

**1995 MINUTES**  
**FACTOR VIII and FACTOR IX SUBCOMMITTEE**

**Saturday 10 June, 1995, 8.00 - 12.00**

Chair: Ian Peake, UK

Co-Chairs: Ernest Briet, Netherlands; Jorgen Ingerslev (secretary), Denmark; Carol Kasper,  
USA;

Christopher Prowse, UK; Yvette Sultan, France

1. Chairman outlined the morning's program and stressed the shortage of time. At present there is one document with the SSC seeking a final approval for publication (In vivo recovery of factor VIII following transfusion, Dr. Christopher Prowse).

**2. STANDARDIZATION**

a) Concentrate standards. Dr. Barrowcliffe announced that the 5th International Standard for Factor VIII concentrate was approved by the WHO, with a potency of 5.4 IU/ml. The 3rd International Standard for Factor II-IX-X concentrate had been assessed with consistency. He discussed the possibility of a common monocomponent factor IX concentrate. Dr. Weinstein reported on the progress of work on the FDA/EP common standard which should be complete by the end of 1995. Regarding a factor IXa standard, Dr. Gray discussed variations associated with different methodologies.

b) Plasma standards. Dr. Barrowcliffe has produced a new Factor II-VII-IX-X plasma standard that will be calibrated this autumn. With regard to the 3rd International Standard for Plasma Factor VIII/ von Willebrand Factor, several large plasma pools have now assayed above 100% for factor VIII. He prompted investigators to send him results from their own laboratories. Dr. Kitchen described progress in assessing the SSC secondary plasma standard. These studies are soon to be completed, with stability testing and a few results to be included.

c) Dr. Barrowcliffe described the Factor VIII and IX Concentrate Field Study involving mainly commercial laboratories. The variation seen was related to use of different assay methods, and analytical approaches. It was noted that the chromogenic assay showed a large variation which was discussed. Further studies will be overseen by a small steering group chaired by Dr. Christopher Prowse.

d) Dr. Lee reported that she had found apparent different recoveries of factor VIII in the same patients with the same infusion by one-stage or chromogenic assay.

**3. LABELING AND PURITY OF FACTOR VIII CONCENTRATES**

Dr. Tuddenham spoke to his paper on this issue which was followed by vigorous discussion. It was the feeling of the meeting that the Subcommittee should recommend that the use of non-scientific purity descriptive phrases should be discouraged in papers and the commercial literature. It should be recommended that products are described in terms of their final constituents and their production technology. The Chairman requested that interested parties write to him so that the final recommendations will be acceptable.

**4. A STUDY TO MONITOR THE VIRAL SAFETY OF PCR SCREENED BLOOD PRODUCTS IN NON-INFECTED PATIENTS (NIPS)**

Dr. Berntorp proposed a new approach for safety assessment of viral transmission by blood products using NIPS in which PCR analysis would be used to monitor infection. It was decided to include this in proposed guidelines for the surveillance of viral transmission in PUPS (see below).

#### 5. THE GENETIC BASIS OF FACTOR VIII INHIBITORS

In the absence of Dr. Hoyer, the Chairman outlined proposals for the study of the genetic basis of inhibitor development in severe haemophilia A with factor VIII gene inversions. This should either be in PUPS or in existing patients where the inhibitor status was known. Along with this, patients HLA class I and II characteristics would be assessed. Dr. Hoyer had proposed a prospective study and in the light of the comments made during the meeting, the Chairman would discuss the proposal further with him. It was clear that problems relating to the numbers of patients and their differing ethnic origins would make these studies difficult.

#### 6. PROGRESS REPORTS AND FINAL REPORTS

a) Dr. Hill (on behalf of himself, Dr. Mannucci and Dr. Ludlam) outlined their proposals for surveillance of viral transmission in PUPS. There was considerable discussion and, following this, the Chairman requested that all interested parties should write to him, or the authors. A draft document would then be circulated to Subcommittee members and others for comment.

b) The following final reports were made (chaired by Dr. Kasper)

Registry of European-derived clotting factor concentrates (Dr. Costa e Silva)

Registry of North American-derived clotting factor concentrates (Dr. Menache)

Registry of North American Immune Tolerance Practices (Dr. diMichelle)

International Registry of Immune Tolerance Studies (Dr. Mariani)

Protocols for surveillance of inhibitor development in PUPS (introduced by Dr. Kasper, expanded by Dr. Klose)

The Chairman requested that these reports be sent to him as soon as available for assessment by the Subcommittee members prior to final submission to the SSC with a view to publication.

#### 7. NEW PROPOSALS

a) Dr. E. Briet discussed the need to monitor inhibitor development in previously treated patients exposed to a new concentrate. Although that it was felt that such practices are being conducted in many centers, it was thought that this could be included in the inhibitor surveillance guidelines proposed by the Subcommittee. Dr. Briet agreed to write to the Chairman outlining his proposal.

b) Dr. Verbruggen described a minor change in the Bethesda inhibitor assay to stabilize factor VIII in the control sample which resulted in a more sensitive and parallel assay. The possibility of a collaborative study was discussed.

c) Dr. J. Lusher proposed a worldwide pharmacokinetic study of factor VIII and IX in infants below the age of five with severe haemophilia. She asked interested doctors to contact either her or Dr. Laurian .

#### 8. CONCLUSION

The Chairman thanked all participants and presenters and said that details of all projects would be sent to the Subcommittee members shortly. The arrangements for 1996 Meeting have yet to be finalized.

## 1996 MINUTES

### FACTOR VIII and FACTOR IX SUBCOMMITTEE

Saturday, 22 June, 1996, 13.00 - 17.00

Room Rubi, Fira Palace Hotel

Barcelona, Spain

Chair: Jorgen Ingerslev, Denmark

Co-Chair: Ernest Briet (secretary), Netherlands

1. The chairman apologized on behalf of Professor Ian Peake who was unable to be present and outlined the program for the afternoon.
2. Drs. Hill and Mannucci presented the final draft of a protocol for the surveillance of viral transmission in previously untreated patients, which includes testing for ALT and serological markers for hepatitis and HIV. PCR may be included for parvovirus B19. A manuscript will be submitted to the subcommittee for circulation and approval.
3. On behalf of Dr. E. Berntorp, Dr. Lou Aledort presented a summary of the previously presented approach for the surveillance of viral transmission by blood products in non-infected patients (NIPS) by PCR technology. A study is being carried out in Germany and Sweden. Results from this study may form the basis of a formal SSC protocol.
4. Dr. Jeanne Lusher, also on behalf of Dr. Ives Laurian, reviewed the pharmacokinetic factor VIII and factor IX study in infants.
5. Genetic basis of factor VIII inhibitors. Dr. Charles Hay presented preliminary data from UK hemophilia centers on Hla class II subclasses as well as factor VIII gene mutations in British hemophilia patients demonstrating a weak over-representation of certain subsets. Dr. Gordon Bray and Dr. Deborah Hearst presented the inhibitor cohort studies in recipients of Recombinate and Kogenate respectively. The formation of a collaborative registry on genetic aspects of inhibitor formation was encouraged.
6. Professor Alan Giles, also on behalf of Dr. Bert Verbruggen, reported on a comparison of the classical and the Nijmegen modification of the Bethesda inhibitor assay. The material appears to be ready for submission as a final report.
7. Dr. Steve Kitchen summarized data on the secondary plasma standard for factor VIII:C, IX:C, vWF:Ag and vWF: activity. This presentation will be repeated at the second meeting of the subcommittee in Dublin on Friday, June 28.
8. Professor Donna DiMichele presented the results of an extensive registry on inhibitor tolerance induction in American hemophiliacs. This report is ready for submission to the subcommittee chair.

9. Dr. Jeanne Lusher made a proposal for the collection of data on the use of porcine factor VIII in pediatric cases of hemophilia. A postal survey is being planned.

10. The meeting adjourned at 17.05.

## 1997 MINUTES

### FACTOR VIII AND FACTOR IX SUBCOMMITTEE

Friday, 6 June, 1997, 8:00-17:00

Raffaello, Fortezza da Basso

Florence, Italy

Chair: I.R. Peake, UK

Co-Chairs: D. DiMichele, USA; J. Ingerslev (Secretary), Denmark;

K. Mertens, The Netherlands; C. Prowse, UK; Y. Sultan, France;

A. Yoshioka, Japan

The audience was welcomed by the chairman who introduced the meeting and outlined the programme.

#### Completed Reports 1996/1997. I.R. Peake.

Collaborative study on assays of activated FIXa by E. Gray et al., *Thrombosis and Haemostasis* 1996, 76, 1114-1117.

#### Final Stages:

Nijmegen modification of the Bethesda assay. A. Giles et al.

Revision of viral safety protocol. F. Hill, North American registry of immune tolerance protocols. D. DiMichele (not yet submitted).

#### Final Report:

The use of porcine FVIII in infants and children - J. Lusher. To be produced after inclusion of further material from the UK.

#### Reports of On-going Activities:

The pharmacokinetics of factor VIII and IX in infants - J. Lusher. Protocol produced. More recruitment needed.

Viral transmission by blood products in noninfected patients by PCR technology - E. Berntorp, one year to go before final recommendation would be possible.

#### New Proposals:

Registry of factor VIII/IX concentrates - C. Kasper. No report given. Chair has received a draft. Final form next year.

Protease inhibitors and bleeding in haemophilia - E. Briët.

Case report presented. Participant meeting arranged for p.m. to consider recommendations and reporting.

Recommendations on the use of PUPs and PTPs - G. White.

Proposal forwarded to study PTP instead of PUP in clinical immunogenicity trials. Comments to Dr. White within four weeks. Proposal to be written.

### **Risk Factor Assessment for Inhibitor Development:**

#### **Product-related Risk.** Y. Sultan, chairman.

Incidence of FVIII inhibitors in PUPs treated with recombinant FVIII: Introduction - Y. Sultan. Dr. Sultan summarized recent French data on inhibitor occurrence.

Data from France and USA - C. Rothschild. Dr. Rothschild gave a detailed report on a French study of 53 PUPs treated with rVIII. D. DiMichele reported on local US and PR cohort comparing two rVIII treated groups with patients shifted from plasma-derived factor VIII to recombinant.

Proposal for a French-American-International Registry - L. Aledort, Y. Sultan. The questionnaire was outlined by Dr. Aledort. Additions to include genetic information and patients treated with a plasma-derived product. Current and past inhibitors should be requested also. Dr. C. Hay also outlined preliminary data on UK PUPs.

Inhibitors in mild and severe haemophilia - F. Rosendaal. Reviewed Dutch patients estimating the spontaneous occurrence of inhibitors and those related to particular product.

#### **Genetic Risk.** L. Hoyer, Chairman

Kogenate inhibitor study - J. Lusher. Outlined proposed study which will commence soon.

Recombinant inhibitor study - A. Goodeve. Detailed the present genetic information on the Recombinate study.

Immunogenetic risk - C. Hay. Outlined situation and stressed that ethnic differences make studies very difficult.

#### **Risk and Assay Variability.** D. DiMichelle, Chairman

Inhibitor assay variability in Canada - A. Giles. A positive inhibitor result may be influenced by methodology adopted, type of equipment, and type of deficient plasma. Chemically depleted FVIII deficiency plasma should not be used.

Inhibitor assay variability in UK - S. Kitchen, E. Preston. Dr. Kitchen reported the UK NEQAS study that showed wide variation.

**Summary and Proposed Inhibitor Risk Guidelines.** I. Peake, F. Rosendaal.

Discussion followed. Nijmegen modification of the Bethesda assay to be endorsed by the SC. Attempts to reduce cost of materials to be explored by Dr. Giles. Working group to meet soon to study in detail the possibility of preparing guidelines and definitions of inhibitors for possible publication to assist in the prediction of inhibitor development. Commercial companies to be asked to contribute.

**Standardization Issues.** C. Prowse (UK), K. Mertens (NL), chairmen.

**FIX Standards.** Dr. M. Weinstein reported on the finalized work on a new FDA/EP standard for Factor IX concentrate, and Dr. E. Gray reported on the development of a new activated FIX standard (FIXa) (potency not yet established).

**FVIII Standards.** Dr. M. Weinstein reported on the ongoing work with a new factor VIII concentrate MEGA standard (MEGA II). Multicentric potency estimation to be performed. Dr. T. Barrowcliffe reported that new WHO standards for plasma and concentrate FVIII will be prepared during 1998 for final release during 1999.

Discussion followed. Members expressed hope that harmonization between US and WHO standards could be reached.

**Collaborative Studies:** results of SSC studies.

Report of 1996/1997 studies. S. Raut reported on two FVIII studies (SSC3 & SSC4) of FVIII at two different potency levels. Recombinant & intermediate purity concentrate show a wide inter-laboratory variation. Factor VIII concentrate study to be continued for another 12 months.

**Plasma and Concentrate Units and *in vivo* Recovery.**

**Summary of the Problem.** T. Barrowcliffe. The problem was outlined. Post-infusion Factor VIII:C levels are subject to variation dependent of the method adopted to measure FVIII (one-stage or chromogenic substrate method).

**A Fractionator's View.** M. Mikaelsson. Data presented indicating that concentrate added to haemophilia plasma (e.g., after injection) does not compare well with a plasma standard but compares more accurately with a concentrate standard.

**A Clinician's View.** C. Lee. Data presented (UK Hemofil M & Recombinate pharmacokinetic study) illustrated that potency assessment of a concentrate as well as the quantitation of VIII:C in post-dosage samples gave higher values with chromogenic substrate methods than one-stage procedures.

**Discussion.** Dr. J. Lusher stressed the importance of assay discrepancies as demonstrable in pharmacokinetic analysis, showing data signifying an increased peak FVIII:C value as well as an apparently prolonged *in vivo* half-life value when the chromogenic assay was used. Dr. Lusher further suggested a clinical review of databases of PUP and PTP recombinant studies to search for a bleeding/dosage relationship.

**Recommendations.** Dr. T. Barrowcliffe argued that the reasons for assay discrepancies are not understood and proposed to the SC

1. that recovery should be based on one-stage assays until more information is obtained;
2. that post-infusion samples should be assayed against the concentrate standard, preferably the same material as was infused; and
3. that the concentrate standard should be diluted in hemophilic plasma (preferably from the patient).

In conclusion, it was hoped that further studies would help to overcome these problems. The possibility of dosage/effect studies will be explored.

**1998 MINUTES**  
**FACTOR VIII AND FACTOR IX SUBCOMMITTEE**

**Part I**

**Saturday, 20 June, 1998, 13:00-17:00**

**Part II**

**Sunday, 21, 1998, 8:00-12:00**

**Cankerjev Dom**

**Ljubljana, Slovenia**

**Chair: J. Ingerslev , Denmark**

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

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

Final Reports

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

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

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

Ongoing Activities

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

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

Dr. Berntorp had reported to the Chair that inclusion of patients was slow for the safety study of non-infected patients studied by PCR technology, and there was no new data to present this year.

On behalf of Drs. P. Mannucci, C.A. Ludlam and himself, Dr. F. Hill presented the latest version of the amended protocol for safety studies in PUPs. Following discussion, it was recommended that this be the final version passed to the members of the F VIII/IX SSC for final comments prior to submission to ISTH for publication as a recommendation.

Standardization Issues

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

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

Dr. T. Barrowcliffe reported on the SSC/5 "Field Type" Collaborative Study on Concentrates, one involving F VIII, the other involving F IX, and proposed a further exercise by October 1998.

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

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

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

#### FVIII Assay Discrepancies

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

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

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

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

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

#### Inhibitors

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

type, the need to define tolerance therapy regimes and the option of linking inhibitor type with specific FVIII gene defects were raised.

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

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

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

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

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

#### Miscellaneous

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

#### SUMMARY OF NEW ACTIVITIES

Formation of new Working Groups on:

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

Next meeting in Washington in 1999.

**1999 MINUTES**

**FACTOR VIII AND FACTOR IX SUBCOMMITTEE**

**Part I**

**Saturday, 14 August 1999, 8:00 to 12:00 PM**

**Part II**

**Saturday, 14 August 1999, 1:00 to 5:00 PM**

**Room 40**

**Washington Convention Center**

**Washington, DC**

**Chair: J. Ingerslev, Denmark**

**Co-Chairs: D. DiMichele, USA; K. Mertens, The Netherlands;**

**C. Prowse, UK; C. Negrier, France; R. Schwaab, Germany;**

**A. Yoshioka, Japan**

PUBLISHED REPORTS

G White et al. Utilization of Previously Treated Patients (PTPs), Noninfected Patients (NIPs), and Previously Untreated Patients (PUPs) in the Evaluation of New Factor VIII and Factor IX Concentrates. Thromb Haemostas 1999; 81: 462.

COMPLETED REPORTS

Revision of the Protocol Recommended for Studies of Safety from Hepatitis and Other Blood Borne Virus Infections of Clotting Factor Products which are Plasma Derived or Contain Plasma-Derived Components. FGH Hill, CA Ludlam, PM Mannucci.

Calibration of the SSC Secondary Plasma Standard for FVIII:C < FIX:C, vWF Activity and Antigen. S Kitchen, TW Barrowcliffe, DL Aronson, JG Lenahan, FE Preston.

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

North American Immune Tolerance Study Report. D DiMichele et al.

REGISTRIES AND STUDIES

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

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

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

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

(Continued)

### STANDARDIZATION ISSUES

Collaborative Study for the 3<sup>rd</sup> International Standard for Factors II and X Concentrates.

E Gray. Approved.

T Barrowcliffe reported on the 1998 WHO ECBS Expert Committee on Biological Standards approval procedure for the 4<sup>th</sup> International Standard for plasma factor VIII and von Willebrand factor, and the 6<sup>th</sup> International Standard for factor VIII concentrate. The decision on the factor IXa standard reportedly was postponed.

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

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

### Factor VIII Assay Discrepancies

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

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

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

## WORKING GROUP ACTIVITIES

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

### Working Group on Definitions and Terminology in Hemophilia

(G White et al.)

Three activities were highlighted:

- the definition of severe, mild and moderate hemophilia, for which the discussion suggested that the definition should be based on biological factor FVIII levels (e.g., <1, 1 to 5 and >5 u/dl) rather than clinical severity,
- a cut off between low and high titre inhibitors, for which a level of five Bethesda units was suggested,
- and, possible experiments in hemophilic dogs or patients to define the minimal level of FVIII required for hemostasis. While the London meeting had been concerned about the ethics of carrying out such studies in humans, the consensus at this meeting was that such a

study was feasible, possibly in countries where the alternative was no therapy, and would probably involve a dose de-escalation design.

(Continued)

### Working Group on Quality Assessment in Measurement of Inhibitors

(S Kitchen for FE Preston et al.)

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

### Working Group on Genetic Aberrations and Inhibitors

(R Schwaab, J Oldenburg et al.)

The group proposed to collect data on FVIII gene mutations, MHC II type (and possibly other immune status markers) in a prospective manner for previously treated patients, both

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

#### Working Group on Protease Inhibitors and Bleeding in Hemophilia

(C Lee et al.)

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

#### Working Group on Pharmacokinetics in Hemophilia Substitution

(M Lee et al.)

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

#### MISCELLANEOUS

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

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

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

(End)

## **FACTOR VIII AND FACTOR IX, Parts I & II**

**15 June 2000**

**08:00 to 12:00; 13:30 to 17:00**

**Auditorium I**

**Maastricht Meeting and Convention Center**

**Chairman: J. Ingerslev--Denmark**

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

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

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

### Reports submitted for publication

Calibration of the SSC Secondary Plasma Standard for FVIII:C, FIX:C, VWF Activity and Antigen.

S. Kitchen, T. W. Barrowcliffe, D.L. Aronson, J.G. Lenahan, F.E. Preston

The North American Immune Tolerance Registry: Practices, Outcomes, Outcome Predictors. D.M. DiMichele, B. Kroner and Members of the Factor VIII/IX Subcommittee.

### Recommendation submitted for publication

The Design and Analysis of Pharmacokinetic Studies of Coagulation Factors.

M. Lee, M. Morfini, S. Schulman, J. Ingerslev and the Factor VIII and Factor IX Subcommittee.

### Finished paper

Nomenclature and Definitions in Haemophilia. G.C. White II, F. Rosendaal, L.M. Aledort, J.M. Lusher, C. Rotschild, J. Ingerslev and the Factor VIII and Factor IX Subcommittee

### Ongoing work with registries and clinical studies in progress

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

Dr. Donna DiMichele reported from the American French Multicenter Comparison of Inhibitor Development on Plasma-derived versus Recombinant Factor VIII in Severe Hemophilia A

Patients. This registry allows for further inclusions of patients, and an extension has been set forth including additional countries.

In the absence of Dr. C. Hay, Dr. D. DiMichele presented the development of the Randomised Immune Tolerance Study Protocol. All of the logistics and funding issues have been clarified, and this randomised, controlled study will begin inclusion of patients within the next few months

On behalf of Dr. I. Warrier and herself, Dr. J. Lusher presented an update on the Registry on Factor IX Inhibitors & Anaphylaxis in Children with Haemophilia B. In total, 39 cases have been included. Of these, 11 cases have suffered a nephrotic syndrome under attempts to induce tolerance, and 9 of these 11 patients had demonstrated anaphylaxis.

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

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

### Standardization

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

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

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

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

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

Dr. M. Lee, on behalf of a larger group of investigators, spoke about his study on Calibration of New Monoclonal-Purified and Recombinant Factor VIII Standards. Standards utilized in the exercise were the MEGA-1, the WHO # 6<sup>th</sup> Standard for F VIII Concentrate, and two in-house

calibration standards, recombinant and monoclonal purified. Authors found a 6-8% lower clotting activity of MEGA-1 as compared to the WHO 6<sup>th</sup>, whereas the chromogenic assays gave similar results.

Dr. M. Weinstein gave an update on the work on the MEGA-II Standard. Vials (100.000) of the standard factor VIII concentrate material have been produced, and the material gave comparable results with one-stage and chromogenic assays. Extensive testing was planned for FDA approval, for Ph.Eur. adoption and material would also be available to the SSC.

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

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

#### Factor VIII assay discrepancies

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

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

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

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

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

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

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

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

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

#### Reports from Working Groups

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

On behalf of the Terminology and Definitions Group, Dr. C. Rotschild gave an introduction to a series of questions that she intended to circulate amongst members of the Factor VIII/IX Subcommittee as a postal interview. The aim of this investigation is to attempt to give a common definition on the term of *transient inhibitors*. The approach selected was approved by the audience.

On behalf of Dr. G. White II and herself, Dr. M. van den Berg presented a draft proposal for a clinical study on critical haemostatic levels of BDD recombinant factor VIII by one-stage and chromogenic assays. The aim of the double-blind cross-over study is to evaluate the efficacy of quite low doses of BDD recombinant factor VIII in knee-joint bleeds in adult patients, and various clinical end-points were proposed. The study proposal was well received. Attendees presented a number of helpful comments and general advice.

#### Other business

Dr. C. Ludlam introduced the audience to documents for possible collaborative review that had been detected in the Cochrane Library. Members wishing to participate or communicate with Dr. Ludlam on Cochrane review issues were invited to do so.

## FACTOR VIII AND FACTOR IX, Part I

**6 July 2001**  
**08:00 to 17:00**  
**Room Salle Malliot**  
**Palais des Congrès**

Chairman: D. DiMichele (USA)  
Co-chairman: J. Ingerslev (Denmark); C. Lee (UK); K. Mertens (The Netherlands)  
C.G. Negrier (France); J. Oldenburg (Germany);  
D. Scandella (USA); A. Yoshioka (Japan)

Dr. Donna DiMichele welcomed the audience and outlined the program.

### **1. Completed/Submitted Reports and Recommendations (2000/01)**

Papers submitted to Thrombosis and Haemostasis for peer-review:

The North American Immune Tolerance Registry: Practices, Outcomes, Outcome Predictors.  
*DM DiMichele, B. Kroner*

Subcommittee recommendations for ISTH web site publication:

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

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

### **2. Notes on the new publications policy**

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

### **3. Registries and Studies in Progress**

*International F VII deficiency registry*

On behalf of Prof G. Mariani and the Registry Steering Committee, Dr. J. Ingerslev presented an update of the International Registry of Congenital FVII Deficiency. This registry now contains 420 patients submitted from 54 centres around the world. Twenty two percent of the subjects have severe deficiency (< 2%) and 52% have FVII levels of 20% or below. The racial background in 85% of pts is Caucasian, with an unexpectedly large distribution of pts from Eastern Europe. A Letter to the Editor was submitted to Blood in an effort to recruit more extensively from the US in an effort to establish more diversity of racial background among

registry subjects. Data being collected includes 1) Method of diagnosis including antigen/activity correlation; 2) Characterization of hemorrhagic and thrombotic symptoms; 3) Treatment complications; and 4) Gene mutation analysis. Of note, 23% of patients are a symptomatic. Mucocutaneous bleeding accounted for 59% of the bleeding symptoms. The registry has documented eight thrombotic episodes and no inhibitors. In 210 patients, an assumed causative mutation has been established. Missense mutations represent the majority of these mutations. An analysis of genotype ó phenotype correlation is planned. Enrolment continues with enrolment forms available through Dr Mariani (contact by email).

#### *Registry on FIX Inhibitors and Anaphylaxis in Children*

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

#### *International Registry of Clotting Factor Concentrates*

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

#### *Gene Therapy Registry.*

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

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

In the second FVIII trial sponsored by Chiron, 13 patients were intravenously infused with a FVIII gene/retroviral vector. The liver was the targeted organ. 6/13 transiently acquired >1% but

<2% FVIII activity levels. The trial has been stopped and the data is to be presented at this ISTH meeting.

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

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

#### *International IT Study.*

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

#### **4. Standardization Issues**

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

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

##### Concentrate working standards:

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

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

##### Concentrate reference standards

The current WHO ref standard 6, the first recombinant, was established in 1999 as the 5<sup>th</sup> IS concentrate standard and the 4<sup>th</sup> IS plasma standard. Replacement in the near future will be

necessary, but the Mega 2 standard is not the likely candidate. At the London meeting, discussion centered on whether the replacement should be recombinant, plasma derived (PD) or both. It is likely that a single standard will continue to be used. A possible candidate could be the FDA ampouled material (N). A decision will be made by the next SSC meeting. A survey of manufacturers indicate that they are using internal standards calibrated against either Mega 1 or the WHO reference. Most regulatory agencies are using either Mega 1 or EP 2.

#### Post Infusion Plasma Measurement

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

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

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

#### *The Mega 2 Standard-M. Weinstein*

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

Six candidate plasma and recombinant materials were screened by the FDA and narrowed to two high purity plasma products. They were sent to 18 reference laboratories for assay correlation studies. Candidate A demonstrated a consistent 5-10% increase in OS compared to chromogenic assay results in all labs. Candidate B showed variable OS/chrom assay ratios, but better correlation in aggregate, and was therefore chosen. Vials (100,000) and ampoules(5000) were prepared under different conditions and sent to 38 international labs for assay using local methodology and balanced assay design. The results are as follows: Mega 2: OS= 11.3 u/ml (10.2-12.6) Chrom = 8.6 (8.1-9.4) For Sample N : OS= 10.5u/ml (9.5-11.8) and Chrom = 10.7(10.1-11.3). Questions that remain to be answered include reasons for the Mega 2 assay

discrepancy; how the working standard potency will be assigned as well as how the potency assignment will affect vial fill, factor supply and clinical outcomes. Further investigation of these issues will be done by the FDA/CBER and a final report submitted to the NIBSC for further consideration. Dr Weinstein also discussed the pros and cons of US manufacturers reverting to the chromogenic assay for potency labeling

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

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

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

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

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

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

### **6. Factor VIII Measurement: Is a New Paradigm Possible?**

Dr DiMichele opened with an explanation for a timely related discussion of optimal FVIII measurement and dosing. Optimal FVIII measurement was necessary because of 1) non-correlation of current assays; 2) the ultimate need to measure FVIII effect on blood clotting and not "drug levels" in plasma; and 3) emerging new paradigms of coagulation. Optimal FVII dosing was required because clotting factor was proving not to be an inexhaustible resource.

Furthermore, an increasing worldwide need for this product necessitated a new cost-effective approach to resource management.

*The Role of FVIII in a TF-based model: Relevance of the APTT- K. Mann*

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

*The role of FVIII in FX Activation- P. Fay*

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

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

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

*The Chromogenic Assay- K. Mertens*

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

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

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

## **7. Factor VIII Dosing: Developing Future Strategies**

### *Historical Perspective on Dosing- H. Roberts*

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

### *Alternative Dosing Strategies- A. Srivastava*

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

### *The Role of TAFI in Hemophilic Bleeding- M. Nesheim*

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

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

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

### *Round Table Discussion: C. Kasper, J. Oldenburg*

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

**Meeting adjourned at 17.30.**

**Attendance**

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

Minutes prepared by Drs Ingerslev and DiMichele

## FACTOR VIII AND FACTOR IX

July 18, 2002

13:00 to 17:00

Georgiana Room

Boston Park Plaza Hotel

Chairman: D. DiMichele--USA

Co-Chairmen: J. Ingerslev--Denmark; C. Lee--UK; K. Mertens--The Netherlands;  
J. Oldenburg--Germany; HM van den Berg--The Netherlands; A. Srivastava--India; JM Saint-  
Remy--Belgium

### ***Completed and Submitted Reports-*** **Chair: D. DiMichele**

*The following report was published in 2002:*

**The North American Immune Tolerance Registry: Practices, Outcomes, Outcome Predictors.** *D.DiMichele, B.L. Kroner. Thromb Haemost 2002;87:52-7.*

*ISTH website publication:*

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

*Completed studies/Publications in progress:*

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

### **International Registry: Congenital FVII Deficiency** *G. Mariani*

Dr. Ingerslev reported on behalf of Dr. Mariani and the FVII Registry Study Group . Currently closed to new subject accrual, the registry now contains 514 subjects from 59 centers. Of these 198 are asymptomatic. Among the symptomatic patients, clinical manifestations are classified as follows: 29% severe; 42% moderate and 30% mild.

There have been DNA samples submitted on 335/514 subjects. All but one mutation have been characterized.

The planned analyses of the data include: 1) clinical phenotype genotype correlation; 2) association of FVII deficiency with thrombosis;3) phenotypic characterization of the severe deficiencies including the incidence of CNS bleeds; 4) natural history of disease; and 5) treatment practices. The first publication on the association of FVII deficiency with thrombosis has been submitted to Blood. Manuscripts in preparation are on the topics of

genotype/phenotype correlation and CNS bleeds. The subcommittee will continue to be apprised of this group's excellent productivity.

***Registries and Studies in Progress***  
*Co- Chairs: M. van den Berg and A. Srivastava*

**Registry on FIX inhibitors associated with anaphylaxis- J. Lusher**

Dr. Lusher gave an update on the FIX inhibitor registry of Dr Warrier. The most recent update in July 2002 included 85 patients were included. Demographics (mean and range) of the group at the time of inhibitor development are as follows:

age	19.5 months	(9-156)
exposure days	11 days	(2-180)
peak inhibitor titer	30 BU/ml	(1-960)
complete gene mutation	17/32	(53%)

All FIX products have been associated with inhibitor development. Among the 32 patients on whom these data are available, 21 attempts at ITI (66%) are noted with only 2/21 successes (9.5%). Data on the other patients are not yet available, so this figure could increase slightly; nevertheless, this is a much lower success rate for immune tolerance than achieved for hemophilia A.

Part of the reason for this registry was to document the prevalence of nephrotic syndrome occurring during ITI. So far three cases demonstrated a clear association. In all, 13 cases have been reported. The nephrotic syndrome occurred after a median duration of ITI of 9 months. Eleven of 13 cases had documented prior allergy to factor IX. Renal biopsy was performed in 2. Both biopsies revealed membranous glomerulonephritis. Immunohistochemical staining was negative in one patient studied.

It was concluded that the prevalence of inhibitors with an allergic phenotype in hemophilia B is low (2.3%), and only 10% of the patients achieved tolerance after ITI. Nephrotic syndrome has been reported in 13 cases. Professor Mannucci commented that although the Milan protocol to study the immunology of this phenomenon is available on the ISTH website, no samples had been received to date. Physicians are again urged to participate.

**Update on the Gene Therapy Registry- K. High**

Dr. K. High gave a gene therapy registry update with respect to the development of databases produced by authorities. The current NIH database on gene transfer is now on the OBA website: [http:// www.4.od.nih.gov/oba/rac/clinicaltrial.htm](http://www.4.od.nih.gov/oba/rac/clinicaltrial.htm) and contains information on 10 trials on gene transfer in X-linked recessive disorders.

During the third and fourth quarters of 2002, the GeMCRIS database - intended for all but with restricted access to the the public and non-investigators through graded built-in firewalls - will

come into operation. It is currently being piloted by the NIH Clinical Research Center. This database will serve MDs with access to rapid reporting of AE's.

Data exchange with the GTPTS (longterm follow up of gene therapy trial subjects) tracking system of the FDA will occur.

#### **International IT study update- D. DiMichele**

Dr. DiMichele gave this presentation on behalf of her two co-principal investigators, Drs. Charles Hay and Evelien Mauser-Bunschoten. Most importantly, after a delay due to the recombinant factor shortage, the study has now officially started (July 2002) and is open for enrollment. A broad outline of the study was presented. It is a two-arm prospective randomized trial comparing IT success in subjects treated with low-dose arm ITT (50 IU/kg 3 times a week) to those treated with a high-dose regimen of 200 IU/kg per week. The anticipated enrollment is 150 subjects. The hypothesis is that the high-dose arm will achieve more rapid tolerance, but may not yield a higher overall success rate. Subjects can be included if they have severe HA, are < 8 years old, have had an inhibitor for < 12 months, have historical peak titers of between 5-200 BU/ml, have a confirmed current titer of < 10 BU. Patients with current titers > 10 BU/ml can be enrolled but will not be immediately randomized until the titer falls below 10 BU/ml in the ensuing 12 months. More information about the trial is available on the Hemophilia Research Society website as well as on the study website: [www.itistudy.com](http://www.itistudy.com). There was a question about whether the study should not also randomize for product type. Dr. DiMichele remarked that the study is not powered to determine the influence of this parameter on IT success; however, using a minimization strategy, both arms would include an equal number of subjects using vWf-containing, monoclonal and recombinant products so that this variable did not confound the analysis.

#### **Proposed registry: FVIII inhibitors developing on continuous infusion- J. Oldenburg**

Dr. Oldenburg briefly reviewed the increasing use of continuous infusion (CI) for factor replacement in hemophilia. He also mentioned that so far, 11 cases of inhibitors developing in such patients had been reported. All except one of them had >50 factor exposure days before developing inhibitors. Most of these patients had mild hemophilia. The basis for this phenomenon is not clear but could be related to altered immunogenicity of the FVIII molecule during the process of infusion. He proposed that it was therefore worth establishing a registry of these cases to gather data in an attempt to better understand its pathogenesis and its natural history.

In the discussion that followed, it was pointed out that unless there were control data available on similar patients treated with bolus infusion, it would be difficult to conclude whether these events were indeed due to CI. Suggestions were also made for modifications to the questionnaire so as to include the time scale in which these inhibitors appeared and their subsequent profile.

#### **Challenges in gene based-diagnosis : Proposed working group on standardization- J. Oldenburg**

Dr. Oldenburg mentioned that gene-based diagnosis of hemophilia was being increasingly used in recent times. Even though a very large number of a variety of mutations had been documented in the databases, one-third of all new reports still included novel mutations. In spite of the many different techniques being used for screening for mutations, including sequencing of the gene, the success rate for mutation detection varies between 85-98%. Establishing causality of disease due to these mutations requires expression studies, which are not widely available. Carrier detection in families with large deletions can be difficult to detect using molecular genetic methods. Dr. Oldenburg presented data on the use of fluorescent *in-situ* hybridization (FISH) with probes for exons 5, 25, 26 of the FVIII gene for detection of such defects. FISH was also useful in documenting somatic mosaicism.

Because of these problems and the need to establish a uniform diagnostic approach, Dr. Oldenburg proposed that a network be established for standardizing the methodology for the gene-based diagnosis of hemophilia. The aim of such a group would be to define the criteria for assigning causality to a particular mutation and also to exchange samples that prove difficult to analyze.

In the discussion that followed, it was mentioned that while this was a good idea, the logistics of establishing such a network, particularly its funding, needed careful planning. It was suggested that a working party be formed to look into the feasibility of this proposal.

***Standardization Issues***  
*Co-chairs: K. Mertens/ JM Saint-Remy*

**The Mega 2 Standard.** *N. Kirschbaum*

Last year an extensive calibration program was completed on the new common working standard of the FDA and the European Pharmacopoeia. 100,000 vials of this material are available as an international, common working standard. In view of this major step toward harmonization in this area between US and Europe, it is unfortunate that it proved impossible to assign one single potency for both 1-stage and chromogenic assays. In Europe, this material is available as BRP batch 3 with a potency of 8.6 IU per vial for the chromogenic assay. In the US the same material is called Mega 2 and has an assigned potency of 8.6 IU per vial for the chromogenic assay and 11.3 IU per vial for the 1-stage assay. Dr. Kirschbaum presented stability studies conducted by the FDA on this material, demonstrating excellent longterm stability in both assay methods. She further reported on the standard performance of Mega 2 in the lot release of 43 batches of plasma-derived FVIII and on various lots of two recombinant products. In general there was good agreement with the labelled potency using Mega 2 in both chromogenic and 1-stage assays, with 90% of batch testing yielding potencies within a range of  $\pm 10\%$  of the label value. The exception involved one of the recombinant products, for which 86% of batch testing by the chromogenic assay was within the  $\pm 10\%$  range, but the use of the 1-stage assay resulted in only 45% of batches testing within this range and another 45% testing in the range of  $\pm 10-20\%$  of the label potency. Dr. Kirschbaum concluded that the introduction of Mega 2 will not imply any shift in product potency. Upon questions from the audience, she further explained that the situation of a dual potency for Mega 2, though apparently confusing, seems workable without causing major inconsistencies.

### **Proposal to replace WHO concentrate standard – T.W. Barrowcliffe**

Together with the calibration of the US/European working standard, another batch of the same plasma-derived product has been processed and filled in ampoules ( N ) to serve as the 7th WHO standard for FVIII concentrate. Dr. Barrowcliffe presented a report of the calibration study involving 38 laboratories. All labs were instructed to follow the ISTH/SSC recommendations, including the use of FVIII deficient plasma as a prediluent. Of the participants, 27 obtained data with the 1-stage assay, while 31 used the chromogenic assay. The candidate standard did not display any significant discrepancy ( $< 2\%$ ) between 1-stage and chromogenic assays, thus allowing the data to be averaged according to the general WHO policy. Interlaboratory agreement was much better than in the calibration of the current 6th International Standard, with an overall GCV value of 8.29%. The new candidate standard has been calibrated against the current 6th IS (recombinant) as well as its predecessor, the 5th IS (plasma-derived) and the two secondary standards, the US Mega-1 and the European BRP-2. Against these four references, the potency of the new IS was found to be 11.40, 10.66, 10.60 and 10.09 IU per ampoule, respectively. For the potency of the new IS, Dr. Barrowcliffe discussed three options: (1) 11.4 IU based on the current WHO 6th IS, (2) 10.8 IU based on all four references, and (3) 11.1 IU based on the mean of options 1 and 2. Option 1 seems preferable in terms of continuity of the FVIII concentrate unitage, but other options allow greater harmonization between other standards that are being used. The various options have recently been sent to the participants as well as to the FVIII-IX subcommittee members for an opinion. Dr. Barrowcliffe expects that a final recommendation will be submitted to the Subcommittee for a vote prior to the Birmingham meeting.

### **Effect of standard and assay method on FVIII measurement in recombinant and plasma-derived concentrates – J.D. Schreiber**

A manufacturer's view on FVIII working standards was presented by Dr. Schreiber from Baxter (Vienna). One study compared the company's recombinant and two plasma-derived products employing 1-stage and chromogenic assays against several standards, including the Mega 2 working standard. Good agreement was found using Mega 2 in chromogenic assays at its established potency of 8.6 IU per vial. In the 1-stage assay, however, values were found suggesting that the potency of Mega 2 seems 15% higher than its established value. In a second study, therefore, an in-house standard was calibrated by six different Baxter laboratories. Good agreement between 1-stage and chromogenic assays could be achieved when the calibration was based on the average of Mega 1 and WHO 6th IS, but not on Mega 2. Dr. Schreiber concluded that this in-house standard can be used within Baxter worldwide with one single potency for both assay methods, and with reasonably low (approx. 8%) interlaboratory variability.

### **Second Refacto lab field study – J. Ingerslev**

This study has been recently concluded and focused on the use of Refacto Lab Standard (RLS) to facilitate the measurement of B-domain deleted FVIII in post-infusion samples. In this study, which involved 35 labs worldwide, hemophilic plasma was spiked with Refacto at levels of 0.2, 0.6 and 0.9 IU/ml, and these samples were assayed against normal plasma and RLS as a

standard. In the chromogenic assay (7 labs) the expected FVIII levels were found irrespective of the standard used. In the 1-stage assay (31 labs) FVIII was underestimated against the plasma standard, and this was totally corrected by using RLS as a reference. Dr. Ingerslev concluded that this approach should eliminate most of the discrepancies reported so far in assaying plasma of patients treated with this FVIII product.

#### **Focus of future SSC concentrate studies – K. Mertens**

The SSC concentrate studies have been running since 1995 and serve to inform participants on the performance of their in-house assay method on a variety of FVIII samples. Seven studies have been performed involving 12 FVIII samples ranging from intermediate and high purity plasma-derived products to recombinant full-length and B-domain deleted FVIII. In these "field studies" inter-laboratory variability was much larger (overall CV's 10-20%) than in controlled calibration studies. A significant number of participants do not follow the SSC guidelines which imply using the chromogenic assay, FVIII deficient plasma as a prediluent and 1% of albumin for all other dilutions. Given the limited extent of improvement over time, the Subcommittee decided at its meeting in Paris to reconsider these activities. A new Steering Committee has been formed, consisting of Drs. U. Oswaldsson, J.D. Schreiber, M. Lee, S. Raut and K. Mertens (chairman). This group proposed to develop a more systematic study design, if possible. The participants' views will be sought as to potential options to expand the program in a way that should allow for the identification of major sources of variability. In the meantime the studies will be resumed and further developments will be discussed at the Subcommittee Meeting in 2003.

#### **General discussion on concentrate issues**

In the general discussion Dr. Kotitschke (Biotest, Germany) presented some data on a collaborative study that will start shortly. This study will focus on the use of prediluents in a standardized chromogenic assay. For this purpose various FVIII deficient plasmas will be compared in the assay of a variety of FVIII products. The main question is how much von Willebrand factor the FVIII deficient plasma should contain in order to be a suitable prediluent. Finding an equivalent to hemophilic plasma would facilitate following the SSC guidelines. Dr. Kotitschke mentioned that 4 additional labs can still join this interesting study.

#### **Proposal to replace WHO plasma standard – A. Hubbard**

Upon the establishment of the current 4th IS, the Subcommittee has expressed some concern on the low FVIII activity content of this material (0.57 IU/ml). Dr. Hubbard presented extensive stability data showing that this plasma displays a FVIII activity loss of < 0.1% per year. Thus, the 4th IS is fully appropriate to be used, together with fresh normal plasma, in the calibration of the next IS. A candidate 5th IS has already been filled, and preliminary estimates indicate that its FVIII activity is approx. 0.7 IU/ml. Recruitment of study participants has recently started, and Dr. Hubbard expects that results will be available at the Subcommittee Meeting in 2003.

#### **FVIII unitage and in vivo recovery – K. Mertens**

This report involved a pharmacokinetic analysis of two plasma-derived products in a bioequivalence study. The study was designed according to the SSC guidelines, including the recommended sampling scheme, and a cross-over design. Post-infusion patient samples were analysed in a rigorously standardized manner in one single lab using both 1-stage and chromogenic assays, employing plasma and product standards in parallel. FVIII levels expressed in plasma units were consistently higher than in concentrate units, the difference being 12%. Results of chromogenic and 1-stage assays were virtually identical, irrespective of whether plasma or the product was used as a standard. As expected, the unitage had no impact on determination of half-life, but did affect the AUC and C<sub>max</sub> values. For some batches there was a slight discrepancy between the label potency and the re-assayed potency according to the SSC recommended method. These discrepancies, though minor, were reflected by apparently different *in vivo* recoveries in the cross-over analysis. Although these discrepancies are minor and without any clinical relevance, this study illustrates the impact of label potency assignment for pharmacokinetic studies, particularly if the infused product is to be used as a reference for post-infusion FVIII assays.

**An improved method for assaying FVIII following B domain-deleted FVIII infusion - C.**  
*Wiseman & J. Lusher*

A comparison was run using freshly prepared or frozen ReFacto Laboratory Standard (RLS). It was shown that more consistent results were obtained with the frozen preparation. For instance, intra-assay CVs of 7.7% were obtained with the frozen RLS as compared to up to 10.8% with the freshly prepared RLS. An inter-assay CV of 6.5% was obtained with the frozen preparation, while 7.5% was observed with the freshly prepared RLS. In addition, using the frozen RLS (kept at -80°C) saved considerable time. The use of a frozen RLS could reduce the discrepancy between the one-stage coagulation and the chromogenic assay and is now planned to be used to evaluate plasma samples of patients under ReFacto infusion.

**Standardization of FVIII assays in post-infusion plasma – T.W. Barrowcliffe**

A few studies have assessed FVIII assay discrepancies in post-infusion samples. A comparison of results obtained from different centres showed CVs of up to 15% for the one-stage coagulation assay and up to 20% when the chromogenic assay was used. One confounding variable could be the use of either plasma or FVIII concentrate standards, which are shown not to be interchangeable. For instance, in a FXa generation assay, it is shown that FVIII concentrates increase the rate of FX activation as compared to plasma, while the reverse is true in a thrombin generation assay. Several draft recommendations were put forward for consideration. For pharmacokinetic studies, the product may be diluted in FVIII deficient plasma in order to serve as a standard that facilitates like-versus-like analysis. For monitoring treatment in clinical situations, plasma standards may be used for patients treated with plasma-derived products, and possibly also with full-length recombinant products. As also suggested by Drs. Ingerslev and Wiseman, product standards may be particularly useful for B-domain-deleted FVIII; however, final recommendations await further discussion of these options at the 2003 Subcommittee meeting.

**UK NEQAS study of FVIII measurement: a 92 laboratory survey - E. Preston**

The evaluation of FVIII:C varies greatly from one laboratory to the other. A number of factors can play a role in this variation: use of different reference plasma samples, calibration and standard curves, as well as the coagulometer to cite but a few such variables. A survey was organized in the UK involving 92 laboratories. A standard reference preparation of FVIII was sent to centres which were asked to run the assay as "field evaluation," namely, using their own reagents and methodology. Large variations were observed. CVs of up to 70% were obtained whenever stored reference samples were used, while CVs of 50% were seen with freshly prepared reference curves. A second survey involved the sending of three plasma samples containing different titres of FVIII to 81 centres in the UK. Again, large variations were observed in FVIII with  $\pm 30\%$  of the laboratories giving results out of the normal variation range. In addition, the interpretation of the results show that in many cases laboratories do not comply with the the ISTH classification of haemophilia A patients as severe (less than 1% FVIII), mild (1 to 5%) or moderate (more than 5%). This misinterpretation of results adds further confusion to diagnosis and may possibly exert an influence upon treatment. The reasons why laboratories do not follow the recommendations of the ISTH subcommittee were discussed, as well as possible remedies to this situation.

#### **Need for Bethesda assay standardization - A. Macartney & G. Savidge**

Many variables can affect the titration of inhibitors in plasma samples. Two of such variables are the source of FVIII and the source of diluent for plasma samples. An assessment of Bethesda titre was carried out using different FVIII preparations, either recombinant (B-domain deleted (BDD) FVIII), or plasma-derived products. Significantly higher titres of inhibitors were obtained using the BDD FVIII in particular. Next, the question of the diluent was examined by comparing reagents obtained from different sources, i.e., plasma of severe haemophilia A patients or plasma artificially depleted in FVIII. It was shown that such diluents vary greatly in terms of content of von Willebrand factor (VWF), and remarkably also of residual FVIII antigen. The IL diluent which contained no detectable functional or antigenic VWF, and no FVIII antigen, gave the highest inhibitor titres using the BDD FVIII preparation. Addition of VWF significantly reduced the inhibitor titre in a dose-dependent manner. This suggests that BDD FVIII might be particularly suitable for the evaluation of inhibitor titres and that VWF may play a role in the detection assay for inhibitor. Further studies are required to understand the precise relationships between these parameters, including the specificity of inhibitor antibodies. The presenters recommended that the Subcommittee pursue the standardization of the Bethesda assay.

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

An NIBSC attempt at standardization of inhibitor assay was carried out by distributing two potential standards, each consisting of a human monoclonal anti-FVIII antibody, and one consisting of a polyclonal rabbit antibody against human FVIII. The two human antibodies were derived from the peripheral memory B cells of two haemophilia A patients with inhibitor. The rabbit antiserum was generated by repeated SC injections of human recombinant FVIII in adjuvant. Three plasma samples from patients with inhibitors were assayed. All three inhibitor samples were sent to 15 centres which were asked to run a standard Bethesda assay. Results demonstrated an intralaboratory variability of between 30 and 50% using each of the two human

monoclonal antibody standards, regardless of whether the one-stage or chromogenic assay was used. The rabbit polyclonal antiserum resulted in decreased interlab variability of  $\pm 25\%$ . The reasons for such variations were discussed and probably relate to the use of different reagents, such as dilution buffer. Dr. Kitchen asked for feedback as to whether a larger study should be launched using the polyclonal rabbit antibody inhibitor standard. If so, he asked for feedback as to the optimal titer for such material. The chair asked Dr. Kitchen to prepare a report containing background information accompanied by his specific questions. The chair will distribute this report to Subcommittee members for feedback in the next few months so that further study can be undertaken prior to the 2003 meeting.

***Factor VIII Measurement: Is a New Paradigm Possible?-***

*Co-chairs: J. Ingerslev and J. Oldenburg*

Dr. K. Mann summarised his work on models of coagulation by introducing the audience to the three principal substrates and models adopted in his lab: 1) purified systems, 2) whole blood, and 3) computerised simulation of coagulation. The series of events in coagulation recorded through the quantification of thrombin activation markers (TAT complexes) all occurred before less than 5% of the clotting reaction had taken place. Levels of anticoagulants such as TFPI and AT modified the time-course of thrombin formation considerably. Dose titration, with the addition of exogenous factor VIII to haemophilia A plasma, demonstrated a tendency toward normalisation of thrombin generation with the addition of very small amounts of F VIII. Early data suggested differences in TAT generation profiles with the addition of similar concentrations of different recombinant clotting factor concentrates. Dr. Mann postulated that, since the clotting response was the composite outcome of multiple genetic and environmental factors, modeling could be used to tailor therapy to the needs of an individual hemophilia patient and alter the current practice of uniform global factor replacement strategies.

Dr. J. Ingerslev, on behalf of his colleague B. Sørensen, introduced their dynamic whole blood clotting method which is based based on thrombelastography using a novel software system for the handling of continuous data during coagulation. Coagulation is activated by small amounts of TF. In this system severe haemophilia A and B patients display a severely diminished coagulation signal; however, heterogeneity is found among patients with less than 1% F VIII clotting activity, some patients demonstrating minimal clot formation late in the process and others showing a much earlier signal of coagulation of higher velocity. The incremental addition of small amounts of F VIII ranging from 0.5% to 5% of normal plasma concentration dramatically improved the coagulation profile to close to normal. Dr Ingerslev was asked about the longterm stability of clots formed in his assay at low FVIII concentrations. He did not have any data at present, but plans to study this question in the future.

Dr. T. Barrowcliffe reported on the results of a joint study from NIBSC and the Royal Free Hospital. The group studied thrombin generation in F VIII deficient plasma, with and without the addition of varying amounts of F VIII concentrate. In general, this model was also sensitive to very low concentrations of F VIII which were capable of generating large amounts of thrombin. The subsequent addition of higher factor VIII concentrations primarily influenced the time to thrombin generation rather than altering the maximum thrombin generation potential. The concentration of FIXA significantly affected this assay.

Dr. A. Giles (Biomerieux), on behalf of Drs. Shima and Yoshioka, updated the Subcommittee on the use of waveform PTT analysis, including a summary of the published studies on hemophilic plasma using this technology. In principle, waveform analysis consists of extracting the photometer data from a clot-based PTT and calculating the first and second derivatives of the signal. Using this technique, derived profiles of coagulation in haemophilia could be established. A large phenotypic variation in the second derivative was seen among severe (<1%) haemophilia plasmas. Dose-response relationships were established using F VIII deficient plasma spiked to various theoretical levels. A small study on the use of this method in the study of the hemostatic response in patients with hemophilia A and inhibitors was also presented. Dr. Giles concluded by saying that with the newest coagulation instruments one can obtain more information than with just an APTT or a PT. Although he agreed that whole blood models of coagulation may prove to be more physiologic in the evaluation of hemostasis in hemophilia, both at baseline and after factor replacement, he believes that there is also a role for the waveform PTT technology in the assessment of the clotting response.

In conclusion, the different methods presented were all consistent in demonstrating that 1) severe haemophilia A plasmas display a heterogeneous pattern of clot formation and thrombin generation; 2) clot formation /thrombin generation is significantly improved by very small incremental increases in F VIII, with some clotting profiles returning to normal or near normal with the addition of less than 5% of factor VIII. All methods demonstrate potential as possible alternative strategies to the currently used plasma-based assay methods. A workshop is planned prior to the next subcommittee meeting to coordinate the systematic study of these techniques. More data on their use in the evaluation of hemostasis in hemophilia is expected in 2003.

### ***Factor VIII Dosing: Developing Future Strategies-***

*Co- Chairs: C. Lee and D.DiMichele*

### **Report on the WHO/WFH workshop on the delivery of treatment in hemophilia - C. Lee**

Dr. Lee reported on the proceedings from the joint WHO/WFH sponsored meeting held in London in February, 2002. The goal of this meeting was to identify the barriers to and development of strategies for extending hemophilia therapy to the developing world. Participants included Drs. C. Lee (UK), E. Berntorp ( pharmacokinetics)(Sweden) and S. Schulman(continuous infusion)(Sweden), K. Fischer (prophylaxis)(Netherlands),M. Escobar (historical data on dosing)(US) and D. DiMichele (immune tolerance dosing)(US), A. Chuansumrit (Thai strategies in health care delivery)(Thailand), A. Srivastava ( global needs and economics)( India), V. Boulyjenkov and J. Emmanuel (WHO) and Line Robillard (WFH). Much discussion at the meeting focused on defining optimal and minimum effective dosing. The rationale for not necessarily normalizing factor levels included 1) redundancy in the clotting cascade; 2) factor shortages; and 3) national economics and health care budget appropriations. The goal of therapy in the developing world was discussed and suggested by Dr. Srivastava to be functionality, not perfect joint health. Identified strategies for optimizing dosing included 1) individual pharmacokinetic analysis; 2) better assays for assessing hemostasis *in vivo*; 3) more frequent dosing; and 4) the concomitant use of antifibrinolytics. It was determined not to be an ethical use of clotting factor resources in the developing world. The identified barriers to optimizing therapy included 1)unavailability of PK variability relative to ethnicity; 2)vial size limitations; and 3) limitations of factor delivery systems. Although no formal recommendations

were possible at this time, ongoing meetings on this topic are planned and will be reported regularly to the ISTH as part of a coordinated project with the FVIII/IX Subcommittee.

#### **A dose-finding study in dogs using B-domainless FVIII- *M. van den Berg***

Although this study will proceed, it was unable to be initiated prior to this meeting. Dr van den Berg plans to present preliminary results at the 2003 Subcommittee meeting.

#### **Dose response in hemophilia: Orthopedic outcome in the developing world- *A. Srivastava***

Dr. Srivastava presented his proposal for a study to be conducted in the developing world. The study proposes to document orthopedic outcomes using existing factor replacement strategies currently practiced in the developing world. The optimal orthopedic outcome identified by this study was functional independence. The goals of the study are 1) to document orthopedic outcome on the various dosing regimens used to treat children in a representative group of participating countries; and 2) to develop a cost effective model for treatment in the developing world. Eligibility criteria will include 1) severe (<1%) hemophilia; 2) age of 2-10 yrs. (currently still being defined however); 3) no inhibitor; and 4) well documented care. An enrollment of 250-500 subjects is expected with a 5 yr. follow-up period planned. Participating countries currently include 1) India, 2) Malaysia, 3) Singapore, 4) Iran, 5) Brazil, 6) Venezuela, 7) Thailand, and 8) South Africa. Dosing strategies among these countries vary from 50-500 u/kg/yr. Data to be collected include 1) clinical joint scores (to be standardized by distributing the CDC video), 2) radiologic joint scores (Pettersson), and 3) functional independence scores (WeeFIM modification). After some discussion, the subcommittee endorsed Dr Srivastava's proposal. An update on the progress of this study will be presented in 2003.

#### **Proposed international survey of FVIII replacement in surgery- *A. Srivastava***

As a follow-up to data presented last year, Dr Srivastava reviewed the published literature on the data supporting the 1995 WHO/WFH guidelines on factor support required for surgical peri- and post operative hemostasis. (Haemophilia, 1995 (suppl 8-13)). He found the data lacking and proposes to conduct an international survey to establish current practices relative to surgical morbidity and mortality, and to ultimately develop a consensus on acceptable outcomes and the dosing strategies needed to achieve these outcomes. The questionnaire will have two components:

Component A: Current standards of practice with respect to a) factor dosing in surgery, b) use of CI vs bolus dosing, c) duration of factor replacement, d) target factor levels for surgery and post-op period, e) frequency of factor level monitoring, and f) variation in practice relative to type of surgery being performed.

Component B: Outcome data using these practices.

He received input from the group with respect to monitoring thrombotic complications and inclusion of data on mild/carrier patients. He will modify the data collection tool and resubmit it to the Subcommittee for final comment prior to sending it out. He will seek external funding for data analysis. An update is expected in 2003.

### **International prophylaxis group- *V. Blanchette***

After a review of the published literature on prophylaxis, Dr. Blanchette reported on the activities of the newly established International Prophylaxis Group, the goals of which are 1) to acquire further data on the optimal delivery of prophylaxis; 2) to disseminate this information worldwide; and 3) to continue to define standards for cost effective prophylactic factor dosing and outcome measurement. Annual meetings on specific topics are planned. These topics will include 1) development of a unified MRI scoring system, 2) development of a unified clinical scoring system, 3) further development of QOL instruments and studies. A Steering Committee and Expert Advisory Group have been identified. The Hospital for Sick Children (HSC) in Toronto will function as the Study Coordinating Center. The group is funded through a multi-industry grant. Dr Blanchette will continue to report on the group's activities to both this Subcommittee as well as to the Pediatric Subcommittee of the WFH.

Dr DiMichele concluded the meeting.

## FACTOR VIII AND FACTOR IX

July 12, 2003

09:00 to 13:00

Hall 1

The International Convention Center, Birmingham

Chairman: D. DiMichele--USA

Co-Chairmen: C. Lee--UK; K. Mertens--The Netherlands;  
J. Oldenburg--Germany; HM van den Berg--The Netherlands; A. Srivastava--India;  
JM Saint-Remy--Belgium

### Completed/Submitted Reports and Recommendations

- ISTH Website Publications: The Design and Analysis of Pharmacokinetics: Studies of Coagulation. M. Lee et al.
- Completed Studies: International FVII Registry-
- Thrombotic Complications in FVII Deficiency: G. Mariani et al.
- Pending publication in J. Haemostasis and Thrombosis
- Complete Report on the International FVII Registry: G. Mariani et al. In the final stages of preparation

### Registries and Studies in Progress: *Co- Chairs: A. Srivistava and J. Oldenburg*

#### 1. Registry on FIX inhibitors and anaphylaxis in children with hemophilia B - I. Warriar

Even though inhibitors occur in only 1-4% of patients with hemophilia B, their special significance lies in the fact that some also concurrently get severe allergic reactions / anaphylaxis with factor IX containing products and may develop nephrotic syndrome when subjected to immune-tolerance induction therapy. The current status of this registry was presented by Dr Roshni Kulkarni on behalf of Dr. Warriar. A total of 88 patient are registered world wide – 52 from within the US and 36 from the rest of the world. Genotypic data on 32 of these patients shows total gene deletion in 24 (75%). The mean age at the time of developing inhibitors is 19 months (range: 9-156) and the median number exposures prior to developing inhibitors was 11 (range: 2-180). The mean inhibitor titer was 30 BU (range: 1-960). 51 (58%) of them have severe allergy to FIX containing products. ITI was successful in only 5/34 patients (15%). Thirteen of these 34 patients developed nephrotic syndrome (38%). Eleven of these 13 (85%) had allergy to factor IX containing products.

Participation in this registry can be initiated through the ISTH website ([www.isth.org](http://www.isth.org)). It was suggested that the ISTH list serve be utilized at least once a year to send a reminder for this registry. The publication of this registry will be discussed with Dr. Warriar.

#### 2. Gene Therapy Registry - K. High

Dr. High informed the subcommittee of the new database being created by the NIH using the Genetic Modification Clinical Research Information System (GeMCRIS) [[www.4.od.nih.gov/oba/rac/clinicaltrial.htm](http://www.4.od.nih.gov/oba/rac/clinicaltrial.htm)]. GeMCRIS is a comprehensive resource and analytical tool for the public, participating investigators, federal government agencies, and others with an interest in human gene transfer protocols. The system allows users to access basic reports on human gene transfer trials registered with the NIH, and to develop specific queries based on their own information needs. The distinctive features of this website include the following: 1. Implementation of controlled medical vocabularies such as Standard Regulatory Medical Vocabulary (MedDRA) and Robust Clinical Trial Data Analysis Vocabulary (SNOMED); 2. On-line reporting of adverse events; 3. An architecture that will allow interface with software developed in future; 4. Very high security of data. The system will allow for three levels of accessing privilege: 1) the general public will be able to review basic information, including protocol title, study phase, clinical indication(s), investigator(s), vector and transgene being used, route of administration, scientific and non-technical abstracts. 2) Registered investigators will be able to access the reporting site and submit any adverse events. Investigators will have access to their own reports at any time to make the necessary amendments. 3) Government agencies (NIH, FDA) will have access to any and all adverse event reports.

Dr High will continue to update the Subcommittee yearly on developments in gene therapy.

### 3. International ITI Study - CRM Hay on behalf of the ITISG

Dr. Hay presented the update on this on-going study. The plan is to recruit 150 patients over a period of 2 years. The inclusion criteria include documentation of presence of high titer inhibitors (>5BU but <200BU). The inhibitor titer should be <10BU before starting ITI. Patients will be randomized to receive either low dose ITI (50iu/kg 3 times a week) or high dose ITI (200iu/kg every day). The efficacy of the therapy and the associated morbidity and cost effectiveness of each arm will be compared. The study was initiated on July 1, 2002 and will include patients from 19 countries. As the last year has been primarily spent finalizing ethical approval and contractual arrangements at many centers, only 15 patients have been recruited so far. Of these, 1 was withdrawn, due to lack of National Health System support for a possible high dose randomization (UK), 5 are enrolled and in the pre-randomization phase of the study, and 9 have been randomized.

The discussion that followed suggested that to avoid selection bias, all patients with inhibitors at the participating centers be reported even if not included in the study. Further information about this study can be obtained through the study website. ([www.itistudy.com](http://www.itistudy.com))

### 4. Proposed Registry of FVIII Inhibitors Developing on Continuous Infusion - J. Oldenburg

Dr. Johannes Oldenburg reported that few data from the literature and a recently performed retrospective survey in Germany suggest an uncommon characteristic of inhibitors developing during continuous infusion (CI) therapy. While the typical inhibitor in PUPS is occurs in severe haemophiliacs with a severe molecular gene defect within the first 50 exposure days, a substantial proportion of inhibitors developing during CI are reported in mild haemophiliacs or

PTPs with more than 100 exposure days. Most of the inhibitors were high responders and required immune tolerance therapy. Therefore it might be worthwhile to document the CI experience with a prospective study including appropriate controls. A proposal that may be coordinated with to the general study of PTP inhibitor (see protocol section of the afternoon session) will be submitted to the SSC.

#### 5. Report of the International Prophylaxis Study Group (IPSG) - V. Blanchette on behalf of the IPSG

The International Prophylaxis Study Group (IPSG) was formed in December 2001. The goal of the study group is to promote the generation and dissemination of new knowledge regarding the administration and outcome of factor prophylaxis in individuals with hemophilia. The coordinating centre for this study group is the Hospital for Sick Children, Toronto, Canada. The study group has linkages to the ISTH through the Factor VIII/IX Subcommittee, and to the WFH through the Pediatric Committee.

Major initial activities of the study group include the development of single, internationally accepted MRI and physical therapy scoring systems for evaluation of musculoskeletal status in persons with hemophilia. Expert MRI and physical therapy working groups chaired by Dr. Holger Pettersson and Drs. Marilyn Manco-Johnson and Pia Petrini respectively assist the steering committee of the IPSG (see below) in these initiatives. Preliminary results of these initiatives will be presented at the first full meeting of the IPSG to be held in Montreal in November, 2003. An expert outcome measures working group (Co-Chairs, Dr. Wolfgang Schramm and Victor Blanchette) has recently been formed. This expert working group will make recommendations regarding an optimal set of outcome measures (other than coagulation or musculoskeletal) for use in clinical trials of factor prophylaxis. Funding for the IPSG is provided by grants from Aventis, Baxter BioScience, Bayer and Wyeth to the Hospital for Sick Children Foundation, Toronto, Canada.

#### IPSG Steering Committee

Chair, Dr. Victor Blanchette (Toronto); Dr. Georges Rivard (Canada); Dr. Brian Feldman (Toronto); Dr. Lou Aledort (USA); Dr. Marilyn Manco-Johnson (USA); Dr. Rolf Ljung (Sweden); Dr. Pia Petrini (Sweden); Dr. Wolfgang Schramm (Germany); Dr. Marijke van den Berg (The Netherlands); and Dr. Alessandro Gringeri (Italy).

#### 6. International Surgery Survey - A. Srivastava

This study was initiated to fill the gap in the knowledge of current practices of factor replacement for surgery in hemophilia. A questionnaire survey was sent to hemophilia treatment centers around the world. This consisted of two parts – the first that looked at over practice policies and the second that required details of specific procedures carried out in the last 1-2 years. Responses were received from 35 major treatment centers from 22 countries (10 from North America, 13 from Europe and 12 from other part of the world). Preliminary analysis of the data showed that 45% of centers used bolus infusions only while 11% used continuous infusion only to administer factor concentrates. Factor VIII and IX concentrates were administered every

12 and 24 hours, respectively, by about 50% of centers while others infused more frequently. Though target pre-operative levels were reasonably uniform at 80-100% at most centers, there was a 10-fold variation in post-operative doses used. The median dose for major surgery was ~ 650 iu/kg with a range of 300-3000 iu/kg. Antifibrinolytic drugs were used by ~ 60% of centers and fibrin sealant was used by ~ 49% of centers. Major complications after surgery occurred in <1% of cases while minor hemorrhage was reported in ~3% cases.

There is need to collect more data and correlate outcome with dosage used in different kinds of surgery. This will be done over the next 6 months. Prospective documentation of data at major centers would provide more accurate data and this is being considered. Further data will be presented at the next SSC meeting.

#### 7. Prospective Role of Age at First Infusion in Inhibitor Development - *M. van den Berg on behalf of the European Pediatric Network*

Dr. Marijke van den Berg (on behalf of the European Pediatric Network) proposed a "prospective study of the role of age at first treatment in inhibitor development". Two recent publications from groups in Spain and the Netherlands reported an increased risk of inhibitor development, if the first treatment with factor VIII concentrates in haemophilic patients occurs under the age of 6 months. Such an association would have considerable implications for haemophilia treatment, however, the patient numbers are still too small for final conclusions. Therefore Dr. van den Berg proposed to address this issue with a prospective study chaired by the European Pediatric Network. The network comprises about 20 European Pediatric Haemophilia Centers with a total of 1600 patients and accrue about 100 PUPs each year. This number will allow the group to study the association of age at first treatment and inhibitor risk within a reasonable time period. This group's progress will be reported at the next SSC meeting.

#### 8. Design for Pre-licensure Studies of Replacement Products for Rare Bleeding Disorders - *A. Shapiro on behalf of MASAC*

In the United States, a rare disorder is legally defined as a disease or condition that affects fewer than 200,000 Americans. Deficiencies of coagulation factors such as VII, II, X, V, protein C, and plasminogen are so exceedingly rare that they are not even listed in rare disease registries. These rare deficiencies pose significant treatment issues. Individuals affected with rare deficiencies may have little or no option for treatment due to barriers to product development including cost of research and development and conduct of clinical trials, both of which often far outweigh potential financial gain due to the limited market. Added to these issues may be regulatory burdens for both the manufacturer and investigator. Therapeutic modalities may be available in one country but not another due to lack of synchronization of regulatory agencies. As such patients with rare deficiencies suffer from limited options for care, and their standard of care is often lower than that of persons with hemophilia with subsequent increased risk of morbidity and mortality. Options for pre-licensure studies for products for treatment of rare deficiencies, such as investigator initiated INDs and use of registry data to support a new indication, with suggested minimal aims for such trials were presented.

Organizations whose mission is aligned and/or who represent these constituents should consider

development of a statement reviewing these issues in treatment of rare coagulation factor deficiencies. Consideration should be given to development of a multi-organizational clearinghouse or resource center for the purpose of:

1. Assisting clinicians
  - a. Searching for treatment options
  - b. In protocol development
  - c. Interfacing with regulatory agencies and manufacturers
2. Development and maintenance of listings of
  - a. Interested private and governmental agencies
  - b. Manufacturers with potentially effective therapies either licensed or in clinical trials
  - c. Companies who may have interest in assuming a product portfolio for a limited indication.

In the discussion that ensued the international community agreed with the need for the strategy proposed. The chair will work with Dr Shapiro to further this initiative with an update to be presented at next year's SSC meeting.

**Standardization: Co-Chair: K. Mertens  
Plasma and Concentrate Standards**

1. WHO 5th IS FVIII/VWF Plasma - Results of Collaborative Study – T. Hubbard

An international collaborative study involving 37 laboratories has been undertaken to calibrate the proposed 5th International Standard Factor VIII/von Willebrand Factor, plasma (02/150). Estimates have been calculated relative to both the current standard (4th IS plasma, 97/586) and locally prepared normal plasma pools. Estimates for FVIII:C, vs the 4th IS plasma, by three methods (1-stage, 2-stage, chromogenic), showed good inter-laboratory agreement with overall geometric coefficient of variation (GCV) of 4.9% and a mean value of 0.68 IU per ml (n=40). Estimates vs the fresh normal plasma pools were significantly lower (mean 0.61 IU per ml, n=18) and more variable (inter-laboratory GCV 12.6%). In consideration of the known stability of the 4th IS plasma and in order to maintain continuity between the 4th and 5th IS it is proposed that the 5th IS be assigned the mean value obtained vs the 4th IS of 0.68 IU FVIII:C per ampoule. Estimates for FVIII:antigen, vs the 4th IS plasma, showed good agreement between laboratories with GCV of 4.7% and an overall mean of 0.94 IU per ml (n=10). This value was not significantly different from the estimates obtained vs normal plasma pools (mean 0.87 IU per ml) which were however more variable (inter-laboratory GCV 14.9%). It is proposed that the 5th IS be assigned the mean value obtained vs the 4th IS of 0.94 IU FVIII:Ag per ampoule. These proposals had already been approved by the study participants. The report had further been distributed amongst the Subcommittee members prior to this Meeting for a vote in writing. In absence of any negative votes, this proposal was considered to be accepted by the Subcommittee and will be presented to the SSC for ratification.

2. WHO 7th IS for Factor VIII:C concentrate (99/678) - S. Raut and T.W. Barrowcliffe

The presentation was the final report of a study that has already been discussed in the Subcommittee at its Meetings in Paris and Boston. On those occasions much discussion has been

devoted to the various candidate materials and the options for potency assessment. The candidate that had been agreed upon was preparation N (99/678). This was assayed and calibrated as the proposed WHO 7th IS for FVIII:C concentrate. There was good agreement between laboratories using one-stage and chromogenic methods (overall potencies differing only by less than 2%) for the comparison of preparation N against the four established standards. Accelerated degradation studies showed that this preparation is very stable (predicted loss of activity per year of less than 0.01% at -20°C). Various potency options for the proposed standard were considered by participants and by members of the FVIII/FIX Subcommittee of ISTH/SSC during its meeting in Boston, 2002. After ample consideration, it was decided to recommend that preparation N (NIBSC 99/768) be established as the WHO 7th IS for Factor VIII:C concentrate with a potency of 11.0 IU/ampoule. This recommendation had been distributed amongst the Subcommittee members prior to this Meeting for a vote in writing. None of the members voted negatively. This recommendation provoked a lively discussion from the audience on assay methodology in general and on the utility of concentrate standards in particular. At the same time, however, it was appreciated that the current proposal should be a major step forward in the harmonisation between European and US manufacturers in terms of standardisation and product labeling. It will be presented to the SSC for approval.

### **Factor VIII product potency assessment**

#### 3. SSC field studies of concentrates – future plans – K. Mertens

At the first meeting of the Steering Committee on SSC field studies, it was concluded that data collection in the 7 SSC studies have been merely anecdotal, without specific assay design. Although this provided many data on potency estimates using various methods and standards, it remained difficult to develop a more specific approach that would justify further studies on behalf of the SSC. It was therefore decided to analyse the large collection of laboratory data first, in order to identify the focus and design of forthcoming studies. This analysis has now been performed on behalf of the Steering Committee by Dr. Martin Lee (see following presentation). This should serve as the basis for the development of future activities on this issue.

#### 4. Analysis of Lab Consistency – M. Lee

The SSC field studies 1-7 comprised in total 731 assays. The data thereof have been subjected to a multivariate analysis, using both regression modes and analysis of variance. Assay variability was relatively large for assays on recombinant FVIII samples, and also dependent on the standard used, the Mega-1 giving the lowest variability. The worst combinations were the use of the WHO standard and predilutions in buffer that did not contain albumin, or the use of the WHO assay in the chromogenic assay. The best combinations were the use of the Mega standard in the chromogenic assay, or the Mega standard plus depleted plasma as the prediluent. In terms of deviation from the overall mean, the worst combinations were the 1-stage assay used with albumin-containing buffer as the prediluent, and the use of the EP standard and albumin-free prediluent. For the best combination, however, there were no obvious candidates. In conclusion, these data display much unexplained variation. Apart from the predilution in hemophilic plasma, no other recommendation seems justified so far.

#### 5. Factor VIII Chromogenic Assay: Report of European Pharmacopoeia Expert Group 6B – R.

*Seitz*

Discrepancies between high purity FVIII concentrate potency values improved with the use of adapted concentrate standard preparations, and the SSC recommendation to use the chromogenic assay, which is currently the official European Pharmacopoeia (EurPharm) method 2.7.4. However, with a B-domain-deleted recombinant FVIII, discrepant values are obtained with different chromogenic assay kits. There are several possibly critical assay variables, notably: thrombin (addition of thrombin or generation of thrombin in the system; final thrombin concentration); phospholipids (concentration and composition); incubation time (measure during linear phase of FX activation ("kinetic") or after FXa activity reached steady state ("plateau")). EurPharm expert group 6B is looking more closely into the assay problems. It is intended to maintain the universal IU for pdFVIII and rhFVIII products, and to maintain the chromogenic assay method, to review, however, the detail aspects of the assay in order to make it more robust. In the longer term, a novel assay might be helpful. A fluorogenic assay developed in the Paul-Ehrlich-Institut (Blood Coag Fibrinol 14:347;2003) is shortly demonstrated. In the discussion Dr. Seitz emphasised that the EP wishes to maintain the current chromogenic method for product labeling, but in the same time remains open for improvements in this area.

#### 6. FVIII Ring Study of the DGTI – R. Kotitschke

This is a collaborative study on the use of various prediluents for the assay of various product types using one single chromogenic assay (Coamatic FVIII). So far 8 laboratories are participating, and another 7 are expected to follow. The study comprises two plasma-derived products and two recombinant products. Two different FVIII deficient plasmas have been included, with different VWF levels. The preliminary data show a major influence of the VWF level of the prediluent, which is also dependent on the standard used. It seems that FVIII deficient plasma that does not contain any VWF cannot be used for the assay of recombinant products against the current (3rd) European working standard. This study is now further being pursued.

#### 7. Specific activity issues with Factor VIII concentrate - Carol K. Kasper

The international "Registry of Clotting Factor Concentrates", begun in 1997 for the Subcommittee, lists specific activity (SA, units of desired clotting factor per mg total protein) for each product, as declared by manufacturers. These specific activities have been quoted to substantiate claims that one product is purer than another. SA, however, does not reflect level of impurity, that is, undesired or irrelevant proteins, which are part of total protein. The major source of impurity in FVIII concentrates is added albumin. The volume of added albumin ranges from 3 to 20 mg/ml (most commonly 4-10 mg/ml) and its level of purification ranges from about 95 to 99%. (The European pharmacopoeia demands more than 96%.) A ml of concentrate might contain 0.03 mg/ml unknown protein (3 mg albumin at 99% purity) to 1.1 mg/ml unknown protein (20 mg albumin at 95% purity.) A typical chromatographically-purified concentrate contains about 1.9 mg/ml of FVIII/VWF and about 0.1 mg/ml of unknown protein before addition of albumin. "Purity" might be judged, in part, by the level of impurities. Dr Kasper agreed to clarify the disassociation between SA and "purity" in future editions of her factor registry.

## 8. Post-infusion Plasma – How to Standardise? - *T W Barrowcliffe*

The problem of variability in assays of post-infusion plasmas has been discussed at several previous Subcommittee meetings. Discrepancies exist both between 1-stage and chromogenic methods and between laboratories using the same method type, and are due to the differences in composition between the test sample, a concentrate "diluted" in a patient's plasma, and the standard, which is usually normal plasma. Concentrate standards have been shown to reduce variability and eliminate methods discrepancies, and are recommended where pharmacokinetic studies are performed for regulatory purposes. For routine clinical use, concentrate standards would be impractical for the wide variety of plasma derived concentrates still used, but are recommended for assessment of B-domain deleted recombinant FVIII (ReFacto) because of extreme variability using plasma standards for this product. Concentrate standards would also be preferable for full-length recombinant post-infusion samples, but their utility remain to be demonstrated in field trials. This presentation was another example of the complexity of biological standardisation and the impact thereof for patient treatment. As such, this issue raised a lively response from the audience that again illustrates the need for continuous input and commitment from the Subcommittee.

## 9. Population kinetics and design of minimal sampling strategies - *T. J. Schouten*

A population pharmacokinetic model was used to simulate different treatment strategies and to determine a limited sampling strategy to individualise and optimise treatment of Haemophilia B. The Non-linear mixed-effects modelling (NONMEM) approach resulted in a population model derived on the pharmacokinetic data of 21 patients. The model was fed with different doses of plasma-derived Factor IX (Nonafact®) simulating prophylaxis and peri-operative administration. Target trough levels of factor IX were: (1) below 1% during less than 12 hours before a further dose, (2) at least 1%, and (3) at least 2% before the next dose. Appropriate targets were also applied for peri-operative simulations. On a population base it was shown that during prophylaxis reduction of the dosing interval from 3 to 2 days could diminish the consumption of factor IX by 25%; adaptation of the dose based on the individual pharmacokinetics could save another 25%. During peri-operative administration similar savings can be obtained, if intervals of 8-12 hours are used instead of 24 hours. With additional techniques a minimal sampling strategy can be derived from the population pharmacokinetic model. This resulted in 4 sampling times instead of the standard 11 samples as recommended in guidelines. Dr. Schouten concluded that by applying population pharmacokinetics and a minimal sampling strategy, treatment of individual patients, including children, with haemophilia B can be optimised and also "economised".

### **Standardisation of Inhibitor Assays**

## 10. Anti-FVIII Inhibitor Standards - *S. Raut and S. Kitchen*

Development of an inhibitor antibody standard was proposed by the SSC/ISTH FVIII/FIX Subcommittee and as such three patients samples and the three standards were assayed in a collaborative study. Results showed that inter-laboratory variabilities continued to be high as observed in previous studies. One standard reduced this variability although CVs were still high

compared to normal FVIII assays. The rabbit polyclonal antibody (99/648) appeared to perform better as a standard compared to the other two monoclonal antibody standards. After discussions at the FVIII/FIX Subcommittee of ISTH/SSC, it was decided to seek feedback from participants on whether a larger study be launched using the polyclonal antibody inhibitor and if so at what optimal titre should such a material be prepared. To date only one laboratory has responded. It is therefore proposed that we seek out further feedback before embarking on a larger study. To help the feedback, it is proposed that a questionnaire be sent out by NIBSC to all participants, recommending that a larger study be carried out using the rabbit polyclonal antibody (99/648) as the primary candidate standard and to design a more controlled study, in the hope that an inhibitor standard may prove useful as a reference reagent. It is envisaged that perhaps after the larger study the candidate material may be suitable to propose as the WHO 1st IS for anti-FVIII inhibitor.

#### 11. Methodology, Old and New - B. Verbruggen

The last presentation of this standardisation session was an overview of assay methodology, with particular reference to the Nijmegen modification of the Bethesda assay. Interestingly, Dr. Verbruggen described two patients who were negative in the Nijmegen assay, but who did have an abnormally short half-life replacement FVIII therapy. By introducing a pre-concentration step of both test and reference, the sensitivity could be considerably increased, and both patients proved positive for a FVIII inhibitor using this method. Although this approach may be difficult to standardise, it suggests, that at least in some patients, it may be beneficial to achieve higher assay sensitivity.

#### **Factor VIII Measurement: Is a New Paradigm Possible? Co-Chairs: C. Lee and D. DiMichele**

##### 1. Report of the Pre- ASH Working Party Meeting/ Proposed Definitions of Clinical Phenotype in Severe Hemophilia A - D. DiMichele on behalf of the Working Party.

Dr DiMichele reported on the proceedings of and recommendations from the FVIII/IX Subcommittee Working Party Meeting on Hemostasis Measurement held in Philadelphia on December 5, 2002. The report included the Working Party's recommendations to the Subcommittee as a whole for the prioritization of ongoing assay development and further collaborative clinical correlation studies among assays. These were accepted without further modification. Part of the presentation also included a proposed definition of clinical phenotype with respect to bleeding severity in patients with biochemically severe hemophilia A (FVIII < 0.01 U/ml). The need to further develop these definitions with the input of the subcommittee was stressed. Anyone with comments should contact Dr DiMichele by email.

The details of this report, including the proposed phenotype definitions are included in Appendix 1.

##### 2. Tissue Factor – Based Models of Coagulation - K. Mann

Unfortunately, Dr Mann was unable to present as he originally intended due to his travel schedule.

### 3. Ex- Vivo Dose Finding Studies: Whole Blood Elasticity - J. Ingerslev

J. Ingerslev reported on the continued work in his group with the dynamic whole blood coagulation assay with the roTEG equipment in which a tiny amount of tissue factor was used as activator. Data showed that severe hemophilia ( FVIII:C < 0.01 IU/ml) phenotype persistently demonstrated a quite wide heterogeneity in terms of the duration of the initiation period as well as the profile of the propagation phase of clot formation. Amongst members of a family with intron 22 inversion as the causative mutation, huge variation was demonstrated in the profile of whole blood coagulation dynamics as demonstrated by the roTEG analysis. Further data from ex-vivo experiments was presented and showed heterogeneity in the response patterns upon addition of factor VIII concentrate to whole blood samples from patients. Blood from some of severe patients produced a normal roTEG profile with as little as 0.10 IU/ml of factor VIII, whereas in others there was still a significant improvement in the profile with 1.00 IU/ml of factor VIII as compared to a level of 0.50 IU/ml. This heterogeneity is at present not well understood, but future work is planned to improve our understanding of the treatment response in hemophilia.

### 4. Measurement of FVIII concentrates and low FVIII levels by thrombin generation. - T. Barrowcliffe

Dr Barrowcliffe presented a version of the thrombin generation test (TGT) that has been developed using FIXa as the trigger and measuring thrombin by sub-sampling into fibrinogen. At high concentration of FIXa the assay was extremely sensitive to low FVIII with high levels of thrombin generated even below 0.01 IU/ml FVIII. This version of the test could be useful for studying phenotypic differences in patients with severe hemophilia and for monitoring prophylaxis and gene therapy.

### 5. Calibrated automated thrombography in the diagnosis and control of the hemophilias - C. Hemker

Dr Hemker reported on the method that he developed in which the velocity of generation of a fluorescent product in the clotting sample is continuously compared to the signal from constant thrombin activity (the calibrator) in a parallel sample. Special software calculates and displays concentration of thrombin in nM in real-time during the clotting of PPP or PRP. Examples of the effect of therapy in hemophilia and von Willebrand disease using this method were shown .

### 6. The thrombin generation assay : In vitro and ex vivo studies - C. Negrier

Dr Negrier reported on his experience with a modified version of the Hemker thrombin generation assay in the monitoring of therapy in hemophilia A patients with inhibitors. Both FEIBA and rFVIIa trigger the formation of thrombin to achieve haemostasis. No simple classical assay shows a real usefulness in the prediction of patients' responses to treatment or in the follow-up and the adjustment of doses. However, the thrombin generation dose-response curve in inhibitor plasma spiked with FEIBA or rFVIIa have shown that thrombin generation could at least be partially restored when the concentrations roughly corresponded to the calculated therapeutic doses. Thus, it has been postulated that this thrombin generation assay

could enable monitoring of the in vivo thrombin generation capacity of FVIII bypassing agents.

### 7. Application of clot wave-form analysis in the management of hemophilia patients with inhibitors. - M. Shima

Dr Shima, on behalf of his collaborators, provided an update on the study of the potential clinical applications of the waveform PTT assay in treatment of hemophilia. The MDA11 is an automated analyser for performing clinical laboratory coagulation assays which uses a variable wavelength photo-optical detection system. It provides the facility to observe and quantify the changes in light transmission which occur during the performance of routine clotting assays – such as the APTT. The waveform analysis is very sensitive to very low levels of FVIII. The usefulness of waveform analysis for monitoring replacement therapy and bypassing therapy in hemophilia patients with inhibitors were studied and the results reported.

Discussion by the group endorsed investigators' ongoing efforts in the refinement of these assays as well as the in the design and performance of studies to further explore the clinical applications of these assays for the diagnosis and treatment of bleeding disorder patients. The chair will expand the membership and mandate of the existing working party to ensure progress in the established priorities for collaborative research. The results of these efforts will be reported at the next SSC meeting.

### ***Inhibitors: The Role of Clotting Factor Concentrate in the PTP: Co Chairs: JM Saint-Remy and M. van den Berg***

1/2: Background on PTP inhibitor development and the previous FVIII/IX subcommittee recommendation to use PTP's to study new product immunogenicity - D. DiMichele on behalf of Drs G. White and J. Lusher who could not attend

Dr. DiMichele introduced the intent of this session which was to address the recent concern expressed by some members of the international hemophilia community about a high rate of inhibitor development in heavily treated PTPs. This has resulted in a voluntary change of labeling of this product by the manufacturers that was subsequently approved by the US and European licensing bodies. These recent events were discussed within the context of the historical data on severe hemophilia A PTP inhibitor development on both plasma-derived and recombinant FVIII products.

The perceived unusual occurrence of inhibitors in PTPs was the basis for the 1999 recommendation from this subcommittee to use PTPs with over 150 exposure days for the pre-licensure study of new product immunogenicity. However, the historical occurrence of this phenomenon in PTPs with both plasma-derived and recombinant FVIII was documented in a review of the literature presented by Dr DiMichele as well as by the presentations that followed.

### 3. Post- Licensure inhibitor development in PTPs

- Dutch/Belgian Experience - *K. Peerlinck*

Two well-documented episodes of inhibitor formation in previously treated patients with severe haemophilia A have occurred in the past decade; both episodes occurred when a pasteurization step was added to the manufacturing process of a previously uneventfully used concentrate.

The first episode was documented in Belgium (1) and the Netherlands (2) in 1990-1991. FVIII-P (FVIII-CPS-P in the Netherlands) was an intermediate purity concentrate (specific activity 1.5 IU FVIII/mg protein) produced by controlled-pore silica adsorption followed by pasteurization (+ 60°C for 10 h in solution). It was manufactured by the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service (CLB) from Belgian (FVIII-P) or Dutch (FVIII-CPS-P) plasma. The introduction of this pasteurized product was followed by an increased incidence of new inhibitors in previously treated patients (> than 200-1000 exposure days (ED)) with severe hemophilia A in Belgium and The Netherlands. In Belgium 5 out of 109 patients receiving the pasteurized concentrate developed an inhibitor and none of the 109 patients receiving another product(1). In The Netherlands 12 patients developed an inhibitor on the pasteurized product, which implied a 5-fold increased risk compared to the time period prior to the introduction of the concentrate (2). Exposure to the pasteurized product before inhibitor detection ranged from 98 to 170 days (Belgium). Withdrawal of the pasteurized product and treatment with another FVIII concentrate resulted in disappearance of the inhibitors (1,3). Characterization of the inhibitors showed predominant specificity for the C2 domain of FVIII light chain. This is highly unusual for FVIII inhibitors in hemophilia A, in that they are usually directed against 2-3 epitopes (light chain AND heavy chain) (4). The pathogenesis of this altered immunogenicity was not found.

The second episode was documented in Belgium (5) and Germany (6) in 1995. Bisinact (Octavi SDPlus in Germany) was a high purity concentrate (200 IU FVIII/mg protein) purified by ion-exchange chromatography and treated with solvent-detergent (0,3% tri(n-butyl)phosphate-1% polysorbate 80), to which a pasteurization step (+63°C for 10 h) was added. Bisinact and Octavi SDPlus were produced by Octapharma AG in Vienna from Belgian and German plasmas respectively. Within 9 to 45 days of exposure to the pasteurized product eight out of 140 multi-transfused patients with severe hemophilia A in Belgium developed inhibitors (5). Several similar inhibitor cases were reported from Germany (6). As in the previous episode the inhibitors disappeared when treatment with the pasteurized product was stopped (5). Inhibitors in the Belgian cases were found specific to the factor VIII light chain (5). Inhibitors in eight of the German cases were found specific to the C2 domain of the light chain of FVIII (6). The cause of the increased immunogenicity of the pasteurized concentrate was not firmly established although some suggested enhanced exposure of the phospholipid binding site (7) or relation to the quality of the source plasma (8).

The major difference between these two episodes of increased inhibitors to FVIII in previously treated patients is the number of exposure days to the new concentrate before inhibitor development, which suggests that the problem may have been batch-related.

- Inhibitor development in Previously Transfused Patients (PTP) with Hemophilia A in Canada.- *M. Carcao, D.Lillicrap, C. Stiles, I. Walker on behalf of the Inhibitor Subcommittee of the AHCDC.*

Virtually all of Canada's 2561 (male) hemophiliacs (2063 (81%) with Hemophilia A and 498 (19%) with Hemophilia B) are followed in one of 24 hemophilia treatment centers. Prevalence of hemophilia among Canadian males is 1 in 5740. Most hemophiliacs receive recombinant (r) factor concentrates. Inhibitor prevalence is 4% for Hemophilia A (12 % for severe) and 0.8% for hemophilia B (2% for severe). 83 Hemophilia A and 4 hemophilia B patients currently have an inhibitor. Inhibitor incidence is not known. Data on Canadian hemophiliacs is entered into and analyzed within a data management software program (CHARMS; Canadian Hemophilia Assessment and Resource Management System). Information on inhibitor development in Canadian PTPs is available from the following:

1) Giles et al (Transfusion Science 1998; 19:139-48) followed hemophilia A patients who switched to rFVIII. At 1 yr and 2 yr post switch 9 patients (1.9%) and 10 patients (3%) respectively, who pre conversion had been inhibitor-negative demonstrated a positive Nijmegen assay. All inhibitors were low titer. Giles et al included PTPs as well as previously un-transfused (PUPS) or minimally transfused patients. As inhibitor development is more common in PUPS the incidence (over 2 years) of inhibitor development in PTPs is therefore no higher than 2-3%. As some patients who pre-conversion were inhibitor positive became inhibitor negative the prevalence of inhibitors did not change with conversion to rFVIII.

2) A Canadian study is currently evaluating the incidence of inhibitor formation in patients switched from Kogenate (K) to K-FS. Baseline inhibitor samples pre-switching (n=375) were collected as were 1-yr (n=235) and 2-yr (n=104) post samples. Preliminary results show that no de-novo inhibitors have as yet developed.

3) There are sporadic reports in CHARMS of inhibitor development in PTPs. There has not been a systematic tracking and reporting mechanism for such patients.

- The UK National Hemophilia Database (NHD)- CRM Hay, on behalf of the UKHCDO

New inhibitors are reported centrally to the NHD. Data from this registry collected between 1991 and 2001 on severe hemophilia A patients was reported to the Subcommittee. Inhibitor risk was reported per patient-treatment-year on the basis of patient age and product used. The database identified 22% of inhibitors in this population as occurring between birth and 9 years of age. Incidence between ages 10 and 50 then remained steady at between 0.3 and 0.6% with a slight rise in inhibitor incidence in patients over 50 years of age (1.2-3.0%). Data analyzed by product for patients under 10 yrs old revealed a similar inhibitor risk per patient treatment year (0.01-0.04) for all products included (Refacto, Recombinate, Kogenate (FS), Method M, Monoclate P and Alphanate) except for 8Y for which the risk was 0.0008. A similar analysis for patients >10 yrs revealed a similar and lower patient treatment year risk for all products of between 0 and 0.0004. Dr Hay concluded that inhibitor development risk in the UK severe hemophilia A population had a bimodal distribution, with advancing age presenting a possible additional risk. However, the risk was similar for all currently-used products among both PUPS and PTPs. Other possible risks for inhibitor development included product change and intensity of therapy. However, further analysis of the database was needed to confirm the latter.

- Refacto : Case Reports of PTP development

- French Experience: *MF Tourget*

Dr Tourget reported on behalf of colleagues at the Cochin and Necker Hospitals, on the observation of 4 adult PTPs (2 HIV positive, all HCV positive) without history of inhibitors who developed inhibitors following introduction of BDDrFVIII.

Three severe haemophiliacs had more than 120 EDs to other FVIII concentrates, one mild haemophiliac had more than 20 EDs. Inhibitors were detected after 28, 19, 63 and 14 EDs to BDDrFVIII because of inefficacy of treatment (n=2), haematuria 6 weeks after prostatectomy (patient treated with Interferon alpha), reduction of circulating FVIII during continuous infusion for surgery (mild patient).

Maximum titres were 1.3, 5.5, 8.9 and 0.9 BU. Further treatment of bleedings consisted in higher doses of BDDrFVIII (n=1), rFVIIa or APCC (n=2). Inhibitors disappeared within 8 months in the patient treated with BDDrFVIII, decreased in two patients treated with rFVIIa but readministration of pdFVIIa for two severe bleedings induced anamnestic responses (40 BU, 300 BU) in the Interferon treated patient. The mild patient's inhibitors disappeared within 4 months after factor VIII withdrawal.

They observed a prevalence of inhibitors of 6.1% (3/49) in their severe PTPs treated with BDDrFVIII (higher than 0.9% (1/113) recorded in the PTP study), and 5% (1/21) in their mild PTPs (no data in the literature). Only two patients had precipitating circumstances: Interferon therapy (severe haemophiliac), FVIII continuous infusion and surgery (mild haemophiliac).

A close prospective follow-up of patients treated with BDDrFVIII was recommended to assess the immunogenicity of this concentrate.

In the discussion, Dr Tourget was asked whether these centers had experienced a similar phenomenon with previous switches from PD to other recombinant products. She said they had, but did not have comparative data with her. She was also asked if the centers were planning to compare the characteristics of the patients switched to Refacto who did and did not develop inhibitors. They had not yet done so but were indeed planning to complete that analysis.

- UK experience-CRH Hay

Included in the above presentation

4. Action: Prospective study and/or long-term pharmaco-surveillance tool for PTP inhibitors - JM Saint-Remy, M van den Berg, J Oldenburg,

The emergence of inhibitors to FVIII in previously treated patients (PTPs) is a relatively newly recognized phenomenon, despite the fact that it may have always been present. It was documented in the early nineties as outbreaks of inhibitors in patients tolerant to FVIII who were exposed to a pasteurized plasma-derived (pd) FVIII from the Belgian Red Cross and, subsequently, to a degraded pdFVIII manufactured by Octapharma.

More recently, it has become obvious that a change of product or possible other circumstances, such as changes in doses or mode of administration could also be associated with the emergence

of inhibitors. However, the precise conditions under which PTPs produce inhibitor antibodies are ill defined. There is therefore a need for a prospective study. A draft protocol was presented, based on both basic and clinical understanding of how inhibitors are produced.

Practical and theoretical considerations of product-related factors were briefly reviewed, including variations in protein sequence, altered pattern of glycosylation or influence of preparation procedures. The stabilization of the final product, as well as the presence of other proteins with putative immuno-modulatory properties was discussed.

Some of the predisposing factors are well established, such as the influence of the gene defect, ethnic origin and severity of disease. The importance of inheritance of some MHC class II alleles should be explored more formally, together with impact of the circumstances under which FVIII is administered, including bleeding, inflammatory syndrome and concomitant therapy. Whether a brisk increase in the doses or the method used (continuous infusion versus bolus injection) are of importance need also to be established.

The discussion that followed was very lively and focused on whether the subcommittee attendees believed the phenomenon of PTP inhibitor development to be a concerning issue from an epidemiological point of view, or simply an interesting one with respect to what an unusual host response could teach us about the process of inhibitor development in general in hemophilia. The group in general suspected the latter, and endorsed the concept of a prospective study as proposed by the co-chairs. This concept was also enthusiastically endorsed by Drs Weinstein and Seitz from the FDA and EMEA, respectively.

On that basis, the chair will put together a working party to finalize the concept for a prospective study. This will be presented and further discussed at a meeting on this issue organized by the FDA to be held on November 21, 2003. This meeting will be attended by all interested parties, including the European regulators, with the goal of harmonizing a prospective surveillance tool.

Dr DiMichele ended the meeting at 18:30 thanking all presenters and participants.

Appendix 1.

**Proceedings of the pre-ASH Working Party  
Meeting on Hemostasis Measurement  
December 5, 2002**

*Attendees:*

**FVIII / IX Subcommittee Members:**

Present: D. DiMichele, J. Ingerslev, K. Mertens, JM Saint Remy, A. Srivastava

Absent: M. van den Berg, C. Lee, J. Oldenburg

**Invited Guests:**

Present: A. Srivastava, T. Barrowcliffe, A. Giles

Absent: K. Mann

**Introduction (*DiMichele*)**

This meeting was convened in an effort to establish the goals and priorities for the FVIII/IX Subcommittee's ongoing work with emerging models of hemostasis measurement. The potential for collaborative research among the models being proposed was explored.

Presentations

1. Hemostasis Measurement: The needs of the hemophilia community / how to meet them (A. Giles)

- Needs in hemophilia for the accurate measurement of hemostasis in patients include:

a) Patient Diagnosis

- correct assignment of clinical severity
- choice of appropriate therapy (e.g. prophylaxis, gene therapy)
- ability to monitor changes in disease severity

b) Patient Treatment

- need to effectively monitor and control replacement therapy w/r/t
  - bleeding prophylaxis
  - complications of therapy (e.g. thrombotic potential with bypass therapy)
  - design of minimum effective dosing protocols for cost effectiveness

c) Standardization of replacement product by manufacturing by more sophisticated analysis

- Suggested ways to meet these needs include:

a) development of assays that can be held to performance standards

- easily performed in a clinical laboratory
- more accurately reflective of in vivo biological clotting activity
- involve dissociation of clotting endpoint and thrombin generation

b) validation of these assays in

- hemophilic dog models
- clinical patient studies

- however clinical outcome efficacy scales need to be improved to allow for objective vs. subjective assessment

### Discussion:

The discussion centered around whether the first priority of collaborative research in this area showed to be either diagnostic, i.e, establish patient's baseline coagulation studies or focused on treatment monitoring.

The questions raised were the following:

- Is there such a state as a patient hemostasis baseline?
- Is a patient hemostasis baseline required for accurate therapy?
- Is a patient hemostasis baseline predictive of clinical bleeding phenotype?
- Does a predictive clinical phenotype exist?

These different assay methods, currently under investigation were then presented and discussed with respect to:

- status of experimentation
- advantages/disadvantages of the individual assay methodology

## **2. rTEG (*J. Ingerslev*)**

- The ideal assay for hemostasis measurement:

- good signal for clot formation
- physiologic, i.e. TF-dependent endpoint
- sensitive enough at the thresholds for both bleeding and hypercoagulability

- Principles of the assay

- clotting velocity is a critical endpoint
- TF is crucial for sensitivity
- system amplitude is affected by cellular components, especially platelets

- Clinical correlation studies

- heterogeneity in biochemically severe (< 1%) hemophilia patients established
- clear dose response curve established in recombinant factor VIII spiking experiments (normal measurements achieved at 0.05 u/ml factor concentration)

- Future studies need to establish

- individual patient baseline variability with serial sampling
- clot stability endpoints in dose response curve experiments

- Potential assay disadvantages

- samples need to be run fresh; therefore it would be difficult to include this assay in collaborative assay comparison studies

### **3. Thrombin Generation Assay (*T. Barrowcliffe*)**

- Interim data (obtained in collaboration with the London Royal Free Hospital Haemophilia Center)
- Principles of the assay

- not TF-based assay
- thrombin generation in the assay modified by concentrations of IXa, Xa, VII and TFPI
- thrombin generation measured by:

- T max reaction kinetics (lag phase and initial rate): (potentially the most sensitive measurement)
- AUC (potentially less sensitive)

- Clinical correlation studies

- factor VIII reconstitution of plasma samples shortens reaction time (T max) but does not alter TG peak, AUC

- Future studies need to establish

- whether this assay may be more useful as a concentrate assay than a patient plasma assay

- Potential assay advantages

- Technically simple

- few reagents required
- adaptable to semi-automated instrumentation or to manual technology
- potentially useful in the developing world

- Flexibility

- can be adapted to different purposes
- applicability in both hemophilia A/B

- Sensitivity

- can factor detect levels of < 1 u/dl

- Potential assay disadvantages

- labor intensity
- time consumption (20 – 30 min/assay)
- difficulty in automation
- decreased precision compared to chromogenic TGT

- Potential applications

- more precise biochemical phenotyping
- post infusion studies/characterization of hemostasis
- monitoring of gene therapy

#### 4. Waveform PTT Analysis (*A. Giles*)

- Main questions: Is there a problem with the PTT-based clotting assay per se or simply in our interpretation of the assay?
- Principles of the assay

- measurement of changes in signal intensity; timing of events; and velocity acceleration of the clotting reaction

- all phases of clotting measured:

- pre-clot formation (a-b)
- clot formation (b-d)
- post-clot formation (d-e)

- Clinical correlation studies (Drs Shima and Yoshioka)

- among 5 severe HA plasmas: different waveform patterns noted, with the highest min-2 derivative seen in the least severe bleeder

- unlike the clot endpoint APTT, correlation of this assay in the hemophilia patient with plasma concentrates to a lower limit of 0.2%

- factor reconstitution of depleted plasma: correlations of waveform pattern with [FVIII] between 0.1 – 1%

- Future studies

- analytical parameters other than min-2 must now be examined for sensitivity/specificity
- further work must be done with hemophilia B plasmas
- effect of different APTT reagents on analytical correlations must be examined
- potential adoption of this method to a PT-based assay

## 5. Defining the bleeding phenotype in "severe" hemophilia A patients prior to clinical/assay correlation studies (*D. DiMichele*)

- Based on 1) the observation that clinical phenotype variability is most commonly observed among these hemophilia patients classified as biochemically severe (< 1%) by current assays; 2) the opinion that the greatest need for phenotype prediction with respect to therapeutic intervention lies within this subgroup of patients and 3) the consequent assumption that this group of patients is a priority target group for clinical/assay correlation studies; the following bleeding phenotype definition was proposed for this population:

- **"Severe" Severe:** > 3 FVIII infusions required for spontaneous bleeding during the first year of life and > 3 joint hemorrhages; prophylaxis started by the age of 3.

- **"Mild" Severe:** ≤ 3 FVIII infusions for spontaneous bleeds during the first year of life and ≤ 3 joint bleeds by the age of 3, in the absence of prophylaxis.

- Discussion

- alternatively, should clinical criteria be developed in the absence of biochemical criteria (i.e. not tied into our current biochemical definitions of severity)?

- what defines a "spontaneous" vs. "traumatic" bleed? (so defined in the proposal to rule out bleeding due to e.g. traumatic venipuncture or circumcision which is more circumstantial than inherently defining of the phenotype)

- should there be an infusion requirement to define a significant bleed? (so defined in the proposal to exclude mild inconsequential bleeding such as non-progressive hematomas)

- should "non-iatrogenic" be used instead of the word "spontaneous"?

- how can cultural differences in bleed recognition/treatment (e.g. developing vs. developed world) be incorporated into the above definition?

## 6. Final Group Discussions/Recommendations to the FVIII/IX Subcommittee

I. Collaborative assay studies should be designed/done, acknowledging that some assays (rTEG) may not be adaptable to multicenter studies.

II. Collaborative multicenter studies could potentially include all of the following assay systems which can utilize frozen plasma samples:

- clotting/chromogenic factor assays
- TGT
- waveform PTT
- computer modeling programs

- III. Limited correlation of these assay data with the rTEG could be done
- IV. Samples of 1 ml of frozen plasma would suffice for (each?) assay
- V. Although the potential for individual patient variability in "baseline hemostatic" status exists and has not yet been studied by any of these assays, the priority for any collaborative studies should be to establish diagnostic and clinically predictive hemostasis baselines
- VI. Hemostasis baselines are critical to the future study of therapeutic intervention studies, including minimum dosing studies
- VII. For hemostasis baseline studies

- hemophilia A is the priority disease given frequency, clinical phenotype variability and greater therapeutic challenges
- study subject age must be considered relative to the developmental coagulation physiology in infants and young children and potential puberty-induced changes in adolescents

VIII. For therapeutic (post-infusion) studies

- both plasma-derived and recombinant products should be studied
- target factor levels of between 50 – 100% should be initially correlated with the proposed alternative assays

Respectfully submitted by D. DiMichele, MD

## Factor VIII and Factor IX

June 17-18, 2004

14:00 to 18:00

Carnelutti Room

Fondazione Giorgio Cini

Chairman: K. Mertens, The Netherlands

Co-Chairmen: D. DiMichele, USA; J. C. Gill, USA; C. Lee, UK; J. Oldenburg, Germany;  
JM Saint-Remy, Belgium; A. Srivastava, India; HM van den Berg, The Netherlands

### Completed/Submitted Reports and Recommendations

- Thrombosis in inherited Factor VII deficiency: *G. Mariani et al. JTH (2003) 1: 2153-2158*
- International Factor VII Deficiency Registry: *G. Mariani, complete report in preparation*
- 7th International Standard for FVIII Concentrate: *S.Raut et al, submitted to JTH*
- 5th International Standard for FVIII-VWF in Plasma: *A. Hubbard et al, submitted to JTH*
- Registry on Factor IX inhibitors and anaphylaxis: *I. Warrier, report in preparation*

**Clinical Studies in Progress: Co-Chairs: C. Lee and A. Srivastava**

### **Update: Gene Therapy Registry: K. High**

Dr. High reviewed the status of gene therapy trials for hemophilia. Five trials (3 for FVIII and 2 for FIX) treating a total of 41 patients have been done. Summary of these trials are available at the US National Institutes of Health, Office of Biotechnology Activities, database called Genetic Modification Clinical Research Information System (Gemcris). The database can be accessed by the public and contains basic information on all gene transfer trials that have been reviewed in the US. It can be searched by disease, vector, or principal investigator. This currently constitutes one of the best resources on hemophilia gene transfer studies, but the data available to members of the public are limited, consisting of scientific and lay abstracts of each trial. Most of the data in hemophilia gene transfer has been published or presented at national meetings.

Overall though no serious toxicity was noted in any of these trials, the major problems have related to poor transduction efficiency and lack or loss of function very soon after infusion. All studies have currently stopped for further evaluation in the laboratory. One approach could be study the effect in larger animals using short course of immunosuppression to avoid rejection and facilitate sustained gene function.

### **Update: International Prophylaxis Study Group: V. Blanchette**

The International Prophylaxis Study Group (IPSG) is a collaborative initiative whose aim is to facilitate generation of new knowledge relating to factor prophylaxis in the hemophilia population. To assist accurate and consistent documentation of the musculoskeletal outcome, the group is also focusing developing new physical examination and MRI based radiological scoring optimized for the detection of early joint disease. Two expert groups were established with this

goal in mind: a physical therapy group (Co-Chair: Marilyn Manco Johnson) and a MRI group (Chair: Holger Pettersson). Scoring systems have been developed (the Hemophilia Joint Health Score and MRI-based joint score) and reliability tests have been conducted. Once this initial process is completed, these scoring systems will be submitted to the Pediatric and Musculoskeletal committees of the World Federation of Hemophilia and the FVIII-IX Subcommittee of the ISTH-SSC for review and endorsement.

#### **Progress Report: Definition of the Clinical Phenotype: *D.M. DiMichele***

Part of the FVIII-IX Subcommittee's focus over the past 3 years has been on identifying more physiologically accurate methods of measuring hemostasis in persons with hemophilia and other bleeding disorders. The intended application of these assays would be in 1) patient diagnosis (accurate assignment of clinical bleeding severity in order to determine optimal therapeutic strategies) and 2) patient treatment (cost-effectiveness monitoring and control of replacement therapy and its complications). The consensus of the Subcommittee Working Party resulted in a recommendation to the Subcommittee in 2003 that included 1) the development and validation of these assays; and 2) major application of this technology in the prediction of hemostatic baselines in severe hemophilia A and B patients.

However, such studies need to be validated by a consensus clinical definition of bleeding phenotype, with particular application to the young child with hemophilia. No such definition yet exists. A definition was proposed in 2003 by Dr. DiMichele for the child with biochemically severe (< 1 U/ml) hemophilia A (HA) that included "mild" and "severe" severe HA phenotypes. At this meeting, Dr. DiMichele proposed that this definition be disseminated among various clinical and scientific groups for comment and validation in hemophilia cohorts around the world. Input will be sought from 1) FVIII-IX Subcommittee co-chairs and members; 2) Pediatric Subcommittee of the WFH; 3) European Pediatric Network; 4) Japanese hemophilia physicians; 5) Canadian hemophilia center directors' organization; and 6) North American Hemophilia and Thrombosis Research Society. Other suggestions were elicited. The subsequent plan is to assemble a working party to review and develop a consensus definition to be presented to this Subcommittee in 2005.

#### **Phenotype versus genotype survey: *H.M. van den Berg***

Dr. Van den Berg presented data of a study done to investigate variability and determinants of phenotypic variability of severe hemophilia. A large cohort of severe haemophilia patients were investigated to determine clinical variability with regard to data on bleeding episodes, treatment characteristics and long-term outcome. Of 285 patients a total of 4737 follow-up years were available (mean follow-up 16.6 years). Clinical variability was shown in age at first joint bleed and a combination of joint bleed frequency and annual clotting factor use.

The determinants of variation in severe hemophilia were further evaluated in a smaller cohort of 38 patients – 20 with mild and 18 with severe phenotype. The clinical difference among these two groups was significant for age at first bleed (4.6 vs 0.7 years), joint bleeds per year (3.1 vs 4.2) and number of joints with arthropathy (0.6 vs 4.5). The half life of FVIII was also studied in these patients. Though there was no statistical difference in factor VIII half-life between patients with a more severe and patients with a milder phenotype, a one hour increase in factor VIII half-

life was associated with 100 IU/kg/yr less annual clotting factor use. Further evaluation of additional markers of defining phenotypic heterogeneity of severe hemophilia is progressing.

### **Final Report: International survey of factor replacement protocols for surgery in hemophilia: A. Srivastava**

This survey, which was initiated to fill the gap in the knowledge of current practices of factor replacement for surgery in hemophilia, has been completed. The two part questionnaire survey (first part for documenting practice policies and the second part for details of specific procedures carried out in the last 1-2 years) received responses from 45 major treatment centers in 23 countries (16 from North America, 14 from Europe, 3 from Australia, 9 from Asia, 2 from South America and 1 from Africa). The data shows that only 15% of centers used continuous infusion exclusively, 42% used bolus infusion only and the other 42% used both with lack of uniformity in the frequency of dosing. The target pre-operative levels were 80-100% at most centers but there was a 5-fold variation in post-operative doses used. The median dose for major surgery was ~ 650 iu/kg with a range of 300-1500 iu/kg. The dosage used in developing countries was 10-30% lower for similar procedures. Antifibrinolytic drugs were used by ~ 70% of centers and fibrin sealant was used by ~ 45% of centers. While most centers reported no major complications, operative bleeding was reported in 3-5% of cases and significant post-operative bleeding occurred in 5-6% of cases at some centers. Wound infection occurred in 3-4% and thrombotic complications in 4-5% patients. These rates appear to be similar in different parts of the world.

The data suggests that there is need for optimization of factor replacement dosage and other practices for surgery in hemophilia. Based on this data, it should be possible to evolve consensus protocols for factor replacement for surgery in hemophilia and test them prospectively.

**Factor VIII Inhibitors: Co-Chairs: D.M. DiMichele and H.M. van den Berg**

### **Factor VIII inhibitor assay standardisation: S. Raut**

Dr. Raut presented an overview of inhibitor assay standardisation. The current gold standard assay is the Bethesda Assay or the Nijmegen Modification thereof. However these assays can be cumbersome, labour intensive and can give inter-lab CVs between 30 - 80% or more. Development of an anti-FVIII inhibitor antibody standard was recently proposed by the FVIII-IX Subcommittee and as such three patients samples and the three standards were assayed in a preliminary collaborative study. Results showed that inter-laboratory variabilities continued to be high as observed in previous studies. One standard reduced this variability although CVs were still high compared to normal FVIII assays. The rabbit polyclonal antibody (99/648) appeared to perform better as a standard compared to the other two monoclonal antibody standards.

After discussions at the FVIII-IX Subcommittee of ISTH-SSC, it was decided to seek feedback from participants on whether a larger study be launched using the polyclonal antibody inhibitor and if so at what optimal titre should such a material be prepared. Dr. Raut proposed that a questionnaire be sent out by NIBSC to all participants, recommending that a larger study be

carried out using the rabbit polyclonal antibody (99/648) as the primary candidate standard and to design a more controlled study, in the hope that an inhibitor standard may prove useful as a reference reagent. It is envisaged that perhaps, after the larger study, the candidate material may be suitable to propose as the WHO 1<sup>st</sup> IS for anti-FVIII inhibitor.

### **Update: International ITI study: *C. Hay***

Dr Hay reported that the ITI study is now in its second year. 63 centres from 20 countries have agreed to participate, 33 patients have been recruited from 9 countries. Recruitment is building up slowly and some administrations have only now completed the administrative steps to start. Six patients have been withdrawn (3 pre-ITI, 2 failed ITI and 1 lost to follow-up). 26 patients subjected to ITI for median 7 months (range 1-20 months). 16 have negative inhibitor (7 with normal recovery and two tolerant). 2 have failed ITI. 48 non-serious adverse events have been reported, including 9 catheter infections and a variety of intercurrent infections and traumatic bleeds. 29 serious adverse events were reported including 9 line infections in 4 patients resulting in 5 line removals. The remaining SAEs were mostly haemorrhagic. Of 29 SAEs reported 10 were study-related. For further information see website: <http://www.itistudy.com>.

### **European Pediatric Network: PedNet Registry and RODIN study: *H.M. van den Berg***

The European Paediatric Network for Haemophilia Management (PedNet) is a collaboration of 23 European paediatricians involved in haemophilia care. The PedNet Haemophilia Registry is an initiative of PedNet members through which a common set of data is collected to improve understanding of the pathophysiology of haemophilia, current clinical management of children with haemophilia, and the safety and efficacy of treatment strategies. Patients with severe (<1%), moderate (1-5%) and mild (5-25%) haemophilia A or B born after January 1<sup>st</sup>, 2000 with well-documented patient history and treatment data from diagnosis onwards are eligible for enrollment. Data are collected using a patient logbook and assessment forms, which are filled out at every regular visit. The assessment forms are sent to the central database by the Internet (<http://www.pednet.nl>). In September 2003 the number of eligible patients at the moment was estimated at 260 patients.

The first study to be performed in the registry is the RODIN study, Research Of Determinants of Inhibitor development among previously untreated patients with haemophilia in Europe. This study aims at examining the roles of potential genetic and environmental risk factors for inhibitor development among PedNet-registered patients with mild, moderate and severe haemophilia A and B. Data collected in the PedNet Haemophilia Registry are treatment characteristics during the first 75 days of treatment with clotting factor (such as age of first infusion of factor VIII, frequency and dose of infusions, type and purity of clotting factor product, possible extravasation of clotting factor), severity and type of bleeds, infections and the use of antibiotics during the first 75 exposure days, allergic diseases, vaccinations, breast feeding, family history of inhibitors, ethnicity and mutations in the genes for the deficient clotting factors. In 5 years we hope to have registered a sufficient number of patients to answer the research questions of the Rodin study. Data can be submitted by the internet site: <http://www.pednet.nl>.

### **Global PTP surveillance study: *D.M. DiMichele***

A possible increase in inhibitor incidence in PTP's using ReFacto in the post-licensing setting prompted an evaluation of the background incidence of inhibitors in PTP's at the July 2003 FVIII-IX Subcommittee meeting. The results of a literature search on inhibitor incidence were presented. The ensuing discussion emphasized the need to more accurately ascertain the rate of new inhibitor formation in heavily treated patients with hemophilia A through post-marketing surveillance. The scientific question of whether this was a host or product phenomenon was raised. This problem was further discussed at the FDA Inhibitor Conference in the USA in November 2003. As a follow-up, Dr. DiMichele presented a potential schema for a universal post-licensure data collection system for all FVIII products at this subcommittee meeting. Her presentation included the following proposals:

- An international database for globally distributed products, and national/multi-national databases for products with more limited distribution.
- PTP's defined by pre-licensure clinical trials, and observed for a period defined by exposure days, not time.
- Two data sets: 1) a minimum data set (MDS) defined by regulatory agencies with industry input; focus on product immunogenicity and the goal of providing an ongoing assessment of a product risk/benefit ratio; and 2) a comprehensive data set (CDS) defined by clinical investigators and scientists with a focus on ascertaining the role of host/treatment interaction in PTP inhibitor formation.
- Both databases should not be industry-driven, but rather compiled and interpreted by physicians and scientists.
- The FVIII-IX subcommittee (PTP Inhibitor Working Group?) could be a possible clearinghouse for the data generated by the MDS, in a role that would include coordination/analysis of the national databases. Proposed funding would come from industry and national governments, where applicable.
- Although the CDS would be largely grant funded in this schema, a reporting relationship between clinical investigators/scientists working on this issue and the FVIII-IX Subcommittee would be optimal.

Dr. DiMichele's proposal received a positive response from the audience. At the same time, however, concerns were raised with regard to the feasibility of such a complex program.

**Proposal to refine the low response inhibitor definition: *J.Gill, presented by D.M. DiMichele***

In 2000, the Factor VIII-IX Subcommittee of the Scientific and Standardization Committee of the ISTH developed standardized definitions to classify the variable clinical course of inhibitors. High response inhibitors were defined as those  $> 5$  BU/ml currently or at any time in the past; and low response inhibitors as those persistently  $\leq 5$  BU/ml despite repeated challenge with substitution factor replacement. An analysis of the long-term follow-up data of the Recombinate PUP study suggests that the low response inhibitor category should be further refined to separate out those patients with inhibitors persistently  $\leq 5$  BU who have a short, benign course that does not require therapeutic intervention. Dr. Gill's proposal was that these inhibitors be termed "transient" and be defined as those whose maximal inhibitor titer is  $\leq 5$  and disappears within 12

months of detection. This group should be analyzed separately when the immunogenicity of new products is being analyzed. In the discussion a more simple definition was proposed depending on whether or not the inhibitor interferes with normal treatment. The issue will be further worked out by Dr. Gill in more detail for presentation to the Subcommittee in 2005.

**Panel discussion: Can we predict product-related loss of tolerance?**

**Animal models of FVIII immunogenicity in the setting of gene transfer: *D. Lillicrap***

Dr. Lillicrap reviewed animal studies on host immune response to FVIII gene transfer. Variables that influence the potential development of antibodies to the transgene product (FVIII inhibitors) include the type of vector, the dose of vector, the site of vector administration, the site of transgene expression, the level of transgene expression and the type and genetic background of animal in which the studies are being performed. At the current time, viral vector-mediated gene transfer is the most effective means of transgene delivery. The immune responses accompanying the use of the different viral vectors, adenovirus, AAV and lentivirus are very different, and are further complicated by the potential of pre-existing immunity to proteins presented on the incoming vector. Overall, while adverse immune responses to gene transfer remain a critical challenge to the success of this therapeutic approach, the utilization of several different animal models is essential to evaluate the likely occurrence of these events. While so far gene transfer has successfully cured hundreds of hemophilic mice and a few hemophilic dogs, it remains clear that more work will be needed to further control the inhibitor risk.

**Transgenic mouse models for FVIII inhibitor formation: *J. Voorberg***

While inhibitory antibodies develop in approximately 25% in patients with severe hemophilia A following treatment with factor VIII, inhibitor formation in mild and moderate hemophilia A is rare. Several reports have described inhibitor development in patients with mild hemophilia A caused by an Arg<sup>593</sup> to Cys mutation. To mimic the immune response in these patients in mice, Dr. Voorberg has created a transgenic mouse model expressing human factor VIII-R593C (hufVIII-R593C mice). Transgenic hufVIII-R593C mice were crossed with factor VIII-deficient mice (E-16 KO mice). Factor VIII-deficient E-16 KO mice develop anti-factor VIII antibodies after five serial intravenous injections with human factor VIII while hufVIII-R593C/E-16 KO mice did not develop an immune response. Apparently, hufVIII-R593C mice are tolerant to human factor VIII which is in agreement with the strongly reduced incidence of factor VIII inhibitors in patients with mild hemophilia A. However, anti-factor VIII antibody development was induced in hufVIII-R593C/E-16KO mice by multiple subcutaneous injections of factor VIII with an adjuvant. These data indicate that partial loss of tolerance can be induced in this novel mouse model for inhibitor development in hemophilia A. Dr. Voorberg suggested that this model might have future potential as an immunogenicity model for assessing novel FVIII products or gene therapy protocols.

**Dissecting immune response to FVIII products: *B. Reipert***

Dr. Reipert reviewed the immunological mechanism of inhibitor formation, including a delicate interplay between antigen presenting cells, B-cells and T-cells as well as co-stimulatory ligands involved in this process. Although we currently know some factors which contribute to the

inhibitor risk of Previously Treated Patients such as type of gene mutation, B-cell response (epitopes), other issues still remain poorly understood. These include the development of FVIII-specific memory cells, and “danger” signals that trigger anti-FVIII response. Dr. Reipert is using an animal model to study these immunological response mechanisms, in which hemophilic mice are challenged with human FVIII. Subsequently, lymphocytes are isolated which can be used in stimulation experiments in vitro. By using different FVIII products for T-cell response it should be feasible to study loss of tolerance if “altered” FVIII is used. In the future, this approach may have potential for detecting neoantigens, for instance due to changes in manufacturing procedure of FVIII products.

**Factor VIII assays and standards: Co-Chairs: K. Mertens and J.M. Saint-Remy**

### **Overview of ongoing activities: K. Mertens**

Dr. Mertens introduced this part of the program. Various standardisation issues have recently been completed, such as the establishment of new International Standards of FVIII in plasma and concentrate. Others have been addressed in two more informal, separate meetings:

- The Steering Committee on SSC Collaborative Studies on FVIII-IX field studies met at NIBSC on November 13<sup>th</sup>, 2003. The committee, currently consisting of Drs. S. Raut, T. Barrowcliffe, M. Lee and K. Mertens, has been working on a new design for these studies to make them more systematic and useful. The first results are planned to be reported at the Subcommittee meeting in 2005.
- The Working Party on novel FVIII Assays met in San Diego, on December 5<sup>th</sup>, 2003. Present were Drs. K. Mann, B. Sorensen (for J. Ingerslev), P. Turecek, D. DiMichele, J. Gill, M. van den Berg, J. Oldenburg, and K. Mertens. After a review of various novel assays, it was decided that some collaborative study would be the next step, and that the thrombin generation assay may be the most promising approach for this purpose. A proposal will be worked out for discussion in the Subcommittee meeting in 2005.

Because these technical issues were already addressed, this year’s standardisation section was devoted to a panel discussion on FVIII assay methodology.

### **The FVIII assay problem: neither rhyme, nor reason: P. Lollar**

Dr. Lollar presented his view on the issue, as published in JTH last year. Discrepancies between one-stage coagulation and chromogenic assays of factor VIII (FVIII) activity and between labeled potency and in vivo recovery of FVIII are two examples of the FVIII assay problem. The chromogenic assay appears to have gained prominence primarily by official decree. Reports that the chromogenic assay is the “correct” assay lack scientific rigor. It has been reported that different FVIII products are comparable because they appear to satisfy the criteria of the parallel line assay. Because of parallelism and because concentrate standards are referenced against a plasma standard, there is no apparent need for concentrate standards or assays of “like *versus* like”. The FVIII assay problem potentially could be solved by making the one-stage assay the official assay for in vitro and in vivo measurements and by adopting a single FVIII plasma standard for all assays.

## **WHO approach on International Standards: *T.W. Barrowcliffe***

Dr. Barrowcliffe summarised the FVIII standardisation strategy followed during the last 25 years. The introduction of WHO International Standards and the principle of “like-versus-like” testing has much reduced interlaboratory variability. For FVIII concentrates the chromogenic assay has proven superior over the 1-stage assay in term of CV values, and for this reason the chromogenic assay is the SSC recommended assay for product potency assessment since 1993. Although Dr. Barrowcliffe acknowledged the simplicity of the use of a single type of assay, he also noted some disadvantages of the 1-stage assay. These mainly relate to the great variability between reagents, which make the 1-stage assay difficult to standardise.

## **Chromogenic assay: background and design: *S. Rosen***

Due to its higher precision and lower interference as compared to clotting methods, the chromogenic FVIII method was recommended by the ISTH-SSC in 1993 for assay of FVIII concentrates and adopted as the Ph Eur reference method 1995. These features were also demonstrated in the recent calibration of the Mega-2 standard. In a further characterization of the chromogenic FVIII method, Dr. Rosen has performed a study on assay of FVIII concentrates vs the 6<sup>th</sup> IS with human and bovine species of FIXa, FX and thrombin or, alternatively, prothrombin and FV. Using fixed concentrations of FIXa, FX, thrombin / prothrombin + FV, calcium chloride, phospholipid, FVIII and one common buffer, no difference in FVIII potency assignments of Octonativ-M, Recombinate and ReFacto was obtained when using either only human or only bovine components. The results thus indicate that FVIII does not show a strict species specificity in the chromogenic assay.

## **FVIII assays: a regulator’s view: *R. Seitz***

Dr. Seitz reported that in Europe potency assessment of FVIII products requires the chromogenic assay. All labelled Medicinal products marketed in Europe have to comply with European Pharmacopoeia (Eur. Pharm) monographs. According to the new monograph for recombinant FVIII, reference preparations should be calibrated against the International Standard. However, with a B-domain-deleted recombinant FVIII, discrepant values are obtained with different chromogenic assay kits. Following a collaborative study verifying the discrepancies, the respective reference preparation was relabelled resulting in a fill of the product increased by approx. 20%. The Eur.Pharm expert group 6B looked into details of the assay and concluded that the FXa generation should be stopped at about half the plateau level. It was decided that the chromogenic method will remain the reference method in Europe, although the assay description will be amended with regard to the optimal incubation times.

## **Thrombin Generation Assays on post-infusion samples: *P. Turecek***

Classification of the severity of haemophilia is generally based on the levels of factor VIII or factor IX levels in the plasma. However these levels do not always match the bleeding tendency of individual patients, especially those with severe deficiencies, i.e. factor VIII or factor IX level <1%. The usual clotting and chromogenic assays have a detection limit of approximately 1-2 %. Thrombin generation assays can assess all activating and inactivating systems of coagulation and

therefore, they might be suitable to monitor coagulation factor substitution therapy with plasma-derived or recombinant FVIII concentrates. Dr Turecek reviewed presented a thrombin generation assay which is suitable for detecting treatment-dependent changes in the kinetic of thrombin generation and thus monitoring the pharmacokinetics of inhibitor-bypassing agents during treatment. The same assay can also be used to assess the thrombin generating capacity of severe haemophilia patients before and after treatment. *In vitro* spiking experiments with severe haemophilic plasma samples using plasma-derived or recombinant therapeutic FVIII preparations showed that the thrombin generation assay is especially sensitive in the low factor activity range, even below 0.01 U/ml. Plasma samples from severe haemophilia patients treated with single doses of FVIII showed a measurable thrombin generation even at time points, when no FVIII activity could be detected.

### **Pilot collaborative study on clinical samples (C. Negrier, also on behalf of A. Srivastava)**

Dr. Negrier showed some data of a collaborative study, in which thrombin generation assays was assessed in samples collected in India. These assays proved particularly useful in a pharmacokinetic study. The study further identified some technical issues that may be subjects for further standardisation. These include the tissue factor concentration, and the need to prepare cell-free plasma samples.

### **General discussion on current SSC guidelines:**

These presentations were followed by a lively general discussion, in which the various speakers and several attendees participated. It was evident that all speakers were in favour of rigorous standardisation, although the proposed solutions were different. Overall, there was no reason to consider the current SSC guidelines for potency assessment (Barrowcliffe, 1993) and for post-infusion assays (Lee et al, 2001) as being inappropriate. At the same time, however, it was felt that the thrombin generation assay, if further standardised, might have the potential of making 1-stage and chromogenic assay obsolete in the future.

### **Rare Bleeding Disorders: Co-Chairs: D.M. DiMichele and J. Oldenburg**

### **SSC Working Group on Rare Inherited Bleeding Disorders: F. Peyvandi**

Dr. Peyvandi's presentation was the kick-off of this new Working Group, which was established as part of the FVIII-IX Subcommittee in 2003. Rare bleeding disorders (RBDs) are typically orphan diseases, relatively neglected until recently by health care providers, advocacy organizations and pharmaceutical companies. They comprise deficiencies of fibrinogen, prothrombin, factors V, combined V and VIII, VII, X, XI and XIII, that together have a global prevalence of around 3% to 5%. Due to the rarity of each factor deficiency, as a consequence of the relative rarity of these deficiencies, type and severity of bleeding symptoms, underlying molecular defects and actual management of bleeding episodes are not established as well as in haemophilia A and B. Also purified factor concentrates are not as readily available as they are for the haemophilias. Various group spread all over the World deal with RBDs, from clinical, phenotypical and genotypical point of view. The development of an International Registry of RBDs through a co-operative network of Haemophilia Centres around the world will help to fill

the gap between data production and their accessibility. Preliminary data obtained from the International Rare Bleeding Disorders Database (RBDD) already present in Hemophilia Centre-Milan-Italy with more than 200 patients affected by each single disorder, indicate the possibility to perform interesting and transversal studies among different centres in order to confirm data obtained by each single Centre. The aim of this registry is to efficiently organize and extract the consistent amount of information on rare bleeding disorders. This would certainly optimise retrospective studies in the field and static reports extracted from the database planned to be made available on the World Wide Web. Another important goal of SSC Working group is the standardisation of laboratory methods for phenotypic diagnosis of RBDs. The last but not the least important goal is to foster the development of orphan drugs for deficiencies with no available therapeutic concentrate, particularly for FV deficiency.

Dr. Peyvandi emphasized that the Working Group on Rare Inherited Coagulation Disorders needs to focus on following issues:

- to develop the International Registry of RCDs
- to standardise laboratory phenotype and genotype diagnosis methods
- to foster the preparation of orphan drugs, particularly for those deficiencies with no available therapeutic concentrate
- to evaluate women's health in RCDs
- to prepare diagnosis and treatment guidelines (on demand and on prophylaxis)

### **Rare Bleeding Disorders: Therapeutic Needs: A. Shapiro, presented by D.M. DiMichele**

This report was on behalf of the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation In the United States. A rare disorder is legally defined as a disease or condition that affects fewer than 200,000 Americans. Deficiencies of coagulation factors such as VII, II, X, V, protein C, and plasminogen are so rare as often not to be listed in rare disease registries. These rare deficiencies pose significant treatment issues. Individuals affected with rare deficiencies may have little or no option for treatment due to barriers to product development including cost of research and development and conduct of clinical trials, both of which often far outweigh potential financial gain due to the limited market. Added to these issues may be regulatory burdens for both the manufacturer and the investigator. Therapeutic modalities may be available in one country but not in another at times due to lack of synchronization of regulatory agencies. As well, some products may have off-label use for some deficiencies, but may not be available in all countries. Off-label use of drugs may lead to difficulty with reimbursement. Thus patients with rare deficiencies suffer from limited options for care, and their standard of care is often lower than that of persons with hemophilia, with subsequent increased risk of morbidity and mortality. Options for pre-licensure studies for products for treatment of rare deficiencies in the United States, such as investigator initiated INDs and use of registry data to support new indications, with suggested minimal aims for such trials, should be explored.

### **Orphan Drugs: the FDA perspective (M. Weinstein)**

The Food and Drug Administration strongly encourages the development of orphan drugs. FDA considers designating a drug (or biologic) as an orphan drug if it will be used to treat any disease or condition that affects less than 200,000 persons in the US, or that affects more than 200,000 persons in the US, but for which there is no reasonable expectation that the cost of developing and making the drug available in the US will be recovered from sales in the US. Incentives for orphan drug development include seven years of market exclusivity following approval of the drug, award of grant funding to defray the costs of clinical testing, and a tax credit on certain clinical testing expenses. The approval of an orphan designation request does not alter the standard regulatory requirements and process for obtaining marketing approval. Safety and efficacy of a compound must be established through adequate and well-controlled studies. Clinical trials should be designed to make a valid comparison with a control, to provide a quantitative assessment of the drug effect. Clinical trial designs for orphan drugs take into account the size of the patient population, prior clinical experience with similar products, whether the endpoint is easily recognized, and whether surrogate markers for efficacy exist, among other parameters. Examples of controls include placebo, dose-comparison, no treatment, or active treatment. In some cases normal volunteers could be used to demonstrate the safety of a product if not enough patients are available. Post licensure (Phase IV) data can be used to gain additional information about safety and efficacy, e.g. registry information. Foreign clinical studies are acceptable for support and/or market approval if they are well designed, well conducted, performed by qualified investigators, and are ethically sound. Examples of recent product approvals employing relatively small patient populations in clinical trials include an immunoglobulin (50 patients), and alpha one protease inhibitor (15 patients). In summary, FDA encourages orphan drug development, requires establishing the safety and efficacy of a compound through adequate and well-controlled studies, and will work with the sponsor to design clinical trials appropriate for the patient population.

### **Orphan Drugs: the EMEA perspective (R. Seitz)**

There are some medicinal products for rare bleeding disorders (e.g. FXIII, FXI) licensed in European countries. Such products have been licensed more than 20 years ago, when the requirements for clinical studies were different. There is nowadays a special EC legislation for orphan medicinal products (OMP), providing Incentives, such as protocol assistance, waiver of fees, market exclusivity for 10 years, and potentially support of research, development, and availability. Criteria for OMP designation are: serious condition affecting <5 in 10,000, and lack of, or superiority over alternatives. The EMEA is willing to give advice to potential applicants, and there is a Blood Product Working Group (BPWG), providing guidelines on clinical evaluation of blood products, taking account of the rarity of the respective disorders. The question is, which OMP would be needed, and whether the industry would be willing to develop such products.

### **Orphan Drugs: manufacturers views on concentrates for treatment**

#### **(1) LFB (Z. Tellier)**

The French laboratory for Fractionation and Biotechnology (LFB) develops and manufactures plasma therapeutic proteins. In consistency with its public health mission, LFB's port-folio includes a large number of Coagulation factors. The majority of these are designed for the treatment of rare and very rare bleeding disorders. It is specially the case for the treatment of

severe types of von Willebrand disease (Wilfactin®, a high purity and low FVIII content von Willebrand Factor); the substitutive treatment of congenital afibrinogenemia (Clottagen®), of inherited Factor VII deficiency (Facteur VII-LFB®) and of inherited Factor XI deficiency (Hemoleven®). A large program of viral safety and protein yield optimisation is applied and updated on an on going basis leading to safe products and to the possibility of an increased availability for patients suffering from these very rare diseases outside of France.

### **(2) ZLB Behring (S. Knaub)**

Hereditary deficiency in fibrinogen (FI) and coagulation Factor XIII (FXIII) are rare bleeding disorders with a prevalence of 0.5 to 1 per million people and 200 to 300 known cases worldwide to date. These disorders are mainly characterized by moderate to severe bleeding; the bleeding events are observed soon after birth or in early childhood. Haemocomplettan P (a fibrinogen concentrate) and Fibrogammin P (a FXIII concentrate) are provided by ZLB Behring as orphan drugs to treat these rare disorders. Both products are made from human plasma. Virus safety is achieved by careful donor selection, testing of plasma donations by NAT/PCR, effective virus elimination and inactivation steps during production and by final product testing.

Haemocomplettan P and Fibrogammin P have been on the market for more than 20 years with an excellent track record of efficacy and safety as documented by data from clinical studies, published data as well as data from ZLB Behring's post- marketing surveillance.

### **(3) BPL (P. Feldman)**

BPL supplies factor VII, factor XI and antithrombin concentrates for the treatment of rare bleeding disorders. These products are manufactured and tested using the same exacting quality standards, facilities and procedures which are used for our other, licensed products. This includes additional testing by the independent WHO laboratory at NIBSC, because all BPL products are manufactured primarily to serve clinical needs in the U.K.. Surplus capacity is then supplied to serve non-UK needs, with an emergency capability to deliver within 48 hours. Even greater access to such products, and the development of new ones, requires progress to eliminate perceived constraints. This may be helped by a new product licence category for orphan drugs which would (a) accept limited patient numbers/outcomes for clinical trial; (b) accept generic validation and risk-assessment to rationalise development costs; and (c) establish a legal framework in which products may be marketed within acceptable bounds of liability.

## **General discussion: treatment of rare bleeding disorders**

A lively discussion ensued when Dr. Mannucci issued the challenge to manufacturers to create novel concentrates for other rare disorders such as factor V deficiency and to the regulators to facilitate and allow development and licensure thereof. The regulators response was positive but cautious on what even good collaboration between Europe and US authorities could accomplish. Dr. C Lee referred to the need of such products in the developing countries as well.

Manufacturers' representatives reminded to the fact that costs may be prohibitive given the complexity of doing extensive pre-licensure evaluation. The general impression was that global harmonisation of regulatory policies in dialogue with manufacturers is needed to open the way for developing and licensing products for rare bleeding disorders. Collaboration on all levels will be needed to achieve this goal.

## **Standardisation issues: assay variability and standards**

### **External Quality Assurance data: factor V assays: *E. Preston and S. Kitchen***

Dr. Preston presented data on factor V assays that demonstrated a significant interlaboratory variability. These discrepancies were caused by variability between available reference plasmas. Dr Preston strongly advocated the establishment of an International Standard for factor V in plasma.

### **International plasma standard for factor V ? *A. Hubbard***

Dr. Hubbard continued on the issue and emphasised the importance of factor V assays in connection with the diagnosis or exclusion of rare bleeding disorders such as "parahaemophilia" (FV-deficiency) or a combined deficiency of FV and FVIII. Estimation of FV is also included in the quality control testing of virus-inactivated fresh, frozen plasma. There is currently no internationally defined unit for FV and hence determinations rely either on local plasma pools or on a variety of independently calibrated commercial reference plasma preparations. This is not surprising considering the lability and wide normal range of FV in plasma. Upon Dr. Hubbard proposal, the Subcommittee was in favour of calibrating a freeze-dried pooled normal plasma preparation as the WHO 1st International Standard for Factor V in plasma, in particular if this calibration would also include the new 3<sup>rd</sup> SSC Plasma Working Standard.

Dr. Mertens closed the meeting at 12.30, thanking all presenters and participants.

## **Factor VIII and Factor IX**

**6 August 2005**

**16:00 to 19:30**

**Ballroom 2**

**Sydney Convention and Exhibition Centre**

Chairman: K. Mertens, The Netherlands

Co-Chairmen: J. C. Gill, USA; C. Lee, UK; J. Oldenburg, Germany;  
JM Saint-Remy, Belgium; A. Srivastava, India; HM van den Berg, The Netherlands

The Chairman opened the Subcommittee meeting at 16.00 for an audience of approximately 200 attendants. He explained that, due to the more condensed format of this year's meeting, not the entire portfolio of Subcommittee activities could be addressed this year. In particular the issue of novel FVIII assays could not be included this time, but the issue will return on the program in 2006.

### **Completed Reports and Recommendations**

In the past year no SSC recommendations have been published. Standardisation activities performed within the Subcommittee have resulted in two full papers in Journal of Thrombosis and Haemostasis:

- Hubbard AR and Heath AB. Standardisation of factor VIII and von Willebrand factor: calibration of the WHO 5<sup>th</sup> International Standard (02/150). JTH 2004, **2**, 1380-1384
- Raut S *et al.* A collaborative study to establish the 7<sup>th</sup> International Standard for factor VIII concentrate. JTH 2005, **3**, 119-126

### **Section 1: Factor VIII: Clinical Issues: Co-Chairs: C. Lee and H.M. van den Berg**

#### **Global PTP inhibitor surveillance study: follow-up: D.M.DiMichele**

The international need for an international PTP surveillance effort was established at the FVIII/IX Subcommittee in 2003, and the project ratified by the Subcommittee in 2004. Donna DiMichele presented a progress report on this ongoing project. In an initial effort to develop a consensus data collection tool, a questionnaire was sent in 10/04 to countries known to have national PTP inhibitor databases (UK, France and Germany) to ascertain both the nature and the scope of data collected. Similar information was also solicited from Italy and Canada in 07/05. As anticipated, data presented from the responses received to date indicate variability in both the nature and scope of ongoing or planned national data collection. A working party currently represented by the US, UK, France, Germany and Italy, but seeking all other interested national participants (contact Donna DiMichele at [dmdimich@med.cornell.edu](mailto:dmdimich@med.cornell.edu)), will begin working on the development of a consensus minimum international data set to satisfy international regulatory requirements. A funded US pilot effort through the CDC to prospectively collect PUP and PTP inhibitor prevalence/incidence data, is in the planning stages

and will incorporate the international consensus guidelines for data collection developed through this working party. A progress report is planned for 2006.

### **Meta-analysis of PUP studies of recombinant FVIII: *H.M. van den Berg***

Dr. Van den Berg, who reported also on behalf of drs Gouw and Van der Bom, addressed the question whether treatment-related factors like early age at first exposure, early start of prophylaxis and a higher intensity of treatment concurrently with tissue damage would increase the risk of inhibitor development. An individual patient data meta-analysis has been performed on four international, multicentre studies of recombinant human factor VIII products (Kogenate Ò , Kogenate FS Ò , Recombinate Ò , ReFacto Ò ) including 236 previously untreated severe haemophilia A (FVIII:C <0.02 IU/ml) patients. The outcome was clinically relevant inhibitor development, defined as at least two positive inhibitor titres combined with a decreased recovery. The analysis included the effect of ethnicity, family history of inhibitors, age at first exposure, start of regular prophylaxis, periods of intensive treatment, duration between exposure days and dosing of FVIII products on the risk of inhibitor development. The databases contained data on patient characteristics and all first fifty exposure days (dates, reason of treatment, dose, bodyweight) and inhibitor tests. However, FVIII gene mutations were not available. Sixty-seven of 236 patients (28.4%) developed clinically relevant inhibitory antibodies against factor VIII. Inhibitors developed at a median of 10 exposure days (IQR 7.5-17 days) at a mean age of 17.6 months. Forty-four were high titre (65.7%) and 23 were low titre inhibitors (34.3%). A black or hispanic ethnicity and a positive family history were positively related to inhibitor development. Dr. Van den Berg concluded that combination of age and intensity of treatment is highly associated with inhibitor development. Also peak moments, surgery and a higher dosing of FVIII were found to be statistically significant determinants of inhibitor development.

### **Prospective evaluation of a uniform factor replacement protocol in surgery: *A.Srivastava***

This report was a follow-up to the international survey done over the last 2 years to document current practices of factor replacement strategies for prophylaxis against post-operative bleeding in hemophilia. The data showed that both continuous and bolus infusion techniques were widely used with doses varying up to 5-fold between centers for similar procedures with no apparently significant difference in complications. This was so regardless of the location of the centers in developed or developing countries. The need for some standardization of practice was obvious. From the various protocols received and based of average figures of factor replacement, consensus protocols were evolved for bolus and continuous infusions and lower and higher doses. Centers were invited to comment on the proposal, choose a protocol that they felt comfortable with and join the effort at collecting prospective information on outcome and complications of surgery. Once identified, funding will be sought for data management and the study initiated over the next 6-12 months. In the discussion, the issue was raised whether this design would be compatible with the notion that individual patients may need individualised dosage. In response to this, Dr Srivastava explained that the advantage of his proposal is its prospective study design.

### **Assessment of inhibitor risk: new approach for trial design and evaluation: *M.L. Lee***

Dr Lee discussed a new analytical method to design and evaluate data from clinical studies of new Factor VIII products where the endpoint is inhibitor development. Current approaches to this problem involve the evaluation of approximately 80-100 patients followed by the calculation of a 95% confidence interval for the inhibitor rate. The upper bound of this interval is then compared with some fixed standard. The basic problem with this approach is that it requires an underlying rate in the order of 1% to succeed. Since confidence intervals are a function of sample data and not fixed quantities, fundamental analytical problems arise. Dr. Lee proposed the use of a Bayesian paradigm, whereby any and all prior information on the safety of the product (or similar products) is employed. The clinical data from a prospective study is then used to update this prior knowledge, resulting in a final calculation of a “posterior” probability that the product meets some acceptable standard. This approach is clinically intuitive and provides a very interpretable outcome from the study data. Moreover it is shown that this approach provides acceptable conclusions for currently licensed recombinant Factor VIII products, but would reject a product with known inhibitor risk (Bisinate). Details of this proposal have been recently published (Haemophilia 2005, vol 11, pp 5-12)

### **Molecular characterisation of haemophilia A patients with undetectable mutations in the FVIII gene: A. Srivastava**

Dr Srivastava presented also on behalf of Dr. Oldenburg. About 2% of patients with hemophilia A do not have detectable mutations in the FVIII gene. This could be due to inadequate sensitivity of current techniques for detecting sequence change in this gene or changes being in parts of this gene or other genes not being analyzed. Significant numbers of such cases have not been systematically analyzed. This proposal intends to initiate an international multicenter effort at identifying such cases, reviewing their clinical and hematological features, reassessing their status for mutations within the FVIII coding regions and then subjecting them to further analysis if found to be negative. This would include analysis of peripheral blood lymphocyte RNA for detection of splice defects or rearrangements. If this was negative, then haplotype analysis would be undertaken for familial cases to see if FVIII deficiency was segregating with the FVIII gene. If so, epistatic factors would need to be evaluated in these cases. For the others, one approach could be a genome wide linkage analysis to identify regions of interest (increased lod scores) followed by further analysis / sequencing of these regions. These issues will be discussed further once the participating centers are identified and resources found for this project.

### **Section 2: Standardisation Issues: Co-Chairs: J.M. Saint-Remy and K. Mertens**

#### **FVIII Collaborative Studies: Phase I field study: S. Raut**

Dr. Raut reported on a new FVIII SSC Collaborative Study. This project has been on hold for a few years, mainly due to limited improvements achieved during the successive studies. Last year the Steering Committee that oversees these activities decided that the program may be resumed provided that it will be performed in a more controlled format. As a first step the number of samples has been extended which should make some more detailed analysis possible. Four samples (3 recombinants and 1 plasma-derived) have been distributed over 31 participants. Data were received from 30 labs. Methods used were the 1-stage assay (12 labs) and the chromogenic assay (the majority). Standards included the EP/Mega-2 standard, and in-house standards

calibrated directly against the WHO standard. Dr. Raut explained that, as in previous studies, the results still were unsatisfactory, with GCVs between 14 and 19%. The data further revealed assay discrepancies, which differed for plasma-derived (1-stage > chromogenic) and recombinant (1-stage < chromogenic) FVIII. This unique data set will be further analysed in more detail. This will further provide an opportunity to analyse potency estimates based on the EP/Mega-2 standard material, which has two different assigned potencies depending on the FVIII assay method used. The extended data analysis will be reported at the meeting in 2006.

#### **ECAT study on FVIII inhibitor assays: *B. Verbruggen***

Dr. Verbruggen reported on the recently concluded ECAT study. Three samples containing respectively 0, 1.5 and about 16 u/ml FVIII inhibitors were sent to 175 laboratories worldwide, with 135 responded (77%). For the negative sample, 4 laboratories reported a positive result. For the high-titer sample, all the reported results were positive, the coefficient of variation (CV) ranged from 32% for the Nijmegen method to more than 60% for the Bethesda method. For the low-titer sample, 16/131 (8%) results were reported negative with similar CVs as for the high titer sample. Based on these results Dr. Verbruggen concluded that the specificity of the used methods is acceptable but the sensitivity of the reported method is rather low. Further standardisation of the assay method seems needed.

#### **Factor VIII inhibitor assay standardisation: *S. Raut***

Three types of reagents have been used in an attempt to standardize an assay for inhibitor detection, namely rabbit polyclonal antibodies, two different human monoclonal antibodies derived from the memory B cell repertoire of patients with inhibitors (one type 1 and one type 2 inhibitor) and human plasma. The latter was found difficult to use for ethical reasons. Results show large variations between laboratories, the less so with rabbit polyclonal antibodies. The reasons for such variation are being examined, including the source of FVIII, dilution buffers and the method actually used by laboratories to carry out the assay. Confounding factors include the possible interference due to the presence of rheumatoid factor or anti-allotypic antibodies in some of the serums. Based on these findings Dr. Raut concluded that a collaborative study would be useful to address assay standardisation. Currently a Steering Committee is being formed in order to decide on the design of the Working Party and to oversee its future activities.

#### **SSC working standard (plasma lot 3): summary of calibration: *S. Kitchen***

Calibrations of the Scientific and Standardisation Committee (SSC) Secondary Coagulation Standard Lot #3 for Factor VIII:C, von Willebrand Factor Ristocetin Cofactor Activity (VWF:RCo), von Willebrand Factor Collagen binding (VWF:CB) and von Willebrand Factor Antigen (VWF:Ag) were carried out against the h World Health Organisation (WHO) International Standard (IS) for Factor VIII/VWF and IX in plasma (02/150). A total of 19 expert laboratories in 11 countries participated in this collaborative study employing the same assay protocol but with different reagents and instruments. The overall geometric mean potencies (\* excluding 1 statistical outlier) were as follows:

- Factor VIII:C\* - 0.80 IU/vial (GCV\* - 4.7%)

- Factor IX:C - 0.94 IU/vial (GCV - 4.6%)
- VWF :RCo/Activity - 0.90 IU/vial. (GCV - 9.7%)
- VWF: Collagen binding - 1.07 IU/vial (GCV - 11.2%)
- VWF Antigen\* - 1.06 IU/vial. (GCV\* - 5.7.%)

These potencies have been accepted by the participating centres and by the SSC Working Group on Coagulation Standards..

### **Human Genome Variation Society: nomenclature recommendations: A. Goodeve**

The Human Genome Variation Society (HGVS) has devised a series of nomenclature recommendations for gene names, nucleotide and amino acid alterations over the past 10 years. Recommendations are available at <http://www.genomic.unimelb.edu.au/mdi/mutnomen/>. These detail how all sequence variation can be described using a standardised system. A number of journals and genetics EQA schemes are now specifying use of this system. Factor VIII and IX genes are denoted *F8* and *F9*. A standardised start point of the first methionine for proteins and the A of the ATG start codon for nucleotide sequence, using the cDNA sequence where possible are recommended for all genes. Dr. Goodeve mentioned that the haemophilia databases can adopt this new system, if accepted. In the discussion it was acknowledged that this proposal would indeed eliminate many inconsistencies in the genetic literature. At the same time, however, it might raise confusion in the protein literature on the FVIII and FIX proteins. In this regard Dr. Mertens referred to the dual numbering for the FIX serine protease domain (FIX and chymotrypsin numbering) which is already confusing sometimes. As such, the merit of a third amino acid numbering remains unclear.

### **Prothrombin complex labelling: R. Seitz**

Dr. Seitz presented in vitro and animal experiments demonstrating that prothrombin overload leads to enhanced thrombin generation and appears to be a major cause of thromboembolic complications during treatment with PCC. The European Pharmacopoeia considers changing the monograph on PCC to make factor II the labelled potency of PCC. Dr. Seitz asked for the input of the subcommittee concerning the following questions: Are licensed PCC products still used for haemophilia B treatment? How would the proposed change of labelling impact on the clinical use? Would there be a need to carry out new clinical studies? During the discussion Dr. Barrowcliffe noted that the prothrombin overload was mainly associated with a single PCC product that is not being used outside Germany. Along the same line, Dr. Mertens suggested that this problem should not impact on other manufacturers whose products do have equivalent FIX and prothrombin content. As PCCs continue to be used for haemophilia B treatment outside Europe and the USA, the Subcommittee does not support changing the current labelling practice.

### **Factor V: calibration of 1 st International Standard: A. Hubbard**

An international collaborative study involving 22 laboratories in 11 countries has been undertaken to calibrate the Proposed WHO 1st IS FV Plasma for FV clotting activity. Each laboratory estimated FV:C in the candidate preparation relative to a local normal pooled plasma which was arbitrarily assigned a value of 1.0 unit per ml. Most laboratories (21/23) used

thromboplastin-based methods rather than APTT-based methods. Estimates relative to the fresh local pools (mean 0.74 IU/ml) were significantly lower than estimates relative to the frozen local pools (mean 0.80 IU/ml) and this could be an indication that some FV:C activity has been lost during freeze-thawing of the frozen pools. It is therefore proposed that the calibration should be based only on the estimates relative to the fresh local pools with a mean value of 0.74 IU/ampoule and inter-laboratory variability (GCV) of 7.6%. Estimation of FV:C in a second freeze-dried plasma (SSC/ISTH Secondary Coagulation Standard) relative to the Proposed WHO 1st IS demonstrated low inter-laboratory variability (GCV 3.5%, n=23); this indicates that the Proposed WHO 1st IS should lead to improved harmonization between laboratories. Estimates of stability based on an accelerated degradation study have predicted a loss of less than 0.01 % per year at -20 oC. All of the participating laboratories have agreed to the proposal to calibrate the WHO 1st IS with a value of 0.74 IU/ampoule. Prior to the meeting, the Chairman had distributed the report among Subcommittee members for a vote per email. This has resulted in acceptance of Dr. Hubbard's proposal by the Subcommittee.

### **Section 3: Rare Bleeding Disorders: Co-Chairs: *A. Srivastava and K. Mertens***

#### **SSC Working Party on Rare Inherited Bleeding Disorders: *F.Peyvandi***

Dr. Peyvandi reported that the main goals of this official SSC-endorsed Working Party are two-fold. The first goal addresses database development, which should serve to (a) identify current national and international databases, (b) identify potential collaborators worldwide, particularly in areas where RBDs could collect data, (c) finalize database tools and data collection protocols and disseminate worldwide and (d) establish a Steering Committee for data evaluation. The second goal relates to product development and licensure. This involves the following step: (a) to finalize common regulatory requirements within FDA/EMEA, (b) to identify already available products (FVII, FXI, FXIII, fibrinogen), (c) to design completed clinical trial in order to get global licensure, (d) to explore new product development (FV) and (e) to design implement post-licensure surveillance. So far an International Database on RBDs (RBDD, [www.rbdd.org](http://www.rbdd.org)) has been developed, with the aim of efficiently collecting and extracting available data. Dr Payvandi has contacted National and International Organizations and Treatment Centres registered in the WFH mailing list with the aim to learn how many Centres would like to participate and what type of intervention needs to be done in each region of the world; so far 22 centres have already responded.

#### **Update MASAC Working Party on US situation: *D.M. DiMichele***

This update was presented by Donna DiMichele on behalf on the Working Party on RBDs of the Medical and Scientific Advisory Council of the NHF, chaired by Amy Shapiro. The report focused on efforts planned by this group in the aftermath of the FDA Workshop on Rare Plasma Protein Disorders held June 13-14, 2005 in accordance with the priorities established by both this meeting and by the FVIII/IX Subcommittee Working Party on RBDs. The efforts planned include:

- National data collection on RBDs (molecular, laboratory, and clinical) through the NATIONAL HEMOPHILIA DATABASE consortium currently being established. The

database will be developed and data collection supervised by a subcommittee of vested parties. The aims of this data collection include a) contribution to the International Data Collection effort; and b) database for industry and investigator-initiated clinical trials for designated product licensure.

- Ongoing dialogue with the FDA to a) pursue FDA/EMA harmonization of regulatory requirements and explore novel clinical trial design paradigms in order to facilitate product licensure for rare disorders; and b) facilitate insurance reimbursement for personal importation and/or off label use of products pending licensure application

### **Treatment of rare bleeding disorders in Europe; the French organisation: *J. Goudemand***

Dr. Goudemand reported that patients affected with rare bleeding disorders in France are included in a national project: FranceCoag Network. This is a prospective multicenter national cohort of patients affected with severe and hereditary haemorrhagic disorders. FranceCoag network is funded by the French health Ministry and coordinated by a public health institution: INVS (Institut National de Veille Sanitaire). Beside the epidemiologic objectives, the aim is to set up a surveillance system able to investigate any unexpected events occurring in this population. Inclusion criteria are defect (<30%) in FVIII or IX, severe defects (<10%) in FII, V, VII, X, XI, XIII, afibrinogenemia, severe VWD. At that time 4049 patients registered in 37 French centres have been included in the project and 3439 record forms analyzed. There are 3103 patients (90%) with haemophilia, 239 (7%) with severe VWD and 97 (3%) with other rare bleeding disorders. Clinical and biological data are collected as part of the regular follow up (main bleeding episodes, surgeries, treatments, coagulation tests...) and monitored by the coordinating centre. The project (<http://www.francecoag.org>) is opened to any interested researcher with the agreement of the steering committee. Regarding the treatment, several specific concentrates (VWF, FVII, FXI, FXIII, Fibrinogen, PCC) are available in France to treat these patients.

### **Complications of management in rare bleeding disorders: *U. Seligsohn***

Dr. Seligsohn presented an interesting report on the management of bleeding with minimum usage of coagulation factor products. Plasma, and plasma derived or recombinant factor concentrates are used for management of patients with deficiencies of factors I, II, V, VII, X, XI or XIII during bleeding episodes or prophylaxis during surgery. Major complications include transmission of infections agents, development of inhibitors and thrombosis. The paradigm of severe factor XI deficiency was used to evaluate by retrospective analyses whether treatment by blood components during surgery and labor can be avoided or tailored. For tooth extractions, only tranexamic acid was necessary to prevent bleeding in 19 patients. A relatively low frequency of bleeding in 62 untreated women during vaginal (24%) or caesarian deliveries (17%) advocates an on-demand policy of replacement therapy. A similar policy is recommended in patients undergoing surgery at tissues with no fibrinolytic activity because only 8/121 (6.6%) of such procedures were accompanied by bleeding, compared to 29/48 (60.4%) during procedures at fibrinolytic sites. Retrospective analyses of patients with other deficiency states are warranted to minimize the deleterious effects of blood components.

### **Profile of rare bleeding disorders in India: *A. Srivastava***

With a population of about 1.1 billion people and significant practice of consanguineous marriages in major parts of the country, India has potentially large number of patients (250-1000) with the rare bleeding disorders. This presentation described the clinical and hematological features and the molecular genetics of 27 patients with fibrinogen, 7 patients with prothrombin, 20 patients with factor V, 25 patients with factor VII, 25 patients with factors V and FVIII, 19 patients with factor X, 3 patients with factor XI deficiency and 59 patients with factor XIII defects / deficiency. There were significant differences in the clinical features noted in this study when compared to the data in the literature. The cause for this was not clear. A wide variety of mutations, including many novel ones, were detected among patients with prothrombin, factors VII, X, XI and XIII deficiencies. Further characterization of these cases for better understanding their biology and management is progressing. A collaboration is being established with Dr. Peyvandi's laboratory.

**Report on FDA workshop “Biological therapeutics for rare plasma protein disorders”: M. Weinstein**

FDA and the Office of Public Health and Science (OPHS) sponsored a workshop entitled, "Biological Therapeutics for Rare Plasma Protein Disorders", in Bethesda, MD on June 13, 14. The focus of the workshop was to facilitate the development of products to treat patients with very rare plasma protein disorders – affected cohorts on the order of 10's or 100's in the US. Presentations from the international, patient, physician, and industry perspectives were made on the need for these products, and challenges to their development. We reviewed opportunities and incentives to foster development that are currently available in the US and Europe. These include regulatory pathways to license products with limited clinical data, orphan drug provisions and incentives, small business and research grant support, and the medicare payment program. Case studies of protein C, factor XIII, antithrombin III, and treatment of Glanzmann's thrombasthenia and Fabry's disease were presented as examples of product development for very small patient populations. Several potential opportunities for enhancing product development were identified. These included:

- Expanding data bases and registries to identify patients for clinical trials and to help understand the natural history of the diseases
- Consider adopting alternate and/or internationally harmonized regulatory pathways
- Improving investment analyses for industry
- Reviewing regulatory options for product development for rare plasma protein disorders in one to one meetings of sponsors with FDA.
- Important communication pathways were established among the regulatory agencies, industry, and stakeholders.

Slide presentations from the meeting are at <http://www.fda.gov/cber/summaries.htm>. When available, transcripts of the workshop will be at <http://www.fda.gov/cber/minutes/workshop-min.htm>. A docket is being prepared for comments and suggestions for further product development.

The Chairman closed the meeting at 20.00, thanking the speakers and the audience for their contribution.

## Factor VIII and Factor IX

Chair: K. Mertens, The Netherlands

Co-chairs: J.C. Gill, USA; C. Lee, UK; J. Oldenburg, Germany; F. Peyvandi, Italy; J.M. Saint-Remy, Belgium; A. Srivastava, India; H.M. van den Berg, The Netherlands

The Chairman opened the Subcommittee meeting at 14.15 for an audience of approximately 170 attendants. He announced a few amendments to the final program and provided the timing details of the various sections in the agenda.

### Section 1: FACTOR VIII CLINICAL ISSUES

*Co-chairs: J.M. Saint-Remy and H.M. van den Berg*

#### **Phenotypic heterogeneity in severe haemophilia A and B: A. Srivastava**

Though it has been recognized that 10-15% of patients with severe haemophilia have clinically mild disease in terms of frequency of bleeds and extent of arthropathy, the basis for this phenomenon has not been fully understood. We hypothesized that the levels of various pro- and anti-coagulant factors and functional polymorphisms in the coagulation protein genes as well as genes of cytokines involved in the inflammatory response may modulate the development of hemophilic arthropathy. A total of 114 minimally treated patients (50-150 IU/kg/year) with hemophilia A (n=94) and hemophilia B (n=19) diagnosed were evaluated as outlined above. They were categorized as 'mild' (<1 affected joint and < 5 bleeds in the preceding year, n=15) or 'severe' (>1 affected joint and >5 bleeds, n=99). Early data suggests that polymorphisms in TNF $\alpha$ -308 AA/AG (pro-inflammatory) (RR-3.4, p=0.037, 95% CI, 1.07-10.7), TGF $\beta$  Codon 10 CC/CT (pro-inflammatory) (RR-2.8, p=0.07, 95% CI, 0.91-8.3) are associated with clinically severe disease while MDM2 GG (RR-0.3, p=0.038, 95% CI, 0.1-0.93) tended to ameliorate severity. These data suggest that the clinical phenotype of severe haemophilia could be influenced by interactions between a variety of hemostatic factors as well as some inflammatory response proteins.

#### **Factor VIII inhibitors and continuous infusion: I. Scharrer**

Dr. Scharrer presented data also on behalf of Drs. v. Auer and Oldenburg and the German Hemophilia Society University Hospitals Mainz and Bonn. They have conducted a retrospective study to investigate the development of inhibitors after continuous infusion of F VIII in Germany. 42 hemophilia centers were questioned. 19 of these conducted 200 continuous infusions in 128 patients. 14 patients developed inhibitors (10 HR, 4 LR). 5 of these patients were suffering from severe, 1 from moderate and 8 from mild hemophilia (age between 7 months and 73 years, 11 PTT's, 3 PUP's). The infused amount ranged between 4300 and > 100 000 U before inhibitor development. Exposure days ranged from 4 to > 100. Regarding the genotype only 2 intron -22-inversions have been found. In Dr. Scharrer's center no inhibitors had been found in 81 patients with major orthopedic surgery and bolus infusions compared to 2 inhibitors in 8 patients with similar surgery. In conclusion the inhibitors developed very often in mild hemophiliacs without the typical gene mutations for inhibitor development.

#### **Feedback from EMEA Workshop on Inhibitors: R. Seitz**

Prof. Seitz reported that in a CHMP class review of recombinant FVIII products and inhibitor

development the data received were heterogeneous and not comparable between products. An expert meeting was convened at the EMEA in London on 28.02. – 02.03.2006. He presented a brief feedback on this workshop; a detailed report is currently prepared. A letter has been sent to the Chair of the FVIII&IX Subcommittee, Prof. Mertens. CHMP notes the ISTH SSC sub-committee on FVIII and FIX initiatives regarding the improvement of the FVIII inhibitor assay and the establishment of the global PTP inhibitor surveillance. These will be complementary to CHMP's ongoing work in revising guidelines on clinical investigation of plasma-derived and recombinant FVIII products. CHMP would appreciate to be informed about the outcome of the ISTH SSC sub-committee on FVIII and FIX discussions on the above topics. In the discussion dr. Mertens indicated that several of the issues are already ongoing actions of the Subcommittee. He proposed that Working Parties could be initiated to address these issues whenever appropriate.

#### **Global PTP inhibitor surveillance study: update : *D.M. DiMichele***

The impetus for this project was derived from regulatory issues identified by the FDA and EMEA associated with potential immunogenicity of new FVIII products studied in pre-licensure PTP clinical trials. The need for harmonized international data collection on the natural history of inhibitors in PTP populations was reiterated at both the FDA (11/03) and EMEA (2/06) workshops, although no consensus definition of the PTP yet exists. International guidelines for PMS studies were reviewed as were the pros and cons of conducting this project through the ISTH FVIII/IX subcommittee. The history of this project in this committee was reviewed. Within this context, this report focused on the feasibility of national PTP database harmonization with respect to a data collection tool. A preliminary effort to do this by two established databases in the US and UK was reported on behalf of its contributors (CRM Hay, chair of the UKHCDO in the UK and Dr. Mike Soucie and the CDC in the US). Dr. DiMichele concluded that based on this effort, such harmonized data collection was feasible. This harmonization project between the US and UK will be further pursued. Furthermore, the proposed schema for collaborative data collection will be shared with other countries planning or engaged in such prospective data collection. Finally, as per the formal request to this subcommittee by the EMEA, the Chair will request approval for this project to proceed a sanctioned FVIII/ IX Subcommittee activity and a formal Working Party will be established to continue this work on an international scale.

#### **Treatment-related inhibitors in PUPs with severe hemophilia: Rodin study : *H.M. van den Berg***

The Rodin study has been developed to determine treatment related risk factors in Pups with severe hemophilia A. The Rodin study will be performed as a satellite study of the Pednet registry and it has been extended to the Rodin study group which consist of other major hemophilia centres from Europe, Israel and Canada which do not participate in the PedNet/Rodin study. The study started to include data of full cohorts of patients with severe hemophilia A born from 1-1-2000. The aim is to collect upto 1-1- 2008 a total of 400 patients in the Pednet/Rodin study and another 400 patients through the Rodin study group. Data will be collected on all first 75 exposure days, which will include every reason for treatment, the dosage, peak treatment, surgery. Besides extensive data on patients characteristics. Further information can be obtained from the websites; [www.pednet.nl](http://www.pednet.nl) and [www.rodinstudy.nl](http://www.rodinstudy.nl).

### **Low-titre inhibitors in Canal study and Recombinant studies : *E. Santagostino***

Dr. Santagostino indicated that our knowledge on the natural history of low-titer inhibitors is mainly based on the data provided by the studies carried out in PUPs with hemophilia A treated with recombinant FVIII. Overall, almost half of the inhibitors detected during these studies showed titers of 5BU/mL or less and remained below this level although FVIII exposure was repeated. More than two third of these low-titer inhibitors were transient and disappeared spontaneously continuing FVIII treatment, while immunetolerance induction was started in the remaining cases. Transient inhibitors have also been reported in hemophiliacs with titers always maintained between 5 and 10 BU/mL. Transient inhibitors may represent a model for understanding the mechanisms of immunetolerance so that immunological studies in this specific setting should be implemented. Many aspects concerning the clinical management of hemophiliacs with low-titer inhibitors are still empirical because few data are available on the behaviour of inhibitor titers, FVIII recoveries and half-lives over time. In particular, the need for immune tolerance induction treatment remains controversial and should be better defined.

### **New methods for the assessment of FVIII inhibitors : *J.M. Saint-Remy***

Factor VIII immunogenicity is not limited to the detection of inhibitors but its evaluation should take into account all antibodies formed towards FVIII. Current ELISAs can easily detect FVIII antibodies but the use of such assays is far too limited. Identifying the precise epitopes recognized by anti-FVIII antibodies is important to understand the reasons/mechanisms by which such antibodies are formed. It could in addition provide some insight into possible new therapies for inhibitors. The combined transcription-translation method using a library of FVIII fragments is well suited for this purpose. The immune memory compartment of the anti-FVIII immune response can also be evaluated at both B and T cell levels. Methods such as the ELISPOT can be used on a routine basis to count the number of memory B and T cells and thereby evaluate the risk of producing an inhibitor. Such methods are also appropriate to compare the efficacy of therapies aiming at the eradication of inhibitor antibodies.

## **Section 2: FACTOR VIII ASSAYS AND STANDARDISATION ISSUES**

*Co-chairs: A. Srivastava, K. Mertens*

### **Factor VIII inhibitor assays: technical issues: *B. Verbruggen***

FVIII inhibitor tests still are assays that show a very high interlaboratory variation in international survey programs despite recent described modifications. There are a number of variables that influence test outcome: pH stability in incubation mixtures, type of control sample, type of used deficient plasma, liquid handling, incubation time, FVIII content of normal pool plasma, type of Factor VIII assay / type of reagent. It is advised to use a FVIII deficient plasma that contains von Willebrand factor as substrate plasma in the assay of residual factor VIII assay in the test and control mixture. With respect to the assay of type II inhibitors the following items are important:

1. Dilutions with at least 50 % residual activity have to be analysed
2. In monitoring type II inhibitors dilutions that give identical residual activity have to be used

To increase sensitivity of inhibitor detection we have developed a FVIII inhibitor test that is ca. 20 times more sensitive than the Nijmegen-Bethesda assay. The test is independent of residual FVIII in the test sample and has a cut-off value of at least 0,04 NBU/ml, but probably better. There is a strong correlation between a decreased half life of infused FVIII and a low inhibitor titre and low titre inhibitors may therefore be of clinical importance. The putative clinical significance of low-titre inhibitors has to be validated in a bigger cohort of patients.

#### **Collaborative Study on 1<sup>st</sup> International Standard for FVIII inhibitors : S. Raut**

The international need for a reference FVIII inhibitor standard was established at the FVIII/IX Subcommittee in 2001, and the project ratified by the Subcommittee in 2003. Sanj Raut presented a progress report on this ongoing project. The aim of this study was to develop a definitive WHO International Reference Preparation for measurement of FVIII Inhibitors in plasma. 5 candidate preparations (rabbit FVIII PAb, 2 humanised MAbs I & II, human low (X) and high (Y) titre inhibitor plasmas) were assessed against 2 human inhibitor patient plasmas. Inhibitor methods and the FVIII assays were also assessed. Samples were distributed to 25 expert laboratories. Data were received from 21 labs. For all samples, results without a reference standard gave CVs between 17-34%. For the overall mean inhibitor titres, comparisons of the Nijmegen vs Bethesda assays, and comparisons of one-stage vs chromogenic methods, the candidate with lowest CVs was preparation Y. On assessing the patients' inhibitor plasma relative to the 5 candidate preparation, once again preparation Y gave the lowest CVs of 17 & 18%. It was interesting that no marked improving in CVs were observed for the Nijmegen method compared to the Bethesda method, but a marked improvement in the CVs was observed for the chromogenic assay compared to the one-stage assay. Overall preparation Y (05/206) was the most suitable candidate material for the proposed 1<sup>st</sup> IS FVIII Inhibitor Plasma standard with an overall Bethesda Titre of 8.2 BU/vial and an overall GCV of 17.5%. Preliminary stability studies show a predicted loss of inhibitor titre potency of < 0.01% per year at

#### **Collaborative Study SSC 8: evaluation of result: M. Lee**

This presentation was intended to provide a basic statistical analysis of the results obtained from the most recent worldwide collaborative study of Factor VIII assays sponsored by the ISTH Factor VIII/IX subcommittee. In this study, four different Factor VIII preparations (three recombinant and one plasma-derived) were evaluated by 31 laboratories using their local assay (primarily chromogenic or one-stage) and pre-diluent (deficient plasma or buffer). Using analysis of variance techniques, buffer pre-diluent typically provides lower potencies for recombinant preparations, but the reverse is true for the plasma-derived material. The chromogenic assay gives a significantly higher result for full-chain recombinant Factor VIII products, but not for the B-domain deleted preparation. The combination of pre-diluent and assay is an important source of variation, particularly for B-domain deleted Factor VIII. For the plasma-derived product only the assay method really matters. This information has been used to help design the next collaborative study, SSC9, where the components of variation for the Factor VIII assay will be examined in greater detail.

#### **Collaborative Study SSC 9: preliminary report: S. Raut**

Dr. Raut reported on the Phase II SSC9 "Controlled" study following on from the Phase I (SSC8) "Field" study carried and reported last year. The aim of this study was to investigate the differences and variability observed in the Phase I study and further identify any potential

sources of variability, by following strict protocol instructions. Furthermore, it tested the ISTH/SSC recommendation by studying the chromogenic method and pre-diluting in FVIII deficient plasma (containing normal levels of VWF) provided. The effect of different operators and different day set-ups were also assessed. The same 4 samples used in the SSC8 study (3 recombinants and 1 plasma-derived) were distributed to 25 participants. Data were received from 21 labs. The results obtained gave a marked improvement in inter-lab variability with GCVs between 4-11% (compared to 14-19% in the SSC8 study). For the 3 recombinant concentrates both the chromogenic method and predilution in FVIII deficient plasma containing normal levels of VWF were particularly important. For the plasma concentrate, predilution in FVIII deficient plasma was not as important. Variability appeared not to be influenced by different operators, assays on different days or by local standards. This unique data set will be further analysed in more detail (components of variation analysis) by Martin Lee and will be reported in 2007. In the discussion Dr. Mertens noted that the results were much better than in previous studies, where other methods than the SSC recommended method were also included.

#### **Genetic reference materials for Haemophilia A: A. Hubbard**

On behalf of Dr. E. Gray, Dr. Hubbard announced a study that will involve reference materials for the detection of the intron 22 inversion. DNA samples are being prepared, and include an affected male, a carrier female, and the appropriate normal controls. The study will start in November 2006. Those who are interested should contact Dr. Gray (egray@nibsc.ac.uk).

#### **Calibrated measurement of thrombin generation in hemophilia: P. Giesen**

Dr. Giesen (Thrombinoscope, The Netherlands) reported that with the calibrated automated thrombogram™ (CAT) plasma is divided in two samples, in one sample thrombin generation is triggered and to the other sample a calibrated amount of thrombin calibrator is added. Both samples receive fluorogenic substrate and the signal is followed in a 96-well plate fluorometer. This allows proper calculation of thrombin in time such that the measured signal is corrected for donor-to-donor differences in plasma color as well as for non-linearities of the signal. It is shown that the same amount of factor VIII in different donors gives substantial different thrombin generation. At the same time a perfect relationship exists between factor VIII concentration and thrombin generation measured in one plasma. Therefore individual calibration in combination with individual measurement of factor VIII are the basis for efficient monitoring of factor VIII treatment.

#### **Thrombin Generation Assay: Application for measuring the haemostatic efficacy of FVIII: K. Varadi**

Dr. Varadi presented assay principles and technical details of a thrombin generation assay (TGA) have been presented. Thrombin generation is triggered by tissue factor-phospholipid complex (TF-PL) and CaCl<sub>2</sub> in the presence of a fluorogenic thrombin substrate. The changes in fluorescence signal are converted to thrombin concentrations using a reference curve, where thrombin is added instead of plasma. The TGA is sensitive to factor VIII levels even below the detection limit of factor activity assays, demonstrated by *in vitro* spiking experiments and in *ex vivo* samples obtained after FVIII administration. The ability to measure the haemostatic effect of factor VIII down to 0.002 U/ml may help to predict the possible risk of spontaneous bleeding episodes and to tailor the prophylactic therapy. Individual plasma calibration could be used in the assay, however it is not essential, and therefore the TGA is easily applicable to any fluorescence

reader having the appropriate excitation/emission filters for the AMC-fluorophore. The assay is commercially available from Technoclone ( Vienna , Austria ).

### **Thrombin generation in hemophilia: proposal for collaborative study: *E. Gray***

Dr. Gray presented a brief overview of the collaborative study on thrombin generation as performed in the Subcommittee on plasma coagulation inhibitors (see minutes of that Subcommittee for summary). The next stage could be to prepare a reference plasma in order to assist in reducing interlaboratory variation. In the next collaborative study Dr. Gray is willing to include plasma samples of thrombophilia and haemophilia, the latter in collaboration with the FVIII&IX subcommittee. In the discussion several attendees stressed the potential of thrombin generation for haemophilia. The view was expressed that substitution therapy might be more appropriately expressed in terms of thrombin generation than in factor VIII levels, and that the Subcommittee should focus on this point.

### **General discussion: future directions**

In the general discussion Dr. Mertens raised the issue how to proceed from here. There was consensus within the audience that a Working Party should be established within the FVIII&IX Subcommittee to focus specifically on haemophilia and low factor levels. This group should work in close collaboration with Dr. Gray in order to prevent any overlap with activities ongoing elsewhere. Dr. Ingerslev suggested that it might be appropriate to also include methods based on thromboelastography in this Working Party.

## **Section 3: FACTOR IX**

*Co-chairs: J. Oldenburg, K. Mertens*

### **Factor IX gene transfer: current status : *A. Nathwani***

Dr. Nathwani reported also on behalf of Drs. Tuddenham, Pasi, Kay, Nienhuis and Davidoff on new technology that may raise new hope for gene therapy of haemophilia A. They proposed a distinct approach to gene therapy of haemophilia B which addresses many of the limitations of previous gene therapy trials. Firstly vectors based on adeno-associated virus (AAV) serotype 8 will be used because of the lower prevalence of pre-existing humoral immunity to this virus in humans. Secondly the genome in our vector will have a self complementary ( scAAV2/8-LP1-hFIXco ) format to enable efficient therapeutic gene transfer with lower titres of vector. Finally, scAAV particles will be administered into a peripheral vein, which is safer and more convenient for patients with a bleeding diathesis. In nonhuman primates their approach has consistently resulted in mean stable plasma human FIX levels of 26% of normal without any toxicity even in animals with pre-existing immunity to AAV. The plan is to establish the safety and efficacy of peripheral vein administration of scAAV2/8-LP1-hFIXco in adults with severe HB. The protocol has received regulatory approval in the UK with plans to commence recruitment in 2007. Further details of this approach were presented and discussed.

### **Factor IX inhibitors: update *J. Lusher***

Dr. Jeanne Lusher gave a brief, near final report on Dr. Warrier's registry concerning FIX inhibitors accompanied by anaphylaxis (or severe allergic reactions) in persons with severe hemophilia B. Fifty-six subjects have been reported (29 from U.S. and 27 from other countries), and a few more were verbally reported to Dr. Warrier at the Vancouver meeting in May 2006,

with the promise that registry forms would be completed. The median number of exposure days was 11; med. age at inhibitor detection 19.5 mos. Among those genotyped, null mutations appear to confer a significantly increased risk. Of those with inhibitors to FIX, roughly one-half also had one or more severe allergic reactions. Of those who were desensitized to FIX and then put on an ITI regimen, success was quite poor (12%), and 1/3 developed nephrotic syndrome within 8-9 months after ITI regimen was begun.

**Factor IX standardisation; replacement of 3rd International Standard: *E. Gray***

Dr. Gray mentioned that the 3rd international standard needs replacement. This will be done in parallel with the EP working standard replacement. Manufacturers are invited to submit materials for the standard, and participants who are willing to perform the calibration study should contact Dr. Gray (egray@nibsc.ac.uk).

**Section 4: ISTH-SSC Working Party on RARE BLEEDING DISORDERS (RBDs)**

**Chair: *F. Peyvandi***

**Co-chair: *C. Lee***

**Overview of ongoing activity of working group on RBDs; *F. Peyvandi***

In 2004, a SSC working group on "Rare Bleeding Disorders" was established. Two years later, 58 centres from all over the world joined the International Database of Rare Bleeding Disorder (RBDD) by including data on 2665 patients. Preliminary information on distribution of affected patients in the world and the available treatment for them are reported at [www.rbdd.org](http://www.rbdd.org).

The next goal of this working group will be to prepare a specific data collection tool which could be used as a homogeneous questionnaire by a majority of the countries around the world. This will contain the most important clinical and therapeutic data useful to answer the missing information that remains unanswered in the field of RBDs. In addition, specific subcommittees will be organized with the aim to prepare evidence-based guidelines using data collected in either RBDD or other available National Registries.

**RBDs in the USA ( US working group on RBDs): *A. Shapiro*:**

The rare bleeding disorder group in the United States will be developing a resource center hosted on-line by the National Hemophilia Foundation that will contain information on many rare plasma protein deficiencies. Links to existing websites, registries, and organizations related to these disorders will be included. The material developed for this resource center will be submitted for publication. In addition, the development of a national database in the United States is presently underway and a uniform platform has been adopted. Development of a database for rare bleeding disorders will be included in this national database effort. The rare bleeding disorder group in the US wishes to collaborate on an international level to further both development of international data collection and research efforts, including expansion of treatment options.

**Glanzmann thrombasthenia: World distribution, mutations and founder effects : *U. Seligsohn***

A preliminary survey was performed in order to estimate the minimal prevalence of the disease in different populations, the mutations so far detected and founder effects. Data were extracted

from reports involving <sup>3</sup> 9 unrelated patients, national and reference center registries, a database of mutations (by Debra French and Alan Nurden) or personal communications. The minimal prevalence ranged from 1:80,000 in Jordanian, 1:143,000 in Israelis and Palestinians to 1:2,800,000 in Indians, with an average global prevalence of 1:1.1x10<sup>6</sup>. As of May 2006, 159 mutations have been reported in peer-reviewed journals, 69 in b 3 and 90 in a IIb. 133 were identified in individual patients and 26 in 2 or more unrelated individuals. For 7 of the 26 mutations, a founder effect was discerned by haplotype analysis: 2 in Iraqi Jews, 1 in Palestinians, 1 in Jordanians, 2 in Indians and 1 in Manouche Gypsies in France . This preliminary survey is a rough estimate of the global problem related to this severe hemostatic abnormality, but is somewhat limited because of lack of data from China , Africa , Latin America , Eastern Europe and densely populated countries like Pakistan , Bangladesh , etc.

### **Menorrhagia in women affected by bleeding disorders- Proposal for an International study: *F. Peyvandi***

A significant number of women affected by blood coagulation diseases present with complications such as menorrhagia and post-partum hemorrhage. Previous studies on this issue are not very informative on this subject due to the heterogeneous group of patients enrolled and the different methodologies used to analyze the data. We therefore propose a multicentre prospective study which enroll a large number of female patients affected by vWD, rare bleeding disorders and carriers of hemophilia. Based on a specific questionnaire designed to collect clinical data in a homogeneous format. In future it could be available online ([www.rbdd.org](http://www.rbdd.org)) for the participating centers. The results obtained will be examined by a scientific committee and then published on the space assigned to on-line studies within the URL: [www.rbdd.org](http://www.rbdd.org). The results of this project will provide information on the incidence of bleeding complications and to assess whether hormone therapy, anti-fibrinolytic and replacement therapy could have beneficial effects on bleeding symptoms and on quality of life of these patients.

### **Long term prophylaxis in afibrinogenemic patients: a rational based on results from single dose PK; *T. Waegemans***

FIBRINOGENE T-I (LFB) is a new concentrate of human plasma fibrinogen. It is derived from cryoprecipitate and its manufacturing process includes three major biological safety steps. A single dose PK study performed in 5 afibrinogenemic patients showed homogeneous results within a one-compartment pharmacokinetic model with IV infusion and first order elimination. This model was applied to simulate PK profiles at steady-state after repeated infusions. A selection of targeted parameters will allow investigators to determine individualized dosage regimens before initiating a long term prophylaxis in afibrinogenemic patients. A design of PK study will be presented, aiming at comparing the values predicted by the simulation with those observed during the prospective follow-up of patients.

### **Getting product to patients: Industry Options: *J. Lloyd*.**

2 years ago, BPL identified market access, regulatory requirement and clinical trial design as constraints to manufacturing products for RBD. Over the past two years there have been some advances made. Registries and databases of rare bleeding disorders have made it easier to establish patient numbers and treatment regimes to support orphan drug designation. Further

development and support of these registries is required to aid industry in deciding whether to develop a product. Orphan drug designation and the parallel scientific advice available from the FDA and EMEA have potentially reduced the need for multiple dossiers and trials. Clinical trial design and support remains an issue, which is overcome by protocol assistance from regulators. There are still some major hurdles for industry to overcome. Further ongoing cooperation between regulatory agencies is required. The maintenance of parallel scientific advice provided by the EMEA and FDA is vital

### **General discussion and concluding remarks**

In the discussion Dr. Peyvandi raised the issue how to proceed with the Rare Bleeding Disorders now her outside funding for this project expires. The Chairman indicated that SSC activities in general would benefit from some level of funding by ISTH. This particularly includes Working Groups or Working Parties that have formally been endorsed by SSC. This issue was discussed in more detail in the audience. It was believed that ISTH, by virtue of its established authority, could drive related national or regional projects under the same umbrella. The general feeling was that ISTH could show leadership in providing funding for those SSC activities that are considered to have appropriate priority. The Chairman mentioned that this issue has been brought to the attention of the SSC Chairman. He concluded the meeting thanking all speakers for their presentations and the audience for attending and contributing to the discussions.

## Factor VIII and IX

Chair: A. Srivastava (India)

Co-Chairs: C. Hay (UK), C. Lee (UK), K. Mertens (The Netherlands), C. Negrier (France), F. Peyvandi (Italy), J. Saint-Remy (Belgium), E. Tuddenham (UK), H. van Den Berg (The Netherlands)

The Chair opened the meeting at 15:45 and welcomed the audience of about 200 attendants. He confirmed that there were no modifications in the agenda and mentioned that all topics of interest could not be included this year due to lack of time.

Completed/Submitted reports and recommendations

In the last year, SSC activities resulted in the following publication in the Journal of Thrombosis and Haemostasis:

- Calibration of the WHO 1<sup>st</sup> International Standard for blood coagulation factor V in plasma, human (03/116). A.R. Hubbard, L.J. Weller, S. Johnes on behalf of the SSC subcommittee on FVIII and FIX of the ISTH. 2007; 5:1318-19.

### **Section 1. SSC Working group on Rare Bleeding Disorders. Chair – F. Peyvandi Co-Chair – C. Negrier.**

Since its inception in 2004 within the FVIII/IX subcommittee, this SSC working group on "Rare Bleeding Disorders" (RBDs) has attempted to improve our understanding of prevalence, diagnosis and treatments of these diseases by developing the Rare Bleeding Disorders database ([www.rbdd.org](http://www.rbdd.org)). Some international data is also available from an annual survey conducted by the World Federation of Hemophilia (WFH) ([www.wfh.org](http://www.wfh.org)). Various national registries have also begun collecting epidemiological data on these conditions (Swiss, North American, United Kingdom, French, Egyptian and other EMBRO countries, Iranian and Indian).

### **RBD in North America – M. Soucie / A. Shapiro**

Dr. Soucie provided information about rare bleeding disorders in the U.S. This data is collected using a public health surveillance system called the Universal Data Collection (UDC) system. The UDC has a national, IRB-approved protocol and collects standardized clinical data and a blood specimen that is centrally tested for transfusion transmitted viruses. Patients in any of 135 federally funded comprehensive care centers with a factor deficiency (<50% of normal) or VWD are included, with informed consent. Data collected include demographic, clinical and treatment information, and a self-assessed quality of life tool. Since May, 1998, over 20,000 patients have been enrolled. The distribution of disorders among enrolled patients is 59% hemophilia A, 16% hemophilia B, 22% VWD, and 3% other factor deficiencies. Dr. Shapiro described the efforts that are also underway to establish a Rare Coagulation Disorders Resource Room, a project of the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation (NHF). This web-based Resource Room will be devoted to a variety of rare blood disorders, each

having a separate manuscript covering clinical, laboratory and genetic aspects authored by an expert.

### **RBD in South America – E. D’Amico:**

Dr. D’Amico presented the South American data. Comprising of 12 countries (total population ~390,000,000) ranging from 458,000 to 191,790,900 inhabitants / country, there is very little published data on RBDs from these countries. Dr. D’Amico had conducted a survey among some of the leading centers in these countries and was able to get data from Brazil, Columbia, Panama, Peru (regional) and Venezuela (national). The reported numbers show wide variability in prevalence of these conditions as documented so far. In all this data, shows the presence of 59 fibrinogen, 62 prothrombin, 41 FV, 132 FVII, 78 FX, 164 FXI, 20 FXIII and 25 combined FV/VIII deficiency patients. The most frequent RBDs are FVII and FXI deficiency. There is limited access to laboratory diagnosis of these conditions. A wide range of products ranging from fresh frozen plasma to factor concentrates are used for treatment, though access is variable depending on the country.

### **RBD - The WFH survey 2006 – P. Bolton-Maggs**

Dr. Bolton-Maggs mentioned that the World Federation of Hemophilia (WFH) had been collecting epidemiological data on hemophilia and other bleeding disorders since 1998 from its national member organizations, which now are over 100 and represent >85% of the world’s population. 56 out of the 101 countries that provide data have some form registry. From 2004, countries have been asked to provide information about the rare bleeding disorders in addition to hemophilia and von Willebrand disease. The number of patients with RBDs reported increases every year and currently runs at more than 17,000. Till 2005, the following numbers of patients with RBD had been reported: fibrinogen-599; prothrombin-167; FV-769; FV/VIII-188; FVII-1689; FX-597; FXI-2446; FXIII-435; platelet disorders-2648. There are many unclassified patients as well. The quality of the data is variable since health care standards vary and the registries may be managed by medical or lay people. This data is accessible on [www.wfh.org](http://www.wfh.org).

### **SSC Working Group on inherited RBD- Proposed plan of Action: F. Peyvandi**

Dr. Peyvandi described the efforts of above mentioned registries and surveys providing data on RBDs ([www.wfh.org/2/7/7\\_0\\_Link7\\_GlobalSurvey2005.htm](http://www.wfh.org/2/7/7_0_Link7_GlobalSurvey2005.htm) and

[www.rbdd.org](http://www.rbdd.org)). The former has information on 6,934 RBD patients in 98 participating countries and the latter has data on 3,017 RBD patients in 64 participating countries. The prevalence of RBDs are similar in the two data sets. However, in both these, only very basic information is available on each patient. About 50% of this data refers to patients in Europe. As a consequence, a network of 10 European treatment centres has been formed to develop a new and homogeneous communication tool for reporting, managing, editing and viewing information on RBD patients ([www.rbdd.eu](http://www.rbdd.eu))

To help focus on the development of the science around each RBD, it has been decided to establish small expert groups to prepare a roadmap for the development of the science of that condition.

## **Section II. F VIII / IX: Clinical issues – I (Assay/EQA/Phenotype) Co-chairs: C.A. Lee and H.M. van den Berg**

### **Optimizing the 1-stage assay – J. Polgar**

Dr. Polgar stated that current factor assays do not optimally cover the clinically relevant range particularly at the lowest levels by a reliable single calibration curve. Further improvement of the one-stage assays would be possible through introduction of a ‘zero calibration point’ and the establishment of calibration curves with an optimization approach. In the optimization approach: A) More than ten mathematical transformations can be used both for time/activity. B) Two curves can create the calibration, by selecting two groups of calibration points. C) Curve fittings can be linear, 2nd or 3rd order polynomials. D) A cut-off point defines the end of one, and the beginning of the next curve on the combined calibration curve with a smooth transition. Factor assays on analyzers, which use calibrations established by an optimization approach, have a wide range for accurate assays (0%-150% activity) with excellent linearity in the calibrated region and increased tolerance for reagent variations. Widespread use of ‘zero calibration points’ and establishing calibration curves by the ‘optimization approach’ could lessen variability in factors results among clinical laboratories.

### **EQA for tests of global haemostasis : ROTEM and TEG – S Kitchen, DP Kitchen, I Walker**

Dr. Kitchen presented data from the UK National External Quality Assessment Scheme (UK NEQAS) for Blood Coagulation that has assessed the use of lyophilised plasma samples for thromboelastography (TEG) and Rotational thromboelastometry (ROTEM). Lyophilised plasmas from a series of normal subjects and patients with deficiency of FVII, IX or XI were analysed in a single centre. These studies confirmed that some parameters were similar to those obtained for whole blood which is the sample material usually analysed for patient study. After this preliminary study, 2 pilot exercises were performed in which 7-10 ROTEM users and 13-14 TEG users received samples from normal subjects and a patient with severe FXI deficiency. The participant group included expert haemostasis centres and anaesthetists in operating theatres. The Coefficient of Variation for clotting times was between 10 and 121% and some clearly outlying results were observed. The clot firmness measurements showed CVs of 8 -33% between centres and were lower for plasma samples with higher fibrinogen concentrations. Overall our data show that lyophilised plasma samples can be used for EQA of TEG and ROTEM, and the degree of variability observed in 2 exercises suggests that such EQA could be of benefit in identifying outlying results. Further exercises are planned.

### **EQA for genetic testing of haemophilia – D. Perry**

Dr. Perry presented the evolution of the EQAS for genetic testing of hemophilia in the UK. A pilot scheme was set up by NEQAS in 1998-2000 using whole blood for the intron 22 inversion.

At that time many laboratories were using southern blotting and only a few long range PCR. In 2003, an advisory group was set up to provide a robust scheme for genetics. It was planned to provide samples, set up immortalised cell lines and set up a scoring scheme which would also depend on the family history. There are now two exercises each year which provide a clinical history and whole blood or DNA samples for analysis. A collaboration has been set up with NIBSC to set up immortalised cell lines. The lyophilised DNA has provided conflicting results but the liquid DNA has performed well. There has been encouragement to laboratories to use standard gene nomenclature – the CMGS (Clinical Molecular Genetics Society) system. Participants are required to 1) identify the individual 2) identify the mutation 3) answer the question and 4) be precise and this is the basis of the scoring system. At present the exercises have been based on FVIII but there are plans to move onto FIX and VWF genetic analysis.

### **Phenotypic heterogeneity of severe hemophilia – Newer players. A. Srivastava**

Dr. Srivastava mentioned that clinical observation of minimally treated patients with severe hemophilia suggested that the phenotypic heterogeneity among them exists not only in terms of the frequency of bleeding but also at the level of the inflammatory response in the joint. It was therefore hypothesized that polymorphisms in the genes of hemostatic factors and inflammatory cytokines could both affect clinical phenotype. Based on this hypothesis, patients with severe hemophilia were classified as mild (<5 bleeds/year, <10 WFH clinical and <10 Pettersson radiological score) or severe phenotypes (all others). Of the 114 patients evaluated, 14 were classified mild by these criteria. Among these patients, ‘severe’ mutations (inversions / deletions) (RR: 4.8), FVII 353 polymorphisms (arg/gln-gln/gln; lower levels) (RR: 5.9), protein C 1476 AT/TT (higher levels) (RR: 4.0) and TNF a 308 GA/AA (higher levels) (RR: 3.9) were found to be associated with clinically severe disease. With plausible biological basis for these polymorphisms affecting clinical phenotype, these data suggest that the clinical heterogeneity of severe hemophilia is not only determined by a balance of various coagulation proteins but also by polymorphisms in inflammatory cytokines. This work is on-going.

### **Section III. Factor III / IX. Inhibitors. Co-Chairs: C.R.M Hay / J. M. Saint-Remy.**

This section of the SSC included reports of completed, ongoing and planned studies of the incidence and risk factors for factor VIII inhibitors in PUPS, MTPS and PTPs. Past research has primarily focussed on the incidence and genetic and non-genetic risk factors for inhibitor development in PUPS. However, the risk factors and incidence of inhibitors in PTPs are poorly understood and require further study. Since regulators are using PTPs as their model to test the immunogenicity of new products, it is against this yardstick that these products need to be measured.

### **Global PTP inhibitor surveillance study: follow-up – D. DiMichele / C.R.M. Hay**

Dr. DiMichele outlined the development and growth of the US registry. Data on 529 patients in 9 sites are being retrospectively and prospectively collected and the database is growing. Inhibitor and genotypic testing is being done centrally and 240 patients have been genotyped so far. Dr. Hay described the UK registry, the planned European Adverse event network and the planned German registry. The UK registry has networked all 108 UK haemophilia centres, covering 6500

patients with haemophilia A and a total of >23000 patients with bleeding disorders of all types. Adverse events, such as new inhibitors, and new diagnoses are reported electronically in real time. Most patients have already been genotyped. A wider dataset is about to be collected prospectively to explore risk factors for inhibitor development in PTPs. A European adverse event surveillance system (EUHASS) is the subject of an EU grant application and is hoped to be operational by early next year. This will involve 45 haemophilia centres across Europe serving a total of 14,500 patients. The German Registry (national data) has also been set up and is due to open later this year. It is expected that these four registries will collaborate closely. Harmonization of datasets is being negotiated and it is hoped to present a common dataset in 2008.

#### **International ITI study update – C.R.M. Hay.**

Dr Hay reviewed the current status of the International Immune tolerance Study. This is an open randomized comparison of low and high-dose ITI in good risk patients. This study investigates factors influencing outcome, morbidity and cost-effectiveness. The study also forms a framework for several other satellite studies. The power calculation indicates that 90 patients are required to demonstrate a 20% difference between treatment arms and 150 patients to demonstrate equivalence. About 80 patients have been recruited and an interim analysis is expected later this year.

#### **Rodin study: Update – H. M. van den Berg.**

Dr. van den Berg explained that this study has been developed to determine the risk factors which cause or prevent inhibitor development in PUPs with severe hemophilia and may have important implications for eventual future prevention of inhibitors in these patients. The RODIN study aims to study potentially modifiable treatment related factors that affect the risk for inhibitors in these patients. It will include a cohort of patients with severe haemophilia who are treated in one of 30 participating European centres for the first 75 exposure days to factor VIII. Clinical data is collected from patients with severe (<1%), moderate (1-5%) and mild (5-25%) haemophilia A or B born after January 1 st, 2000. Data will be collected from patients born between 1-1-2000 and 1-1-2008. In total, 400 patients with severe haemophilia will be collected through the PedNet registry.

#### **Section IV. FVIII / IX Standardization issues. Co-chair: K. Mertens**

##### **FVIII collaborative studies: Phase II field study. M. Lee**

Dr. Lee reported on the statistical analysis of the SSC 9 th field study. This study included both recombinant and plasma-derived FVIII products. All participants had been asked to perform testing using two different operators on two separate occasions. The aim of this was to find an explanation for the high interlaboratory variation in the field studies, including the 9 th. Multivariate analysis revealed that variability was mainly due to the assay as such, and not to other factors such as operators or days. As in the 8 th SSC study, the main factor introducing variability was the prediluent (FVIII-deficient plasma or buffer) used. This study confirms the previous SSC recommendation that predilution in FVIII deficient plasma should always be

applied. In the discussion, Dr. Lee mentioned that the current activities are in full support of the previously published SSC recommendations for the assay of FVIII in concentrates. As such, it seems appropriate to work toward completion of these field studies, if appropriate, by publishing the current conclusions.

### **First International Standard for Factor VIII inhibitor - update. S. Raut**

Dr. Raut provided an update on the collaborative study on proposed reference standard for FVIII inhibitor. Preliminary data of this study had been presented in 2006 and a final report was subsequently distributed to participants, requesting feedback from them. The study highlighted that although one candidate preparation Y (pooled inhibitor patient plasmas- 05/206) had the lowest overall CV (17.7%) with a mean Bethesda titre of 8.2 BU/vial, inter-laboratory variability within the study was relatively high (CVs 17-33%). The intra-laboratory variability also gave high CVs (0.5-36%). Furthermore, when the results were recalculated relative to the 5 candidate preparations, only a slight improvement in inter-laboratory CVs was observed for the patient test plasmas and only relative to sample Y, with minimal further improvement for the Nijmegen modification and hybrid inhibitor methods compared to the classical Bethesda assay when assaying patient / plasma inhibitor samples. Major improvement in the inter-laboratory variability was also observed when assaying residual FVIII activity using the chromogenic assay (CVs: 2.5-20.1%) compared to the one-stage assay (CVs: 17.9-32%), although only 3 laboratories used this method. Following further discussions at a FVIII Inhibitor SWP meeting in Amsterdam (May 2007), it was decided to defer finalization of these results till the reasons for such large inter-laboratory variability have been precisely delineated.

### **Replacement of the 7th International Standard FVIII concentrate – S. Raut**

Dr. Raut further announced the forthcoming replacement of 7 th FVIII concentrate standard. Stocks of the current WHO 7 th IS (99/678) are running low and could be exhausted by 2009. This standard is used for the potency measurement/estimation of FVIII in therapeutic concentrate products, both recombinant / plasma derived FVIII by manufacturers and clinical laboratories. Approximately 600 - 800 ampoules are despatched each year from NIBSC. The current 7 th IS is a plasma derived material. Material (both plasma derived FVIII and recombinant FVIII) for its replacement will be sourced from product manufacturers. The materials to be selected as candidates will be discussed and decided after carrying out in-house comparative assessments (trials fills, accelerated degradation studies and potency estimations). Calibration will be performed by clotting assays and chromogenic assays relative to the current WHO 7 th IS in an international multi-centre study involving manufacturers, clinical laboratories and regulatory authorities. Objective will be to submit to ECBS in October, 2009.

### **Replacement of the International Standard for Factor VIIa – A. Hubbard**

Dr. Hubbard reported that the stocks of the current WHO 1 st IS Factor VIIa concentrate (89/688) are low and a replacement preparation is required. Candidate materials are currently being collected and definitive fills should be completed by the end of 2007. The original calibration of the 1 st IS was performed by one-stage clotting assay, relative to the WHO 1 st IS Factors II, VII, IX, X, plasma (84/665), using the same thromboplastin reagent in all

participating laboratories. This was necessary since potency estimations of FVIIa concentrate relative to plasma FVII show considerable variation depending on the thromboplastin reagent used. Since the original thromboplastin reagent used to calibrate the WHO 1 st IS FVIIa concentrate is no longer available it is proposed that calibration should rely on a direct comparison of the proposed 2 nd IS relative to the 1 st IS FVIIa concentrate. This approach is supported by accelerated degradation and real-time stability studies which have indicated that the 1 st IS FVIIa concentrate has not degraded since it was calibrated. Depending on the availability of suitable candidate materials it is planned to complete the multi-centre collaborative study by spring of 2008.

### **Thrombin generation tests- Report of the 2nd collaborative study – E. Gray**

On behalf of the SSC Working Party (WP) on Thrombin Generation Tests, Dr.Gray referred to the fact that this WP was set up in 2004 under Plasma Coagulation Inhibitors subcommittee with the remits to investigate, standardize and validate methodologies for the quantitation of results to facilitate good intra and inter laboratory agreements. As the results from the first study in 2006 indicated, the use of a reference-plasma would lower both intra- and inter-laboratory variability, this second study was carried out to investigate the feasibility of establishing a reference plasma for thrombin generation tests. Six freeze-dried samples including three candidate normal pooled plasmas were sent to 110 laboratories and 128 sets of results were returned for analysis. The majority of the labs used commercial kits (CAT, Dade-Behring-ETP, Technothrombin and In-TDT). Four labs used in-house methods. The results confirmed data from the first study and show that calibration against reference plasma improves intra- and inter- laboratory agreement. All 3 candidates reduce variability, but 2 of these materials were better. The WP is now discussing how a reference plasma should be used and also how it can be established as a SSC reference plasma for thrombin generation tests. The next task for the WP is to investigate the application of thrombin generation tests for use in the study of haemophilic plasma. The WP is requesting collaboration with experts from the FVIII/FIX Subcommittee who are interested in the standardization of thrombin generation tests.

In his concluding remarks, the chairman thanked all the co-chairs, speakers and the audience for their participation and closed the meeting at 19:45 hours.

## Factor VIII and Factor IX

4-5 July 2008  
Vienna, Austria

Chair: A. Srivastava (India)

Co-Chairs: C. Lee (UK), H. M. van den Berg (The Netherlands), C. Negrier (France), F. Peyvandi (Italy), J.-M. Saint-Rémy (Belgium), C. Hay (UK), J. Oldenburg (Germany), E. Tuddenham (UK)

The Chair opened the meeting at 14:00 and welcomed the audience of about 200 attendants. He explained the structure of the program mentioning that apart from standardization issues in each of the four sections of the agenda, there was one review lecture intended as a CME. He also informed the changes in the presentations.

### Completed/Submitted reports and recommendations

There were no completed reports, recommendations or publications to mention this year.

### Section I - Rare bleeding disorders

Chairs: F. Peyvandi (Italy) and C.A. Lee (UK)

#### Overview. Flora Peyvandi

Since its inception in 2004, the SSC working party on Rare Bleeding Disorders (RBDs) has attempted to improve the understanding of prevalence, diagnosis and treatments of RBDs by developing the Rare Bleeding Disorders Database (RBDD, [www.rbdd.org](http://www.rbdd.org)). At present, the RBDD contains clinical, phenotype, genotype and treatment data of more than 350 patients coming from 21 countries and collected in the last 10 years at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre in Milan. The RBDD project has already obtained the collaboration of 66 centres from all over the world that are willing to provide epidemiological information on over 3,000 RBD patients. Recently, data pertaining to two specific projects, designed to answer to particular issues of RBDs, were published online (<http://www.rbdd.org/studyonline.htm>): a study on menorrhagia and other gynaecological problems in women affected by bleeding disorders and a report on the prevalence of central nervous system bleeding and their therapeutic approach in patients affected by RBDs. To further improve the quality of data being obtained from these centres, there is a need for a good communication tool that will allow inserting, managing, editing and viewing information on RBD patients. The development of such a tool was made possible by a recently funded European project (EN-RBD network: [http://ec.europa.eu/phea/documents/2006\\_Health\\_Information.pdf](http://ec.europa.eu/phea/documents/2006_Health_Information.pdf)), in which 10 centres would collaborate ([www.rbdd.eu](http://www.rbdd.eu)). In November 2007, the first workshop of the EN-RBD working group was held in Milan. The testing phase, where partners started to insert specific data about their severely affected patients is on-going. By the end of June 2008, ~10% of patients records were inserted. Queries and reports allowed us to derive preliminary results about clinical manifestations, treatment and identified mutations ([www.rbdd.eu](http://www.rbdd.eu)). In conclusion, the on-line database resulted to be a powerful tool to improve the quality of data collection.

#### Defining clinical severity of rare bleeding disorders. Flora Peyvandi

The rare bleeding disorders (RBDs) are associated with a wide spectrum of clinical bleeding manifestations. In order to understand the correlation of bleeding with the level of factor deficiency, it is necessary to have tools to quantify clinical bleeding in these patients. Recently, two different tools were proposed to quantitatively bleeding history. The bleeding score proposed by Rodeghiero and Tosetto *et al*, principally applied to type 1 von Willebrand Disease (VWD) patients, summarises both the number of episodes and their severity. However, this score was validated only in type 1 VWD and, in a small study, on type 3 VWD obligatory carriers. The bleeding score (BS) proposed by Srámek *et al*, and modified by Podda *et al*, accounts for the number of bleeding symptoms and their nature, severity, duration, localization, frequency. This score was only tested in 128 patients with suspected abnormalities of haemostasis, where a BS >12 was associated to severe bleeders. Unfortunately, up to now, none of these scoring systems was specifically applied to RBDs. We tested them on a group of 35 women affected by RBDs. The results showed that both the scoring systems are good to

distinguish even mild RBDs patients from controls; however, there was no significant differences in the bleeding scores of patients with different levels of phenotype severity. The mean BS increased from patients with a mild to those with a severe deficiency in the analysis of the only women affected by FVII deficiency (n=14), whereas an opposite trend was observed in a small group of women affected by FXI deficiency. Tools for quantifying clinical bleeding need to be tested and validated in people with RBDs. The assay methods for measuring these factor levels need to be standardized and their quality assessed.

### **Defining Severity for Rare Factor Deficiencies: Insights from the NARBDR. D. DiMichele, S. Acharya**

The FVIII/IX Subcommittee posed two questions: How can disease severity be defined for rare bleeding disorders (RBDs)? Could the North American (NA) Rare Bleeding Disorders Registry (NARBDR) database (Acharya, JTH,2004) offer potential answers to this question?

Among 284 subjects in the NARBDR, 78% had FII, FV, FVII, or FX deficiency for which we could not establish homozygosity / heterozygosity on the basis of molecular studies because only 5.4% subjects underwent gene-based diagnosis. Factor activity levels, available for 96% subjects, proved more useful. Based on a similar strategy by Roberts (T&H, 2003),  $\geq 0.20$  u/ml ("hemostatic") factor activity was originally used to define heterozygosity for between 40 and 60% of each RBD. However, since other RBD registries had published alternative severity schema, the investigators performed a reanalysis of the original dataset to correlate FII, FV, FVII, FX activity levels with: age at first bleed, bleeding trigger and site of bleeding. Bleeding site / trigger data were examined according to two-disease severity schema: mild disease was defined by either the original NARBDR designation ( $\geq 0.20$ u/ml) or the more traditional hemophilia model ( $\geq 0.05$ u/ml). The NARBDR provided limited but interesting data on the association between bleeding parameters and disorder severity as ascertained by factor activity. The authors concluded that: 1) Rare deficiencies may not be amenable to a single severity categorization as attested to the differences between the Vitamin K- dependent factors and FV; and 2) Similar analyses of larger more informative national and multi-national RBD prospective databases will be useful in defining clinical severity for these disorders.

### **Factor assays in patients with rare bleeding disorders - Data from UK NEQAS. S. Kitchen**

Data from clotting factor assays of FII, V, VII, X and XI performed in 230 to 330 laboratories participating in the UK National External Quality Assessment scheme (UK NEQAS) are discussed. Lyophilised samples from the same donor were analysed using whatever method was in local use. The CVs of results for samples from normal subjects were between 9 and 17%. For samples with levels of 20 - 27 IU/dl CVs were higher at between 23 and 39%. These figures are similar to those obtained by the same laboratories when performing assays of FVIII and IX in both groups (normals and mild deficiency). The variables which contribute to this imprecision include the use of normal plasma unit in the case of FV and FXI where the IU has not yet been adopted by a number of manufacturers of reference plasmas used for assay calibration. Some variability occurs as a consequence of use of different reagents, for example in the case of different thromboplastins in FVII assays. A major contributor to assay variability is the use of poor assay design which often does not follow recommendations available in the literature. Regular external proficiency testing of assays for rare bleeding disorders should help to draw attention to these problems and educate centres in the need for more standardised assay design.

### **Replacement of the 3<sup>rd</sup> International Standard for Blood Coagulation Factors II, VII, IX and X, Plasma, Human. E. Gray**

The current WHO 3<sup>rd</sup> International Standard for Blood Coagulation Factors II, VII, IX and X, Plasma, Human, NIBSC code 99/826 was established in 1999. The stock of this standard is expected to be exhausted by December 2010. This is an important primary standard used not only by clinical laboratories and manufacturers of plasma derived blood products, but also for calibration of secondary standards such as the SSC/ISTH Secondary Coagulation Standard. A replacement standard is essential for the continuity of units of the four coagulation factors. The value assignment of the replacement standard will involve assays of all four factors against the current 3<sup>rd</sup> International Standard and locally collected normal pooled plasma. As the SSC/ISTH Secondary Coagulation Standard Lot#3 will also require replacement at approximately the same time, it is envisaged that this exercise will also include the calibration of the SSC/ISTH Secondary Coagulation Standard Lot#4. The collaborative study will be initiated in September 2009 with a final study report

submission date to SSC in July 2010. Upon approval by the SSC, the proposed candidate will be recommended to the Expert Committee on Biological Standards for establishment as the 4<sup>th</sup> International Standard for Blood Coagulation Factors II, VII, IX and X, Plasma, Human in October 2010.

#### **Replacement of the WHO International Standard for Factor VIIa Concentrate. *A. Hubbard, L. Weller, A. Heath***

The current WHO 1<sup>st</sup> International Standard (IS) Factor VIIa Concentrate (89/688) is used for the potency estimation of activated factor VII (FVIIa) concentrates intended for therapeutic use and also for FVIIa estimation in plasma and prothrombin complex concentrates. Stocks of the WHO 1<sup>st</sup> IS are extremely low and a replacement preparation is required. Calibration of the replacement WHO IS has been achieved through an international multi-centre study involving 23 laboratories. Two candidate preparations (sample B: recombinant and sample C: plasma-derived) were assayed directly relative to the WHO 1<sup>st</sup> IS (sample A) using the conventional one-stage clotting method (175 assays), the chromogenic method (30 assays) and the clotting assay specific for activated factor VII using soluble recombinant tissue factor (21 assays). Estimates by conventional clotting assays using a common "provided" thromboplastin reagent and local thromboplastin reagents were extremely similar and gave combined mean values of 655.63 IU/ampoule (n=43) for sample B and 679.77 IU/ampoule (n=43) for sample C. For sample B, estimates by the chromogenic method and activated factor VII clotting method differed by 1% and 2% respectively from the conventional clotting method and this emphasises the similarity of sample B with the WHO 1<sup>st</sup> IS which were both prepared from the same product. In contrast for sample C, the estimates by the chromogenic method and activated factor VII clotting method differed by 7% and 22% respectively from the conventional clotting method. Estimates of stability based on accelerated degradation studies, after 6 months storage, together with long-term experience with other FVIIa preparations, indicate that the candidates should be very stable when stored at -20 °C. In the interests of continuity and, in consideration that sample B represents the only licensed Factor VIIa therapeutic concentrate available at the present, it is proposed that sample B (NIBSC code 07/228) is accepted as the WHO 2<sup>nd</sup> IS Factor VIIa Concentrate with an assigned potency of 656 IU/ampoule.

#### **Licensure of products for therapy of rare bleeding disorders - EMEA perspective. *R. Seitz,, G. Silvester***

In the European Communities (EC), products are available for rare coagulation disorders, e.g. Fibrinogen, FVII, FXIII, but still some are missing. The European Orphan Medicines Legislation provides incentives, such as free protocol assistance (scientific advice), fee reductions, and market exclusivity for 10 years, as well as EC programs to support research, development and availability. In the area of blood products, some new and follow-on products have been designated. The EMEA has also installed an office for support of small and medium-sized enterprises (SME). There is the possibility of parallel EMEA and FDA scientific advice meetings. There are regulatory mechanisms to license with limited clinical data: Conditional Authorisation and license under Exceptional Circumstances, where the applicant can show that he is unable to provide comprehensive data on efficacy and safety. Another important mechanism is Compassionate Use. An example is Protein C, where in response to requests from physicians, after years of compassionate use, the product was authorised in May 2001 under 'Exceptional Circumstances', and after the company supplied additional information 'Exceptional Circumstances' ended in July 2006. The CHMP Blood Products Working Party (BPWP; Chair R. Seitz) elaborates guidance on clinical assessment of products. Since not enough patients are available to perform statistically meaningful pre-licensing studies, it is understood that pre-licensing clinical studies alone will not provide full assurance of safety, and post-marketing studies and registries play an important role. How can we obtain more products in the EC? We need the demand expressed by physicians and/or patients to have access to products which are so far lacking, to motivate companies to develop a products taking advantage of the available regulatory assistance (e.g. orphan medicine designation, protocol assistance) throughout the licensing process.

#### **Licensure of products for therapy of rare bleeding disorders – US FDA perspective. *M. Weinstein***

FDA promotes the development of products that demonstrate promise for the treatment of rare diseases. Generally, the disease or condition for which the candidate orphan drug is intended affects fewer than 200,000 people in the United States. Incentives available to encourage orphan drug development are 7 years of market exclusivity following approval of an orphan drug; 50% tax credit on certain clinical testing expenses; clinical study grants; and waiver of the Prescription Drug User Fee Act application fee. Adequate and well controlled

clinical trials are necessary to demonstrate the safety and efficacy of orphan drug products. FDA will work with sponsors to develop clinical trial protocols that are appropriate for the size of the patient population. A recent example is the approval of Protein C Concentrate (Human), a human plasma derived product. Approval was based on treatment of acute thrombotic episodes including purpura fulminans (PF) in an open label non-randomized study in 18 subjects with severe congenital Protein C deficiency compared to the efficacy ratings for 21 episodes in an historical control group. FDA also encourages international cooperation in orphan product development through such programs as the EMEA – FDA parallel scientific advice meetings. This continuing program, initiated in 2004, provides a mechanism for EMEA and FDA to exchange their views on scientific issues during the developmental phase of new medicinal products. This program can optimize product development, and avoid unnecessary testing replication or diverse testing methodologies. FDA is also interested in establishing and using patient registries and databases to increase understanding of the rare plasma protein disorders. Registries and databases have the potential to help in product development; to make available information about the natural history of the disease; to provide guidance in dosing and frequency of dosing; and to aid in surveillance, and adverse event reporting.

**Section II - Factor VIII:IX: Clinical issues - I (Assays / EQA / Phenotype)**  
**Chairs: C. Negrier (France) and H.M. van den Berg (The Netherlands)**

**Phenotypic heterogeneity of severe hemophilia – Defining the issues. A. Srivastava, India**

It has been long recognized that 10-15% of patients with 'phenotypically characterized' severe hemophilia (<1% clotting factor activity) have relatively mild disease clinically. Not all these patients have frequent spontaneous bleeding and even among those who bleed, the extent of joint damage tends to vary considerably. The basis for this difference has not been completely understood. Clinical observation and laboratory data from animal models suggests that this heterogeneity exists at several levels. Differences in overall hemostatic efficiency could account for some of the differences in bleeding frequency. Preliminary data from tests of global hemostasis carried out in this population suggests that these tools can be informative but further evaluation is needed particularly among patients with <1% FVIII:C but clinically heterogeneous disease. The second levels of heterogeneity probably occurs at the level of the inflammatory response to the iron in the synovium. With the central role of hemosiderin deposition in initiating inflammation in the joint following hemarthroses as well documented in animal models, it is tempting to predict that functional polymorphisms in the immunomodulatory cytokines could affect this response. In fact we have some early data from patients with minimally treated severe hemophilia to suggest that this could be so. Finally, the extent of synovial hypertrophy could be affected by polymorphisms in the cytokines affecting angiogenesis. This needs further evaluation. One of the major issues in the management of hemophilia today is to decide on ways in which therapy, particularly the initiation and intensity of prophylaxis, can be individualized. A detailed understanding of all factors that may contribute to joint damage in severe hemophilia could help us in tailoring therapy for these individuals.

**Thromboelastography – An International Study on reproducibility and consistency. M. Chitlur**

Thromboelastography is a global assay of haemostatic function, from the beginning of clot formation to fibrinolysis. Two systems utilizing this technology include Thrombelastograph/ TEG® (Haemonetics, Braintree, Massachusetts, USA) and ROTEM (Pentapharm GmbH, Munich, Germany). They are simple to use and proven to be an effective methods of intraoperative monitoring to optimize blood product selection and utilization and improve outcomes during complex surgical procedures such as cardiothoracic surgery and liver transplantation. Case reports or small case series have addressed the usefulness of thromboelastography in the management of bleeding or monitoring of treatment with bypassing agents. In order to establish the usefulness of this test a standardized methodology needs to be developed and reproducibility and consistency have to be demonstrated. In an attempt to initiate this process, the TEG-ROTEM Working group was established in 2006 and investigators from several countries joined hands to perform studies. Here we present the results of the first international study, wherein 9 laboratories from 6 countries (Canada, Denmark, Israel, Norway, UK and USA) participated. Standard pooled normal plasma as well as FVIII deficient plasma was activated using either kaolin (TEG) and INTEM (ROTEM). Clotting parameters i.e. clotting time (R/CT), time to clot formation (K/CFTR), Maximum clot firmness (MA/MCF), clot strength (G) and rate of clot polymerization (Angle) were measured. The CV's varied from 6 to 60% and varied significantly for the different parameters, with the lowest CV's being seen with the R/CT and MA /MCF. The inter laboratory variance was also significant

with CV's greater than 10%. Even though these results are not satisfactory, this has been the first effort to standardize this methodology and significant work remains to be done to improve the reliability and reproducibility. These studies were performed on PRP and the results may be more reliable when performed on whole blood samples. Future studies will use whole blood; determine the effect of anticoagulants and the effect of different activators on the clotting parameters, helping us to optimize the methodology and improve the reliability characteristics.

#### **ROTEM – Usefulness in predicting response to bypassing agents. E. Tuddenham**

Thromboelastography has been proposed as a means to measure and predict response to bypassing agents ( rVIIa and aPCC). In our laboratory we have investigated in-vitro addition of bypassing agents to whole blood from patients with FVIII or FIX antibodies. Blood is taken into citrate with corn trypsin inhibitor and coagulation is initiated with CaCl<sub>2</sub> and 1/50,000 (~1pm) dilution of rTissue Factor. Clot stiffness was measured with ROTEM<sup>R</sup>. In three cases subjected to surgery the predicted effect of invitro addition of bypassing agents was confirmed by adequate haemostasis during procedure. Six further cases were also tested but have not yet undergone surgical challenge. Three patients had normal ROTEM parameters prior to addition of bypassing agent. Further studies with intraoperative (ex vivo) analysis are needed to validate the assay for its clinical usefulness, but results so far are encouraging.

#### **Thrombin generation test - Usefulness in monitoring therapy. C. Negrier**

Since haemostasis is considered to be a combination of multiple proteins which can assemble in complexes at the cellular surface, global evaluation of this process may be more relevant in some situations. Thrombin generation assay (TGA) is one of the candidate which may theoretically be used to further precise the phenotype of a particular patient and to measure *in vitro* and *ex vivo* the effects of bypassing agents used for the treatment of inhibitor-developing haemophiliacs. We have established at our institution the normal range for various parameters of the TGA and have demonstrated that these parameters were significantly impaired in patients with various severities of haemophilia A and B. The TGA parameters were completely normalised upon substitution with factor VIII or factor IX, but needed more subtle dose adaptation when bypassing agents were used. Because of the inter-individual variations in terms of biological response to bypassing agents, we have designed a 3 step-protocol where both the therapeutic agent and the dose are chosen according to TGA results for challenging situations such as planned surgeries. High risk surgeries have been conducted in consecutive patients with results that matched the predictions of the *in vitro* and *ex vivo* assays performed pre-operatively, suggesting that TGA may be useful to predict the effective dose of bypassing therapies and to follow up the haemostasis profile during and after the surgical procedure. Our results strongly suggest that this assay could represent a surrogate marker for monitoring bypassing therapies in surgical situations, and this approach needs further validation. A working party is proposed with the aim to correlate the results from the assay with clinical parameters.

#### **External Quality assessment of ROTEM. S. Kitchen, D.P. Kitchen**

Data were presented from 3 external quality assessment surveys through the UK National External Quality Assessment scheme. These included results from 14 users of TEG and 10 users of ROTEM who analysed lyophilized plasma samples from normal subjects and patients with severe FVIII and FXI deficiencies. Each sample was analyzed once as per routine practice. The CVs for Extem and Intem tests on the ROTEM were 25%-121% and 10% – 37% respectively. Clot firmness was better for the sample with 5g/l fibrinogen than those with lower levels. The CV of results on the TEG were in the range 15 -33%. The data indicated that plasma could be successfully used for EQA purposes and it was possible to identify centres with outlying results.

#### **Assay discrepancies between one and two stage assays –**

##### **a)Discrepant assays in mild haemophilia. S. Kitchen, M. Makris**

Three main methods are available for FVIII:C activity estimation. The one stage clotting assay is used by the vast majority of laboratories internationally, whilst a small number use the chromogenic and two stage clotting assays. All of these assays give equivalent results in severe haemophilia and following most FVIII replacement.

In mild haemophilia however, where the structure of the molecule is abnormal, significant differences between the assays are seen in some cases. In Sheffield, UK we use the two stage clotting assay as our standard assay. We have recently started re-examining all our mild haemophiliacs and found significant discrepancy in around 30% of our patients. The two stage clotting assay results were largely similar to chromogenic results. We defined discrepancy when the ratio of the one stage: two stage results were outside the 0.5-2.0 ratio. From 56 mild haemophiliacs we identified 10 with discrepancy in which two stage levels were significantly lower than one stage ones. Seven (12% of the study group) of these patients with definite bleeding histories and genetically confirmed haemophilia had FVIII:C that were normal by the one stage assay and the diagnosis of haemophilia would have been missed. 6 patients (11%) in our study group had FVIII:C that were reduced by one stage but normal by two stage assay. With referrals we have now identified 16 individuals from 10 families with this pattern all of whom had the Tyr346Cys mutation and largely negative bleeding histories. In all our patients chromogenic assay results are broadly in agreement with the 2 stage clotting method. In the Sheffield experience, the two stage clotting assay reflects the severity of haemophilia better than the one stage assay which can fail to diagnose some and over diagnose other patients as mild haemophiliacs.

**b)FVIII assay discrepancy - Hemophilia A variants escaping the two-stage assay. H. Brondke, J. Oldenburg**

Factor VIII activities are mainly determined by either the one-stage clotting assay or by a two-stage chromogenic assay. Although these tests give similar Factor VIII activities in the majority of hemophilia A patients, a small subset of the currently known mutations are characterised by discrepant results in these assays. The larger fraction of these mutations lead to higher FVIII concentrations in the one-stage assay, while the two-stage assay displays an activity, which is congruent with the clinical picture of the patients. However 6 mutations (Glu321Lys, Tyr346Cys, Ile369Thr, Glu720Lys, Arg1689His and Phe2127Ser) show a reverse effect, in which the two-stage assay is higher than the one-stage. In these, mainly mild hemophilia A cases, the effect of the underlying mutation is only apparent in the one-stage clotting assay, as the two-stage assay yields normal activities. A common feature of the above mentioned mutations, except for Phe2127Ser, is that they cluster in the vicinity of thrombin cleavage sites.

**Section III - Factor VIII/IX: Clinical issues – II: Inhibitors  
Chairs: C. Hay (UK) and J.M. Saint-Remy (Belgium)**

**Biology of the development of inhibitors: Current understanding focusing on B cells. JM Saint Remy**

The bone marrow produces millions of new B cells every day with high risk of producing B cells capable of reacting with FVIII. FVIII exposure is by definition a recurrent event, driving B cells into process of affinity maturation. The B cell pool at one given moment in time is made of very heterogeneous population of cells with different sensitivity to signaling events. The purpose of this short review is to discuss the ways by which B cells interact with their environment and to present a clearer understanding of how this could be manipulated for the benefit of patients with inhibitors.

B cells interact with their environment via 3 types of receptors: the antigen-specific receptor (BCR), the CD40 molecule and Toll-like receptors (TLR). The CD40 molecule allows B cells to interact with T cells via CD40 ligand and will not be considered here. The BCR is coupled to a complex network of intra-membrane and intracellular factors, the aim of which is to transmit surface activation into metabolic events. Depending of the stage of maturation of the B cell, the BCR-mediated events will depend on the format of the ligand (FVIII), the BCR avidity and the localization of the B cells. TLRs bind pathogen-derived factors and the consequences of TLR binding vary dramatically from cell to cell. Thus, for a same stimulus, B cells and dendritic cells will react in opposite directions. This offers possibilities to manipulate B cells without touching upon the normal capacity of the immune system to react to pathogens. The molecular events that are decisional for B cells to enter either in an differentiation process to produce antibodies or to recycle within germinal centers are well established. At transcriptional level, BLIMP-1 is a master regulator, which, upon activation, drives B cells into plasma cells. Numerous factors, some of which can be manipulated, either activate or suppress BLIMP-1 transcription, providing another stage at which the production of inhibitors could be counteracted. The recent generation of a BCR-transgenic mouse strain makes it possible to evaluate at molecular level every step leading to activation and/or suppression, thereby providing a new tool to explore therapeutic approaches for eradication of inhibitors.

## **Analysis of inhibitors in clinical trials: Where are we now? N. Jain**

During the 2003 FVIII inhibitor workshop sponsored by FDA, problems related to evaluation and assessment of inhibitors in FVIII deficient patients were identified. These were: definition of an inhibitor, cut off value of a positive inhibitor, definition of high and low inhibitor and clinical implications of a transient inhibitor. In order to obtain uniform data on inhibitor developments in a clinical trial, FDA recommends a standard way of analyzing inhibitors in a clinical trial(s). Based on recent publications on difference in rate of inhibitors between pd and recombinant products, FDA investigated its AERS database. No conclusion could be drawn on rates of inhibitor development between pd and rFVIII products because of the limitation of the above database. FDA has attempted to collect a uniform data on inhibitor development during the development phases of a new and /or product undergoing major manufacturing changes.

### **Rodin study update. H. Marijke van den Berg**

Observational cohort studies are an important design to investigate the etiology of inhibitor development. A large number of genetic factors like large gene mutations of the factor VIII gene have been recognized as important factors. Apart from the genetic factors, treatment related factors have an impact in inhibitor development. There is still a large debate on a different impact for plasma or recombinant products on inhibitor development. Recently in the Canal study the large influence of a peak treatment moment on inhibitor development have been clearly demonstrated. Patients that were treated at their first treatment episode with a peak moment had an increased relative risk of 6.8 that they were going to develop an inhibitor. In contrast patients that started with early prophylaxis had a decreased risk to develop an inhibitor. The hypothesis is that danger signals like intensive treatment and infection or surgery are mechanism that can induce inhibitor development in a particular patient. This also is an explanation why for instance in identical twins there can be discordant inhibitor development. In the Rodin study data are collected from all children with severe hemophilia A born between 1-1-2000 and 1-1-2008. Patients will be followed until they have received 75<sup>th</sup> exposure days. In structured base line and follow-up forms every reason for treatment, the dose, mode of infusion etc will be captured along with data on vaccinations, infection and surgery. At the moment 28 centres have IRB approval and already >270 patients with severe haemophilia A have been included. The estimated number of patients to be included is a total of at least 800. More information on the study can be obtained on the [www.rodinstudy.eu](http://www.rodinstudy.eu).

### **Update: International ITI Study. D. DiMichele**

D. DiMichele presented an update on the International ITI Study on behalf of co-PI, Charles Hay, and the I-ITI study group. The study is a prospective randomized multi-center trial of the safety and efficacy of high (200 u/kg/d) vs. low (50 u/kg thrice weekly) dose ITI in a good risk cohort of high titer hemophilia A inhibitor patients. The data for the cohort as a whole was presented; investigators remain blinded to interim arm-specific results. To date, 106 of the targeted 150 subjects have been enrolled from 96 centers in 25 countries. As of 4/08, 91 have been randomised at a median age of 23.4 months) and a median pre-ITI titer of 5.0 BU (0.6 - 9.4), achieved after a median 5.0 (0 – 23) months from inhibitor diagnosis. This cohort has been on ITI for a median 13.0 (0.8 – 33.0) months. To date, 44/91 achieved negative titers; 38 achieved normal FVIII recoveries; and 35/91 became tolerant after a median of 14.9 (3.5– 30.4) months from ITI start. Forty-three subjects have reached a study endpoint; a total of 11 subjects have failed ITI, based on one of two failure criteria. Two interim data safety analyses are mandated by the protocol when 50 and 100 subjects completed the study. In May 2008, the DSMB conducted its first *in camera* interim comparative analysis of study treatment arms with respect to safety and efficacy, and concluded that it was safe to continue the trial. Independent investigators are conducting 7 satellite studies in conjunction with the trial. The PIs continue to welcome any additional participating centres until target enrolment is achieved.

### **Harmonisation of National Databases for investigation of Inhibitor development in Haemophilia A. C.R.M. Hay**

CHMP and EMEA requested ISTH to facilitate harmonization of data collection by national databases to enable them to share anonymous data. This would permit analysis of large numbers of patients to investigate risk-factors for inhibitor development in previously treated patients (PTPs). Possible risk factors for investigation include treatment factors such as product switching, product type, treatment pattern use of immunomodulatory therapy and intense treatment episodes and surgery, and host factors such as factor VIII genotype, age of the

patient, ethnicity and first degree family history. For such data to be aggregated in the form of a meta-analysis, the contributing databases would have to collect similar data using similar methods from a similar sample of their patient population. This process aims to achieve adequate harmonization of data collection to make such collaboration a practical proposition. To this end, data-fields have been exchanged and conference calls conducted and a detailed questionnaire circulated between those running the UK National Haemophilia Databases and the National Databases in the USA, Germany, Italy and Canada. Informal discussions have also taken place with several other databases and it is hoped that they may also become involved in this process. The emerging European Adverse Event Surveillance System (EUHASS) has also been involved in this discussion but may not be able to participate because it has a different model and considerable overlap with several other European networks.

All the other participating databases aspired to be complete National Networks though country coverage was incomplete for the US and Italian networks. The German database is only currently being established and is too new to evaluate from this perspective. Participation is voluntary in each case, with some central governmental compulsion in the UK and Germany. Patient consent was required by all countries but Canada, though all databases but the UK database had varying degrees of patient anonymity, usually falling short of the requirements of the European Data Protection legislation. Only three had a national network although all but the Canadian Databases used similar or identical software and hardware organization. Staffing varied in different countries. The UK and US databases were well staffed but all the others were run by one or two people, raising doubts about data quality and capacity to collaborate. With the exception of the Canadian database, there was a very high degree of harmonization of data collected, though all databases were at an early stage in collating genotypic data. All databases committed informally to the principle of sharing aggregated anonymous data according to a pre-agreed protocol. Merging of national databases is not anticipated and has not been discussed. Further conference calls and meetings will be arranged to push the process forward.

#### **Role of Immune Response Genes in Inhibitor Development. *J. Oldenburg, J. Astermark, E. Berntorp***

The formation of alloantibodies against factor VIII (FVIII) or factor IX (FIX) is the most severe complication of replacement therapy in patients with haemophilia. In the last decade, genetic factors have been shown to constitute a decisive risk determinant for the development of inhibitors. In severe haemophilia A and B, mutations that result in an absent or truncated FVIII/FIX protein are associated with a 20–80% risk of inhibitor formation. Missense mutations represent the main mutation type with a low inhibitor prevalence of 5%. Recently, a significant association between inhibitor formation and polymorphisms in genes coding for cytokines (IL-10) and other immunoregulatory factors (TNF- $\alpha$ ) has been shown. These genetic factors constitute the individual genetic risk profile of a haemophilic patient. This risk is imprinted and fixed; however, environmental factors such as treatment schedule may increase or decrease the inhibitor risk in an individual patient. Improved understanding of these complex interactions may lead to the development of preventive measures to minimize inhibitor formation. Why some haemophilic patients develop inhibitors while others do not and whether it may be possible to predict their development, are two major issues that still need to be resolved. However, a genetic predisposition to inhibitor development to FVIII/FIX proteins is clearly present. In part, this vulnerability depends on the underlying FVIII/FIX gene defect (inversions, nonsense mutations, etc.). For most gene alterations in haemophilia A, the inhibitor risk ranges from 20% to 40%, corresponding to an OR of about 2, while for some FVIII gene defects, the OR can be 10–30 (nonsense mutations vs. missense mutations and large multidomain deletions vs. missense mutations). However, immune response genes may play a decisive role in inhibitor formation, as has been reported for IL-10 and TNF- $\alpha$ , and may prove to be even more important than the FVIII/FIX gene defects. The ongoing challenges are to fully discover and characterize these variables and their interactions with environmental factors, and then to apply that information to prevent inhibitor formation, thus overcoming this most serious complication of haemophilia treatment.

#### **Section IV - Factor VIII/IX: Standardization issues**

**Chairs: E. Tuddenham (UK) and J. Oldenburg (Germany)**

#### **Factor VIII at 4.5 Angstroms - Insights from the new crystal structure. *E. Tuddenham***

Two papers had been published in the last 6 months reporting medium resolution X-ray crystallographic structures of B domain less rFVIII. (Structure. 2008 Apr;16(4):597-606; Blood. 2008 Feb 1;111(3):1240-7) The

structures are very similar and differ from earlier low resolution electron diffraction structure mainly in the position of the C2 domain, which is side by side of the C1 domain. Two Cu<sup>++</sup> atoms are present in the A1 and A3 domains and two Ca<sup>++</sup> atom are also visualized. Three carbohydrate side chains were also well visualized even though partly buried. PDB coordinates for FVIII are available online to download and use for interpreting mutations, antibody epitopes and novel FVIII designs, as well as many other applications.

#### **SSC - Factor IX concentrates “Field” studies: A new proposal. S. Raut, M. Lee**

Previous SSC “Field” studies on FIX concentrates have shown high inter-lab variability [CVs 9% - 23%; TG tests ~110%]. Recent SSC “Field” studies on FVIII concentrates have shown that high inter-laboratory variability can be significantly reduced by following SSC assay recommendations. The last FIX study was carried out 1997 and since then a number of (new generation) recombinant FIX concentrates are under development (Phase I/II Clinical Trials) and it is important to ensure correct potency measurement of these new and older FIX products. Following discussion at meetings of the SWP on field studies (ISTH/SSC FVIII/FIX Sub-Committee), it was decided to investigate causes of variability observed. Two studies are proposed: *Phase 1* - Field Study using routine methodologies (to evaluate current status in assaying FIX concentrates); *Phase 2* - Controlled Study following strict protocol instructions, to assess the effects of different, methods, pre-diluents, operators and assays on separate days. In addition, components of variation analysis will be carried out by Martin Lee. Four materials are proposed to be included in the studies (2 plasma derived & 2 recombinant). In addition to potency assessments, participants are requested to carry out thrombogenicity tests also (e.g. NAPTT, thrombin/fibrinogen clotting time (TFCT), FIXa etc.) Samples and Protocol for the Phase I study is scheduled to be dispatched in Feb 2009 with participants returning data by 1<sup>st</sup> May 2009. It is proposed to present preliminary data to the SSC FVII/FIX Sub-committee in July 2009.

#### **Collaborative Study to Establish the 4<sup>th</sup> International Standard, the EP BRP and the FDA Standard for Factor IX Concentrate. E. Gray, W. Pickering, J. Hockley, P. Rigsby, E. Terao, KH Buchheit, M. Weinstein, T Lee and R. Drews**

Thirty laboratories from 14 countries took part in a collaborative study to assign potency values to a World Health Organisation (WHO) replacement international standard (IS) for Blood coagulation Factor IX, concentrate, Human, a replacement European Pharmacopoeial (EP) Biological Reference Preparation (BRP) and a replacement FDA Standard. Three candidates, one of recombinant origin and two of human plasma derived origin, were assayed against the 3<sup>rd</sup> International Standard for Blood Coagulation Factor IX, Concentrate, Human (96/854). The 3<sup>rd</sup> International Standard for Blood Coagulation Factors II, VII, IX and X, Plasma, Human (99/826) was also included to evaluate the relationship between the factor IX plasma and concentrate units. Thirty-two sets of clotting assay results and two sets of chromogenic assay data were analysed. There was significant difference in potency estimates by these two methods for sample B, the recombinant candidate and sample P, the plasma IS. Similar potency values were obtained for samples C and D, the plasma derived products by clotting and chromogenic assays. For the clotting assays, intra-laboratory variability (GCV) was found to range from 0.5 – 21.7%, with the GCV for the majority of laboratories being less than 10%. Good inter-laboratory agreement, with the majority of the GCV being less than 10% (GCV range = 4.7 – 10.6 %) was also obtained. The estimated potency of the 3<sup>rd</sup> IS for plasma factor IX relative to the 3<sup>rd</sup> IS for factor IX, Concentrate was found to be 6% lower than with the assigned value. This discrepancy appeared to be a carry-over from the calibration of the 3<sup>rd</sup> IS for factor IX, Concentrate. Considering the preliminary stability data, the intra- and inter-laboratory variability, and the differences between the clotting and chromogenic assay results, it is proposed to recommend that sample C, 07/182, to be established as the 4<sup>th</sup> International Standard for Blood Coagulation Factor IX, Concentrate, Human (96/854), the EP BRP for Human coagulation factor IX concentrate, batch 2 and the FDA reference standard for Human coagulation factor IX concentrate, with a clotting potency value of 7.9 IU/ampoule.

#### **Factor VIII inhibitor study: Status update: K. Mertens**

The development of a FVIII inhibitor standard was initiated at the 2003 Subcommittee meeting. The need for this reference was also endorsed by WHO and, more recently, by EMEA. Dr. Raut (NIBSC) has been running the study, and a progress report of the study has been presented at the SSC meeting in Oslo in 2006. The study had been performed by 22 expert laboratories, and addressed 5 candidate preparations for the International Standard, including a rabbit polyclonal inhibitor, 2 human monoclonal antibodies, and 2 human

inhibitor patient plasmas. The study highlighted that although one candidate preparation (inhibitor patient plasma 05/206) had the lowest overall CV (17.7%) with a mean Bethesda titre of 8.2 BU/vial, both inter- and intra-laboratory variability were relatively high (CVs up to 36%). Moreover, when the results were recalculated relative to the 5 candidate preparations, only a slight improvement in inter-laboratory CVs was observed, suggesting that the new standard would be of limited benefit. The progress report has raised numerous concerns, including the viral status of candidate 05/206 (HCV and HIV positive), the high CVs, and uncertainty with regard to statistical validity of assays for residual activity. In 2007, a Working Party was established (Chair: K. Mertens) with the aim to assess the feasibility a 'post-hoc' statistical validity check in order to identify the most reliable inhibitor assays. This, however, may be possible for only part of the data. Dr. Mertens discussed several options to proceed from now, ranging from using data from a limited number of participants only, to performing a new, smaller study under more controlled conditions. The audience was invited to send suggestions to Dr. Mertens ([k.mertens@sanquin.nl](mailto:k.mertens@sanquin.nl)) for further discussion in the FVIII inhibitor Standardisation Working Party.

#### **Calibration of the replacement WHO International Standard for FVIII/VWF Plasma. A. Hubbard**

The current WHO 5<sup>th</sup> IS Factor VIII/von Willebrand Factor, plasma was established in 2003 with assigned values for FVIII:C, FVIII:Ag, VWF:RCo, VWF:Ag and VWF:CB. With an average despatch of approximately 750 ampoules per year it is expected that stocks will be exhausted in 2009. A candidate replacement preparation consisting of approximately 20,000 ampoules was filled in March 2008. Calibration of the proposed WHO 6<sup>th</sup> IS will involve assays for all 5 analytes relative to the WHO 5<sup>th</sup> IS and locally collected normal plasma pools. The former comparison is essential for continuity between standards and the latter comparison provides a check on the value of the IU relative to the "fresh plasma unit". The collaborative study is scheduled for September – November 2008 with analysis completed early in 2009. The study will be submitted to SSC for endorsement in July 2009 prior to submission for formal establishment by the Expert Committee on Biological Standardisation of WHO in October 2009.

#### **Proposed 8<sup>th</sup> International Standard for FVIII Concentrate: Update. S. Raut**

The current WHO 7<sup>th</sup> IS Factor VIII concentrate (99/678) was established in 2004 with assigned values for FVIII:C. This standard is used for the potency estimation of FVIII in therapeutic concentrate products, both recombinant as well as plasma derived FVIII, which are used for the treatment of haemophilia. Approximately 600 - 800 ampoules are despatched each year and it is envisaged that stocks of the current WHO 7<sup>th</sup> IS (99/678) will be exhausted by late 2009. Although the current 7<sup>th</sup> IS is plasma derived, materials for its replacement have been sourced from product manufacturers of both plasma derived and recombinant FVIII concentrates. Following assessment of numerous trial fills, four candidates (2 plasma derived & 2 recombinant) have been selected and 20,000 ampoules of each have been filled (March - April 2008). Calibration of the proposed WHO 8<sup>th</sup> IS will involve assays for all 4 candidates (using one-stage APTT & chromogenic methods) -relative to the current WHO 7<sup>th</sup> IS and BRP batch 3/Mega 2US standards. These comparisons are essential for both continuity and harmonization between the 3 different standards. Furthermore, as the BRP batch 3 also requires replacement, this will be a joint project between NIBSC & EDQM to replace both standards. Participants (35 laboratories) have been recruited and the joint collaborative study has been launched (June 2008) with analysis scheduled to be completed in late 2008. The study for the proposed 8<sup>th</sup> IS will be submitted to SSC for endorsement in July 2009 prior to submission for formal establishment by the Expert Committee on Biological Standardization of WHO in October 2009.

#### **Collaborative Study on a Candidate International Genetic Reference Panel for Haemophilia A, Intron-22 Inversion, Human gDNA. E. Gray, W. Pickering, M. Hawkins, J. Boyle, R. Hawkins, P. Metcalfe**

Fourteen laboratories participated in an international collaborative study to assess the suitability of a panel of four genomic-DNA (gDNA) samples as the 1st International Genetic Reference Panel for Haemophilia A, Intron 22 Inversion, Human gDNA, NIBSC Code 08/160. The panel consists of gDNA from a normal male (06/186), a normal female (06/200), a female carrier (06/204) and an affected male (07/116). The participants evaluated the panel against their in-house controls which were characterised patient samples. In total, 166 genotype tests were carried out on the panel, with an error rate of 1.8 %. The findings of this study indicated that this panel is suitable to be used as a reference material for genotyping of Haemophilia A intron 22 inversion mutation. The participants all agreed with the recommendation that this panel (08/160) of four gDNAs should be proposed to

be established as the 1<sup>st</sup> International Genetic Reference Panel for Haemophilia A, Intron 22 Inversion, Human gDNA.

**Closing remarks** – In his concluding remarks, the chair invited the audience to submit work related to standardization issues relevant to the mandate of this subcommittee for possible presentation in the next meeting. He thanked all the co-chairs, speakers and the audience and closed the meeting at 12:00 noon on the 5<sup>th</sup> of July, 2008.

*Submitted by A. Srivastava*