Therefore, calibration of a high fibrinogen plasma preparation of the type discussed at this meeting is critical to its use as a reference material. The meeting recommended that Dr. Weinstock should submit a report to the Subcommittee on the material coded S2 in his presentation. The Subcommittee would then consider whether or not the manufacture, assay performance and in particular, calibration of this preparation are suitable for its recommendation as an International Standard. Dr. Weinstock agreed to do this.

**Standardization and Fibrin Sealant**

Dr. Trevor Barrowcliffe (UK) presented results from the recently completed International Collaborative Study on the establishment of a fibrinogen concentrate standard. The meeting agreed that, subject to a satisfactory report on the study, the preparation coded "A" and "C" in the study should go forward to be recommended as the First International Standard for Fibrinogen Concentrate. It was also agreed that when reviewing the study report the Subcommittee should consider whether or not to recommend a reference method for use with this material.
It has been decided previously (1999 minutes) that studies on the standardization of the measurement of other components in fibrin sealant should continue with the Fibrinogen Subcommittee. Following on from this decision, the present meeting agreed that the priorities for these studies should be thrombin, Factor XIII and a functional measurement of fibrin sealant (i.e., the product resulting from the combination of the thrombin with fibrinogen) if it can be agreed that this is required.

Characterization of Fibrin Sealants

Dr. Peter Feldman (UK) presented "options for change" to the Current European Monograph on Fibrin Sealant. Dr. Rainer Seitz (FRG) outlined the aspects of the monograph being considered for a draft revision that he has been asked to prepare by the expert group 6B of the European pharmacopoeia. Dr. Gerhart Dickneite (FRG) described animal experiments on the role of Factor XIII in fibrin sealant and Dr. Ronald McIntosh gave an update on responses to an international survey asking for proposals to change the monograph.

Although it was agreed that the monograph should contain details of how Factor XIII should be measured in fibrin sealants there was no agreement on whether the Factor XIII content should be declared by the manufacturer or if this should be optional. This question turns on whether or not Factor XIII is always an essential component of fibrin sealant.

The Subcommittee agreed to set up a working party to formulate its views on revisions to the monograph to be submitted to group 6B through Dr. Seitz. Dr. Peter Feldman, Dr. Hubert Metzner (FRG), Dr. Anne Walton (UK) and Dr. Per Bengtsson (Sweden) volunteered to participate. The subcommittee permitted the working party to seek other expert views as required.
**Fibrinolysis**

Chairman: N. A. Booth--UK  
Co-chairmen: P.J. Declerck--Belgium; C.-E. Dempfle--Germany; O. Matsuo--Japan

**D-Dimer**

This topic was first discussed by the Subcommittee in 1999, when it was clear that there were important issues on measurements in different assays. Four speakers presented data. Dr M Nesheim discussed his studies on an *in vitro* model of fibrin degradation, which showed the presence of essentially the same products over the time course of degradation, and stressed their large size, consisting of variable numbers of repeating units. Dr Dempfle presented the results of his fibrin assay comparison trial (FACT), in which all manufacturers of kits for the measurement of D-Dimer had measured the same set of 86 samples, consisting of modified plasma samples containing various fibrin derivatives, and plasma samples from patients with DVT or DIC. The variations in response could be normalized by an approach previously used by Dr W Nieuwenhuizen. Concordance of assay results could be improved by using fibrin derivatives comparable to those found in clinical plasma samples for calibration instead of low molecular weight fibrin degradation products. The FACT trial will continue with the aim of developing calibrators usable for all D-Dimer assay systems. He now proposed to build on this work, with more detailed analysis of a limited number of samples. Dr Nesheim offered to provide well-characterized fibrin derivatives for inclusion in this exercise, and it was agreed that this would strengthen the study. The clinical utility of different assays was reviewed by Dr J J Michiels, who discussed the diagnosis of DVT, and the variation in these assays was discussed by Dr P Meijer on behalf of the ECAT group. These presentations underscored the differences between assays and the large inter-assay and intra-assay variations in data, further encouraging Dr Dempfle’s efforts in defining methods. It was agreed that these continuing studies should be presented at the next meeting of the Subcommittee.

**Standards for proteins of the fibrinolytic system**

Dr C Longstaff presented information on three issues about standards. The first arose from the report on the replacement standard for plasminogen activator, tissue type (commonly called tPA), presented and accepted at the meeting in Washington, 1999. The recommendation of the Subcommittee was that the new standard should be adopted, with an agreed potency of 10000 IU per ampoule. The standard was approved by ECBS/WHO in 1999, which decided that it should be called the 1st standard, reflecting the change to recombinant tPA (melanoma tPA was used in the 2nd international standard); they also included Alteplase in the title.

There was concern that this name might cause confusion among users, possibly causing them to view this standard as appropriate only for the measurement of recombinant material. These issues were discussed at a small meeting in May, as a result of which members of the Subcommittee were asked by Dr Booth for their views. All 15 responses agreed that the continuity of the standards was a more important issue than the source of protein, and supported the request that the standard be named the 3rd international standard. 14/15 favoured or were
neutral on the inclusion of the word recombinant. The same large majority (14/15) felt that inclusion of the word Alteplase was undesirable. These responses were presented to the meeting by Dr N A Booth. The meeting approved the recommendation that the name of the standard be amended to "3rd International Standard (Recombinant) for Plasminogen Activator, Tissue Type (Enzymatic Procedure)". This wording is based on the nomenclature adopted by ISTH/SSC (Thromb Haemost 71: 375-384, 1995) but could be shortened for convenience to "3rd International Standard (Recombinant) for tPA". Information should be included with the standard on: the source of tPA in this standard and in the 2nd international standard it replaces; information on the assay used for the standardization (fibrin clot lysis); suitability of the assay for measuring all natural sources of tPA as well as Alteplase.

Dr Longstaff went on to explain that supplies of the current international standard for streptokinase were running low. The same is true for the current British standard for plasminogen, but there is an international standard for plasmin, adopted by the Subcommittee in 1998. Members were asked to inform him of any requirement for replacement standards. His final topic was the stability of standards. He showed data on stability on standards for tPA and plasminogen activator, urokinase type (uPA) and indicated his desire to hear from any user who had information on stability or problems with any standard (clongstaff@nibsc.ac.uk).

**PAI-1 in animal models**

Dr P J Declerck presented data on the plasma and platelet pools of PAI-1 in several species, most of which were broadly comparable with human, the concentrations in mice and rats being lower in both plasma and platelets. Rabbit platelets contained no detectable PAI-1; this has important implications for studies using animal models.

**Procarboxypeptidase U / TAFI**

This protein has been a topic for discussion by the Subcommittee since 1998, Dr D Hendriks playing the major role in organising the discussions. Nomenclature was discussed by Dr Hendriks, who made the case for its name being in line with those of other carboxypeptidases. The discussion that followed accepted the need for a common name but there was a reluctance to drop the name TAFI, for reasons including bibliographical searches. It was agreed that all authors should include both names at first mention, including also the EC number. Data on current antigen and activity assays were presented by Dr L Mosnier and Dr D Hendriks. Considerable progress has been made over the last year and there was a lively discussion. There was agreement that normal plasma contains no detectable active enzyme. There was good correlation between antigen and total activity generated by thrombin. The data on normal plasma concentrations differed between groups, and it was agreed that groups should exchange standards to achieve harmonization of values.

**Secondary SSC standard**

Dr Booth explained that assay methods for measuring functional plasminogen (Dr J Sidelman) and plasmin inhibitor (α 2-antiplasmin) (Dr P Meijer) were now in place. Laboratories ready to
participate in measuring this material were asked to make contact with her 
(n.a.booth@aberdeen.ac.uk).

Abbreviations for plasminogen activators

Dr Booth had explored the question of whether the abbreviations for the plasminogen activators 
should be hyphenated or not, and had sought the views of members of the Subcommittee. There 
was a perception that the hyphen was the official abbreviation, based on discussions in 1984. The 
responses suggested a majority (9/14) in favor of tPA rather than t-PA, but some people strongly 
preferred t-PA, and there were some concerns about confusion in bibliographic searches with the 
phorbol ester and with tissue polypeptide antigen. The nomenclature adopted by ISTH/SSC  
(Thromb Haemost 71: 375-384, 1995) does not recommend any abbreviations, so it appears that 
authors may use whichever version they prefer. Dr Booth recommended that at first use in any 
manuscript, the correct name should be given, followed by the chosen abbreviation. This would 
make it unnecessary to get agreement (which in any case appeared unlikely) for an official 
abbreviation.

Plans for 2001

Suggestions for topics were requested but none were made; attendees were encouraged to send 
suggestions to Dr. Booth (n.a.booth@aberdeen.ac.uk), Dr. Declerck 
(Paul.Declerck@farm.kuleuven.ac.be), Dr. Dempfle (dempfle@verw.ma.uni-heidelberg.de) or 
Dr. Matsuo (kr9o-mto@asahi-net.or.jp). It was agreed that discussion at the meeting in Paris 
should include the topics of PCU / TAFI, D-Dimer and Standards.

The meeting was attended by about 120 people, including all the co-chairs. Dr Booth expressed 
her thanks to them and to all participants and closed the meeting at 5.20.
Hemostasis and Malignancy

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

I. The chairmen welcomed participants to this annual meeting of the Subcommittee.

I. Basic and Laboratory Studies

Dr. D. Ornstein made a presentation on the value of melanoma as a model for the assessment of the role of tissue factor in tumor biology. She discussed the evaluation of Lovastatin in the prevention of melanoma based upon a proposed mechanism of TF inhibition.

Dr. A. Falanga presented an overview of the current status of laboratory markers of haemostasis in cancer patients. Few prospective cancer studies have included routine analysis of haemostasis markers and it is not possible to say which of these markers has any predictive value for venous thromboembolism in cancer patients.

It was resolved that the Subcommittee wishes to create a database of studies in which haemostasis markers have been analysed in cancer patients and perform a pooled analysis of data.

Work-in-Progress

A study in 1° brain malignancy was presented by Dr. D. Walsh. This study will evaluate the coagulation status in patients undergoing operation for glioma and meningioma perioperatively and for 2 months after operation.

A laboratory study which has developed an endothelial cell based model for assessment of the effects of various anti-cancer drugs on coagulation markers was presented by Dr. A. Falanga. This model may provide valuable mechanistic data on the interaction between cytotoxic drugs and the coagulation system.

I. Evidence-based medicine in cancer and thrombosis

Mr. Kakkar presented the limited data on the clinical management of venous thromboembolism specific to cancer patients. Four areas were identified for future clinical trials of anti-thrombotic agents in cancer patients:

Prophylaxis in cancer surgery

Prophylaxis in patients receiving chemotherapy
Prophylaxis of central line associated thrombosis

Prevention of recurrent thrombolism in cancer

A number of specific issues were raised with regard to the dose and duration of prophylaxis in cancer patients, and the influence of tumor histology and stage of disease on the risk of thromboembolism.

The Subcommittee would wish to endorse well-designed clinical trials in the above topics.

Work-in-Progress

Dr. M. Prins presented the SOMIT study preliminary results. This study demonstrated that screening for cancer in patients with spontaneous VTE did result in the detection of more cancers than in a group of patients who were only observed. Difficulty in recruitment resulted in termination at 1/5R of the proposed sample size.

It was felt future studies should concentrate on a cohort of spontaneous VTE patients to determine the most effective screening strategy.

Mr. Kakkar presented an update on the CLOT study (DVT on treatment of cancer) on behalf of Dr. Levine. The study has recruited 196 patients to date.

Dr. Von Templehof presented the TOPIC study (Prophylaxis of VTE in breast cancer receiving chemotherapy). He also presented data suggesting thrombosis as a risk factor for poor outcome in breast cancer. The mechanism was unclear.

Mr. Kakkar presented an update on FAMOUS and Dr. S. Smorenburg presented an update on MALT ó both studies are evaluating survival in cancer patients receiving LMWH. Both studies are endorsed by the Subcommittee.

Mr. Kakkar presented a study evaluating LMWH in the prevention of central line associated thrombosis in cancer patients which is being undertaken by Pharmacia and will include 435 patients.

Ms. A. Young presented the WARP study ó a 1400 patient evaluation of fixed dose vs. adjusted dose (INR1.5-2) vs. no warfarin for the prevention of line thrombosis in cancer patients. 127 patients have been recruited to date.

Dr. S. Schulman presented late follow up data from the DURAC study. This demonstrates a late benefit (up to 6 years) for patients who received six months rather than six weeks of warfarin for treatment of DVT in terms of the development of cancer. There was no difference between two warfarin groups in first 2 years.
A joint registry of clinical data and various anticoagulant regimens and cancer survival was proposed.

II. Registry of clinical trials

Dr. Zacharski presented an overview of coagulation mechanisms in cancer growth and metastasis.

It was resolved to establish a registry on the ISTH Website to include trials of antithrombotic agents in the 1) prevention and 2) treatment of thrombosis in cancer patients and of trials evaluating the effects of these drugs on cancer survival.

A policy paper on the trials to be included, access to raw data from investigators, location and responsibility for the database, ownership thereof and the analysis and publication policy will be distributed within the Subcommittee.

It was resolved to reconvene the Subcommittee during the XVIIIth ISTH Congress in Paris.
Lupus Anticoagulants/Phospholipid-Dependent-Antibodies

Chairman: J. Arnout, Leuven

Number of attendees: 125 - 150

I. Diagnosis of antiphospholipid antibodies

Mr. P. Meijer (Leiden), program manager of the ECAT surveys on thrombophilia testing, reported on the compliance of laboratories with the SSC criteria on LA testing. He recently sent out a simple questionnaire to the ECAT participants to evaluate how they perform the screening for lupus anticoagulant. About 80% of the participants perform at least 2 different screening tests. APTT and DRVVT are mostly used as screening tests (about 75%). Furthermore KCT (10%), dAPTT (5%) and dPT (7%) are used, while a few laboratories follow a different strategy (e.g. immediately starting with mixing studies). Fifty-three per cent of the laboratories perform a mixing study for each type of screening test. Of the 20% who only perform 1 screening test, 52% perform a DRVVT test. Only sixty per cent of these perform a mixing study on this single screen assay. About 60% of the participants do not perform a confirmation test for each type a screening test. These results show clearly that the criteria published by the SSC on Lupus Anticoagulant are not completely fulfilled by all laboratories. One of the possible reasons is the availability of the so-called integrated systems today. It seems that to many labs rely to much on so-called integrated test systems.

Dr. J. Arnout reported on the relative responsiveness of different LA screening assays for beta-2-GPI and prothrombin dependent LA. His presentation was also based on results obtained from the ECAT survey on LA testing. A normal plasma pool, and the same pool spiked with a LA positive anti-ß2GPI antibody (27G7) and a LA positive antiprothrombin antibody (28F4) were recently used as control materials. The majority of the 105 laboratories that participated found the two LA samples to be positive (87.1 % for the antiprothrombin LA and 91.5 % for the anti-ß2GPI). The submission of a normal sample together with the LA samples enabled a comparison of the relative responsiveness for this two types of LA of the different screening assays used. As most laboratories used more than one assay, a total of 177 screening assay results could be used for analysis. Clotting time ratios varied from 1.06 to 2.96 for the prothrombin-dependent LA and from 0.99 to 4.69 for the ß2GPI-dependent LA. Further analysis of the results allows the following led to the following conclusions:
a) the KCT seems to be somewhat more sensitive for prothrombin-dependent than for ß2GPI-dependent LA. b) most aPTT reagents have a rather weak sensitivity for ß2GPI-dependent LA and a somewhat higher sensitivity for prothrombin-dependent LA. Among the aPTT reagents, PTT-LA shows the highest responsiveness for both LA types. c) the DRVVT from Gradiopore, DadeBehring and IL has a high sensitivity for ß2GPI-dependent LA and a slightly lower sensitivity for prothrombin dependent LA. The DRVVT from Am Diagn shows a weak sensitivity for ß2GPI-dependent LA but a good sensitivity for prothrombin-dependent LA. d) the dPT with Innovin is highly sensitive for ß2GPI-dependent LA and moderately sensitive for prothrombin-dependent LA. Recombiplastin (IL), shows a poor sensitivity for ß2GPI-dependent LA but a moderate sensitivity for prothrombin-dependent LA.
These data show that LA positive monoclonals are useful for the production of LA control specimens and suggest that reagents might be produced with which LAIs can be differentiated into specific subtypes.

Dr. P. Sie (Toulouse) presented data from a French study by the groupe d’étude sur l’hémostase et la thrombose in which the LA monoclonal antibodies developed by Dr. Arnout were used for calibration and quantification of LA assays. A pool of normal plasma spiked with varying concentrations of a combination of a LA positive anti-β2GPI monoclonal and a LA positive antiprothrombin antibody was used as calibrator. A panel of plasmas from 69 patients (40 patients with APS and 29 asymptomatic patients) with persistent LA diagnosed using the SCC-ISTH criteria was used. The LA activity in the samples was quantified with 8 assay systems using a variety of reagents and instruments. These assays included 4 dRVVTs marketed by different companies, 2 aPTTs (PTT LA and Synthasil, selected because of their reported large difference in responsiveness to anti-β2GP1), and 2 dPT(Innovin and Thrombomat).

- Assay sensitivity, calculated as the percent of LA positive samples, varied between assays, reflecting the mode of screening LA in this study, which favored PTT LA and dTT. There was no relationship between responsiveness and sensitivity.
- LA activities of the patients plasmas, expressed in monoclonal antibody equivalents, varied significantly between assays (p<10^-4), but homogeneous subgroups could be identified. Among the dRVVT, LAC Screen (Instrumentation Laboratory) and LA1(Dade-Behring) gave similar results, distinct from a second subgroup comprising DVV test (American Diagnostica) and Bioclot LA (Biopool). aPTT (PTT LA) and dTT (Innovin) formed a third subgroup and dTT (Thrombomat) and aPTT (Synthasil) a fourth. The titers obtained using the dTTs and aPTT Synthasil, a group of assays relatively more responsive to antiprothrombin than to anti-β2GP1, were about twice those obtained using the dRVVT assays (order of magnitude of the mean: 40-50 versus 15-20 AU/ml respectively).
- There was a trend towards higher LA titers in symptomatic, compared to asymptomatic, patients, but the difference was significant (p<0.01) only for a group of dRVVT assays.

Dr. Sie concluded that quantification of LA activity in clinical samples by using calibrator made from plasma spiked with mAbs against β2GP1 and/or anti-prothrombin is feasible.

Dr. Arnout proposed to extend this study with an international study where these monoclonals could be used. He will prepare a protocol and discuss it with the co-chairs. People interested in participating were invited to sign-up via e-mail: jef.arnout@med.kuleuven.ac.be

Drs. S. Donohoe (London) discussed standardisation and clinical relevance of antiprothrombin antibodies as measured in an ELISA system. They set up optimisation experiments for this assay and identified an array of possible pre-test and analytical variables that had an influence the sensitivity and specificity of the aPT ELISA. Some of the variables are: the microtitre plate type, the antigen type, purity and concentration, the buffer used throughout the assay, the sample type
and the way reference ranges are defined. The investigators used an in-house optimised assay to screen various populations of patients and assess the clinical relevance of these antibodies. An association between aPT and a history of thrombosis but not miscarriage was observed. Dr Donohoe however was prudent in her conclusions as the restricted numbers of patients and the referral pattern of clinics from which their aPS patients were recruited may contribute to the clinical associations detected. She proposed that an international, multi-centre standardisation study to be performed, preferably in employing an international reference preparation, so that future reports of clinical associations are directly comparable. A first draft of such a multicenter study was presented and the participants to the session were invited to collaborate. The contact person for this multicenter study is Dr. S. Donohoe who can be contacted by e-mail: s.donohoe@ucl.ac.uk

Dr. MC Boffa (Paris) critically reviewed the existing literature on the detection of antiethanolamine antibodies (aPE) via ELISA and their potential clinical relevance. Ethanolamine differs from other phospholipids used to detect aPL by the fact that it does not bind β2GPI. Antibody binding to this phospholipid depends (in about 50% of the cases) on the presence of HMW kininogen, kallikrein, factor XII. Prothrombin has also been shown to bind to PE but it is unclear whether this can result in antibody binding. It is possible that in some cases antibodies bind directly to PE. About 15 percent of SLE of APS patients have aPE usually in addition to aCL.

Many variables affect the aPE assay results such as the blocking agent (adult bovine serum or plasma) and the source of PE (brain, egg yolk or plant). The content of free HMWK in the blocking agent seems important. Given the large variability of assay results further work should be done on the standardization of this assay before it can be recommended to be widely used by non-expert laboratories.

II. Mechanism of action of antiphospholipid antibodies

Dr. Ph. de Groot (Utrecht) presented data on the importance of bivalent-β2GPI interaction with negatively charged phospholipids for its lupus anticoagulant effect. He showed a new model on the three-dimensional structure of β2GPI based on crystallography. β2GPI appears to be much more rigid than originally thought. β2GPI is composed of 5 SCR domains of which the first are lined up in a straight line. Domain four is bended and domain V is more positively charged at the outside than originally thought. Through this positive charges, β2GPI interacts with the polar headgroups of negatively charged phospholipids. There is a small hydrophobic loop that enters into the hydrophobic part of the membrane. He further presented data on recombinant dimers of β2GPI. He attached a dimerization motif at the N-terminal end of the molecule (domain 1). The dimerization motif that he used consisted of the apple 4 domain of factor XI which is important for the dimerization of factor XI. The β2GPI dimer prepared in this way behaved as a lupus anticoagulant in several LA assays such as the dilute prothrombin time (dPT) using Innovin, in the dRVVT and in aPTT with PTT LA. His results are in line with findings from others that the β2GPI-dependent LA activity in human plasma is due to formation of bivalent β2GPI-antibody complexes on phospholipid surfaces with increased affinity for phospholipid so that competition with clotting factors for this catalytic surface can occur. This material offers new possibilities to study the pathogenesis of aPL: are the antibodies needed for the pathogenicity or is it the increased affinity of dimer-β2GPI for PL alone that is important?
Dr. V. Pengo (Padova) presented data that LA positive anti-beta-2-GPI isolated from patients with APS may have procoagulant properties. He tested the effect of 6 affinity-purified preparations of anti-β2-GPI antibodies on the PT of normal pooled plasma and of plasma pooled from patients on oral anticoagulant treatment. The 6 affinity-purified preparations had LA properties in conditions where low phospholipid concentrations were used. However, instead of prolonging non-diluted PT, the anti-β2-GPI antibodies shortened the PT of both normal plasma and anticoagulated plasma by a mean of 2.4 seconds and 5.6 seconds, respectively. This shortening was observed with several tissue thromboplastins and was lost upon removal of β2-GPI from the plasma. The procoagulant effect was not as evident in an assay that used stimulated monocytes as a source of thromboplastin. This is the first time that somebody could show a direct procoagulant effect of aPL in a static system. The mechanism however is not yet elucidated.

III. Which assays should we recommend

Dr. Galli (Bergamo) presented data on a meta-analysis of the sensitivity and specificity of different aPL assays towards arterial and venous thrombosis. In her meta-analysis based on 12 prospective, cross-sectional and case-control studies, including 1608 patients, revealed a sensitivity (Sens.) and specificity (Spec.) of LA for arterial and/or venous thrombosis ranging from 8.5 to 14% and from 98 to 100%, respectively. The association of LA positivity with thrombosis was si
The chair, all co-chairs and approximately 40 Subcommittee members were present. Issues discussed were as follows:

1. Recommendations for laboratory evaluation of children with thrombosis: This project has been written as an official recommendation of the Subcommittee. M. Manco-Johnson presented the paper to the Subcommittee and each major point was voted upon. Minor additions or changes were agreed upon. The finished product will be sent to Dr. Francis.

2. LMWH dosing in infants and children: Data from four very small pharmacokinetic studies were presented and evidence for increased dose and monitoring requirements in children were discussed. The Subcommittee members agreed that there is sufficient evidence to warrant formal study. A working group was constituted including M. Manco-Johnson (Colorado, USA), E. Grabowski (Massachusetts, USA), U. Nöwak-Gottl (Germany), U. Peters (The Netherlands), R. Liesner (London, UK), J. Tusell (Barcelona, Spain) and M. Williams (Birmingham, UK). The working committee will develop a data collection instrument to be used in an international collaborative study of LMWH dosing based upon plasma anti-Xa level measured at 3 to 4 hours after a subQ dose (4 hours is preferred as this represents the dominant clinical practice). A report will be due at next yearís meeting.

3. Arterial versus venous thrombosis in children. A stimulating presentation was give by E Grabowski in which evidence was presented to substantiate low flow currents around arterial catheters, damaged endothelium and arterial prostheses. These localized low-flow currents would promote "venous-like" red cell clots in children. This data supports application of the recommendations developed for thrombophilia evaluations (as discussed in #2, above) to children with arterial thrombosis.

4. Lp(a) levels in children were discussed by U Nöwak-Gottl. Lp(a) was shown to contribute a six-fold increased risk for thrombosis. Familial thrombotic disease has been related to higher levels of Lp(a) (> 30 mg/dl) and isoforms consisting of fewer kringle 4 repeats (14 to 22 versus greater than 28). Neonates were found to have lower levels of Lp(a) and a skewing toward the isoforms comprised of more kringle repeats. By twelve months of age, results in children are similar to that of their parents. African children have higher levels of Lp(a) but this does not appear to confer an increased thrombotic risk in that population. The IFCC conducted an evaluation of 39 assay systems and found substantial problems with half of the assays. The inadequate assays did not show a linear dose-response of Lp(a) and dilution curves of unknowns were not parallel to the standard curve. The ELISA assays performed better than immuno-nephelometric or immuno-turbidometric assays. Recommendations for assays included use of a universal standard and two-antibody ELISA assays. Thawed and refrozen samples should not be used for assay and prolonged storage affected assay results. Consensus was reached that large sample sizes are needed to develop ethnic specific-normal values and to investigate the relationship of Lp(a) to neonatal and pediatric venous and arterial thrombosis. Dr.
Nöwak-Gottl will develop a proposal to compare results generated in different laboratories, develop appropriate control groups and correlate levels measured in children with venous and arterial thrombosis.

5. Diagnosis and management of thrombosis during pregnancy: the Anticoagulant and Perinatal Subcommittees jointly developed this project. Drs. J Connard and M Hellgren read the report. Each major point was discussed and voted upon by the members present. The document will be edited to include suggestions made, circulated to a list of members who registered willingness to review the document and will be sent by M Manco-Johnson to Dr. Francis within two months.

6. A possible inclusion of women's coagulation problems into the Perinatal/Pediatric Subcommittee was discussed. It was decided that the scope of work by such an expanded subcommittee would be too broad and would dilute the efforts of both groups. A formal recommendation will be sent to Dr. Francis suggesting that a new subcommittee be established for coagulation problems in women and that coagulation disorders during pregnancy be transferred to such a subcommittee. It was recommended that collaborative activities be conducted between the Perinatal/Pediatric Subcommittee and a new subcommittee for Coagulation in Women.

7. ITP in children: J Bussell (New York, USA) presented the major points of an SSC paper that has been written comparing recommendations and/or consensus developed independently by the UK and the ASH (US). A Sutor presented German recommendations on childhood acute ITP. S Davidson (London, UK) and C Van Geet (Leuven, Belgium) volunteered to review the final draft of the manuscript and suggest any changes needed.

8. Severe protein C deficiency: B Moritz (Vienna, Austria) reviewed the development and compassionate use of a human plasma-derived protein C concentrate. Plans to complete formal pharmacokinetic studies and begin phase II/III treatment trials were outlined. The Subcommittee approved these plans.
1. **Protein S: Genotype and phenotype**

After the presentation of Tomio Yamazaki from the group of Bjorn Dahläck and other papers recently published, it appears that type III protein S deficiencies, characterized by low free protein S levels and subnormal total protein S levels, are due in most cases to a protein S mutation moderately affecting the gene expression. Thus type I and type III are respectively severe and mild-protein S deficient phenotypes. The Ser 460 Pro mutation (protein S Herleen) also results in type III phenotype, although in this case the mild decrease in circulating protein S level is not due to a decreased gene expression. The reduced stability of the mutant protein suggests an increased turnover.

Sophie Gandrille took advantage of a new monoclonal antibody recognizing an epitope encompassing gene 61 to 72 to show that 10 to 20% of circulating protein S is cleaved after Arg 60. This might influence the assays measuring activated protein C cofactor activity.

Piet Meijer reported a Dutch study of the variability of protein S concentrations among different laboratories. Three types of calibrators were used, all made from human plasma. In some cases, universal pooled plasma from healthy donors (excluding women receiving oral contraceptive treatment) yielded the lower variability. It would be interesting to test recombinant uncleaved protein S as a calibrator.

2. **Factor V gene and new mechanisms of APC resistance**

The R2 polymorphism was described in Ferrara by the group of Francesco Bernardi. Elisabeth Castoldi reviewed the biochemical consequences of the different polymorphisms associated to the R2 haplotype (hré OR fv Ferrara) and Elena Faioni reviewed the association of HR2 with APC resistance, low FV levels and deep venous thrombosis (DVT). Overall HR2 contributes to APC resistance and might increase the risk of DVT when associated with other genetic risk factors such as FV Leiden.

3. **Tissue factor pathway inhibitors (TFPI)**

Theo Lindhout gave a review on the recent advances on TFPI mechanism of action.

Several monoclonal based assays are now available to measure total and free circulating TFPI, such as those presented by Hisao Kato and by Marc Grimaux. The clinical significance of the variations in TFPI levels remains to be extensively studied. Pierre Morange reported associations of TFPI levels with cardiovascular risk factors in a large population.
Since during the past years, most subjects discussed by our subcommittee were extended to all modifications of the coagulation balance (such as APC resistance), we propose to change the name of the "Plasma Coagulation Inhibitors" subcommittee which could now be the "Thrombophilic Risk Factors" subcommittee.
ALLOIMMUNE THROMBOCYTOPENIA

For the past three years the Subcommittee has been engaged in discussion of possible areas for consensus regarding the clinical management of alloimmune thrombocytopenia (AIT) caused by maternal alloimmunization against fetal platelets. Clinical management of the first affected infant in a family is reasonably standard in most developed countries and was included in last year’s Subcommittee report. In AIT, the affected fetus can suffer from serious hemorrhage antenatally as well as during the perinatal period. Clinicians take a number of different approaches to management of these pregnancies including weekly administration of IgG IV, steroid therapy, platelet transfusions given to the fetus, and monitoring without therapy. At the present time, there is not sufficient consensus to warrant preparation of a consensus statement. However, the Subcommittee will continue its consideration of this topic.

Nomenclature standardization for the platelet alloantigens is a major goal of the Subcommittee. Platelet allotyping is being evaluated more extensively as a possible risk factor for cardiovascular diseases. We plan to work with the Working Party on Platelet Serology of the International Society of Blood Transfusion (ISBT) to achieve a consensus on this issue. Nomenclature systems that have been proposed include a molecular designation published by Newman (Blood 83:1447, 1994) and the human platelet antigen (HPA) system published by von dem Borne and Decary (Vox Sang 58:176, 1990). Both were discussed at the meeting, and a proposal was made to seek a compromise utilizing the most useful features of both. A detailed proposal will be circulated among the membership of the Subcommittee and voted upon at the next meeting.

The Subcommittee requests that the ISTH-SSC ask the World Health Organization (WHO) to participate with us in a three year plan to facilitate the process of platelet alloantigen nomenclature, to standardize platelet allotyping and antibody measurement, and to maintain international standard allotyping reagents, both polyclonal and monoclonal. The proposed timeline for completion of the individual components of this project would be as follows:

2001 -- Develop a mechanism to designate new platelet alloantigens


2002 -- Acquire and prepare for standardization polyclonal alloantibodies for the most clinically significant platelet alloantigens
2003 -- Notify all pertinent journals of the final nomenclature system, requesting conformity in future publications

2003-- Include monoclonal platelet allotyping reagents as international standard reagents when they become available

AUTOIMMUNE THROMBOCYTOPENIA

Dr. Bussel presented a summary of the clinical guidelines for management of children with immune thrombocytopenia (ITP) prepared for the Subcommittee on Perinatal/Pediatric Hemostasis. It was clear that most of the decisions about clinical management of ITP are based upon individual judgement rather than results of clinical investigations. Members of the Subcommittee enthusiastically supported the proposal for a registry of available ongoing clinical trials for ITP therapy to be posted on the ISTH website. Information on each open study should include the principal investigator and study coordinator contact information, questions being addressed by the investigation, industry or organization sponsoring the study, and inclusion and exclusion criteria. Such a listing should facilitate clinical investigation in this area.

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

Stem cell transplantation (autologous and allogeneic) for immune thrombocytopenia is being reviewed by Co-chair, Dr. Ertem, although there was no formal presentation of the topic at this yearís meeting. For other autoimmune diseases, transplantation is promising with long term remissions in 87% of cases after allogeneic stem cell transplantation and in 33% of cases after autologous transplantation. The Platelet Immunology Subcommittee continues work to establish recommendations regarding which patients with immune thrombocytopenia would be appropriate candidates for clinical trials of stem cell transplantation. Follow up after autologous stem cell transplantation for patients with ITP revealed relapse in the majority of patients treated with autologous transplantation. These findings are consistent with reports of allogeneic and autologous stem cell transplantation in animal models of immune thrombocytopenia. A draft of a consensus statement will be completed soon and distributed to members of the Subcommittee prior to submission for approval.

DRUG RELATED IMMUNE THROMBOCYTOPENIA

Over the past year Dr. Chong organized and completed an international wet workshop to assess results of laboratory testing for heparin induced thrombocytopenia (HIT). The aims of the workshop were 1) to survey the methods used for HIT antibody testing, 2) to evaluate the sensitivity and specificity of the methods, and 3) to compare the results of the different methods and different laboratories including specialist and non-specialist laboratories. Participants included 24 international specialist laboratories and 23 Australian non-specialist laboratories. Sensitivity and specificity were excellent for the specialist laboratories and good for the non-specialist laboratories. The specialist laboratories performed well with both ELISA and functional assays, while the non-specialist laboratories did well with the ELISA but poorly with...
platelet activation test methods. Dr. Chong will prepare a Brief Report of the workshop for review by members of the Subcommittee and approval at the 2001 SSC meeting.
Tremendous strides have been made in our understanding of the aberrant platelet mechanisms in some groups of patients with inherited platelet dysfunction. However, in a substantial number of such patients, the specific abnormal mechanisms are unknown. There is convincing information that at least some of these patients have defects in the early events of platelet activation/signal transduction. The theme of the present session was to review aspects of normal platelet signaling mechanisms and evidence for specific abnormalities in signal transduction events with the overall goals: a) to develop approaches applicable in defining the molecular mechanisms in patients with impaired platelet function; and b) to foster a collaborative approach involving a network of investigators with the required expertise.

Dr. C. Gachet, S. Levy-Toledano and M. Jandrot-Perrus reviewed the existing information on platelet ADP, thromboxane A₂ and collagen (GPVI) receptors respectively. Dr. J-W Akkerman reviewed the information on protein kinase C. Each of the speakers reviewed data from available specific knock-out mouse models as well. The second part of the session focussed on defects in patients with impaired platelet function. Dr. A.K. Rao provided an overview of the platelet signal transduction defects with specific emphasis on deficiencies of platelet phospholipase C-β 2 and Gαq. Drs. P. Nurden, M. Cattaneo and M. Hoylaerts described their studies on defects at the level of the ADP receptors. Drs. S. Watson and K. Clemetson presented information on abnormalities in platelet responses to collagen and in the involved receptors. Dr. C. van Geet presented studies on an inherited defect in Gαs hyperfunction.

Following presentations, there was an extensive discussion regarding the approaches needed to delineate the platelet mechanisms in the larger group of patients with abnormal aggregation responses and impaired hemostasis. It was concluded a working group be established on inherited platelet signal transduction defects that would evaluate the methods and develop guidelines regarding the laboratory studies needed to define the abnormal mechanisms in these patients. This group would address issues such as the specific platelet responses to be studied and the agonists involved. There was also a discussion regarding the need for a data base/registry and a Web site focussing on patients (and mouse knock-out models) with signal transduction defects and the investigators involved in these studies. It was concluded that a working group be established to address this. Other points of discussion included the need for assessing platelet adhesion and the importance of studying flow-related aspects of platelet function in such patients.
Predictive Variables in Cardiovascular Disease

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

The number of people attending this subcommittee meeting was approximately 50.

**Homocysteine.** Dr. A. Tripodi presented results of a collaborative study evaluating different methods (by HPLC, enzyme immunoassay ó EIA, and fluorescence polarization immunoassay ó FPIA) for measuring homocysteine levels. The conclusions of the study were that the FPIA method gave a lower coefficient of variation than the other methods and that the performance characteristics of the FPIA and HPLC methods compare favorably to one another. The comparability of standards is a problem and it was felt that establishment of a plasmatic standard would be helpful in the standardization of assay methodologies. Dr. M. Cattaneo presented data showing that high post-methionine load total homocysteine levels and low plasma vitamin B6 levels are independently associated with an elevated risk for deep venous thrombosis. Low folic acid levels were less strongly associated with risk.

**Meta-Analysis of Haemostatic Variables in Coronary Heart Disease.** Dr. J. Danesh presented the results of meta-analyses for fibrinogen, the G/A ó455 beta-fibrinogen gene polymorphism, fibrin D-dimer, von Willebrand factor, lipoprotein (a), and C-reactive protein as markers of coronary risk. Using the British Regional Heart Study as well as many other published studies, data was presented for incident disease associations in apparently healthy populations as well as for disease versus undiseased cohorts. All of the aforementioned variables with the exception of the beta-fibrinogen gene polymorphism were found to be significantly associated with coronary heart disease.

**Effect of Factor XIII-Val34Leu on Fibrin Structure and Function.** Dr. R. Ariens presented data showing that the catalytic efficiency of thrombin-mediated factor XIII activation is doubled by the presence of the Val34Leu polymorphism as compared to wild type. Early covalent cross-linking of factor XIII-Val34Leu produces more gamma-gamma and alpha-alpha crosslinks, thereby inhibiting lateral aggregation processes. This leads to formation of a fibrin clot with a finer structure, thinner fibers, and smaller pores as compared to fibrin formed using wild type factor XIII.

**Role of Hemostatic Gene Polymorphisms in Venous and Arterial Thrombotic Disease.** Dr. P. J. Grant gave an overview of a large amount of information on this topic that was recently published by he and Dr. D. Lane in BLOOD. While clear associations between genetic polymorphisms, intermediate phenotypes, and disease are found for deep venous thrombosis (e.g., factor V Leiden leads to resistance to activated protein C), this has frequently not been the case for polymorphisms reported to be associated with arterial thrombotic disease. The results of different studies seeking associations between hemostatic gene polymorphisms and arterial thrombotic disease have largely given inconsistent results with respect to the predictive value of most genetic variants. A discussion ensued regarding future prospective studies in this field and the ultimate utility of such data given the data to date.
Emerging Candidate Genes for Cardiovascular Disease. Dr. L. Iacoviello presented new data that a ó511 C to T polymorphism in the IL1-beta gene is independently associated with an increased risk of myocardial infarction in a young Italian population with a positive familial history. The monocytes of patients homozygous for the CC polymorphism exhibited increased release of IL1-beta after 24 hours of stimulation by lipopolysaccharide. Data was also presented on the role of various factor VII gene polymorphisms (Arg353Gln, decanucleotide insert, and ó402 G to A) and CHD risk in this population. The Arg353Gln polymorphism and the decanucleotide insert are in strong linkage disequilibrium. Arg353Gln has been reported to be protective against coronary heart disease by Dr. Iacoviello. It was pointed out that the allele frequencies for these two polymorphisms are significantly different between Italian and Northern European populations, thereby providing a potential explanation for the very different results that have been reported. Data was also presented that the ó402 G to A polymorphism was not associated with an increased risk for premature myocardial infarction.
von Willebrand Factor

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

The Subcommittee met Friday, June 16th, at Auditorium I at the Maastricht Meeting and Congress Center, Maastricht, The Netherlands, from 08:00 to 12:00 and from 13:30 to 17:30. Presiding chair was Dr. Francesco Rodeghiero; Dr. R.R. Montgomery and Dr. J. Rand could not attend. Attendance was approximately 95.

PART I

CLINICAL ASPECTS, DIAGNOSIS AND EPIDEMIOLOGY (F. Rodeghiero, Chair)

Dr. F. Rodeghiero (Italy) presented, also on behalf of Dr. A. Srivastava (India), the early results of a survey on VWD prevalence and impact in developing countries. The study was undertaken following an extensive discussion on the impact, prevention and control of VWD, held at the Joint WHO/ISTH meeting in London on October 12-14, 1998. It was decided that detailed and precise data on the impact of VWD in developing countries was an important preliminary step to improve the awareness and management of this disorder. A full written report summarizing this meeting appeared in the issue of August 2000 of Thrombosis and Haemostasis (Vol. 84, p. 160-174). The present survey is an extension of a previous one, conducted by Dr. A. Srivastava, which includes also data on the incidence of severe Hemophilia A from the same referral area, and mortality data. In this way, prevalence on VWD could be normalized to that of severe hemophilia A. The expected ratio of clinically significant VWD/severe hemophilia A is around 4 (assuming 25/million the prevalence of severe hemophilia A and 100/million the prevalence of symptomatic VWD). Preliminary data were available for 9 developing countries (Paraguay, Iran, Thailand, Panama, Zimbabwe, Serbia, S. Africa, Latvia and Malaysia) with a median ratio of 0.43 (range 0.09 - 0.9). For comparison, the ratio VWD/severe hemophilia A in Italy is 1. In all these countries a minimum of diagnostic facilities and treatment products, including DDAVP, was available, but some deaths in VWD, preventable with better facilities, were reported by some of these countries. These preliminary data show the feasibility and reliability of this survey for the monitoring of VWD diagnosis and management in developing countries. A gross underestimation of VWD and, to a lesser extent, of severe hemophilia A, is becoming apparent.

Dr. P.M. Mannucci (Italy) reported on the spectrum of bleeding symptoms in patients with severe type 3 VWD, based on an analysis of 385 patients from Iran. In contrast to an estimated prevalence in the Caucasian population of 0.55 to 3.2 per million, this recessive bleeding disorder is more prevalent in countries such as Iran where consanguineous marriages are frequent (6 per million). He collected data on the clinical manifestations of type 3 VWD by examining 385 patients from 300 Iranian kindreds, who were compared with 100 age-matched patients with severe hemophilia A. Joint and muscle bleeding was less frequent than in hemophiliacs, perhaps because factor VIII levels were in general higher (median value 4% vs. 1% or less). Mucosal-type bleeding symptoms such as epistaxis and menorrhagia were the most prevalent symptoms in VWD. Post-circumcision and oral cavity bleeding occurred frequently.
when prophylactic replacement therapy was not carried out or was inadequate. Ten of 385 (2.6%) of these polytransfused patients developed an alloantibody to VWF and 55% are chronically infected with the hepatitis C virus.

**Dr. I. Peake (U.K.)** presented an outline of a European study, funded by the E.U., on "Molecular and clinical markers for diagnosis and management of type 1 VWD". The main objective of the study is to determine the relative value of a series of clinical and molecular markers in the diagnosis and management of type 1 VWD. The study will recruit 200 families with type 1 VWD from nine countries in Europe. A detailed clinical history will be obtained and VWF:Ag, FVIII:C, VWF:RCo, VWF multimeric profile will be confirmed by re-assay in expert laboratories. Additional tests will include VWF:FVIII and VWF:CB, PFA-100 assay and intraplatelet VWF measurement. Analysis of VWF gene (exons, exon/intron boundaries, 3' and 5' non-coding regions) will be the molecular core of the project. Analysis of clinical expression of the disease and DDAVP responsiveness as a function of genetic and laboratory data will be the key part of the study.

**Dr. F. Rodeghiero (Italy)** presented the preliminary results of the "Multicenter study on the validation of the diagnostic criteria of type 1 VWD", decided during the Florence SSC VWF Subcommittee meeting in 1997. The aims of the study were the standardization of the clinical and laboratory assessment in order to reduce measurement biases and the evaluation of the discriminant power (sensitivity and specificity) of the clinical and laboratory procedures in the diagnosis of type 1 VWD. These very preliminary data were focused on the bleeding history, a critical issue in the diagnosis of VWD. Bleeding symptoms were analysed in obligatory carriers (transmitters) in families with type 1 VWD and in carriers of type 3 VWD, as diagnosed by specialized centers in a learning set of 30 type 1 and 35 type 3 families. Controls were normal subjects matched for age and sex. As an example, three symptoms (epistaxis, menorrhagia and bleeding after tooth extraction) were considered, and a score system for their severity (before diagnosis) was presented. It was apparent that the distribution (frequency and score) of bleeding symptoms was similar in obligatory carriers of type 3 and in normal subjects. An higher score was present in carriers of type 1 VWD, in older and younger affected members of type 1 and in affected members of type 3 families, without further distinction in these affected groups. These preliminary data suggest that more families are needed. There is a confounding factor by bleeding symptoms in normal subjects and no single symptom seems discriminant. The time of exposure before diagnosis is critical and it should be taken into account for the analysis of the bleeding history. Of course, the combination of symptoms, or its absence, will be considered to increase the diagnostic discriminant power. Correlation of bleeding symptoms with VWF-related measurements is in course, whereas a correlation with the type of mutation and mutation analysis is planned for the next year.

**Dr. W.L. Nichols (USA)** talked about the distinctive laboratory features and the higher than expected prevalence of VWD Vicenza. The Mayo group reviewed all the VWF multimer analyses (autoradiograms), the associated interpretations and the supporting lab data (VWF:Ag, VWF:RCo, FVIII:C) performed during 1987-1998. Among ~8927 multimer analyses (~90% of plasma samples referred from other US laboratories), 78 "Vicenza" VWD cases were identified: 48 definite, 22 probable and 8 possible cases. Most cases (~78%) were apparently genetically unrelated. Based on the relationship of Vicenza VWD cases (definite & probable) to types 2A,
2B, 3 and acquired VWD cases (as determined by VWF multimers and associated tests), it was estimated that Vicenza VWD is ~10% of types 2, 3 or AVWD, and therefore it was suggested that Vicenza VWD may be ~2-3% of clinical VWD. Reflecting its prevalence and unique features, Dr. Nichols' study concludes that Vicenza VWD merits consideration for designation as type 2V VWD, rather than inclusion in the type 2M variants as in the current (1994) VWD classification. Discussion issues included the subtlety of Vicenza VWF multimer aberrations, the difficulty of laboratory diagnosis, the evolution of DNA-based mutation detection, and the pathophysiology of Vicenza VWD. Confirmation of these findings is needed before any change in the current classification is considered.

Dr. J.J. Michiels (The Netherlands) reported on the clinical implications of correct typing in Acquired von Willebrand Syndrome (AVWS). AVWS has been described in association with monoclonal gammopathy, lymphoid, myeloproliferative, auto-immune and metabolic or hormonal disorders, tumors, infection, or the use of drug. AVWS type 2A in systemic lupus erythematous (SLE) responds poorly to DDAVP and FVIII concentrate but responds transiently good to high dose gammaglobuline intravenously. AVWS type 2A in SLE is cured by appropriate treatment with prednisone and/or immunosuppression.

Multimeric analysis of the VWF in AVWD associated with lymphoproliferative disorders usually show a type-2A AVWD due to the absence of large VWF multimers as the consequence of the rapid clearance the anti-VWF-factor VIII/VWF complex from the circulation. AVWS type 2A in benign IgG monoclonal gammopathy poorly responds to intravenous DDAVP and to factor VIII/VWF concentrate infusion, does not respond to prednisone and/or chemotherapy. High dose intravenous gamma globulin (1g/kg for 2 days) usually induces a transient correction of the factor VIII/VWF parameters for 1 to a few weeks. In contrast, AVWS type 2A in benign IgM benign monoclonal gammopathy does not respond to any treatment including high-dose gamma globulin. AVWS type 2B and 1 in myeloma responds well to chemotherapy with melphalan/prednisone or combination chemotherapy with complete restoration of RIPA and the VWF/FVIII parameters respectively.

AVWS in thrombocythemia is characterized by prolonged bleeding time, normal factor VIII coagulant activity and VWF:Ag concentration, a very low VWF:RCo and collagen binding activity (VWF:CB) and absence of large and intermediate VWF-multimers simulating a type 2A von Willebrand disease. Reduction of platelet count to about 1000 x10^9/l results in the disappearance of the bleeding symptoms, improvement of VWF:RCo activity to low normal levels and significant improvement of the VWF multimeric pattern. Correction of the platelet counts to normal (<400 x10^9/l) is associated with complete correction of the VWF-multimeric pattern and correction of all VWF-parameters to complete normal values.

The AVWS in hypothyroidism is a typically type 1 VWF deficiency due to decreased synthesis of the VWF protein. Treatment of hypothyroidism with thyroxine was associated with the disappearance of the AVWS and the bleeding diathesis.

The AVWS in Wilms' tumor is featured by undetectable or very low levels of VWF:Ag and VWF:RCo and moderate factor VIII:C deficiency. Multimeric analysis of VWF show a normal pattern consistent with type 1 VWD or type 3 VWD. A plasma factor, hyaluronic acid, secreted
by nephroblastoma cells of the Wilms' tumor may be responsible for the atypical or spurious AVWS in Wilms' tumor. Drug-induced AVWS has been described in association with the use of valproic acid, ciprofloxacin, griseofulvine, tetracycline, pesticide, thrombolytic agents and hydroxyethyl starch.

**MOLECULAR ASPECTS (J.E. Sadler, Chair)**

**Dr. A. Goodeve (U.K.)** presented a proposal for a standard nomenclature for von Willebrand factor gene mutations and polymorphisms. Examination of the entire VWF gene for mutations, particularly in type 1 VWD, is becoming more widely practiced and the sequence of the entire VWF gene is now available (although it has not yet been compiled as a single sequence). A common nomenclature to use for numbering the VWF nucleotide and amino acid sequence is therefore required. Two schemes have been used in the past for numbering VWF cDNA nucleotide sequence; from the mRNA cap site as +1 and from the A of the initiator ATG as +1. The latter is now recommended for future use. Amino acid numbering has previously been from the initiator ATG as the +1 position with sequential numbering of amino acids throughout VWF as in Bonthron et al (1986) and Mancuso et al (1989). Many authors working on type 2 VWD have however utilised an alternative scheme, with numbering of the mature VWF initiating from serine 763 of pre-pro VWF, and the use of a (c) prefix to denote propeptide numbers. It is envisaged that the latter scheme will cause increasing confusion as investigators examine the entire gene for mutations. The use of single letter amino acid codes, with sequential amino acid numbering for the whole of VWF is therefore recommended.

**Dr. R. Schneppenheim (Germany)** addressed the issue of a molecular approach to the classification of VWD. The classification of von Willebrand disease (VWD) is complicated by its pronounced heterogeneity both clinically and with respect to the underlying defects. For the clinician it is sufficient in many cases to differentiate between mere quantitative defects, represented by VWD type 1 and VWD type 3 and some functional defects in VWD type 2. However, this classification does not adequately describe the many subtypes of VWD type 2A which comprise all phenotypes with a lack of high molecular multimers (HMWM), except VWD type 2B. Furthermore, the current classification disregards compound phenotypes of different types or subtypes. According to the different mechanisms resulting in the lack of HMWM and in response to the description of new VWD phenotypes, an extension of the current classification seems necessary. This could be done by re-introduction of subtypes from the former classification, e.g. by designating VWF multimerization defects as VWD type 2C and VWF dimerization defects as VWD type 2D, respectively, and by creating new designations e.g. for novel functional deficits, impairment of intracellular transport and subcellular distribution of VWF.

**COFFEE BREAK**

**LABORATORY ASPECTS (F. Rodeghiero, Chair)**

**Dr. A.B. Federici (Italy)** The VWF:RCo/Ag and/or VWF:CB/Ag ratios: by which assays?
Despite the multiple functional domains of the VWF, only one basic assay has been widely and routinely used in the diagnosis of VWD so far, i.e. the ristocetin cofactor activity (VWF:RCo). Besides VWF:RCo, the collagen binding assay of VWF (VWF:CB) has been also proposed to measure VWF activity. However, there is no consensus about the best type of VWF:RCo assay and about the type of collagen (type 1 versus type 3 or equine versus human) to be used. To approach this issue, a brief retrospective survey was organized among ISTH Members searching for the tests of VWF activity routinely used by them. Information about this retrospective analysis was presented and discussed. Moreover, the results of an intra-laboratory study organized by the Hemophilia and Thrombosis Center of Milan to compare several assays for VWF:RCo and VWF:CB was also reported and a new diagnostic flow chart for type 2 VWD discussed. All these data emphasized the utility of a future large International Study on VWD diagnostic tests on behalf of the SSC-ISTH on VWF.

**Dr. J.C.J. Eikenboom (The Netherlands)** showed data on the FVIII/VWF ratio as a phenotypic predictor of the genotype. It is often stated that changes in plasma concentrations of VWF result in corresponding changes in the factor VIII levels. This may not be always the case and the ratio between factor VIII and VWF may depend on the genetic defect causing VWF deficiency. Data were collected from literature and from the VWF mutation database on heterozygous carriers of VWF null alleles, carriers of VWD type 2N mutations, and carriers of type 2B mutations. Data were found on 126 heterozygotes for null alleles, 29 heterozygotes, 15 homozygotes and 12 compound heterozygotes for type 2N mutations, and 81 heterozygotes for type 2B mutations. Carriers of null alleles had a FVIII:C/VWF:Ag ratio of two, carriers of type 2B mutations a ratio of one, and heterozygous carriers of type 2N also had a ratio of one, whereas homozygotes and compound heterozygotes for 2N mutations had a decreased ratio. There was no difference in this ratio related to blood group O and non-O in controls and carriers of null alleles. These data indicate that the change in factor VIII is not always concordant with the change in VWF, and that the ratio depends on the cause of the VWF reduction. The ratio may predict the genetic defect: reduced biosynthesis of VWF ó ratio increased; increased clearance VWF ó ratio unchanged; reduced factor VIII binding affinity ó ratio reduced.

**Mr. M. Mitchell (U.K.)** reported on differential diagnosis of VWD type 2N. Type 2N VWD, as a clinical disorder, is prone to considerable diagnostic error, due to similarities in phenotypic parameters between this form of VWD and the more common mild hemophilia A. Misdiagnosis is possible where laboratory investigations are incomplete and more sophisticated methodologies are unavailable. The complete and accurate diagnosis of this disorder is of major importance in selecting the most effective treatment options and in providing patients and their families with relevant information e.g. on genetic transmission. The recent publications on combined genotypes and atypical "type 2N" mutations have emphasised the need for a structured approach to diagnosis. The differential diagnoses of clinically similar diseases such as type 2N VWD, type 1 VWD, mild haemophilia A, and combined Factors V and VIII deficiency necessitate a co-ordinated approach involving traditional coagulation techniques, FVIII binding methodology, and molecular genetics.

**Dr. K. Friedman (USA)** presented an update of the international standardization study of VWD variants. The main findings were:
a. There is a considerable inter-laboratory variation, and adjustment of the results using a normal pooled plasma sample that was supplied did not improve the results.

b. Compared to the other techniques used, there may be a systematically lower VWF:Ag using Laurell immunoelectrophoresis. Similarly, VWF activity is possibly systematically underestimated by BCT device, and systematically higher results may be obtained with the "VWF-activity epitope" ELISA. These are preliminary data based on the 2-3 labs that reported these techniques.

c. Sending frozen samples from USA to some Europe locations was very expensive.

A follow-up study was proposed to determine if systematic variation by technique of assay is an issue. Participants would be chosen by assay technique used in their lab; Dr. Federici is conducting a survey of labs and he could provide this data. Participants would analyze several, well-characterized samples of type 1, 2A, 2B and 2M variants. Five - ten labs performing each of the three types of VWF antigen assay (Laurell immunoelectrophoresis, LIA test, and ELISA) and the four types of activity assay (Aggregometry, BCT automated technique, Collagen binding assay, and activity-epitope ELISA) will test the hypothesis that the assay technique may introduce a systematic bias.

**Dr. R. Seitz (Germany)** reported on the development of a collagen-binding assay as new European Pharmacopoeia method. In the current version of the European Pharmacopoeia (EP) monograph on factor VIII (1998:0275), it is stated that for products intended for treatment of von Willebrand Disease the von Willebrand factor (VWF) activity has to be determined by a suitable method, e.g. the ristocetin cofactor activity or the collagen binding activity. However, so far no EP method has been fixed for a collagen-binding assay (CBA). The expert group 6B considered two alternative CBAs and evaluated comparative data obtained by members of the group. The methods using different types of collagen differed in their sensitivity to VWF multimer fractions. The method using fibrillary type 1 collagen (based on a method published by K.B. Thomas et al, 1994) reflected more closely the high-molecular multimer content. The group 6B felt that this would be better correlated with VWF activity as relevant for therapy, and decided to further evaluate this assay as candidate EP method in a collaborative trial.

**Dr. U. Budde (Germany)** introduced a proposal concerning the standardization of multimeric assay in VWD. Since the first descriptions of methods for the evaluation of VWF multimers by Ruggeri and Zimmerman and Hoyer and Shainoff (1981) numerous modifications have been published. These methods differ in the type of agarose used, electrophoresis chambers (flat bed, vertical, submarine and Phast system), transfer (tank blot, semidry blot, vacuum blot and diffusion) and detection systems (radioactive, colorimetric and luminescence). Thus, standardization may be as hard to achieve as with the thromboplastin time. Dr Buddeís proposal was to define the common patterns of patients with type 2 VWD and set standards that should be met in diagnosis of these subtypes. In 1999, 166 patients with type 2 were diagnosed in Hamburg. 73% of type 2A, 13% of type 2M, 10% of type 2B and 4% of type 2N. Out of the patient with type 2A only 27% showed the ñclassical" pattern, while 29% had prominent inner sub-bands, and 26% resembled the previous type 1B.
Dr. C. Mazurier (France) addressed the issue of the nomenclature of VWF related abbreviations. In 1985, the Subcommittee on Factor VIII and von Willebrand factor of the International Committee on Thrombosis and Haemostasis published recommendations for a standard nomenclature for von Willebrand factor (Thromb. Haemost., 1985, 54, 871-2). The two proposed abbreviations "VWF" and "VWF:Ag" for the protein and its respective antigen have endured for more than two decades but many other abbreviations have been used for the transmission of laboratory data and in publications. Furthermore, no abbreviation was recommended for von Willebrand factor function. Recently the abbreviation "VWF" (all-capitalized) has been proposed for the von Willebrand factor protein (1999 Annual Report of Subcommittee on von Willebrand factor). If this abbreviation is adopted for general use, the abbreviation for von Willebrand antigen would be changed. Taking this opportunity, some abbreviations not only for von Willebrand antigen but also for von Willebrand factor pro-peptide and for well-established von Willebrand factor functions (ristocetin cofactor activity, collagen binding assay and factor VIII binding capacity) were proposed to the members of the subcommittee. All the suggested abbreviations were discussed in order to have an approval from the majority of the members of the VWF subcommittee before the submission of an official recommendation.

VWF-CLEAVING PROTEASE ASSAY (P.M. Mannucci, Chair)

Dr. P.M. Mannucci (Italy) reported on the VWF cleaving protease levels in health and disease. It has been recently recognized that thrombotic thrombocytopenic purpura (TTP) is due to the congenital or acquired deficiency of a metalloprotease that cleaves VWF physiologically. The deficiency of the VWF cleaving protease reduces or abolishes the removal from plasma of supranormal VWF multimers, that aggregate platelets intravascularly and thereby cause the thrombotic microangiopathy typical of TTP. Although the behavior of the VWF cleaving protease has been extensively studied in TTP and in the hemolytic uremic syndrome, there is little information in other physiological and pathological conditions. Using a recently developed method based on the preferential binding of large VWF multimers to collagen, Dr. Mannucci and his group measured the protease in four groups of healthy individuals of both sexes in the age groups 20-35, 36-50, 51-65 and over 65. While there was no difference between men and women, lower values were found in the elderly groups compared with groups of younger age (mean values expressed in % of overage normal plasma (96±22 vs 102±27). To evaluate whether the cleaving protease is synthesized in the liver, 18 patients with decompensated liver cirrhosis were studied: mean values were definitely lower than in normal individuals (43±28 vs 102±27). To evaluate whether the protease behaves as an acute phase reactant, it was measured in individuals with inflammatory states defined by serum levels of C reactive protein higher than 5 mg/dL. Data obtained so far in 10 patients would indicate that the VWF cleaving protease behaves as a negative acute phase reactant, being low in most patients (median value 55%, range 31-84). These data provide preliminary information on the behavior of the VWF cleaving protease in health and disease.

Dr. A. Veyradier (France) presented a prospective study of VWF-cleaving protease in different types of microangiopathies. Specific von Willebrand factor (VWF)-cleaving protease was reported to be a new potential biological tool to distinguish thrombotic thrombocytopenic purpura (TTP) from hemolytic uremic syndrome (HUS) since retrospective studies demonstrated
that its activity was decreased in TTP while normal in HUS. To further analyse this observation, she designed, together with her group, a multicentric national prospective study involving patients during acute phase of TTP or HUS. Forty adults, 25 TTP and 15 HUS diagnosed using usual clinical and biological criteria, were included over a one year period and tested for plasma VWF antigen (Ag), multimeric distribution and -cleaving protease activity (home-made method, Obert et al, Thromb Haemost 1999). VWF:Ag levels were increased in about 90% of patients from both groups with values close to 200 IU/dl in most cases. Ultralarge multimers of VWF were present in 60% of TTP and 27% of HUS. In the TTP group, VWF-cleaving protease activity (normal range > 50%) was normal in 3 patients and decreased in 22 patients including 17 patients with nil values related in 11 cases (65%) to an inhibitor. In contrast, in the HUS group, all patients demonstrated a normal VWF-cleaving protease activity, except one patient with a typical HUS whose protease activity was nil. Multivariate analysis using logistic regression showed that a decreased activity of VWF-cleaving protease was linked to the diagnosis of TTP (p = 0.0004), thrombocytopenia (p = 0.003), schizocytosis (p = 0.02) and the presence of ultralarge multimers of VWF (p = 0.04). The involvement of VWF-cleaving protease in the pathogenesis of TTP is probably important but rare exceptions in HUS make the specificity of its defect questionable.

PART II

MEASUREMENT AND STANDARDIZATION OF VWF CONTENT IN THERAPEUTICAL CONCENTRATES (C. Mazurier, Chair)

Drs. C. Mazurier (France) reported on the present state of the collaborative work between NIBSC (T. Barrowcliffe), FDA (M. Weinstein) and SSC VWF Subcommittee (C. Mazurier), initiated after the SSC Lubiana meeting (1998). Dr. A.R. Hubbard (NIBSC, U.K.) summarized the stability data (VWF:Ag, VWF:RCo, VWF:CB, electrophoretic profiles) obtained on the five VWF concentrates provided by different manufacturers and ampouled in NIBSC. Dr. A. Chang (FDA, USA) reported the results from the characterization of the five concentrates and on the assessment and rationale for selecting two candidates for the next phase of the study. Two of the preparations were proposed and accepted as suitable candidates for the WHO 1st International Standard VWF concentrate. Over the next 12 months a calibration will be carried out for VWF:Ag and VWF:RCo activity, using the 4th International Standard Factor VIII/VWF plasma (97/586) except for VWF:CB where participants will be asked to include local plasma pools in their assays.

Dr. A. Inbal (Israel) addressed the issue of correlation between ristocetin cofactor and collagen binding assay in patients with various types of VWD following VWF concentrate 8Y using pharmacokinetic analysis. Ten patients with various types of VWD underwent pharmacokinetic analysis. The patients received 30-50u/kg 8Y and VWF:RCo, FVIII:C, VWF:Ag and VWF:CB (measured by home-maid assay using type III collagen) were measured before and at constant time intervals after the infusion. Using the model-independent method, the recovery, clearance, volume of distribution and half-life of the above parameters were calculated. For 4 patients with type 2A VWD the recovery, clearance and half-life of 8Y were 1.99 ± 0.06 %/kg/U; 2.84 ± 0.70 ml/hr and 12.7 ± 5.93 hours, respectively. For 3 patients with type 3 VWD the recovery, clearance and half-life of 8Y VWF:RCo were 1.42 ± 0.11%/kg/U; 5.08 ± 1.10 ml/hr and 7.88 ±
0.69 hours, respectively. For 2 patients with type 1 VWD the recovery, clearance and half-life of 8Y VWF:RCo were 2.24 ± 0.52 %/kg/U; 2.59 ± 0.96 ml/hr and 14 ± 1.17 hours, respectively. In 1 patients with Normandy type VWD the recovery, clearance and half-life of 8Y VWF:RCo were 2.65%/kg/U; 3.08 ml/hr and 32.85 hours, respectively. Different values of VWF:CB recovery, clearance and half-life were obtained. Strong correlation was observed between pharmacokinetic parameters of VWF:Ag and VWF:CB (r: 0.78 ó 0.87) in patients with types 1, 2A and 3 VWD. Similarly, very strong correlation was observed between VWF:RCo and VWF:CB in type 3 patients (r = 0.9). In contrast, no strong correlation between pharmacokinetic parameters of VWF:RCo and VWF:CB was observed in patients with type 2A and type 1 VWD (r: 0.47 ó 0.62). In conclusion, Following 8Y infusion there is a strong correlation between VWF:CB and VWF:Ag in patients with type 1, type 2A and type 3 VWD. In contrast, the correlation between VWF:RCo and VWF:CB was observed only in type 3 VWD.

**Dr. D. Varon (Israel)** reported on the monitoring of VWF replacement therapy (Hemate-P) in patients with various types of von Willebrand Disease using Cone and Plate(let) Analyzer (CPA). The aim of this study was to apply the cone and plate(let) analyzer (CPA) method in monitoring VWF replacement therapy among VWD patients of various types, and to compare the CPA and the RCo % methods. In the CPA method whole blood platelets (200 μl) are tested for adhesion and aggregation on a thrombogenic substrate under arterial flow conditions (shear rate of 1800 sec⁻¹) utilizing a cone and plate device. The degree of adhesion is determined by an image analysis system and expressed as the % of surface coverage, (SC%, normal range 9% - 16%). Factor VIII and VWF:RCo activity was determined by standard methods. Eleven patients underwent pharmacokinetic studies. Seven patients (severe VWD-4, acquired VWD-3) received Haemate-P while four patients (type 1 VWD) received DDAVP. Changes in mean (± se) CPA values with time reflected closely the change in mean (± se) RCo %. Simple linear regression revealed a statistically significant correlation between the two parameters. The CPA was found as a useful method for testing VWF activity. The small volume and the short testing time (4-5 min) make the CPA method an attractive alternative for testing and monitoring VWF replacement therapy.

**Dr. P. Turecek (Austria)** addressed the issue of determination of VWF activity in plasma samples and in concentrates by a commercially available collagen binding assay. Collagen Binding Activity (CBA) reflects the physiological hemostatic activity of von Willebrand factor (VWF) in plasma and in VWF concentrates and, thus, describes a major function of the protein. A new collagen binding assay has been developed for the determination of the functional activity of human von Willebrand factor based on the following principle:

type III collagen from human placenta was covalently immobilized on a microtitre plate. Binding of collagen to the microtitre plate was carried out in neutral phosphate buffer within one hour. A collagen concentration of 3 µg/ml was sufficient to achieve optimal coating. After drying, the coated microtitre plates remained stable for months without losing their VWF-binding properties and could be incorporated into a ready-to-use kit and has been commercialized under the brand name Immunozym VWF:CB. The assay thus comprises the following steps: Serial dilutions of a VWF reference preparation and VWF-containing samples are prepared and bound to a microtitre plate, which is pre-coated with collagen; VWF is detected with a polyclonal antibody; The substrate reaction is photometrically measured with an ELISA reader. Currently the Immunozym
VWF:CB is under evaluation in clinical investigations of VWD. The use of a standardized commercially available test kit could minimize inter-laboratory variability sometimes seen with in-house assays lacking reproducible reagent preparation. Thus, the collagen binding assay may represent a suitable replacement for the common ristocetin cofactor method.

PAST, PRESENT AND FUTURE STUDIES IN FEMALES WITH VWD (P. Kouides, Chair)

For what concerns the British perspective, Dr. C. Lee (U.K.) presented data on the obstetric complications of menorrhagia. Menorrhagia is both a common symptom in those patients already diagnosed as having von Willebrand's disease (VWD) and is a common presenting symptom of the condition. The diagnosis of menorrhagia is >80 ml of blood loss per month and this is equivalent to a score of 100, using a pictorial bleeding assessment chart (PBAC). This chart has been applied to make assessments in individuals with VWD, in order to develop a rational treatment program. It has also been used to screen women presenting with menorrhagia at gynecological clinics, in order to diagnose VWD. In view of the limited data on the prevalence of VWD, it would be possible to use the PBAC to screen populations of young women. Both primary and secondary post-partum hemorrhages have been shown to have a higher incidence in women with VWD. It would be helpful if protocols could be developed both for the management and treatment of menorrhagia and the post-natal management of women with VWD.

Dr. P. Giangrande (U.K.) reviewed the management of pregnancy in VWD, with a particular reference to the use of desmopressin in pregnant women. In a recent review of obstetric management in VWD, 16% of VWD females had bleeding with vaginal delivery and 25% experienced post-partum hemorrhage. Current data indicate that the risk of hyponatremia represents a contraindication to the use of DDAVP in pregnant women, since the response to DDAVP is exaggerated in pregnancy and osmolality falls by 10 mOsm/kg in pregnant women. DDAVP may however be used after delivery, with cord clamped. UK guidelines on management of pregnancy in women with VWD recommend that FVIII/VWF should be checked at 34-36 weeks and vaginal delivery should be regarded as safe if VWF activity is above 40 IU/dL; Cesarean section should be considered as safe if VWF activity is above 50 IU/dL.

As to the American perspective, Dr. P. Kouides (USA) presented a review of on-going US VWD prevalence studies in menorrhagia and Center for Disease Control Project for the management of VWD related menorrhagia. Retrospective studies of the obstetrical and gynecological complications of female VWD were reviewed, particularly a survey of 99 female VWD patients in New York USA that were compared to a cohort of "normal" menstruating volunteers. Questions generated from such studies were reviewed. In terms of menorrhagia therapy: (i) How effective is hormonal therapy for VWD-related menorrhagia? (ii)

Is intranasal or subcutaneous DDAVP home therapy of VWD related menorrhagia clearly effective; (iii) Should anti-fibrinolytic therapy be "front-line" therapy for VWD-related menorrhagia. In terms of peri-partum management: (i) Is the miscarriage rate in type 1 patients significantly greater than the normal population? (ii) Should post-partum type 1 patients be empirically treated at home with intra-nasal or subcutaneous DDAVP for several days after delivery (iii) Should type 2A,B patients have third trimester FVIIIc and VWF level testing and
not receive therapy peri-partum if the FVIIIc level is > 50% or just empirically be given peri-partum VWF-containing concentrates (even if the FVIIIc level is >50%)? (iv) What is the safety of DDAVP ante-partum? (v) What is the risk, if any, of excessive bleeding with circumcision in a type 1 VWD neonate? An ISTH registry of obstetric complications and the attendant management will be proposed with the intent to collect an adequate number of cases internationally in answering these questions. Lastly, prospective studies of screening for VWD in women with menorrhagia were presented in the context of the Swedish study by Edlund et al, the British study by Kadir/Lee et al and 2 American studies nearing completion (CDC-Atlanta, Rochester NY). The need to "pool" data with the intent to determine the degree menorrhagia is related to Blood type O and to develop a screening tool by history to predict VWD in women presenting with menorrhagia was discussed.

With regard to the Italian perspective, Dr. A. Tosetto (Italy) presented a project on the prevalence of VWD among women discharged with a diagnosis of menorrhagia. The objective of the study is to evaluate the etiologic fraction of VWD in menorrhagia, as ascertained by an epidemiologic investigation in a well-defined cohort of females. The pros and cons of epidemiologic surveys vs. observational studies in patients were discussed.

**SUMMARY OF SUBCOMMITTEE ACTIVITIES**

**Issues voted:**

- A consensus nomenclature concerning gene mutations and polymorphisms of VWF has been approved and will be submitted to SSC for publication as an official recommendation.
- VWF-related abbreviations were approved in principle. A detailed letter with the suggested abbreviations will be circulated among the members of the Subcommittee for formal approval and submission to SSC for publication as an official recommendations.
- Creation of a new Working Party on the standardization of basic diagnostic tests (Friedman), including VWF:CB (Federici) and multimeric assay (Budde), aimed at implementing a new more comprehensive collaborative international study, was approved.
- Creation of a new Working Party on VWD in females and related issues was approved.

**Ongoing projects:**

- Survey of VWD prevalence and impact in developing countries.
- Multicenter, retrospective study for the validation of the diagnostic criteria of type 1 VWD.
- The WP for VWF assay in concentrates will continue to cooperate with FDA and NIBSC and set up a collaborative study for the potency assignment against the WHO 4th International Standard Factor VIII/VWF plasma (97/586) to two selected concentrate preparations to be used as the first concentrate standard for VWF content in therapeutical concentrates.