

1996 MINUTES

VON WILLEBRAND FACTOR SUBCOMMITTEE

Sunday, 23 June, 1996, 8.00 - 12.00
Room Vivaldi, Fira Palace Hotel
Barcelona, Spain

Chair: J. Evan Sadler, USA
Co-Chairs: Paul Foster, USA; David Ginsburg, USA;
Dominique Meyer, France; Francesco Rodeghiero, Italy

The Subcommittee met Sunday, June 23, at the Fira Palace Hotel in Barcelona, Spain, from 8:00 to 12:00.

Presiding chair was Dr. Dominique Meyer. Dr. Sadler and Dr. Ginsburg did not attend. Other co-chairs were present.

Attendance was approximately 80.

Recent developments in VWD were presented in a session chaired by Dr. Dominique Meyer. Dr. Claudine Mazurier summarized the discovery of new mutations in VWD during the past year. Dr. Meyer reported on the use of the PFA-100™ (Dade International) for screening in VWD, and on an automated latex bead immunoassay for VWF (Diagnostica Stago). Dr. Emmanuel Favaloro described the use of a collagen binding assay (VWF:CBA) in the laboratory assessment of VWD.

During the past year, the Subcommittee has developed consensus criteria for the diagnosis of VWD type 1. Dr. Pier Mannucci chaired a session in which these criteria were presented and discussed. Dr. Francesco Rodeghiero summarized the criteria for the use of patient history and family history. Dr. Giancarlo Castaman summarized the consensus approach to laboratory tests. Dr. Mannucci presented the specific criteria for the diagnosis of VWD and possible VWD. Several suggestions for modification of the criteria were offered. By a vote of the members these criteria were APPROVED for further evaluation in patient populations after consideration of the suggested modification by the working party.

Dr. Paul Foster chaired a session to consider Subcommittee projects for the next year. Dr. Rodeghiero proposed a retrospective study of consensus criteria for VWD type 1. A proposal for the retrospective validation of the diagnostic criteria in two patient populations was made and the creation of a Working Party on VWD Diagnosis for this purpose was proposed and members recruited. Dr. Foster discussed issues of standardization for patient populations and laboratory tests. Creation of a Working Party on VWF Assays was also proposed and members recruited. This Working Party will address the standardization and evaluation of laboratory tests in VWD, including VWF:CBA and other candidate assays in the diagnosis of VWD.

The following article will be published during 1996:

Mazurier C, Meyer D: Factor VIII binding assay of von Willebrand factor and the diagnosis of type 2N von Willebrand disease. Results of an international survey. *Thromb Haemostas*, in press.

1997 MINUTES

SUBCOMMITTEE ON VON WILLEBRAND FACTOR

Saturday, 7 June, 1997, 8:00-16:30

Botticelli, Fortezza da Basso

Florence, Italy

Chair: J. E. Sadler, USA

Co-Chairs: P. Foster, USA; D. Meyer, France; F. Rodeghiero, Italy

Attendance was approximately 200.

Dr. Augusto B. Federici discussed a proposal for an international registry on acquired von Willebrand syndrome (AVWS). Based on the preliminary analysis of responses to a survey questionnaire on AVWS, mailed by Dr. Federici and Dr. Jacob Rand, creation of a Working Party on AVWS was APPROVED by voice vote.

Dr. William C. Nichols presented an update on the VWD mutation and polymorphism databases. These resources continue to be used heavily. In the absence of specific financial support, the currency and accuracy of the database is dependent on voluntary entries by the scientific community. Efforts are underway to incorporate the VWF database into other more general mutation database projects.

Dr. David L. Aronson discussed proposed labeling recommendations for plasma products for use in VWD. A survey of 38 treatment centers yielded 33 responses, 31 of which favored labeling factor VIII products for VWF content.

Dr. Mark J. Weinstein (Acting Chief, Hemostasis Laboratory, U.S. Food and Drug Administration) discussed the benefits and liabilities of using the ristocetin cofactor activity as a means of defining VWF activity and proposals for standardizing VWF-containing concentrates. These issues will be addressed in detail at a workshop on von Willebrand factor to be held at the National Institutes of Health on September 26, 1997. Members of the Working Party on VWF Assays will attend. A proposal was APPROVED by voice vote that manufacturers should be encouraged to meet the requirements to label Factor VIII products for treatment of VWD with VWF content. Measures to establish this practice will be considered by the Working Party on VWF Assays.

Dr. Dominique Meyer presented a report on the activities of a network of 32 hemostasis centers throughout France, supported by INSERM. Its aims are to improve the diagnosis of VWD, to assess the frequency of the different types of VWD, and to identify new mutations in the VWF gene.

Dr. Federici gave a progress report on the Italian Registry of VWD. A computer database was developed to collect information from 33 hemophilia centers at four-month intervals. An analysis of data for the first 637 patients from eight centers was presented.

Dr. Sadler reviewed the activities of the Subcommittee during 1995-1996 to develop consensus guidelines for the diagnosis of VWD type 1. The consensus guidelines for diagnosis of VWD type 1 were APPROVED for further evaluation through a retrospective collaborative international study by the Working Party on VWD Diagnosis.

Dr. Robert R. Montgomery chaired a session of the Working Party on VWF Assays. Dr. Trevor Barrowcliffe addressed issues concerning VWF standards, including the replacement of the current WHO plasma standard. In further discussion the topics considered were: (1) the production and distribution of VWF standards for plasma and concentrates; (2) the use of standards to evaluate assays for VWF:Ag, VWF:RCo, and multimers; and (3) the development of specific recommendations for the use of assays in diagnosis and product standardization.

Dr. Francesco Rodeghiero chaired a session of the Working Party on VWD Diagnosis. He proposed a collaborative international study to retrospectively assess the value of bleeding history and laboratory measurements in the diagnosis of VWD type 1. In response to a preliminary feasibility questionnaire, at least 20 centers around the world have agreed to participate so far. Dr. Alberto Tosetto briefly presented some methodological issues relating to the design of this study. Dr. Giancarlo Castaman discussed the relationship between phenotype and genotype based on available data.

SUMMARY OF SUBCOMMITTEE ACTIVITIES

Issues voted:

1. Creation of a Working Party on AVWS was APPROVED.
2. The proposal, that manufacturers should be encouraged to meet the requirements to label Factor VIII products for treatment of VWD with VWF content, was APPROVED by voice vote.
3. Consensus guidelines for diagnosis of VWD type 1 were APPROVED for further evaluation by the Working Party on VWD Diagnosis, through a retrospective collaborative international study.
4. A kit of VWF plasma and concentrate standards, and plasma samples from VWD subtypes and controls, will be assayed by collaborating laboratories to test the ability to diagnose VWD subtypes. This study was APPROVED by voice vote.

Completed projects:

C. Mazurier, D. Meyer
Factor VIII Binding Assay of von Willebrand Factor and the Diagnosis of Type 2N von Willebrand Disease. Results of an International Survey. *Thrombosis and Haemostasis*, vol. 76, 1996, p. 270.

Ongoing projects:

1. Issues relating to the labeling of factor VIII products with VWF content will be addressed in detail at a workshop on von Willebrand factor to be held at the

National Institutes of Health on September 26, 1997. Members of the Working Party on VWF Assays will plan to participate.

2. The Working Party on VWF Assays will address the standardization and evaluation of laboratory tests for the labeling of blood products and for the diagnosis of VWD subtypes.
3. The Working Party on VWD Diagnosis will conduct a collaborative, international retrospective study of diagnostic criteria in VWD type 1.

1998 MINUTES
SUBCOMMITTEE ON VON WILLEBRAND FACTOR
Sunday, 21 June, 1998, 13:00-17:00
Cankarjev Dom
Ljubljana, Slovenia
Chair: F. Rodeghiero, Italy
Co-Chairs: A. Federici, Italy; C. Mazurier, France;
R. Montgomery, USA; J. E. Sadler, USA

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

Attendance was approximately 80.

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

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

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

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

Dr. Rodeghiero presented the final version of the project for a multicenter retrospective study on VWD type 1 diagnosis. The study implies direct reinvestigation of carriers of type 1 and 3 and their relatives. Interested centers are invited to participate.

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

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

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

SUMMARY OF SUBCOMMITTEE ACTIVITIES

- Issues voted:

The information provided by available national registries or surveys has been considered sufficient. Uniform entering criteria are needed before fostering additional national registries
Creation of a WP to prospectively evaluate diagnostic criteria for AVWS

Creation of a WP for molecular diagnosis of type 1 VWD

Further studies are needed before the "functional" ELISA can be proposed as a substitute of VWF:RC of activity

- Ongoing projects:

Updating of the International Registry on AVWS.

The WP on AVWS will prepare a protocol for prospective evaluation of AVWS diagnosis.

Activation of the multicenter, retrospective study for the diagnostic criteria for type 1 and type 3 VWD.

Creation of a WP to test the ability to diagnose VWD subtypes through a kit of VWF plasma and concentrates standards, and plasma samples from VWD subtypes and controls.

The proposal that the 4th IS be assigned the mean of the estimates vs. the 3rd IS and the local plasma pools for VWF:Ag and VWF:Rcof will be submitted to the members of the Subcommittee.

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

1999 MINUTES
VON WILLEBRAND FACTOR
Sunday, 15 August 1999
8:00 to 12:00 PM
Room 40
Washington Convention Center
Washington, DC
Chair: F. Rodeghiero, Italy
Co-Chairs: A.B. Federici, Italy; C. Mazurier, France;
R.R. Montgomery, USA

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

Attendance was approximately 350.

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

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

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

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

Dr. C. Mazurier (France) chaired the session of the Working Party on the measurement of VWF content in therapeutic products. Dr. A. Hubbard (U.K.) and Dr. A. Chang (U.S.A.) reported on the joint NIBSC and FDA study for the identification of a suitable concentrate preparation to be used as a future standard. Dr. T. Barrowcliffe (U.K.)

presented data on potency assignment of the 4th International Standard of FVIII:VWF adopted by WHO. Dr. R. Seitz (Germany) presented two methods for VWF:CBA in concentrates.

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

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

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

SUMMARY OF SUBCOMMITTEE ACTIVITIES

Issues voted:

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

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

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

Ongoing projects:

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

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

Survey on VWD in developing countries.

Proposals for genetic and molecular terminology of VWF/VWD.

1999 ANNUAL REPORT
SUBCOMMITTEE ON VON WILLEBRAND FACTOR
Sunday, 15 August 1999, 08:00-12:00
Room 40, Washington Convention Center
Washington, USA
Chair: F. Rodeghiero, Italy
Co-Chairs: A.B. Federici, Italy; C. Mazurier, France; R.R. Montgomery, USA

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

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

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

<u>AvWS (total 221):</u>	<u>LPD(98)</u>	<u>MPD(40)</u>	<u>CVD(27)</u>	<u>NEO(14)</u>	<u>OTH(32)</u>
sex (% of male)	59	38	56	50	46
age at onset (yrs)	63	46	57	61	62
bleeders (%)	70	38	11	50	50
in follow-up (%)	58	18	59	50	67
VWF:Ag (U/dL, mean)	25	68	120	32	31
VWF:RCof(U/dL,mean)	8	22	68	17	7
FVIII:C (U/dL,mean)	21	33	131	23	25
pos anti-FVIII/VWF (%)	14	2	n.t.	14	12

Effective therapy with:

ddavp (%)	31	15	7	21	19
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fVIII/VWF conc (%)	38	5	7	43	22
immunoglobulin (%)	16	0	0	14	3

The data from this International Registry suggest that AvWS is highly associated with certain disorders such as LPD, MPD, CVD. A practical flowchart for the diagnosis and the management of AvWS in these underlying disorders was discussed and approved during the meeting. An official document of the SSC-VWF will be prepared by Federici, Rand, Mannucci, Budde, Mohri, van Genderen, Bucciarelli and Rodeghiero. This report will be submitted to Thrombosis and Haemostasis.

REPORT FROM THE JOINT ISTH/WHO MEETING IN LONDON

(J.E. Sadler, Chair)

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

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

Assessment and Monitoring: National Patient Registries

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

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

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

Laboratory Methods and Quality Assurance

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

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

VWD in the Developing Countries

Dr. A. Srivastava (India) reported on the magnitude of the problem of VWD in developing countries, which is undefined due to the lack of awareness of this disorder, inadequate facilities for diagnosis and limited resources for treatment. In preparation for the joint WHO/ISTH meeting on this disorder in 1998, a questionnaire survey was conducted by Dr. Srivastava to record the available information on the prevalence, diagnosis and treatment of VWD in as many developing countries as possible. Responses were received from 22/56 countries (39%) that were approached. The results are shown in the table. The salient features are: 1.) Majority of patients are unidentified; 2.) There is a higher proportion of patients with severe disease, particularly in those countries with significant levels of consanguinity; 3.) At least one center capable of

performing VWF:Ag and/or RiCof exists in most countries but facilities for multimer analysis are uncommon; 4.) Entire range of products from FFP to purified factor concentrates are used for replacement; 5.) Countries where treatment is supported by government/insurance, "on-demand" replacement is provided, while in others only major bleeds are treated with factor replacement; 6.) Surgeries, both minor and major, are performed in most countries.

While this survey provided a glimpse of VWD in developing countries, more detailed and precise data would convey better the impact of VWD in these countries. At the joint WHO/ISTH meeting in London, it was suggested that a system be established to collect data on VWD from more developing countries. In collaboration with Dr. F. Rodeghiero, the previous questionnaire was modified to include data on mortality and on severe hemophilia from the same referral area to assist in estimation of the true prevalence of VWD in that population. This survey will be conducted over the next 12-18 months. ISTH could help the cause of VWD in developing countries with a training program and by promoting joint collaborative projects of mutual interest between centers in developed and developing countries. ([See Table, attached.](#))

MOLECULAR DIAGNOSIS OF VWD (I. Peake, Chair)

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

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

times per week in 1998, and 161 times per week in 1999. The current rate of access is approximately 8,400 hits per year.

Other Internet resources also contain information on VWD mutations. The Human Gene Mutation Database Cardiff at the location <http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html> lists approximately 115 VWD mutations.

The Genome Database (GDB) contains approximately 28 VWF polymorphism entries. Online Mendelian Inheritance in Man (OMIM) includes VWD as MIM #193400 and lists 28 mutations. These resources overlap to some extent but each contains some unique information.

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

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

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

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

Dr. T. Barrowcliffe (UK) described the final results of the collaborative study designed to establish the 4th International Standard (IS) for FVIII and VWF plasma. For VWF:Ag and

VWF:RiCof, mean potency estimates against the previous IS exceeded those against fresh plasma pools by 14 and 20 % respectively, indicating a possible drift of the International Unit away from normal plasma. It was proposed to make a partial correction for this drift by assigning potencies as the means of the two estimates. This proposal was supported by members of the Subcommittee to whom the report had been circulated, and was accepted by the Expert Committee on Biological Standardisation of WHO, which accordingly established plasma 97/586 as the 4th IS for FVIII/VWF plasma.

Dr. R. Seitz (Germany) asserted that a reliable assay of VWF potency in concentrates is a prerequisite for their clinical use for treatment of VWD. The European Pharmacopeia Expert Group 6B currently evaluated two candidate VWF collagen binding methods: (1) commercial method (Baxter), using pepsin-treated soluble type III collagen of human placenta, and (2) in-house method (Paul Ehrlich Institut, modified after Thomas et al, 1993), using fibrillary type I collagen of equine tendon. Method (1) is robust and reproducible and is sensitive also to low and medium MW multimers, while method (2) gives lower values in concentrates containing predominantly low MW multimers. The question is which method would more adequately reflect the clinically relevant VWF activity. The Expert Group 6B will go on with their evaluation of the methods and would appreciate the advice of the SSC on this matter.

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

WORKING PARTY ON LABORATORY DIAGNOSIS OF VWD

(R. Montgomery, Chair)

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

On the basis of the encouraging results of this study, Dr. R. Montgomery (U.S.A.) made specific recommendations on behalf of the Working Party on Diagnosis of VWD. A number of different international laboratories should study a group of molecularly defined VWD variants with ratios of VWF:RiCof/VWF:Ag, VWF:CBA/VWF:Ag, and FVIII/VWF:Ag to determine agreement of assays. These assays should be compared to internal standards within that laboratory and between the study laboratories. A cohort of type 1, heterozygous type 3, and type 1 patients with different ABO blood groups should be compared by a small number of multinational

laboratories. Multiple methods for ristocetin cofactor and collagen binding should be compared using multiple participating laboratories. All of above should be directly compared to the 4th International Standard. Commercially available standards should be compared to the 4th International Standard in multiple laboratories. Using the 4th International Standard, several laboratories should determine VWF antigen levels in 50 unselected individuals to determine the mean and standard deviation of normal individuals with this standard.

Shipping costs should be borne equally by participating laboratories.

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

Individuals(n):	[A] VWF:Act/Ag	[A vs B]	[B] VWF:RCof/Ag	[C] VWF: CBA/Ag	[C vs B]
Normals (25)	0.90 (0.16)	ns	0.97 (0.20)	1.01 (0.16)	ns
1 PN (22)	1,03 (0.24)	ns	0.99 (0.17)	0.99 (0.16)	ns
1 PL (13)	0.88 (0.65)	ns	0.67 (0.25)	0.78 (0.29)	ns
1 PD (7)	0.79 (0.40)	0.015	0.34 (0.17)	0.97 (0.17)	<0.001
2 A (10)	0.89 (0.34)	<0.001	0.31 (0.18)	0.47 (0.29)	0.07
2 B (16)	0.96 (0.31)	<0.001	0.56 (0.20)	0.75 (0.22)	0.004
2 M (13)	1.11 (0.63)	ns	0.94 (0.50)	0.86 (0.44)	ns

(Vicenza)

The home-made VWF:RCof [TEST B] is more sensitive than VWF:ACTIVITY kit [TEST A] and VWF:CBA [TEST C] to identify defects due to abnormal VWF multimers (1PD, 2A and 2B).

Dr. A.B. Federici (Italy) reported VWF:(RCo/Ag) ratio in the different types of VWD. According to the study of different VWF functional assays previously shown, Dr. Federici

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

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

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

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

Dr. C. Miller (U.S.A.) reported on difficulties with ristocetin cofactor measurement using commercial kits. A number of laboratories in the U.S. have had difficulties with the ristocetin cofactor assay over the past 2-3 years when using commercial reagents. The problems consist of inability to produce an acceptable standard curve, erratic results for the same sample during a

single assay, and poor reproducibility of results from day to day. Quality control was unacceptable for patient care, causing many labs to abandon the assay. Reagents from three companies gave similar results, in two different brands of aggregometer. Dr. Miller has tracked the problem and found the common factor to be ristocetin, all of which comes from a single source. The U.S. companies have been reconstituting and then re-lyophilizing the material to be packaged in their kits. The ristocetin as originally supplied performed well in our hands, with over 250 tests on study subjects all with excellent results. The problem apparently lies with the quality of the processed ristocetin. Recommendation was made that lots of ristocetin be characterized for activity and stability prior to packaging to assure that the consumer gets a reliable product.

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

VWF-CLEAVING PROTEASE ASSAY

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

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

CLOSING REMARKS

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

SUMMARY OF SUBCOMMITTEE ACTIVITIES

Issues voted:

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

Ongoing projects:

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

2000 ANNUAL REPORT

SUBCOMMITTEE ON VON WILLEBRAND FACTOR

Friday, 16 June 2000, 08:00-12:00 and 13:30-17:30

Maastricht Meeting and Congress Center

Maastricht, The Netherlands

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

FRIDAY MORNING

(08:00 TO 12:00)

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 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 erythematosus (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 $1000 \times 10^9/l$ 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 ($<400 \times 10^9/l$) 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 δ ratio increased; increased clearance VWF δ ratio unchanged; reduced factor VIII binding affinity δ 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. Participant labs 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 Budde's 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 "classical" 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 average 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

FRIDAY AFTERNOON

13:30 TO 17:30

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

Dr. 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-made 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 ($r: 0.78 \text{ } \acute{0} \text{ } 0.87$) 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 ($r: 0.47 \text{ } \acute{0} \text{ } 0.62$). 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 (\pm se) CPA values with time reflected closely the change in mean (\pm 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 $\mu\text{g/ml}$ 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 Immunozytm 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 Immunozytm 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 recommendation.
- 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

VON WILLEBRAND FACTOR

7 July 2001

08:00 to 12:00

Room Salle Maillot

Palais des Congrès

Chairman: R.R. Montgomery--USA

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

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

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

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

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

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

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

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

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

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

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

Dr. Goodeve presented the approved plans by the European Union on centralized molecular testing in Europe. The study on molecular and extragenic causes of VWD or low VWF was discussed.

Dr. D. Lillicrap (Canada) reviewed polymorphic haplotypes in type 1 VWD. These studies are going to be done collaboratively with the EU Study presented by Dr. Goodeve.

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

Dr. Montgomery introduced the third session on Laboratory Standardization.

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

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

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

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

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

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

SUMMARY OF COMMITTEE ACTIVITIES

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

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

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

Ongoing projects:

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

Study of general availability of VWF testing in developing countries.

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

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

Study of VWF in more diverse ethnic groups.

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

Continue standardization of the VWF-cleaving protease assay.

Laboratory partnering for greater availability of VWF testing worldwide.

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

Von Willebrand Factor

July 19, 2002

14:00 to 18:00

Georgian Room

Boston Park Plaza Hotel

Chairman: R. Montgomery, USA

Co-chairs: G. Castaman, Italy; J. Eikenboom, The Netherlands; A. B. Federici, Italy;
A. Goodeve, UK; P. A. Kouides, USA; C. Mazurier, France; J. H. Rand, USA

The VWF Subcommittee was attended by about 125 attendees at the first session and 100 participants for the second session. They actively participated in the discussion of the various talks provided by the program. The following represents a full summary of the program.

Cooperative Studies (J. Eikenboom, Chair)

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

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

Dr. Rodeghiero reported the final results of the "Validation of the Diagnostic Criteria of Type 1 von Willebrand Disease: an International Multicenter Study" that was initiated by the SSC in 1997. The aim of this study was to investigate the contribution of bleeding history to the

diagnosis in a sample of obligatory carriers of type 1 (n=42) and type 3 VWD (n=70). Bleeding history was compared with that of affected members and age and sex-matched controls (n=224). A standardized questionnaire was used to evaluate each hemorrhagic symptom at presentation, using a score system (0: no symptoms, 1: mild symptoms; 2: symptoms requiring medical attention only; 3: symptoms requiring hospitalization, replacement therapy or blood transfusion). The severity of epistaxis, menorrhagia and bleeding after tooth extraction is similar but not equal in obligatory carriers of type 3 VWD and controls; affected subjects in type 1 and 3 families show a clear prevalence of score 1, 2 and 3. For type 1 VWD obligatory carriers vs. controls, menorrhagia and epistaxis have a low sensitivity, whereas cutaneous bleeding is the most sensitive symptom. For type 3 VWD obligatory carriers vs. controls, menorrhagia and bleeding after tooth extraction have a low sensitivity, whereas bleeding after surgery is the most sensitive symptom. Laboratory screening in subjects with at least two hemorrhagic symptoms seems a reasonable option.

Database and Classification (A. Goodeve, Chair)

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

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

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

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

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

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

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

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

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

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

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

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

Nomenclature (G. Castaman, Chair)

Dr. Mazurier summarized the current [recommended abbreviations for VWF](#) activities as published in *Thrombosis and Haemostasis* in 1991. All authors, reviewers, and Editors were requested to use this nomenclature.

Dr. Mazurier proposed an abbreviation for VWF multimers as VWF:Mult or VWF:Mers. Since these were not quantitative measurements, it was decided to refer to these as VWF multimers rather than any further abbreviation. A group will be appointed to make recommendations by next year on methods to quantitate the relative concentration of the various size multimers.

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

publications the propeptide should be referred to as the VWF propeptide.

Standardization Issues (A. Federici, Chair)

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

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

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

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

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

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

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

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

Dr Han Mou Tsai reviewed the recent identification of the VWF-cleaving protease as ADAMTS13 and reported on his assay methodology. The protease has been purified from plasma and the gene has been mapped to chromosome 9q34. Other studies report that some

patients without TTP have low (<30%) levels of the VWF-cleaving protease and suggest that the decrease may have pathophysiological implications. A review of his data reveals that mildly decreased ADAMTS13 levels are detected in some patients with various pathological conditions, however, the decreases are generally not as profound as described by others. The discrepancy most likely results from difference in the assay methods, some of which generate broad ranges among normal individuals. Since the decrease does not correlate with the severity of the underlying diseases and is not associated with an increased VWF size, the significance of the observed decrease is unclear and should be interpreted with caution. He also compared the various current assay methodologies.

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

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

Final recommendations

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

Robert R. Montgomery, Chair

Von Willebrand Factor

July 12, 2003

14:00 to 18:00

Hall 11

The International Convention Center, Birmingham

Chairman: R. Montgomery, USA

Co-chairs: G. Castaman, Italy; D. Lillicrap, Canada; J. Eikenboom, The Netherlands;

A. B. Federici, Italy; A. Goodeve, UK; P. A. Kouides, USA;

C. Mazurier, France; F. Rodeghiero, Italy

Summary of VWF Subcommittee Approvals and Working Parties

1. The Subcommittee unanimously approved the FVIII VWF standard proposed by A. Hubbard and the IBSC.
2. The working Party study of intra laboratory assay standardization of the ADAMTS13 protease. All but 2 centers have completed their assays and we expect a final report at the next SSC Meeting. (P.M. Mannucci, Chair)
3. The working party on diagnosis of VWD and variant VWD is in active progress and should be able to send lyophilized patient plasmas to study centers this fall and expects to have accomplished this by next SSC Meeting. (A. Federici, Chair)
4. The working party on response to DDAVP has developed a web site and will be operational soon. (A. Federici, Chair)
5. Approved a new working party to re-examine the current adequacy of VWD classification as a 10 year follow-up to the VWF Subcommittee's current classification. A formal report is expected by the next SSC Meeting. (J.E. Sadler, Chair)
6. Approved a Joint Working Party with the Platelet Immunology Subcommittee to develop a registry of type 2B VWD and Platelet-type VWD because of their association with thrombocytopenia. (Jim Bussel, New York)

Issues Identified as Requiring Further Study or Standardization

1. Type 1 VWD as a discrete disease or disorder versus VWF as a continuous risk factor and how this should be approached internationally.
2. Standard characterization of VWF concentrates and the importance of multimer size in clinical efficacy. (Work in progress, U. Budde and C. Mazurier)
3. Definition of genetic modifiers that affect hemorrhagic expression in type 1 VWD.
4. Further identification and understanding about FVIII levels in treatment products and the impact on thrombosis with VWF concentrate administration. (Work in progress, P.M. Mannucci)
5. Potential for standardization of the VWF:RCo assay with recombinant, well-defined ELISA assays.
6. Consideration of PFA or other shear-related endpoint assays and their use as replacements for bleeding times in clinical evaluation and clinical efficacy of treatment.

The VWF Subcommittee was attended by about 375 attendees at the first session and 275 participants for the second session. They actively participated in the discussion of the various talks provided by the program. This is the final VWF Subcommittee meeting under R. Montgomery as Chair. The following represents the minutes of the program.

Multinational/Epidemiological Study Updates *Anne Goodeve, United Kingdom*

Dr Goodeve presented the current ISTH VWF SSC electronic database. The database replaced the original version, maintained in the USA, during 2002. All data from the original was transferred. The database now lists 291 mutations, 72% of which are missense. These are located throughout the VWF gene from exons 3-52, the majority (55%) are in the A domains. The database also lists 143 polymorphisms. Both mutation and polymorphism listings can be queried. The large sequence resource section has complete human genomic and cDNA, many other species cDNA and alignments between cDNA and amino acid sequence for human and mouse, and for human gene-pseudogene. Additionally the database has example multimer gels, information for patients, A1 and A3 domain molecular models and recommended nomenclature for mutations and polymorphisms and for VWF and its activities.

Dr Goodeve then presented a brief update on the progress of the collaborative European study Molecular and clinical markers for the diagnosis and management of type 1 VWD. 154 families comprising 743 family members plus 1143 normal individuals were recruited into the study. Mean levels of VWF:Ag and VWF:RCo in index cases and in affected family members were approx 42 and 40 IU/dl respectively, compared to close to 100 IU/dl in unaffected family members and controls. Multimer analysis in 101 families indicated that 51 had a pattern consistent with type 1 VWD, 28 had a 2A (IIE) pattern and 8 had a 2M pattern. 2 index cases had reduced VWF:FVIIIIB consistent with 2N VWD and 4 index cases had a heterozygous pattern. Mutation analysis initiated in 150 index cases has identified 100 candidate mutations to date in 83 individuals. 71% are missense mutations, splicing errors, small insertion and deletions, nonsense mutations and 5'UTR changes were also identified in small numbers of patients. The study aims to provide a comprehensive analysis of patients diagnosed with type 1 VWD and to make recommendations for future enhanced diagnosis.

Working Party Updates *Claudine Mazurier, France*

A. Federici (coordinator with K. Friedman) presented an update of the Working Party on VWD diagnosis. The main achievements since SSC Boston July 2002 are: 1) Financial support from several companies (Account name = ISTH) to cover the costs, 2) Data of the pilot study on the comparison of VWF:Ag, VWF:CB, VWF:RCo levels and VWF multimers before and after lyophilization of a small plasma volume from 2 (type 2A and 2B) VWD patients, 3) London Ethical Committee approval for plasmapheresis (600 ml from 6 VWD patients and 2 controls). Enrolment of 30 labs (30 or 40 % in developing countries) is open. The list of participating labs is foreseen to be available by the 15th September 2003 and the lyophilized samples by 15th January 2004.

A. Federici (coordinator with C. Lee and S. Lethagen) reported on the Working Party on

desmopressin (DDAVP) in the management of VWD. The prospective observational study is organized on behalf of ISTH-SSC on VWF and AICE (Italian Association of Hemophilia Centers) to evaluate worldwide the clinical efficacy of DDAVP in type 1 and 2 VWD patients. The enrolment started in Milan on May 15th 2003. The files for inclusion of centers and the final CRF version will be available on the www.ddavp-invwd.it web site.

Diagnostic Testing of VWF David Lillicrap, Canada

In the first session relating to diagnostic testing for VWD, Tony Hubbard presented details of the new 5th International Plasma Standard for FVIII/VWF. This plasma has been collected from a pool of 22 normal donors and after immediate freezing has been aliquoted into 5,500 ampoules. The 5th International Plasma Standard has a FVIII:C value of 0.68 U/mL, VWF:Ag of 0.91 U/mL, VWF:RC₀ of 0.78 U/mL and a VWF:CB of 0.94 U/mL. The multimer profile of the Standard is normal. The 5th International Plasma Standard received unanimous approval from the sub-committee.

Connie Miller presented data from studies in African Americans highlighting ethnic differences in VWF indices and in platelet function. In the first of two presentations relating to multimeric analysis of VWF, Ulrich Budde described a comparative quantitative study of multimer testing performed in his laboratory and that of Claudine Mazurier. Although the ranking of samples for their HMW multimeric content was the same in both laboratories, the absolute values for HMW multimeric content was significantly different. Dr. Budde has proposed a prospective study of this issue in 15 laboratories. Dr. Trong next described a novel method for multimer analysis involving electrophoretic quasi-elastic light scattering. This methodology can currently provide a quantitative assessment of multimer size that has a 3-fold resolution improvement over current gel-based electrophoresis and has the potential of providing results from a plasma sample in 20 seconds.

Presentations from Drs. Federici, Favaloro and Seitz provided more information concerning the utility of the collagen binding assay in VWD testing. There is growing evidence to indicate that this test would provide a complement to the VWF:RC₀ and multimer analysis in evaluating type 2 VWD variants. Finally, Hans Deckmyn described a novel GpIb binding assay for VWF in which the GpIb fragment is provided by plasma glyocalicin. With intra- and inter-assay CVs of <10% and a sensitivity of 0.0005 U/mL this assay appears to have significant promise as an alternative to the VWF:RC₀ assay.

Potpourri Session Giancarlo Castaman, Italy

G. Castaman presented the ad interim results with PFA-100 evaluation in the European study MCMMDM-VWD1. So far, data from 263 members of 96 families with type 1 VWD recruited in 9 different EU Centers were available. By analysing the data with ROC methodology, it appears that probably PFA system is able to identify a significant greater proportion of patients with type 1 VWD in comparison with bleeding time. ADP and Epinephrine cartridges were similarly effective to this purpose. C. Hayward presented the Hamilton experience with PFA-100 in the screening of inherited bleeding disorders. The system was superior to BT in diagnosing VWD,

but it appeared to have low specificity and sensitivity in some platelet disorders. Furthermore, clinicians feel further testing would be required, regardless of CT results. Cost is also an important issue. D. Nugent presented data on PFA-100 system in the USA. The system is used to screen not only for inherited coagulation or platelet disorders, but also for monitoring effective inhibition of platelet function with antiplatelet agents in some clinical situations. Probably, a refinement of the more appropriate use of the system appears necessary. A. Tripodi presented the methodology for a multicenter study for the standardization of ADAMTS 13 assay. A preliminary survey on the methodology used in several laboratories showed a large heterogeneity of the methods used. First results are anticipated to be available for the next SSC meeting. P.M.Mannucci reported an additional case of venous thromboembolism in an older VWD patient treated with VIII/VWF concentrate during high-risk surgery without anti-thrombotic prophylaxis. Very high FVIII:C levels were attained during the post-operative period, despite a RiCof of 100 %. D. Bowen presented results on the relationships between blood group and VWF proteolysis in plasma. Blood group O shows enhanced proteolysis, while A and AB groups showed reduced time-course proteolytic pattern. The Tyr1584Cys change in VWF seems to be associated with an increased susceptibility to proteolytic attack by ADAMTS 13.

VWD – A disease or a risk factor *Jeroen Eikenboom, Netherlands, Chair*

In this session the clinical significance and appropriate management of low VWF levels were discussed. Evan Sadler proposed a two-tiered approach, dividing subjects into a small group with "VWD type 1" and a much larger group with "low VWF." The diagnosis of VWD type 1 would apply to patients with exceptionally low VWF levels who are likely to experience significant bleeding symptoms and frequently have mutations in the VWF gene. The category of low VWF would apply to persons with modest decreases in VWF level that may associate coincidentally with bleeding, and in whom a genetic basis for the low VWF level usually cannot be identified. This risk management strategy would be analogous to that applied already to risk factors for cardiovascular disease. Implementation of such a strategy will require more data on the relationship between VWF level and the risk of bleeding in specific clinical settings. Francesco Rodeghiero argued that VWD type 1 is a clinically relevant diagnosis when based on family investigation. The high prevalence (up to 10-20 %) of isolated, often trivial, very mild bleeding symptoms in normal population together with a 2.5 % prevalence of low VWF level by definition predicts that about 0.5 % (20 % X 2.5 %) of such cases in normal population could be detected by chance. However, this situation is not representative of the average patient referred to a specialized center (reasonably less than 1 % of normal). In this case, the rate of false positivity by chance falls below 1 / 4,000. For a definite diagnosis, family investigation is required. We propose that appropriate selection of patients on the basis of personal and/or familial history for laboratory diagnosis of VWD is crucial for producing a clinically relevant and useful diagnosis.

Diagnosis/classification issues *Augusto Federici, Italy*

Enigmas in VWD diagnosis was presented by U, Budde He reported that by using his sensitive multimeric assay more type 2 defects can be identified among previously type 1 VWD: a large number of 2A especially with the subtype 2A/IIIE are present. Issues to be addressed: 1b/2A;

1/2M. VWF gene linkage with low VWF levels was presented by J. Eikemboom. He described the results of recent published studies in VWD Italian and British families and he showed that linkage cannot be demonstrated in all of them. European and Canadian studies ongoing can provide additional information. Genetic Modifiers of VWD was presented by J. Di Paola. He described his methodology to apply specific platelet polymorphisms in different racial population. T. Kunicki presented his recent data on platelet SNPs in a group of 15 well characterized families with type 1 VWD enrolled in Milan, as a part of the families of the European Study MCMDM-1VWD. Some of these SNPs seem to correlate with the bleeding score. At the end of the session A.B. Federici proposed the WP on VWD classification with the aims to update the previous classification by J.E. Sadler. J.E. Sadler agreed to chair this WP together with the Chairman and Co-Chairmen of this SSC on VWF

Clinical Assessment Tools and Treatment Issues *Peter Kouides, USA*

Drs. Castaman, Miller, and Kouides summarized their work with clinical data assessment forms. In light of the on-going discussion/topic in this SSC of von Willebrand factor as a risk factor for bleeding as opposed to a being a marker of disease, this session was convened in order to clarify, establish and address the future development of clinical tools in assessing the degree and risk of bleeding. An international perspective was presented beginning with a study of VWD families presented by Dr. Alberto Tostetto of bleeding symptoms in obligatory Type 1 carriers and affected Type 1 patients; surprisingly cutaneous bleeding proved to be the most discriminatory symptom followed by dental related bleeding then surgical related bleeding; equally surprising was the relative low sensitivity of menorrhagia. Studies are on-going in validating algorithms for diagnosis and management of VWD based on this analysis. Dr. Connie Miller then presented the data from the Centers for Disease Control in the study of 102 women registered at Hemophilia Treatment Centers in the US. Four bleeding symptoms were more prevalent in the VWD patient than controls: heavy menstrual bleeding, bleeding after minor injuries, bleeding after surgery and excessive gum bleeding. Based on this a predictive tool has also been developed and is undergoing validation as is a similar predictive tool (based on multi-regression analysis incorporating age, history of anemia, history of dental or surgical related bleeding, decreased quality of life during menses) from studies in Rochester NY USA presented by Dr. Kouides. Dr. Kouides also presented data from Rochester NY, CDC Atlanta and Royal Free London on bleeding symptoms in menorrhagia patients. It was emphasized that there is a need to pool data of symptoms from the various VWD menorrhagia prevalence studies worldwide in correlating those symptoms with the measurement of menstrual flow and the VWF level in predicting menorrhagia risk in relation to the VWF level. Dr. Michiels reviewed correlation of response to DDAVP based on the VWF:Ag/VWF:RCo ratios in confirming further the diagnosis of VWD. Labeling of VWF concentrates was also reviewed with the point made that there is need of further study not necessarily of laboratory parameters after infusion of VWF containing concentrates but of clinical efficacy. Dr. Kessler made several recommendations for clinical efficacy of VWF concentrates: use a central laboratory independent of industry, need for back-up samples, importance of multimeric analysis over surrogate tests, inclusion of genotype in response analysis, screening for hypercoagulable states if positive family history and need for accurate surgical assessment., Lastly, Dr. Bussell presented a joint proposal on behalf of the Platelet subcommittee on a study of Type 2B VWD in accruing further clinical data such as the severity of symptoms and treatment data such a the safety of DDAVP.

Revised and submitted 7-27-03
Robert R. Montgomery, Chair

Von Willebrand Factor

June 18-19, 2004

14:15 to 18:15

Carnelutti Room

Fondazione Giorgio Cini

Chairman: A. B. Federici, Italy

Co-chairs: G. Castaman, Italy; J. Eikenboom, The Netherlands; E. Favaloro, Australia;
A. Goodeve, UK; P. A. Kouides, USA; D. Lillicrap, Canada; C. Mazurier, France;
R. Montgomery, USA; R. Schneppenheim, Germany

Summary of Approvals and Working Parties:

1. Continuation of WP on VWF Assays in VWF in VWD Diagnosis: The lyophilized samples are ready to be sent out to labs and the survey will start by September 2004 (A.B. Federici, C.A. Lee, R.R. Montgomery)
2. Continuation of the WP on Standardization of Multimeric Analysis, with more laboratories (U. Budde and C. Mazurier)
3. A new WP on the use of suitable reagents for VWF:CB (collagen binding assay) has been approved (L. De Marco, E. Favaloro and A. Hubbard)
4. Continuation of the WP on VWD classification has been approved with presentation of a final report during the next ISTH-SSC meeting in Sydney (E. Sadler & the panel of VWD experts)
5. The WP on Standardization of methods for mutation and expression studies will continue (A.Goodeve, D. Lillicrap, J Eikenboom, R. Schneppenheim)
6. A WP on development of new improved assays for ADAMTS-13 has been proposed, as a continuation of the previous inter-laboratory assay standardization study (J.E. Sadler & R. Schneppenheim)
7. A WP on requirements for shear-stress related VWF assays to be used in clinical diagnosis of VWD and drugs interfering with VWF-platelet interactions have been proposed (Y. Ikeda & Z.M. Ruggeri)
8. An updated version of International Registry on Acquired Von Willebrand Syndrome has been proposed to be organized on line in a specific Web site (A.B. Federici, U. Budde, H. Mohri, J.H. Rand)

Two co-chairs, Emmanuel Favaloro and Peter A. Kouides were not present. The VWF Subcommittee was attended by about 190 attendees at the first session and by about 140 at the second session. They actively participated in the discussion of the various topics provided in the program. The following represents the minutes of the program.

1) WP on VWF Assays (Claudine Mazurier, France)

Christine Lee presented an update of the Working Party on VWF assays in VWD diagnosis (<http://www.vwfassays-in-vwd.com/>). The main achievement since SSC Birmingham July 2003 is the plasmapheresis and preparation of lyophilised plasma samples from 2 donors and 6 VWD

patients. Preliminary results showed that no changes in VWF:Ag, VWF:RCo and multimers were induced by the lyophilization.

Giancarlo Castaman then presented his experience with LIA test for VWF:Ag. This test based on latex technology was used in the context of the European MCMDM-1 VWD study on type 1 VWD. The VWF:Ag values are: 112.4 ± 40.9 IU/dl in 1049 controls and 38.3 ± 23.3 IU/dl in 127 patients. In comparison with ELISA this high precision turbidimetry test is 2 to 4 times cheaper and adapted to emergency situation.

Ulrich Budde reported on the Working Party on Standardization of VWF multimers. He presented the data from six laboratories having tested 5 VWF concentrates and plasma samples of 2 VWD patients. Using or not transfer, radioactivity, enzymes or luminescence for revelation, the patterns were consistent. For quantification the multimers were classified in the following categories: LMW: multimers 1 to 5, IMW: multimers 6 to 10 and HMW: multimers >10-mers. For a given sample the range of the values was rather wide from one lab to another but the rank of the samples based on their HMW content was consistent in the majority of labs.

Don Gabriel presented a EQELS (Electrophoretic Quasi Elastic Light Scattering) method for multimeric analysis. The effect of temperature, ionic strength and hydrophobicity was studied on partially purified VWF preparation. Results obtained with plasma of a type 3 and a type 2B VWD patient were also presented. The results obtained within 1 hour showed excellent reproducibility.

Ronald Kotischke summarized the data of a collaborative study on automated VWF:RCo assay organized by the German society on Transfusion Medicine and Hemotherapy (DGTI). Thirteen labs not only from Germany but also from Austria, France, Italy and UK, tested 8 reconstituted and frozen concentrates (5 plasma-derived VIII/VWF, 2 plasma-derived VWF and 1 recombinant VWF product). The CV were ranging between 10.9 and 23.5 %.

2) VWF collagen binding versus VWF:RCo activities (Giancarlo Castaman, Italy)

Augusto B. Federici presented data on the relationships between VWF:CB and VWF:RCo in a sample population from the MCMDM-1 VWD study. It appeared that about 10 % of subjects had both VWF:CB/Ag and VWF:RCo/Ag ratios below 0.6. All these subjects had VWF gene mutations clustered in the D', D3 and A1 domains of VWF.

Luigi De Marco presented preliminary data on a new VWF:CB assay using human collagen VI. This test is able to exploit both A1 and A3 domains of VWF. The test has been so far used for flowing experiments and some data should be available after testing in patients for the next subcommittee meeting in Sidney.

Anthony Hubbard presented data on a possible standard for VWF:CB. He suggested that no potency assignment could have been made as to VWF:CB activity due to extreme heterogeneity obtained among different labs and the uncertainties of the results obtained by using collagen type I or III.

Tobias Suiter presented data on VWF:CB and VWF:RCo in several VWF concentrates.

Conclusions The general feeling was that some guidelines on the use of VWF:CB should be provided by the Subcommittee prior to its definite use in clinical practice. At the present, the role of time-honoured tests for VWD diagnosis remains fundamental. A WP on the use of VWF:CB has been proposed to be organized by Luigi De Marco, Emmanuel Favaloro and Anthony Hubbard)

3) Working Party on VWD Classification (Jeroen Eikenboom, The Netherlands)

The session started with a presentation of the **proposal for a new VWD classification** by **Evan Sadler**. The Subcommittee on VWF approved the current classification of VWD at the meeting of the SSC in New York, on July 4, 1993, and it was published the next year. The 1994 classification was based mainly on differences in pathophysiology, and was intended to correlate with clinical behavior, response to therapy and the need for genetic counseling.

A working party was authorized at the 2003 SSC meeting in Birmingham to consider revising the current VWD classification. This working party will continue through the 2005 ISTH meeting in Sydney, to allow the incorporation of results from ongoing Canadian and European studies of VWD type 1. An interim report was made to the Subcommittee in Venice. Members of the working party are: Ulrich Budde, Jeroen Eikenboom, Augusto Federici, Emmanuel J Favaloro, Frank Hill, Lars Holmberg, Jørgen Ingerslev, Christine Lee, David Lillicrap, Pier Mannuccio Mannucci, Claudine Mazurier, Dominique Meyer, Robert R. Montgomery, William L. Nichols, Masato Nishino, Ian Peake, Francesco Rodeghiero, Zaverio M. Ruggeri, J. Evan Sadler (chair), Reinhard Schneppenheim, and Alok Srivastava.

Concepts and suggestions for a revised VWD classification were presented and discussed. There was broad agreement that the general principles outlined in the 1994 classification should be retained. In particular, the classification is intended primarily to guide VWD patient treatment and genetic counseling, and therefore must be clinically relevant. In addition, the classification should be simple, with a minimum number of formal categories, and implementation should depend mainly on laboratory tests that are widely available. The classification should be separated conceptually from specific laboratory testing protocols, so that the development of new assay methods will not render it obsolete. During the following year, the working party will resolve some issues relating to the boundaries between some VWD variants, and the annotation of certain subtypes. Final recommendations for VWD classification will be presented and discussed in Sydney, with the expectation that the VWF subcommittee will then vote on their approval. If so approved, a revised VWD classification will be published.

Alberto Tosetto presented data on **Bleeding scores in VWD**. The bleeding score was retrospectively evaluated in two multicenter studies. The bleeding score may be useful to identify subjects with a possible bleeding diathesis and to quantify the severity of the affected individuals. The bleeding score showed a good correlation with unaffected, affected, and index cases. It was also correlated with the VWF:RCo level (lower levels, higher bleeding scores). The score could be useful for biological correlations. However, prospective evaluation is needed. Implementation of the bleeding score in the VWD classifications has to be discussed.

Margareta Blombäck illustrated the problem of diagnostic criteria for VWD by the presentation of a case report.

Conclusions The revised classification for VWD will be further debated during the next year and the results from ongoing multicenter studies on type 1 VWD will be implemented. Final recommendations will be presented at the SSC in Sydney 2005. The role of the bleeding score has to be evaluated in a prospective study.

4) Methods for genetic analysis of VWF defects (Anne Goodeve, UK)

Anne Goodeve presented an update of VWF gene defects from the ISTH VWF website, <http://www.shf.ac.uk/vwf>. A total of 307 mutations are listed. Mutations comprise type 1, n=14; type 2A, n=71; 2B, n=52; 2M, n=18; 2N, n=37 and type 3, n=85. 80% of the mutations are missense. Whereas type 3 mutations are located throughout the VWF gene, type 2 mutation distribution reflects the functions that they disturb. The summary has been added to the VWF website.

David Lillcrap discussed the use of the terms polymorphism or mutation to describe two sequence alterations in the VWF gene newly recognized as associated with increased risk of low VWF and bleeding, but which demonstrate incomplete penetrance. Y1584C and R924Q were previously referred to as polymorphisms as they are found at a frequency of >1% in the normal population. However, both are enriched in the type 1 VWD population. Both result in enhanced intracellular retention. The findings suggest no simple distinction between the terms mutation and polymorphism.

Jeroen Eikenboom discussed the relative merits of SNP and STR polymorphisms for linkage analysis within VWD families. STR polymorphisms benefit for enhanced informativity and therefore are useful within single pedigrees. However, their mutability renders them less useful for population based studies, and SNPs may be preferred for such studies. Polymorphisms can then be utilized with parametric analysis to determine inheritance mode or in non-parametric analysis to examine quantitative trait loci (QTL).

Reinhard Schneppenheim described mutation scanning techniques that have been utilized to seek sequence variation in the VWF gene; CSGE, DHPLC, CCMA, SSCA and DNA sequencing have all been used. The EU MCMDM-1VWD study enabled comparisons of sensitivity of various methodologies to be made. DNA sequencing was the most sensitive technique, with a single false negative result of 60 individuals analysed. Other techniques were up to 90% sensitive. The variety of techniques used to express VWF *in vitro* were also described, and the importance of expression of both homozygous and heterozygous recombinant mutant VWF in comparison with wild type VWF was emphasized.

5) ADAMTS-13 Assays (Reinhard Schneppenheim, Germany)

Armando Tripodi reported in detail the results of the large international laboratory assay standardization of the different assays available until now. He described the blind design of the study showing the type of samples with different levels of ADAMTS-13 sent to the participating labs. Eleven international expert laboratories for ADAMTS13 participated in this study with five different methods: a) Multimer analysis of plasma or recombinant VWF as substrate incubated

with patient's plasma and quantitative evaluation (2); IRMA (1); Immunoblotting of recombinant VWF A1-A3-fragment as substrate incubated with patient's plasma and quantitative evaluation (1); Collagen binding assay (VWF:CB) of plasma or recombinant VWF as substrate incubated with patient's plasma (5); Ristocetin-Cofactor assay (VWF:RCo) of plasma VWF as substrate incubated with patient's plasma (1); Cone and platelet aggregometer test after incubation of VWF with patient's plasma (1)

Results were as follows: In general the performance of tests was of considerable variance even between comparable methods. Test linearity was excellent for VWF:RCo assay, multimer analysis and VWF:CB (not in all cases of the latter). Reproducibility was excellent for VWF:RCo assay, and VWF:CB with a CV < 10 % (not in all cases of the latter). Identification of severe deficiency was excellent for multimer analysis, VWF:CB, VWF:RCo and IRMA. Discrimination between 0 and 10 % activity was excellent for RCo, multimer analysis and in one center for CBA. Overall the best suitable methods were VWF:RCo assay, multimer analysis and VWF:CB.

Derrick Bowen presented the data on the effects of ABO blood group and of specific VWF polymorphisms in relationship with ADAMTS-13 effect, discussing the results of his recent publications in JTH and Blood. He described enhanced proteolysis of VWF in the order of O, B, A, AB with bg O-VWF as being the most susceptible. He also presented data on enhanced proteolysis in patients with the mutation Y1584C. Most patients had blood group O. Enhanced proteolysis was detected by decreased VWF:CB. Enhanced proteolysis is considered a pathogenic mechanism that may have implications for therapy, i.e. with DDAVP. The issue of standardization of assays for ADAMTS-13 was then discussed by **Flora Peyvandi, J.Evan Sadler** and **Reinhard Schneppenheim** who presented preliminary data on new assays developed in their laboratories.

Flora Peyvandi reported on an ongoing project for the development of new ADAMTS13 assays both quantitative as well as functional. Her group produced 12 monoclonal antibodies directed mainly towards the carboxyterminal of ADAMTS13. These monoclonals are now tested for their usefulness in an ADAMTS13-ELISA. A functional assay shall be developed on the basis of a small fragment of VWF containing the proteolytic site flanked by a fluorochrome and a quencher. The method would detect the activity of the protease directly by measuring the increasing fluorescence.

Evan Sadler presented experiments on the proteolysis of VWF fragments containing the proteolytic site flanked by antigenic tags. He could show that fragments lacking the VWF A1-domain are more easily proteolysed than fragments including it. However, if the A1-domain was present, proteolysis could be enhanced by GpIb. He also introduced a VWD type 2A mutation into the A2-domain that further increased proteolysis. He concluded that functional ADAMTS13 assays that employ VWF fragments with the A2 domain may be a suitable method. Such assays may, however, not reliably reflect the situation in vivo.

Reinhard Schneppenheim presented data on the interaction between recombinant ADAMTS13 and diverse VWD type 2A mutants, with a recombinant fragment comprising the A1-A2-A3 domain as substrate flanked by antigenic tags. He could differentiate between mutants with

enhanced proteolysis, characterized by cleavage in the absence of urea, those with normal proteolysis compared to the wildtype fragment (cleavage only in the presence of urea) and a mutant fragment that was not cleaved neither without nor with urea. He considered the method as suitable for the study of mutants providing both enhanced or decreased resistance against proteolysis. The limitation of the method is, however, its performance as a static assay without the influence of shear.

The **session was concluded** with the statement that fast, sensitive and reliable assays to measure ADAMTS13:Ag and ADAMTS13 functional activity, respectively, are highly desirable. It was emphasized that there is still a deficit in good routine assays to detect ADAMTS13 antibodies in patients with acquired TTP, a condition that requires specific therapy. There was a general agreement in the audience to continue the efforts to develop additional and more standardized assays for ADAMTS-13 evaluation. A WP on ADAMTS-13 will be organized by E. Sadler & R. Schneppeheim and will include all the interested experts working in this field.

6) Shear-stress related VWF Assays: new tools of VWF activities ? (Augusto. B. Federici, Italy)

Zaverio M. Ruggeri discussed the general concepts for the use of shear-stress tests as a measurement of VWF function. He described the most recent data about the basic mechanisms on VWF-platelet interactions in different shear-rates conditions, from relatively low to high shear rates. He is positive about future applications of these methods in a more clinical setting to determine VWF defects.

Dr. Ikeda reported about his experience in Japan on shear-stress assays. In particular he reported the data obtained with these assay in collaboration with cardiologists who evaluated with him the effects of different anti-platelet agents, aspirin, ticlopidine and different monoclonal antibodies against anti-IIb IIIa receptors. His assay can distinguish very well the effects of these different drugs (aspirin versus ticlopidine versus monoclonal antibodies) and therefore it can be useful in the clinical setting to monitor the effects of these drugs.

Catherine Hayward reported her experience with the use of PFA-100 in diagnosis and monitoring treatments of VWD. She reported that the assay is very sensitive but not specific for VWD and therefore she has not introduced the PFA-100 as a standard routine test for primary hemostasis in her coagulation laboratory at the Mc Master University in Canada. Due to the low costs of VWF panel tests she prefers to perform specific VWF assays instead.

David Varon presented results on VWD cases with the improved version of the Automated Cone and Plate(let) analyzer. His device can show reduced thrombus formation in different types of VWD patients followed in Israel and in Italy, at the Angelo Bianchi Bonomi Hemophilia Thrombosis of Milan.

Conclusion: After a fruitful discussion it was proposed that Dr. Ikeda and Ruggeri will organize a WP on Shear-stress related VWF assays to determine the requirements for such assays to be used in a more clinical setting.

7) Clinical Studies on Management of VWD (D. Lillicrap, Canada)

The session relating to clinical studies in von Willebrand disease (VWD) was introduced by **Professor Mannucci**, who addressed the issue of mucosal bleeding in VWD. He re-emphasized the fact that we still do not know which measures of VWF function best determine the risk of mucosal bleeding in patients. The optimal treatment of gastrointestinal bleeding (especially from angiodysplastic lesions) and menorrhagia, with VWF concentrates, will require further evaluation of VWF dosing using different potency assessments. Professor Mannucci encouraged the development of proposals to investigate this issue in prospective clinical trials.

Giancarlo Castaman next discussed the preliminary information available from the EU Type 1 VWD Study relating to the correlation between DDAVP-responsiveness and VWF genotype. He showed data to indicate that missense mutations in the propeptide and D4/CK domains of VWF were associated with prolonged elevations of VWF post-DDAVP, whereas, in contrast, missense mutations in the D3 domain were associated with more rapid clearance of VWF post-DDAVP. To continue the DDAVP theme, **Stefan Lethagen** presented the current status of the DDAVP biological response and clinical assessment study. This study will evaluate the biological response to DDAVP infusion at 4 time points in type 1 and 2 VWD patients, and in those patients with a positive biological response (>3-fold increase in VWF levels and a post-DDAVP level of >0.30 u/mL) a subsequent clinical assessment of efficacy can be made. In addition, there are plans to enroll 40 VWD patients in a more extensive post-DDAVP pharmacokinetic study in which VWF and FVIII levels will be measured at 8 time points up to 24 hours post-therapy. Currently, 29 patients have been enrolled on the biological study. The study is recruiting patients through the web site – <http://www.ddavp-in-vwd.com>. Finally, in this session, **Peter Lenting** presented data on the clearance of recombinant normal and type 1 VWD mutant VWF in a mouse model. He showed data to indicate that several of these VWF mutants, including C1130F in the D3 domain, are associated with significant shortening of the circulating mutant VWF half-life. This preliminary data suggests that premature clearance of mutant VWF may play an important role in pathogenesis in some cases of type 1 VWD.

8) Other reports and proposals, concluding remarks (Augusto B. Federici, Italy)

Derrick Bowen presented on behalf as his colleagues of UK Hemophilia Centers the preliminary results of molecular pathology study in VWD type 1.

Jacob H. Rand presented the proposal of an updated version of the International Registry on Acquired von Willebrand Syndrome. The results of previous registry were published in 2000 with data collected until December 1999. The new registry will enroll directly on line with an appropriate web site (www.intreaws.com) all cases with AVWS diagnosed after January 1st, 2000. Aims of this new updated registry on AVWS are also to develop better diagnostic tests for AVWS, to promote prospective studies in specific underlined clinical conditions usually associated with AVWS and to determine clinical responses with new therapeutic approaches (WP organized by A.B. Federici, U. Budde, H. Mohri, JH Rand)

James Bussel was not present during this session but he sent a letter to the Chairman with a proposal of joint study with platelet Subcommittee on 2B VWD frequency on non-autoimmune thrombocytopenia.

Augusto B. Federici presented the concluding remarks of the entire two sessions, summarizing all studies and proposals to be reported next year in Sidney.

Von Willebrand Factor

August 6th , 2005

11:00 to 14:30

Ballroom 2

Sydney Convention & Exhibition Centre, Sydney

Chairman: A. B. Federici, Italy

Co-chairs: G. Castaman, Italy; J Di Paola, USA; J. Eikenboom, The Netherlands; E. Favaloro, Australia; A. Goodeve, UK; D. Lillicrap, Canada; C. Mazurier, France;
R. Montgomery, USA; R. Schneppenheim, Germany

Summary of Approvals and Working Parties:

1. Continuation of WP on VWF Assays in VWF in VWD Diagnosis: The lyophilized samples were sent out to labs and the results will be collected by September 2005 (A. Hubbard, A.B. Federici, C.A. Lee, R.R. Montgomery)
2. Continuation of the WP on Standardization of Multimeric Analysis, with more laboratories (U. Budde and C. Mazurier)
3. Novel suitable reagents for VWF:CB (collagen binding assay) have been prepared and will be tested during the next year (L. De Marco, E. Favaloro and A. Hubbard)
4. The WP on VWD classification has approved that the final report will be presented during the next ISTH-SSC meeting in Oslo (E. Sadler & the panel of VWD experts)
5. The WP on Standardization of methods for mutation and expression studies will continue and will prepare a written report before Oslo (A.Goodeve, D. Lillicrap, J Eikenboom, R. Schneppenheim)
6. The WP on development of new improved assays for ADAMTS-13 will continue and report update results in Oslo (J.E. Sadler & R. Schneppenheim)
7. The WP on requirements for shear-stress related VWF assays to be used in clinical diagnosis of VWD and drugs interfering with VWF-platelet interactions decided to postpone the report in Oslo (Y. Ikeda & Z.M. Ruggeri)
8. A specific Web site for an updated version of International Registry on Acquired Von Willebrand Syndrome is now available and the first interim report will be presented in Oslo (A.B. Federici, U. Budde, H. Mohri, J.H. Rand, I. Sussman)

A) Progress reports on different Working Parties (Chairs: J. Eikenboom and E. Favaloro)

The session started with a report on **VWF assays in VWD diagnosis** by **A. Hubbard & C.A. Lee**. The objective of the working party is to determine the best diagnostic repertoire for VWD diagnosis and thereby produce guidelines for the minimal requirements for correct VWD diagnosis. This will be achieved through a review of methods currently in use and a multi-center international study in which plasma samples covering a range of VWD variants will be dispatched for diagnosis. Trial fills on VWD plasma have indicated that lyophilisation does not affect the estimation of VWF. A panel of plasma samples from patients with known VWF mutations (and viral marker negative) were lyophilised in 2004. Thirty four laboratories from 17

different countries have agreed to participate and dispatch of the coded samples is almost complete. Participants are requested to return information on their methodologies as well as laboratory data to support their diagnosis of the lyophilised samples. Analysis of the results is planned for the last quarter of 2005.

L. De Marco & E. Favaloro reported on the results of the **WP on different collagen reagents**. It is intended that the VWF:CB working party undertake a study to help evaluate the utility of different collagen preparations in the diagnosis of VWD, and in the discrimination of qualitative VWD Types, as well as for identification of functionality of VWF in therapeutic factor replacement products. The VWF:CB study will entail (i) retesting of an identical plasma set by a smaller select number of expert laboratories using methodologies currently existing in those laboratories as well as by additional methodologies, including commercial options, (ii) additional testing of in-house well characterised plasma material with all methods, (iii) testing of therapeutic VWF concentrates. Companies producing commercial VWF:CB kits have agreed to participate and different collagen preparations will also be evaluated. Proposed members and participants of this exercise will be Dr Favaloro, Dr De Marco, Dr Hubbard, Dr Federici & Dr Mazurier, plus other interested parties to be identified. It is proposed that this study commences after completion of the above mentioned diagnostics study. In the interim, the members of the VWF:CB working party will formulate a study protocol that identifies inclusions and requirements.

U. Budde & C. Mazurier reported on the progress of the **WP on multimeric analysis**. Since the session in Venice we had requests from 3 more laboratories to analyse our samples. Only 1 of these did send results and one laboratory analysed their results in a way compatible to the others. This brings up seven results that can be evaluated statistically for most of the 10 samples. While for the 4 samples with a full or near full content of large multimers the coefficient of variation for the content of >10 multimers was acceptable (below 15%), it was much too high for those samples that missed greater parts of the large multimers: between 52% and 81%. Thus the method is still far from standardized. We await results from a WP of VWF assays (results from 30 laboratories) and from the type 1 study before proceeding further in our aim to standardize the multimeric analysis.

The **WP on VWD classification** has produced a report with recommendations for VWD classification, which was presented by **J.E. Sadler**. As of the SSC meeting in Venice in 2004, the Working Party on the classification of VWD had evaluated recent published advances in our understanding of the pathophysiology of VWD and made significant progress in revising the classification to reflect this progress. Substantial changes were made to the definition of VWD and of VWD type 1. At this time, a preliminary manuscript for the revised classification has been written and extensively revised. Before it is submitted for publication, the Working Party would prefer to include the major findings of the Canadian and European studies of VWD type 1, which will be relevant to the changes proposed for VWD type 1. The first papers from these studies should be submitted for publication with the next few months. During the next year, the working party will review the published results of Canadian and European studies of VWD type 1, and other ongoing studies of VWF assays and multimer analysis. With the benefit of these data, a manuscript entitled "Update on the classification of von Willebrand disease" will be submitted for publication no later than the next SSC meeting in 2006.

The session ended with a report by **L. Hilbert & D. Lillicrap** on a new **WP on VWF molecular biology and expression studies**. The aim of this working party is to complete a review on the methods used for the identification and expression of molecular abnormalities in VWD. A questionnaire was sent to laboratories involved in two parallel projects on type 1 VWD: the European MCMDM-1 VWD project and the Canadian project. The major conclusions from this pilot survey were presented and indicated that there is some consensus in the methods used for the identification of a VWF gene abnormalities and for the construction of mutated expression vectors harbouring a VWD mutation. However, transient transfections were performed using different procedures, in different cell types and this results in recombinant VWF antigen levels that may vary by a 10-fold factor according to the laboratory. In order to conduct a second larger survey, this questionnaire will be sent to other laboratories willing to participate. Those interested should send an e-mail to hilbert@lfb.fr or lillicrap@cliff.path.queenu.ca .

B) Progress reports on ADAMTS13 assays & clinical applications (Chairs, Anne Goodeve & Reinhard Schneppenheim)

1. Role of chloride ions in the VWF-ADAMTS13 interactions

R. de Cristoforo reported on the kinetics of VWF cleavage by ADAMTS13 at different concentrations of NaCl. Low concentrations of NaCl significantly enhance cleavage of VWF compared to physiologic NaCl concentrations. Testing of different cations and anions, respectively, revealed that the inhibitory effect of higher salt concentrations was dependent on anions rather than on cations. It was suggested that the inhibitory effect was due to conformational changes of VWF that could be reversed by ristocetin. Accordingly, anions should stabilize a more closed structure of the VWF A2 domain, whereas shear should stabilize an open form.

2. The fluorogenic substrate for ADAMTS13

H. Kokame reported on normal values for ADAMTS13 of a large Japanese control population of 3,822 individuals obtained by the commercially available ADAMTS13 FRET assay developed by the group. They observed a slow decrease of fluorescence at zero activity. The lower limit of detection was estimated as 3 % of normal, and the assay was able to differentiate between values of 3 %, 0 % and 5 %. The minimum value was 43 %, and the reference range was 52 – 172 %.

3. Novel ELISA assay for ADAMTS13 activity using MoAbs directed against the N-terminal decapeptide of VWFA2 domain

M. Matsumoto reported on a new ELISA based on MoABs directed against the N-terminal decapeptide that is exposed after ADAMTS13 digest of VWF. This assay is very sensitive and specific with a detection limit of 0.5 % which would be superior to all other assays to date. The correlation with classical assays like the multimer method was good.

4. ELISA for ADAMTS13 autoantibodies in TTP patients

J. Kremer-Hovinga reported on her experience measuring the titer of autoantibodies against ADAMTS13 in 74 patients with TTP/HUS by a new ELISA (Technozyme ADAMTS13 Inh, Technoclone, Vienna). The group detected antibodies in 93 % of cases with ADAMTS13 activity < 10 %, in 39 % of cases with ADAMTS13 between 10 and 25 %, in 8 % of cases with ADAMTS13 between 26 and 50 %. The limit of detection was estimated as < 1BU which would be an improvement compared to the standard Bethesda method.

5. Action on ADAMTS13 on different VWF mutants

R. Schneppenheim and U. Budde showed experimental data about the action of ADAMTS13 on different VWF mutants. The group reproduced the enhanced susceptibility of classical VWD type 2A (IIA) mutants for ADAMTS13 proteolysis not only for the group 2 mutations, previously defined as being susceptible for enhanced proteolysis but also for a group 1 mutation in the A2 domain (S1506L) for which previously intracellular retention of large VWF multimers was made responsible for their observed lack. The group could also show by in vitro mutagenesis involving single and multiple substitutions of amino acids that Y1505 and M1506 flanking the VWF proteolytic site are not essential for recognition by ADAMTS13.

6. Clinical experience on Italian Registry of TTP

F. Peyvandi reported on the Italian experience of TTP in 140 patients. Plasma level of ADAMTS13 was reduced to < 10% in only 57 % of patients in acute phase. Measurements of ADAMTS13 in remission phase of recurrent patients more often revealed lower levels than in patients with a single episode. Severe ADAMTS13 deficiency (<10 %) was associated with a high prevalence of neutralizing inhibitors (61 %). 12 % of patients without measurable neutralizing antibodies had non-neutralizing antibodies on Western blotting. In about 1/3 of patients with severe ADAMTS13 deficiency no neutralizing antibodies were found which could not be explained by diagnosis of the inherited form of TTP predisposition (Upshaw-Schulman syndrome, USS) alone, since only 5/140 (3 %) patients had USS.

C) Other reports and proposal for projects and surveys (Chairs: J. Di Paola and D. Lillicrap)

Update of the Working Party on DDAVP in VWD : Dr. Lethagen summarized the current status of this project. He introduced the project web site and re-iterated the main points of the project. In addition to the aim to enroll 150 VWD patients for the main biological response and clinical efficacy part of the study, there is also a plan to assess a detailed pharmacokinetic profile in 40 patients.

Long-term prophylaxis of VWD : Dr Abshire introduced this new activity. He described the rationale, organization, objectives and entry criteria for the proposed VWD prophylaxis project. This project will be organized by the newly created VWD Prophylaxis Network and will be sponsored by an investigator-driven grant from ZLB-Behring.

Update of the Registry on Acquired von Willebrand Syndrome : Dr. Budde presented a brief update on the activities associated with this registry. He provided details on the new Registry web site (www.intreavws.com) and the information that could be entered at this site.

Protocol on non-immune thrombocytopenia and type 2B VWD : Dr. Bussel introduced this project and discussed the details of a proposed questionnaire that would be sent to interested investigators. He encouraged anyone with an interest in this area to contact him.

US NHLBI VWD Document : Dr. Nichols presented information about the recently completed US NHLBI “clinical guidelines” project on VWD.

Von Willebrand Factor

Chairman: A. B. Federici, Italy

Co-chairs: T. Abshire, USA; G. Castaman, Italy; J. Di Paola, USA; J. Eikenboom, The Netherlands; E. Favaloro, Australia; A. Goodeve, UK; D. Lillicrap, Canada; R. Schneppenheim, Germany

Summary of Approvals and Working Parties:

1. Final report of the WP on VWF Assays in VWF in VWD Diagnosis; data to be reported on line www.vwfassays-in-vwd.com (C.A. Lee & A. Hubbard)
2. Continuation of the WP on Standardization of Multimeric Analysis, with more laboratories (U. Budde and C. Mazurier)
3. Continuation of the WP on the use of suitable reagents for VWF:CB (collagen binding assay) has been approved (L. De Marco, E. Favaloro, A. Hubbard, R. Seitz)
4. Final report of the WP on VWD classification; publication submitted to JTH (E. Sadler & the panel of VWD experts)
5. Final report of the WP on Standardization of methods for mutation and expression studies will continue (A. Goodeve, L. Hilbert, D. Lillicrap, R. Schneppenheim)
6. Progress report of the WP on development of new improved assays for ADAMTS-13 (R. Schneppenheim)
7. Progress report of the WP on requirements for shear-stress related VWF assays to be used in clinical diagnosis of VWD (Z.M. Ruggeri & A. Reininger)
8. Progress report of the International Prospective observational study on Biological response and clinical efficacy of DDAVP in VWD type 1 and 2 with reported data directly on line into the web site www.ddavp-in-vwd.com (A.B. Federici, G. Castaman, S. Lethagen)
9. Progress report of the WP on Acquired Von Willebrand Syndrome: standardization of pro-peptide and auto-antibody assays together with the enrollment of new cases on line: www.intreavws.com (U. Budde, A.B. Federici, J. H. Rand)
10. New WP on pre-analytical variables in VWD diagnosis (U. Budde & E. Favaloro)
11. New WP on the use of prophylaxis in VWD (T. Abshire & E. Berntorp)
12. New WP on the clinical & molecular markers of VWD type 3 with frequency of alloantibodies: a joint project of ISTH-SSC and WFH (A.B. Federici & P. Giangrande)

The VWF Subcommittee was attended by about 160 attendees at the first session and by about 130 at the second session. They actively participated in the discussion of the various topics provided in the program. The following represents the minutes of the program.

1) WP on VWF Assays (J. Eikenboom, The Netherlands) **Final report of the Working Party on VWF assays in VWD**

The organization of the study was reported by **Dr C.A. Lee**. Thirty two laboratories from 17 countries have participated in a study to evaluate VWF assays in the diagnosis of von Willebrand Disease (VWD) with the objective of determining the best diagnostic repertoire for VWD. Each laboratory received 11 or 12 individually coded samples comprising 6 VWD patient samples

with known genotype (types 1/2N, 2A, 2B, 2M, 2M smear, 2N) and 2 normal control samples (Group O and non-Group O) with 3 or 4 coded duplicate samples. All plasma samples were lyophilized in 0.5 ml aliquots and comparisons with frozen aliquots have indicated that the multimeric profile has not been affected by the lyophilization procedure. Laboratories were requested to perform the following 5 assay methods: VWF:Ag, VWF:RCo, VWF:CB, VWF:FVIII B and multimeric analysis. Samples were dispatched in May/June 2005 and results were returned by November 2005. Laboratories returned details of methodology together with a diagnosis and supporting laboratory data for each sample. The results have been decoded, sorted according to sample and dispatched for analysis by expert laboratories designated for each method.

Dr K. Friedman reported on the VWF:Ag assay. A diversity of reference calibrators was used (3 rd-5 th WHO standard). There was also variation in the reference ranges used (population based or ABO blood group stratified), in the size of the population surveyed for range definition, and in the lower limit of the range. Furthermore, the use of concordance ratios is unreliable when the VWF:Ag is low.

Dr E.J. Favaloro reported on the VWF:CB assay. The VWF:CB assay is effective in identifying loss of HMW multimers associated with type 2A and 2B VWD. VWF:CB (especially type I/III mixture) collagen based) is in this respect better than VWF:RCo. The results are, however, dependent on the source of the collagen used. VWF:RCo is better in identifying type 2M with a functional defect. VWF:CB does not replace VWF:RCo, but could possibly replace the need for multimers in selected cases.

Dr C.A. Sabin reported on the statistical aspects of the study. The diagnostic accuracy was high for 2A, 2N and normal individuals, but poor for 1/2N compound heterozygote, 2B, 2M and 2M smear individuals. The diagnostic accuracy was higher in the labs performing all assays. There was a good agreement (kappa value) between repeated diagnosis, however this is a measure of agreement and not of accuracy as consistently incorrect diagnoses give a high kappa value. Additional information was provided by **Dr. Budde** and **Castaman** on VWF Multimers and VWF:RCo assays.

VWF collagen binding versus VWF:RCo activities

Dr. A.B. Federici informed the audience that Dr. De Marco could not attend but he is still working on the isolation and characterization of human collagen type VI: he probably will report on this issue in Geneva . Dr. Federici presented data on the relationships between VWF:CB and VWF:RCo in a sample population of patients with VWD types 2A, 2B, 2M followed at the Angelo Bianchi Bonomi Hemophilia Thrombosis Center of Milan, all characterized by RIPA and by specific mutations. Patients with VWD type 2M showed RIPA > 1.2 mg/ml, VWF:RCo/Ag < 0.7 VWF:CB/Ag > 0.7; patients with VWD type 2B showed RIPA < 0.8 mg/ml, VWF:RCo/Ag < 0.7 VWF:CB/Ag < 0.7; patients with VWD type 2A showed RIPA > 1.2 mg/ml, VWF:RCo/Ag < 0.7 VWF:CB/Ag < 0.7. Collagen type I seems more sensitive than collagen type III in this setting of experiments. Therefore, differential diagnosis of VWD 2A, 2B, 2M can be obtained by using these tree tests together.

Conclusions. After discussion, there was agreement to publish the data of the WP on VWF assay in VWD diagnosis in a peer review Ms (future submission to JTH after presentation for formal approval by the ISTH-SSC on VWF in Geneva) and to go on with the WP on multimers and on collagen to standardize the minimal requirements and the appropriate collagen reagents for these two assays.

2) Proposal of WP on standardization on pre-analytical variables in VWF assays (G. Castaman)

Dr U. Budde and E. Favaloro presented data on the role of different temperature and processing-freezing-thawing of blood/plasma samples on VWF:RCo, VWF:CB and VWF multimers. After discussion there was a consensus that it is important to provide written recommendations about the best procedure to process blood and to prepare and store plasma samples to be devoted to VWF tests.

3) Final report of the WP on VWD Classification (David Lillicrap, Canada)

The final **proposal for an updated VWD classification** was presented by **Evan Sadler** and was formally approved by a unanimous vote **Dr. Sadler** will submit the Ms to JTH within two weeks.

4) Progress report of the WP on Molecular Biology and Expression Study (Anne Goodeve, UK)

Dr. L. Hilbert presented results of a survey; SSC/ISTH Working Party on VWF Molecular Biology and Expression Studies. The objective of this Working Party is to make a survey on the methods used for the identification and expression of von Willebrand disease (VWD) mutations. In 2005, 11 laboratories involved in 2 multicenter studies on type 1 VWD (European MCMDM-1VWD and Canadian projects) filled out a four page questionnaire to collect data on methodology. The major results from this pilot survey were presented during the SSC meeting in Sydney. A second questionnaire, with additional questions on both transient and stable transfection, was then sent not only to the participants from the pilot survey, but also to 21 other laboratories who had published data in this field. 22 laboratories from 15 countries responded and 19 laboratories completed the questionnaire. This international survey allows recommendations to be made on the conditions for the identification of a von Willebrand factor (VWF) mutation and the construction of a mutated expression vector. At present, no recommendations may be drawn for stable transfection because little data (4) is available. The 12 responses concerning transient transfection of VWF were heterogenous, so new participants must be recruited, and further discussion must take place before the best conditions are fixed. All interested parties, please contact hilbert@lfb.fr to participate. **Dr. Hilbert** will prepare a written report to be published as SSC Communication

Dr. S. Haberichter presented VWF pro-peptide assay for identification of type 1 VWD patients with decreased VWF survival. The decreased survival of VWF in plasma has recently been identified as a novel mechanism for type 1 VWD. We report four families with moderately severe type 1 VWD characterized by low plasma VWF:Ag and FVIII:C levels, proportionately low VWF:RCo, and dominant inheritance. A decreased survival of VWF in affected individuals

was identified with VWF half-lives of 1-3 hours, while the half-life of VWF propeptide (VWFpp) was normal. DNA sequencing revealed a single (heterozygous) VWF mutation in affected individuals, S2179F in two families, and W1144G in two families. We report preliminary data establishing the normal range of VWFpp and VWFpp/VWF:Ag for each blood group. We demonstrate that the ratio of steady-state plasma VWFpp/VWF:Ag can be utilized to identify patients with a shortened VWF half-life. An increased ratio distinguished affected from unaffected individuals in all families. A significantly increased VWFpp/VWF:Ag ratio together with reduced VWF:Ag may indicate the presence of a true genetic defect and decreased VWF survival phenotype. This phenotype may require an altered clinical therapeutic approach and we propose to refer to this phenotype as type-1C VWD.

Proposal for the future work of this WP; Molecular screening recommendations in type 1 VWD
Discussants: **A Goodeve, D Lillicrap, P Collins**

Dr. P. Collins presented the UK Haemophilia Centre Doctors' Organization investigation into the molecular pathogenesis of type 1 VWD. Of 40 families diagnosed by UK Hemophilia Centers to have type 1 VWD 8 (20%) families were found not to have type 1 VWD following review. Six families were re-diagnosed to have type 2 VWD, 1 family was found to have a platelet storage pool disorder, and 1 family was determined to be unaffected. In the remaining 32 families direct DNA sequencing of the essential regions of the *VWF* gene identified a total of 11 different candidate mutations in index cases of 20 of the 32 confirmed type 1 VWD families. These included R1205H (4 families), Y1584C (8 families), R924Q (3 families), 2 other missense changes, 4 candidate splice site mutations and a single nt deletion. No candidate *VWF* gene mutation was identified in 12 of the 32 type 1 VWD index cases (38%). Linkage analysis showed that in 13 of the 32 families it was likely that VWD segregated with the *VWF* gene. In 8 families, VWD did not segregate with the *VWF* gene. Y1584C was present in 8 families but did not co-segregate with VWD in all cases. It was associated with blood group O in 95% of cases. In the rest of the cohort 70% of patients were blood group O.

Dr. A. Goodeve presented results of the MCMDM-1VWD study. 150 families with type 1 VWD were analyzed for mutations, and were identified in 105 (70%). A total of 124 candidate mutations were identified, of which 75 were different and of which 55 were novel. 18 (12%) index cases had 2 or more candidate mutations. 80% of the mutations were missense alterations, and these were located throughout *VWF*. Several changes were recurrent; R854Q (5 cases), R924Q (4 cases), C1130F/G/R (7 cases), R1205H (10 cases), R1374C/H (6 cases) and Y1584C (13 cases). Blood group O contributed significantly to low VWF, being present in 65% of index cases. In families lacking an identified mutation, blood group O was present in 78%, and in 95% of 19 families lacking a mutation and demonstrating non-linkage to *VWF*. Only a small proportion of families had candidate mutations demonstrating fully penetrant VWD.

Dr. D. Lillicrap presented a summary of the Type 1 VWD Canadian Cohort Study. One hundred and twenty three index cases and their families were studied and putative mutations identified within the *VWF* gene in 63% (n=78) of index cases leaving 37% (n=45) with no identified changes. These changes comprised 50 different putative mutations; 31 (62%) missense mutations, 8 (16%) changes involving the *VWF* transcriptional regulatory region, 5 (10%) small deletions/insertions, 5 (10%) splicing consensus sequence mutations, and 1 nonsense mutation.

Twenty-one of the index cases had more than one putative *VWF* mutation identified. Although a total of 50 different mutations were identified in this study, twelve of the mutations occurred in multiple index cases. These twelve mutations were found in 63 (51%) of the index cases and, with one exception, these were missense mutations.

Presenters suggested that mutation screening of the *VWF* gene has limited general utility in genetic diagnostic and family studies in type 1 VWD. If genetic studies are performed, the incomplete penetrance and variable expressivity of type 1 VWD must be taken into account. Unless linkage of VWD phenotype with the *VWF* gene can be clearly demonstrated, the results of any genetic family studies should be interpreted with caution.

5) Progress report on Shear-stress Assays in VWD (Emmanuel Favaloro, Australia)

Dr. Z. M. Ruggeri discussed the general concepts for the use of shear-stress tests as a measurement of VWF function. He described the most recent data about the basic mechanisms on VWF-platelet interactions in different shear-rates conditions, from relatively low to high shear rates and about the role of VWF with different multimers on platelet adhesion (only dimeric VWF A1 domain) and platelet-platelet interactions (all the VWF multimers). He is positive about future applications of these methods in a more clinical setting to determine VWF defects in patients with VWD and suggest to make a joint WP with the SSC on biorheology.

Dr. A. Reininger in his presentation suggested that future diagnostic tools for the clinical application in VWD patients building on these new insights may therefore include various blood flow assays at elevated shear rates, and – although complicated – direct, real-time visualization and quantification.

Conclusion: Contacts with the SSC on Biorheology and vascular biology should be organized to make final statements about the application of these tests on VWD diagnosis

6) WP on ADAMTS-13 Assays (Reinhard Schneppenheim , Germany)

Dr. R. Schneppenheim organized a very interesting session with presentation of the most recent work of several laboratories actively working on ADAMTS-13 assays.

- a. Comparison of FRETTS-VWF73 to full-length VWF as a substrate for ADAMTS13 activity measurement in human plasma samples presented by Dr **A Veyradier**

The FRETTS-VWF73 fluorescence assay was compared with a home made IRMA using full-length VWF for the measurement of ADAMTS13 activity in plasma samples from a cohort of 64 patients with thrombotic microangiopathies (TMA) including 41 acquired thrombotic thrombocytopenic purpura (TTP), 3 inherited TTP and 20 hemolytic uremic syndrome (HUS) and also from 20 normal subjects. Both methods were correlated ($y = 0.94x + 1.71$, correlation coefficient 0.97). With both methods, all normal subjects and most HUS patients showed ADAMTS13 activity higher than 50% although all TTP patients exhibited an ADAMTS13 activity lower than 5%. Compared to the IRMA method, the FRETTS-VWF73 assay exhibits reliable results to measure low, moderately reduced as well as normal levels of ADAMTS13

activity. Accuracy and reproducibility of the assay combined with its short incubation time make the method highly attractive and appropriate for the clinical screening of patients with TMA.

- b. ADAMTS13 activity measurement: comparison of FRETTS-VWF73 and other static assays presented by **JA Kremer-Hovinga**

Comparison of a slightly modified FRETTS-VWF73 assay with older static assays using a full-length VWF substrate showed a good agreement between the ADAMTS-13 activity determined by FRETTS-VWF73 and the older assays (Kremer Hovinga, Mottin and Lämmle, J Thromb Haemost. 2006;4:1146-8). However, the presence of hemoglobin in a patient's sample constituted a problem in the FRETTS-VWF73 assay and preliminary data indicate that discrepancies in ADAMTS-13 activity between different assays may be observed in rare cases of acute thrombotic microangiopathies.

- c. Japanese experience of novel ADAMTS13 activity-ELISA on patients with TMA and liver transplantation, presented by **Dr M. Matsumoto**

The group developed a convenient and highly sensitive ELISA for ADAMTS13 activity. This ELISA precisely determined the half-life of infused plasma ADAMTS13 activity in USS patients to be between 2.3 and 3.5 days. A rapid fall in the level of ADAMTS13 activity after liver transplantation was found. Platelet transfusions in respective patients are therefore not recommended, but plasma infusions to supply ADAMTS13 after liver transplantation.

- d. ADAMTS13 activities and genetic polymorphisms in the Japanese general population, presented by **Dr. K. Kokame**

Plasma ADAMTS13 activities in the Japanese general population (N = 3,616) were measured by the FRETTS-VWF73 assay. The values did not show a Gaussian distribution, suggesting that some kind of factor should affect the activity. The activities in males were lower than those in females. In both genders, the activities became lower with age. The plasma VWF level was not related to the ADAMTS13 activity. Comprehensive search revealed that Japanese people have six common missense polymorphisms. The P475S polymorphism significantly lowered plasma ADAMTS13 activity.

- e. Biosynthesis, secretion and regulation of ADAMTS13 protease in human vascular endothelial cells-relevant to TTP presented by **Dr X. Long Zheng**

Zheng and his colleagues have shown that ADAMTS13 is synthesized and secreted from arterial, venous and microvascular endothelial cells; ADAMTS13 is secreted apically in polarized endothelial and epithelial cells; the apical secretion of ADAMTS13 depends on the CUB domains and their association with lipid rafts. Mutations in the CUB domains of ADAMTS13 may disrupt the signal directing polarized secretion, thereby leading to congenital ADAMTS13 deficiency and thrombotic thrombocytopenic purpura. Our data suggest that ADAMTS13 produced in the vascular endothelial cells may contribute significantly to the plasma levels of ADAMTS13 protease.

- f. Podocytes express ADAMTS13 in normal renal cortex and in a patient with TTP presented by **Dr.M. Manea**

This study showed intracellular ADAMTS13 expression in podocytes in normal renal tissue and in the kidney of a TTP patient with the compound heterozygous mutation P353L and P457L. Expression of the mutants revealed impaired secretion and activity. Podocyte-derived ADAMTS13 may serve as a local protective mechanism in the high-shear microcirculation of the glomerulus. Deficient activity could result in the formation of platelet plugs in the glomeruli as seen in TTP.

- g. Action of recombinant ADAMTS13 on recombinant mutant VWF: VWF type 2A with decreased proteolysis and VWF type 2B with enhanced proteolysis presented by **R. Schneppenheim**

The enhanced proteolysis of VWF in patients with classic VWD type IIA is due to an intrinsic susceptibility of mutant VWF to ADAMTS13. VWF type 2B is proteolyzed to a similar extent, however the underlying mechanism has not been experimentally elucidated. The results of a study on ADAMTS13 proteolysis of recombinant VWF 2B and VWF IIE, the latter being a phenotype with decreased proteolysis suggest that in a static assay both mutants are proteolyzed roughly normal. Therefore, the different phenotypes are most probably due to different function under flow conditions: enhanced proteolysis of VWF type 2B as a result of enhanced affinity to platelets and decreased proteolysis of VWF IIE due to its poor functional activity in platelet dependent hemostasis, respectively.

- h. Could Chloride binding to VWF explain the occurrence of TTP in subjects with normal levels of ADAMTS13 ? presented by **Dr R. De Cristoforo**

Physiological concentrations of NaCl inhibit the hydrolysis of VWF by ADAMTS-13. It was demonstrated that chloride ions specifically bind to the A1 domain of VWF. Cl⁻ ions stabilize a “super-folded” conformation of VWF through binding to the A1 domain. The affinity of Cl⁻ for the VWD R1306 mutant was reduced and its hydrolysis by ADAMTS-13 accelerated. These findings showed that Cl⁻ ions control allosterically the availability of the Y1605-M1606 bond to the proteolytic attack of ADAMTS-13.

After a fruitful discussion among the participants and the audience, **Dr. Schneppenheim** proposed issues of ADAMTS13 and VWF of current interest:

- a. to define the ideal ADAMTS13 functional assay to be equally applicable for individual samples and for large cohorts of patients, respectively
- b. to further characterize ADAMTS13 with respect to i) other substrates apart from VWF, ii) the biological significance of alternative ADAMTS13 splice products, iii) its local distribution in different tissues and cells
- c. the role of ADAMTS13 in conditions like sepsis, stem cell transplantation and other microangiopathies
- d. therapeutic issues like the development of rhuADAMTS13

7) Progress report on WP on Acquired von Willebrand syndrome (J. Di Paola, USA) Dr U. Budde presented the technical problems on the measurements of auto-antibodies in AVWS: he showed that the use of plasma-derived or recombinant VWF used to capture auto-antibodies from the patients serum or plasma can be critical for the assay due to the role of blood groups. He proposes to use recombinant VWF from insects. **Dr. C Mazurier** proposes to use plasma-derived VWF from donors with blood group O.

Dr. K. Mertens, on behalf of **Dr. J van Mourik** , reported on the significance of the measurement of the plasma concentration of the pro-peptide of VWF (VWAgII) in the diagnosis of acquired von Willebrand syndrome (AVWS). An increased steady state pro-peptide/VWF ratio is clearly indicative of decreased survival of mature VWF, as encountered in AVWS or certain congenital VWF defects, and useful as an index to assist in the diagnosis of abnormal VWF catabolism. The molar concentration of plasma pro-peptide was assessed by calibrating pooled normal plasma against recombinant pro-peptide purified to homogeneity. This approach allows straightforward standardization of pro-peptide assays.

Dr R.R. Montgomery reported his experience with his assay with VWF pro-peptide that was tested together with native VWF:Ag in normal individuals with different blood groups. This assay will be commercially available soon. After a general discussion, a standardization of these assays was proposed for the use in AVWS and also in patients with different forms of inherited VWD.

8) Progress reports and new proposal of WP and clinical surveys (T. Abshire, USA)

Dr. G. Castaman presented the progress report of the WP on Biological response versus clinical efficacy of DDAVP in VWD type 1 and 2; 128 patients have been enrolled. The dead line for enrollment is postponed to 31 st December 2006. The study is recruiting patients through the web site – www.ddavp.in.vwd.com.

Dr, A.B. Federici proposed a new WP on the clinical & molecular markers of VWD type 3 with frequency of alloantibodies to be organized as a joint project of ISTH-SSC and WFH

Dr. T. Abshire proposed a new WP on the use of prophylaxis in VWD. This project is composed by a retrospective survey and by a prospective study on the use of different VWF concentrates in the secondary long-term prophylaxis of hemarthrosis, epistaxis and G I bleeding in severe VWD patients with a VWF:RC₀ < 20 U/dL.

9) Other reports and proposals, concluding remarks (A. B. Federici, Italy)

Dr. A. B. Federici presented the concluding remarks of the entire two sessions, summarizing all studies and proposals to be reported next year in Geneva.

von Willebrand Factor

Chair: D. Lillicrap (Canada)

Co-Chairs: T. Abshire (USA), G. Castaman (Italy), J. DiPaola (USA), J. Eikenboom (The Netherlands), E. Favaloro (Australia), A. Federici (Italy), A. Goodeve (UK), B. Lämmle (Switzerland), R. Schneppenheim (Germany)

335 people in attendance

Working Group on VWF assays in the diagnosis of VWD (Christine Lee, UK)

Plasmapheresis of 6 patients with different types of VWD. Prospective study involving 32 laboratories worldwide. Laboratories from 17 countries participated. Laboratories reported a median 6 correct diagnoses. 16 laboratories could perform all of VWF tests. The Lyophilized plasma samples collected for this study maintained their multimeric patterns. All 5 assays are required for optimal diagnostic evaluation. The more tests performed the better. At low levels of VWF the RCo:Ag ratio is problematic.

VWF collagen binding assays (Emmanuel Favaloro, Australia)

A number of laboratories performed VWF:CB in the recent prospective assessment of diagnostic tests for VWD. Discussed the relative merits of the VWF:CB in the VWD diagnostic samples. Bovine and equine tendon material is preferred (type 1 collagen). Can give results that are more sensitive than the VFW:RCo. Discussion of the potential of alternative forms of collagen.

VWF propeptide (Bob Montgomery, USA: Jan van Mourik, Netherlands)

The VWF propeptide can identify patients with accelerated clearance of VWF. Should the measurement of the VWF:pp and the VWFpp/VWF_{Ag} be standardized? Prior assessment of the VWF:pp with other existing ISTH and WHO standards for VWF has shown good consistency. The pp:Ag ratio is also consistent. There is an influence of the ABO blood group on the pp:Ag ratio. There is a need to involve more laboratories to extend the assessment of the VWF:pp assay. We need to agree upon the accepted nomenclature for the VWF:pp. Measurement of the VWF:pp and VWF:Ag also allow assessment of endothelial cell perturbation. The respective half-lives of the VWF:pp and VWF:Ag are 2.5 and 12 hrs. The assays of VWF:Ag and VWF:pp can be used to diagnose acquired VWD where VWF is cleared prematurely from the plasma. The plasma level of VWF:pp is 5.5 nmol/L.

VWF Standards (Tony Hubbard, UK)

The current VWF plasma standard is the 5 th WHO FVIII/VWF plasma standard (202/150 established in 2003). 700 ampoules of this standard are distributed each year. There is a need to replace this plasma standard by end of 2009. The 1 st WHO VWF concentrate standard (00/514) was established in 2001. There have been problematic discrepancies using collagen binding assays with the VWF concentrate standard. Collagen binding assays are very sensitive to the HMW multimers. It is important to correlate concentrate and plasma units for diagnosis and

therapeutic purposes. The requirement of the highest MW VWF multimers is still uncertain in therapy. Proposal for a 2nd VWF concentrate with a lack of HMW multimers to try to address the issue of the collagen binding assay discrepancies.

Standardization of VWF propeptide using SSC Lot#3 standard. It is proposed that this initially be undertaken by a small number of laboratories – 5/6 labs. Future international assessment of the VWF:pp standardization. There are currently also two different assays that have been developed in the laboratories of Drs. Montgomery and Van Mourik. There appear to be some pre-analytical factors that will alter the assay results. Working groups need to be established for propeptide measurement and for the use of collagen binding assays in standardizing VWF concentrates. The committee voted in favor of the formation of these working groups.

Acquired von Willebrand Syndrome - aVWS (Augusto Federici, Italy)

There was an initial discussion of the diagnostic background and the associated diseases and the assays needed to make the diagnosis. There is a need for better assays to identify anti-VWF autoantibodies. A working group is already in place to evaluate this diagnosis. An online registry has been established for Acquired VWS. 3 centers have enrolled 12 patients into the online registry. There is an interest in using the registry for assessment of the frequency of aVWS. MGUS and ET were the most frequent accompanying conditions. Also need to assess incidence of aVWS in association with anti-phospholipid syndrome. Finally, there is a need to evaluate novel therapies for aVWS.

ADAMTS13 assays (Koichi Kokame, Japan)

There was a discussion of the various ADAMTS13 assay methodologies that have been developed. All are difficult to use in the clinical laboratory. An international interlaboratory study has been performed and reported but there still needs to be advances for the reliability of this test. There was a description of the VWF 73mer substrate assay using a 73mer peptide sequence around the ADAMTS13 cleavage site. This assay incorporates a FRET readout and is a single step assay. In a large population study, women had higher levels of ADAMTS13 than males. The cause of this variance is as yet unexplained.

ADAMTS13 resistance (Reinhard Schneppenheim, Germany)

The effect of variation of the VWF substrate on ADAMTS cleavage was discussed, with a consideration of the concept of a more resistant VWF molecule. Sequence variation in the A1 region has been shown to produce ADAMTS13 resistance. Type 2B rVWF appears to be ADAMTS13 resistant. In some type 2B mutants this ADAMTS13 resistance is profound. The type 2B New York/Malmo variant is ADAMTS13 resistant. There is a possibility that this could also enhance VWF clearance. This phenotype should be considered in TTP patients in whom ADAMTS13 deficiency is not present. In these patients, A1 domain VWF sequencing should be performed.

TTP - ADAMTS13 mutation registries (Johanna Kremer Hovinga and Bernhard Laemmle, Switzerland and Yoshihiro Fujimura, Japan)

There was a description of the Berne and Japanese experiences with Upshaw-Schulman Syndrome and other forms of thrombotic microangiopathy. Both presenters summarized the clinical experience they had documented. There is now an extensive molecular genetic experience with this condition. There is a proposal for an international registry to improve international awareness, to improve genotype-phenotype correlations and to learn more about the natural history of TTP. Beware the re-reporting of the same patients in the literature. Some patients are studied repeatedly. The committee voted in favor of establishing an international TTP registry. There was further discussion of thrombotic microangiopathic pathologies in 783 patients from Japan. There had been extensive investigation to evaluate the causative pathologies in these cases. Note that acquired TTP can occur in children. The outcome of these cases is poor. Diagnosis is not easy and sometimes wrong.

Standardized Bleeding scores (Francesco Rodegheiro, Italy and Paula James, Canada)

There was a review of the history of bleeding scores by the SSC and other bodies. There have been previous publications on this issue from the SSC. There is already a bleeding score questionnaire available at the VWF ISTH website. Ideal features of a bleeding score were described. There is no current ‘gold standard’ for mild bleeding in particular. The aim of this initiative is to improve diagnosis, treatment and communication about bleeding within the community. In Canada, the original Vicenza survey was condensed to be able to administer this in 5-10 mins. This maintains a -1 to +4 scoring system and has positive scores of >+3 in males and >+5 in females. Data on >230 subjects was presented from 3 ongoing studies to assess the application of this bleeding score. There was a proposal for the establishment of a joint working group (with the pediatric SSC) to develop guidelines for a quantitative bleeding score. The committee voted in favor of this proposal.

Report on DDAVP study in VWD (Giancarlo Castaman, Italy/Stefan Lethargan, Denmark)

The initial part of this international prospective study has been completed. 245 patients have been enrolled. The study will continue as the enrolled patients are followed with therapeutic administration of DDAVP. There are all types of VWD enrolled in the study

Report on Prophylaxis in VWD (Tom Abshire, USA and Eric Berntorp, Sweden)

This is a prospective international multicenter study. Central data collection center will occur in the USA and there is a central laboratory site in Sweden. The study will evaluate the effects of prophylactic therapy on joint bleeding, mucosal bleeding, epistaxis and menorrhagia. This is a prospective non-randomized study. Any licensed VWF product can be used for the prophylactic therapy. The treatment strategy will involve a dose-escalation approach. Dose escalation will be carried out based on defined bleeding events. IRB review currently in progress at several centers. Enrolment will start later in 2007.

Report on type 3 VWD Registry (Augusto Federici, Italy and Paul Giangrande, UK)

There is need for a much better estimate of the prevalence of severe type 3 VWD. We also have no idea of how many of these patients have anti-VWF antibody development. In some of these antibody +ve patients episodes of anaphylaxis have been reported with the infusion of VWF concentrate. There is a significant need for a standardized anti-VWF antibody assay. Proposal for a multicenter, multinational study involving both developed and developing countries. This will involve an outreach study in which larger expert centers will assist smaller centers in the diagnosis and therapy of this condition. There is an aim to enroll approximately 500 patients for analysis.

VWF mutation and polymorphism database (Anne Goodeve, UK and Dan Hampshire, UK)

A summary was provided of the updated web-based VWF mutation database. There has been a recent significant increase in the reporting of VWD mutations. These include all types of VWD. Mutations are found across the VWF gene and involve all types of mutation. Some new categories of sequence variation have been added to the database. There was a call for the submission of newly acquired data.

VWD type 2B and Platelet type VWD Registry (Maha Othman, Canada)

There was a description of the background of the problem. The clinical implications of the two diagnoses was highlighted. There is no idea what the frequency is for PT-VWD. Discrimination can be done with phenotypic studies or by genotype. A proposal was made for a new international registry for PT-VWD. The committee voted to support the establishment of this registry.

Von Willebrand Factor

3-4 July 2008
Vienna, Austria

Chairperson: *David Lillicrap (Canada)*

Co-Chairs: *Thomas Abshire (USA), Giancarlo Castaman (Italy), Jorge DiPaola (USA), Jeroen Eikenboom (Netherlands), Emmanuel Favaloro (Australia), Anne Goodeve (UK), Bernhard Lammle (Switzerland), Reinhard Schneppenheim (Germany)*

VWF Assays for VWD Diagnosis:

Multimer Standardization: Ulrich Budde (Germany)

- 4 population studies used for standardization purposes. Many methodologic variables.
- No significant effects of lyophilization of samples – multimers preserved.
- CVs vary significantly between methods especially for the type 2 variants.
- Multimer patterns are sometimes mutation-specific.
- Medium resolution gels are recommended as “universal” gels for multimer profiles.
- Useful to have a supra-normal multimer control.

Collagen Binding: Emmanuel Favaloro (Australia)

- Still major problems with the VWF:RCo assay. ~40% CV in many EQA settings.
- VWF:CB improves diagnostic precision. Also adds a complementary functional assay.
- Current VWF:CB assays too good for binding VWF. Often, just type 3 collagen – this may be too sensitive.
- Need better optimized type I/III VWF:CB assays. Better plasma and concentrate standards also required.
- No point in further field testing until we have a more optimized assay.

Alternative Functional Assays: Bob Montgomery (USA) and Hans Deckmyn (Belgium)

- The current aggregometry-based VWF:RCo assay is poorly reproducible and has a poor sensitivity <10%.
- We need a better functional platelet-dependent assay.
- Instead, Deckmyn assay uses soluble wild type GPIb in an ELISA protocol. Still needs ristocetin to promote binding. Avoids use of human platelet variability.
- In the Deckmyn assay – better CV and much better sensitivity (0.0005 U/ml). Correlates with VWF:RCo results.
- Needs production of recombinant soluble GPIb.
- Potential for using glyocalicin as the soluble GPIb fragment. Need to “re-set” glyocalicin. Good CV <10% sensitivity 0.0005 U/ml. Correlates with soluble GPIb and VWF:RCo assays.
- In Montgomery assay – PT-VWD mutants used (enhanced VWF binding) – expressed in HEK293 cells with GPIbbeta and GPIX. Using the GPIb expressing 293 cells, good correlation with VWF:RCo results. No requirement for ristocetin in this assay. Multimer size sensitive. Enhanced by microtiter plate vortexing.

Flow-regulated Assays: John Heemskirk (Netherlands)

- Need to take into account the key role of flow in mediating VWF-GPIb interaction. Recent publication in JTH by Biorheology SSC.
- PFA assay one example of a flow-mediated assay. But there are some limitations.
- Cone and plate analyzer also an alternative but again with limitations.

- Potential of establishing a plasma bank of abnormal functionality for assessment in flow systems.

VWF Propeptide Assays: Bob Montgomery (USA) and Bas De Laat (Netherlands)

- VWF:pp is a marker of endothelial perturbation. Can also be used as an indirect marker of VWF clearance. Half-lives – VWF:pp 2.5 hrs: VWF:Ag 12 hrs.
- Subset of type 1 VWD patients with increased VWF clearance. Higher VWFpp/VWF:Ag ratio.
- Sanquin and GTI commercial test comparisons show initial very good correlation of the two VWF:pp assays.
- Measurement of molar units or IUs.
- Need for a VWF:pp standard. Possibility of ISTH #3 as the standard – assigned as 1.06 U/mL. Assume a VWF:pp to VWF:Ag ratio of 1.
- Need to finalize, agree upon, an abbreviation for the propeptide. ?VWFp

Acquired VWD: Ulrich Budde (Germany)

- A rare disorder but may be under-diagnosed.
- Disease associations – lymphoproliferative disease, myeloproliferative disease, cardiovascular disease.
- Bleeding tendency is high.
- Some functional defects measured by especially VWF:CB.
- In cardiovascular associated cases often loss of HMW multimers.
- Testing for the anti-VWF autoantibodies is problematic. High background binding.
- Web site available for acquired VWD registry.

VWF Antibody Testing: David Lillicrap (Canada)

- A rare complication of a rare disease (type 3 VWD). Prevalence ~5%. Incidence unclear. Not in any other form of VWD.
- Predictors of antibodies. VWF genotype – deletions and nonsense mutations.
- No standardized methodology. Both binding and functional (FVIII binding/platelet binding) inhibitory assays.
- Sometimes associated with anaphylactic responses or delayed hypersensitivity.
- Therapy possible with rFVIII and/or platelets.
- Formalized antibody testing program being established for introduction of rVWF.

Standardized Bleeding Scores:

Adult bleeding scores: Francesco Rodeghiero (Italy), Alberto Tossetto (Italy)

- Different aims in different cases. May be a challenge to have a single score. Should we aim for a universal scoring system or should this be disease-specific?
- Main aim is to develop the tool for mild bleeding disorders.
- Development of a structured questionnaire. This should be standardized. Should have good inter-observer reproducibility. A score should be developed. Then validate the scoring system prospectively in patients vs controls.
- Presentation of some example pages from the Rockefeller on line bleeding database (Barry Collier).
- Differentiation of severity in severe bleeding disorders may be difficult due to early score “saturation”.

Pediatric bleeding scores: Paula James (Canada)

- Special issues for children with bleeding. Less chance of hemostatic challenges. Different patterns of bleeding.
- Previous studies in children have been semi-quantitative in type.

- Discussion of Pediatric Vicenza Bleeding Questionnaire (-1 to +4).
- Three study groups evaluated: normal children (positive score >2), most frequent symptoms minor wounds; prospectively assessed outpatient children (135 subjects) – some mild type 1 VWD cases. The pediatric scoring system showed a very good ROC performance (0.86): final group – children with previous diagnosis of a bleeding disease (average scores of >6).

Preoperative bleeding scores: Christoph Bidlingmaier (Germany)

- Pre-operative bleeding assessment. Large country-wide survey. 555 bleeding reports. 8% bleeding complications in tonsillectomy cases. 2% bleeding in adenoidectomy.
- Multitude of ways to screen – assays (PT/PTT etc) and many different questionnaires.
- In prospective study 42% +ve bleeding history but normal PTT. 30% +ve bleeding history with a defined bleeding disorder.
- A normal bleeding history still missed 10% of bleeding problems.
- History problems – language, memory, ethnic contexts.
- Now, prospectively using the Pediatric Vicenza scoring system pre-operatively.

ITP bleeding scores: Cindy Neunert (USA)

- Rationale for disease-specific scoring system. Example of ITP vs marrow failure-related thrombocytopenia.
- Discussion of prior ITP scoring systems. Some only semi-quantitative but some much more quantitative.
- Inverse correlation between platelet count and incidence of bleeding. Correlation between skin and oral bleeding.
- Prior studies have not been designed with vigorous methodology.
- For future studies – more rigorous methodology required. Validity, reliability and responsiveness should all be included in future plans. Planned to incorporate into the SSC Working Party.

VWF Plasma and Concentrate Standards: Tony Hubbard (UK)

- Currently, 5th International Plasma VWF/FVIII Standard and 1st International VWF/FVIII Concentrate Standard
- Both will need to be replaced in next 2 years.
- For Plasma Standard already 20,500 ampoules filled. Looking for endorsement by SSC in Boston 2009.
- Concentrate standard ampoules being used - x 400-600/year.
- Concentrate standard will need endorsement by SSC in May 2010.
- Still problems with collagen binding assay for the VWF/FVIII concentrate.

Concentrate functional testing: Andreas Hillarp (Sweden)

- Consideration of automated ristocetin cofactor assays for VWF concentrates.
- Different reagents and different equipment.
- With the automated testing the RiCoF levels of concentrates are all low (~50%). This improves with increasing ristocetin concentration in the assay (up to 1.8 mg/ml). Beware ristocetin precipitation.

Classification of VWD: Javier Battle (Spain) and David Lillicrap (Canada)

- Should types 2A and 2M be differentiated?
- Some variants have been variably classified.
- Multimer abnormalities are often very subtle.
- There have been rare cases of distinct collagen binding defects (ie. 2M collagen type)..
- Differentiation between severe type 1 and type 3 VWD remains problematic.

- Practical advantages of type 2M differentiation?

ADAMTS13

ADAMTS13 Polymorphisms and Mutations in Japan: Koichi Kakame (Japan)

- 11 patients with Upshaw-Schulman Syndrome. R193W most common ADAMTS13 mutation.
- Now more than 80 ADAMTS13 mutations worldwide. No hotspots - throughout ADAMTS13 gene.
- Six ADAMTS13 polymorphisms prevalent in Japanese.
- ADAMTS13 activity levels lower in males.
- Q448E and P475S polymorphisms both have an influence on the ADAMTS13 levels.
- Subgroup (1%) of general population with low ADAMTS13 levels – sequenced ADAMTS13 gene. In this group there are some new, not previously-described ADAMTS13 mutations.
- Calculation of frequency of inherited ADAMTS13 deficiency – 1 in 1.14 million (80-150 USS patients in Japan). We do not know how many of these cases have yet to be identified.

TTP registries: Yoshihiro Fujimura (Japan)

- Central referral center at Nara University Medical Center. 882 thrombotic microangiopathy syndromes referred to date. 4% are due to USS.
- ADAMTS13 assays (activity and antigen) and ADAMTS13 genetic analysis.
- 37 Upshaw-Schulman Syndrome patients - clinical features described. Recurrent thrombocytopenia in childhood (78%) and 100% incidence of pregnancy-related thrombocytopenia.
- Half-life of plasma ADAMTS13 - 2.4 days. Some USS patients develop severe reactions to plasma. Will need rADAMTS13. No ADAMTS13 antibodies seen.
- One patient with ADAMTS13 level of 5% but no symptoms by adult life.

ADAMTS13-VWF interactions: Reinhard Schneppenheim (Germany)

- Variants of VWF with differential ADAMTS13 proteolysis. Can be either increased (type 2A and 2B VWD) or decreased.
- Will often be identifiable by abnormal multimers.
- Can also demonstrate this with the use of either the full-length or smaller VWF substrates (ie.A1-A3 domains).
- Beginning to test some of the VWF substrates under shear influences. There should be a stringent analysis of the qualitative features of the VWF being evaluated.

Standardized Animals Models of VWD: Cecile Denis (France) and David Lillicrap (Canada)

- Type 3 VWD dogs and VWF KO mice. For in vivo evaluation of VWF variant function.
- In VWD animal models FVIII:C levels are higher 20-50%.
- Species specificity for GPIb binding and ADAMTS13 processing.
- VWF reconstitution with murine VWF - hydrodynamic delivery.
- With pLIVE vector (liver-specific) promoter – VWF expression for 4+ weeks.
- Differences in multimer profiles in the animals expressing from hydrodynamically-delivered plasmid.

DDAVP Biological Responsiveness: Stefan Lethagen (Denmark)

- International study – study update – study is ongoing. Two stage study – biological response followed by clinical response. Two year follow up. 13 centers have enrolled 247 patients.
- 182 type 1 VWD patients. 23 Type 2A VWD and 21 type 2M VWD.
- 174 patients have completed biological response evaluation.

- Clinical evaluation in approx. 45 cases to date. Variable results. Bleeding cessation/DDAVP efficacy in 67/77 patients. 78 bleeding events in 25 patients. Mainly epistaxis and menorrhagia. Evaluation further complicated by use of antifibrinolytics.

Type 3 VWD ISTH-WFH Initiative: Augusto Federici (Italy)

- International study involving interaction with World Federation of Hemophilia.
- Type 3 mutational background – heterogenous. No clear founder mutations.
- Many unresolved issues for this rare disease. Goal to collect and analyze 500+ patients. Multiple clinical and laboratory parameters to be collated.
- Even in type 3 VWD ~48% of patients may not bleed in one year. Unclear why there is clinical heterogeneity.
- Initial funds obtained from Bayer Hemophilia Awards program to start this project. Will need additional funds to complete the project.

Prophylactic Treatment of VWD: Tom Abshire (USA)

- Organization of Prophylaxis Study Network. International multicenter study.
- Multiple outcome analysis – clinical and quality of life assessments. Will look at a number of types of bleeding – menorrhagia, epistaxis, joint bleeds and GI bleeding.
- Non-randomized dose escalation approach - starting dose 60 VWF:RCo units/kg then going up if bleeding continues.
- Will include type 1 and type 2A VWD that are non-DDAVP responsive and type 3 VWD.
- Strict bleeding type and frequency inclusion criteria.
- Also some retrospective analysis from past 1-2 years.
- Current status - 22 centers enrolled. 37 patients enrolled to date from 6 clinical centers.

VWF and VWD Registries and Nomenclature: Anne Goodeve (UK) and David Lillicrap (Canada)

- ISTH VWF Registry. Dan Hampsire is the University of Sheffield maintenance person.
- Platelet-type VWD registry now on-line. 55 patients assessed. Type 2B VWD confirmed in 25-60% of cases.
- Encouragement to keep submitting new information to the database. In particular the VWF SNPs need to be added.
- Currently, 26% of mutations in the Database are type 1 VWD. 19% 2A, 6% 2B.
- Encouragement to reduce number of “unclassified” variants.
- Proposal for change in Reference sequence – use chromosome 12 reference sequence deposited in GenBank.
- Proposal for SSC publication on the database. Hopefully to be published in the coming year.
- Nomenclature issue – use of Human Genome Variation Sequence (HGVS) convention.

Submitted by D. Lillicrap