A retrospective study on the influence of the type of FVIII concentrate on the incidence of FVIII inhibitors in previously untreated patients with severe haemophilia A has been published by J. Goudemand et al. after the French workshop. C. Ratignier reported on the results of this study in her presentation.

Discussion and Conclusions

The discussion was opened by two brief presentations.

P. Mannucci presented the protocol of SIPPET (Study on Inhibitors in Plasma-Product Exposed Toddlers). The aim of this international, prospective, controlled (open-label) and randomized clinical trial is to compare the immunogenicity of plasma-derived-VWF-FVIII products with that of recombinant FVIII products, by determining the frequency of inhibitor development in PUPs and MTPs less than 6 years of age. Prophylaxis or on-demand regimens will be chosen by the clinician according to local guidelines and patient preferences. The screening assessment is to be done at the central laboratory, and the patients will be followed-up until 50 EDs or 6 months after the inhibitor occurrence (for discussion see session 5.1).

A. Kallas gave a presentation on the role of VWF in plasma-derived FVIII preparations in the development of FVIII-specific antibodies in a FVIII-deficient mouse model. The ability of FVIII antibodies to interfere with functional FVIII activity (Bethesda assay) was lower in the VWF-treated mice.

Experts agreed there is a clear need for further clinical studies and data on inhibitor formation. Reliable information on the incidence of inhibitor development in PTPs cannot be obtained from post-marketing spontaneous reports. There is the need to standardise the requirements, definitions and methods in pre-and post-marketing as well as in observational studies with PTPs and PUPs. In view of the low patient numbers the need for international collaboration was stressed.

No robust conclusions could be made on the relative risk of inhibitors regarding plasma-derived or recombinant products for the time being even though some signals may be seen.

SESSION 3 METHODOLOGY

The scope of this session, chaired by K. Mertens, was to discuss the current status of the FVIII inhibitor assay including information regarding its optimisation and standardisation as well as new scientific approaches on the detection of inhibitor development. This topic was addressed in seven presentations. In an open discussion a number of specific questions were answered.

Presentations

K. Mertens gave an introductory presentation on inhibitory and non-inhibitory antibodies against FVIII asking what may cause differences. Seventy five percent of detectable FVIII antibodies do not inhibit FVIII activity and are therefore not quantified by the currently used Bethesda assay and its Nijmegen modification. K. Mertens reported on his own results obtained with inhibitory and non-inhibitory anti-FVIII light chain antibodies cloned by phage display technology. A single point mutation in the epitope of one inhibitory antibody resulted in the loss of its inhibitory function. While the specificity of the antibody was not affected by the point mutation, its affinity was reduced by 100 fold. It was concluded that affinity maturation of antibodies might cause a switch from non-inhibitory to inhibitory antibodies or vice versa. The absence of a detectable inhibitor in the Bethesda assay does not exclude an immature antibody directed to a functional FVIII epitope with affinity in nanomolar levels possessing a potential inhibitory effect. Furthermore, the dissociation of the FVIII/FVIII antibody complex may influence residual FVIII activity and could have an impact on the haemostatic effect.

S. Raut presented unpublished data on the step-by-step standardisation of the FVIII inhibitor assay obtained in an exercise during a workshop at the National Institute for Biological Standards and Control (NIBSC). The data showed a stepwise improvement of the performance of the FVIII inhibitor assay with reduction of CVs from 53-80% to 14-20%. An International Collaborative Study to

Standardise anti-FVIII Inhibitor Assays showed a high variability of test results. Critical for the standardisation of the FVIII inhibitor assay are the antibody dilutions, the incubation stage, the choice and standardisation of FVIII activity assay (chromogenic CVs < one-stage CVs) and the need for an international inhibitor standard. NIBSC/ISTH/WHO are currently working on the establishment of a WHO international FVIII inhibitor standard (panel of monoclonal and polyclonal antibodies) and progress will be reported at the ISTH SSC Subcommittee for FVIII and FIX meeting in June 2006.

P. Meijer reported on the outcome of the ECAT Foundation collaborative study, which underlines the NIBSC findings concerning the need for standardisation of the FVIII inhibitor assay. 135 clinical laboratories participated in this study, 69% used the Bethesda assay, 22% the Nijmegen modification of the Bethesda assay and 9% other assays. Interlaboratory CVs ranged from 28% (Nijmegen assay) to 41 % (Bethesda assay). There was a broad range of cut-off values (0-2 IU). The specificity of the assays was acceptable, while the sensitivity was rather poor. This is of interest for the detection of low titre inhibitors, which may or may not be of clinical relevance. In conclusion, further improvement of the specificity, sensitivity, inter- and intra-lab CV is necessary. Laboratories need to strengthen standardisation as shown by conflicting information on assay type and conditions used.

C. Miller reported that the Centres for Disease Control and Prevention (CDC) is setting up a centralized FVIII inhibitor testing program as part of a surveillance project. The testing program includes the Nijmegen modification of the Bethesda assay and the assessment of the impact of various parameters, e.g. collection and transport of blood sample, and sample treatment for removal/inactivation of previously infused FVIII. Furthermore, the project investigates the clinical importance of non-neutralizing antibodies, the possibility to use ELISA methods to screen for FVIII inhibitors, and to compare the influence of chromogenic versus one stage FVIII activity assay on inhibitor test results.

J. Goudemand addressed clinical conditions known to influence FVIII inhibitor measurement. Among these, the time interval since the last infusion of FVIII product is of importance because the infused residual FVIII may mask an inhibitor in the assay. Therefore, anti-FVIII antibodies should be tested pre-infusion when the FVIII level is at its lowest. In addition, especially in children secondary effects due to viral infection (e.g. Lupus anticoagulant) may have a major impact on the inhibitor measurement. Furthermore, occurrence of new FVIII inhibitors was reported in HCV positive haemophilia A patients undergoing treatment with interferon as well as in HIV positive haemophilia A patients having an immune reconstitution inflammatory syndrome associated with anti retroviral therapy.

B. Verbruggen, addressing variables influencing the performance of the Nijmegen modification of the Bethesda assay, concluded in line with previous speakers that further standardisation, e.g. the improvement of sensitivity and the validation of the assay are needed. Specificity and sensitivity are individually influenced by various parameters (pH stability; type of FVIII deficient plasma; vWF content of control sample; FVIII content of normal plasma; chromogenic or one-stage FVIII activity assay). The level of cut-off values is influenced by factors such as the VWF content of the control plasma and the choice of the FVIII activity assay (chromogenic or one-stage assay). This especially affects low titre or borderline inhibitor detection.

J.M. Saint-Remy presented new methods, which can detect the full range of FVIII antibodies in the patient plasma. These include memory B cell epitope mapping and T cell epitope identification. However, only 25% of the currently detected FVIII antibodies are suspected of having an inhibitory effect with clinical correlation. Therefore, the experts agreed, that these immunological methods to detect FVIII antibodies might be of greater use to assess the specific antigenicity of a new or modified FVIII product, rather than representing tools for clinical monitoring of FVIII inhibitor development.

Discussion and Conclusions

The questions below were addressed in the discussion in session 3 and the following consensus reached:

- *What are the critical experimental parameters/conditions for the standardisation of the Bethesda assay for the detection of type I and type II inhibitors?*

The use of the Nijmegen modification of the Bethesda assay is recommended for pre-authorisation studies and also for post-marketing surveillance. The Nijmegen modification requires further optimisation/standardisation and validation for performance improvement. The following parameters were identified as critical for valid inhibitor results: The buffering of the normal plasma and its FVIII content; the type of control plasma (chemically or immune depleted or severe congenital FVIII deficient) and its VWF content; the incubation time; the type of FVIII activity assay (chromogenic or one-stage assay) and its reagents (e.g. phospholipids). The measurement of low-titre inhibitors is recognised as a difficult task. The results of inhibitor measurements should always be interpreted in relation to clinical observations. It was agreed to refer this consensus to the ISTH SSC Subcommittee meeting for FVIII and FIX products in June 2006 for further considerations and input.

Development of standards / reference materials was considered as necessary. ISTH / NIBSC are currently working with the WHO on the implementation of a standard and progress will be reported at the ISTH SSC Subcommittee meeting for FVIII and FIX products in June 2006.

The optimisation of the FVIII inhibitor assay should also address the detection of type II FVIII inhibitors. Type II FVIII inhibitors are mostly linked to acquired haemophilia A, while type I FVIII inhibitor reactivities mainly occur following treatment of severe haemophilia A. However, a modified FVIII plasma-derived product (double virus inactivated) induced type II FVIII inhibitors in PTPs with severe haemophilia A in the past.

- *Are modified tests / in-house methods acceptable and if yes, under which conditions?*

  The Nijmegen modification of the Bethesda assay is acknowledged as a gold standard.

- *Is there value in FVIII inhibitor measurements being carried out in specialised central laboratories? Should testing laboratories be encouraged to participate in proficiency studies?*

  It is recommended to perform the inhibitor assay in a centralised laboratory for pre-licensing studies. The participation of laboratories in proficiency and collaborative studies is essential.

- *How should FVIII inhibitors be defined in terms of borderline/low titre/high titre inhibitors; type I/type II inhibitors; intermittent inhibitors? With regard to the various thresholds applied in different studies (≥ 0.6 BU, 1.0 BU or ≥ 1.0 BU), how should the threshold be defined? Will a single sample / single positive test result qualify for an inhibitor diagnosis?*

  It was emphasised that the diagnosis of an inhibitor should primarily be based on clinical observations and be confirmed by FVIII inhibitor testing in the laboratory. A close collaboration between clinical and laboratory staff is important.

  Consensus was reached on the threshold of ≥0.6 BU for the diagnosis of an inhibitor and of >5 BU for a high titre inhibitor. A confirmatory test on a second, separately drawn sample should always be performed. This second sample should be taken prior to any change of treatment and shortly after the previous positive test (within a month).

- *What are the clinical conditions that influence the results of inhibitor measurement (e.g. significant amount of FVIII in the sample due to timing between last FVIII administration and sampling for FVIII inhibitor measurement)? How to define the clinical parameters / conditions under which inhibitor testing will bring reliable results?*

  Reference is made to the discussion of residual FVIII plasma levels and lupus anticoagulants in session 4.

  Inhibitors have been detected in patients with chronic viral infections (e.g. HIV, HCV) during anti-viral therapy.

- *What is the added value of FVIII antibody measurement by ELISA?*

  The added value of FVIII antibody measurement by ELISA is not yet defined, but is an interesting area of research.

SESSION 4 CLINICAL RELEVANCE OF FVIII INHIBITOR OCCURRENCE

The purpose of session 4, chaired by J. Ingerslev, was to discuss the clinical relevance of FVIII inhibitor occurrence. Three main questions were addressed by experts, dealing with the interpretation of inhibitor results, clinical signs of inhibitor occurrence, and the case of low titre FVIII inhibitors.

In order to answer these questions, a round table discussion was convened with the participation of five experts - G. Auerswald, R. Lassila, C. Lee, P.M. Mannucci and M. van den Berg.

Discussion and Conclusions

- *How to interpret inhibitor results, which are obtained under clinical conditions that may interfere with the FVIII inhibitor measurement?*

P. Mannucci pointed out that clinical conditions could interfere with the FVIII inhibitor measurement but not with the clinical signs of inhibitor occurrence.

This question was addressed under three aspects: content of FVIII in the sample for testing, lupus anticoagulant, and use of heparinised ports. Summaries of these discussions, as well as the consensus reached with all participants, are presented below.

FVIII content in the sample for testing

Presence of FVIII in plasma samples, due to a recent infusion of FVIII or to endogenous FVIII in the plasma of non-severe haemophilia patients, can interfere with the results of the test for FVIII inhibitor measurement. For routine inhibitor testing, preferably no clotting product should have been given in the previous 3 days. The inhibitor test should be performed when the plasma FVIII level has reached a pre-substitution nadir rather than at a specific time point.

The inhibitor assay should be validated for the impact of residual FVIII on the test results. In test samples the residual FVIII level should be low. Heating of the sample in order to inactivate residual FVIII content could circumvent the possible interference, while keeping immunoglobulins intact. (For example, H. Verbruggen uses a one-hour pre-treatment at 58°C; in the French Expert Group Report, a pre-treatment of 30 min at 56°C is recommended if the plasma contains FVIII). The heating pre-treatment needs to be validated. This point should also be referred to ISTH SSC Subcommittee for FVIII and FIX products for review.

Lupus anticoagulant (LA)

LAs are a heterogeneous group of immunoglobulins directed against negatively charged phospholipids in complex with proteins (β_2-glycoprotein I (β_2-GPI), prothrombin and others). Although LA and anti-FVIII inhibitors are dissimilar in terms of specificity and clinical presentation, they are somewhat related; as both of them prolong the phospholipid-dependent coagulation tests. Differentiation of LA from anti-FVIII inhibitors is crucial, because of different therapeutic consequences (i.e. antithrombotic agents in the former or haemostatic agents in the latter). LA is apparently rare in adults, but so is the occurrence of inhibitors in PTPs. Therefore, immediate signs of a positive inhibitor test in a PTP might be due to LA. Small children suspected of having an inhibitor should be also tested for LA, which especially in children are associated with viral infections and are difficult to differentiate from FVIII inhibitors.

Heparinised ports

In general, it is recommended for FVIII inhibitor testing to take blood from a vein and not from a port, as flushed heparin has an impact on FVIII inhibitor measurement.

- *What are the clinical signs for the occurrence of FVIII inhibitors to be monitored in clinical studies (e.g. increased bleeding tendency, high consumption, lack of response or efficacy, decreased recovery, shortened half life)?*

PTPs

It is much easier to detect changes from the patient's usual condition in PTPs as compared to PUPs. All the following parameters are relevant: increased bleeding tendency, high consumption, lack of response or efficacy, decreased recovery, shortened half life. High consumption was a clinical sign in the cluster of inhibitor cases (type II) in PTPs seen in Germany with modified plasma-derived FVIII product (double virus inactivated).

Infants and young children

It is much more difficult to diagnose inhibitor development from clinical signs in infants and young children.

> ***Pharmacokinetics:*** It is not feasible to perform extensive pharmacokinetic investigations in infants and young children. If an inhibitor is suspected, it is recommended to look at the recovery. If the recovery appears greatly reduced and/or no FVIII is detected, an inhibitor is suspected.
>
> It should be kept in mind that the normal pharmacokinetic range may be age-related, with children exhibiting different kinetics (shorter half-life and lower recovery) compared to adults.
>
> ***High consumption:*** This parameter is not a reliable indicator for FVIII inhibitors in infants and young children as there is no "usual condition" to compare with. Furthermore, there are many differences in lifestyle of children (careful or accident-prone), which may lead to dose modifications.
>
> ***Increased bleeding tendency:*** This parameter is unreliable as a clinical sign. Bruising can be observed, but it is difficult to assess objectively.
>
> ***Lack of response or efficacy*:** Poor response to treatment, including prophylaxis, is useful as a clinical sign of inhibitor occurrence. High titre inhibitors may be signified by an increased bleeding tendency (e.g. joint bleeds), while treated under a FVIII prophylaxis regimen. Effectiveness of treatment of a fresh joint bleed is measurable. Low titre inhibitors will probably not be detected under FVIII prophylaxis regimens.

Nevertheless, there are inhibitor patients who do not bleed. Therefore, there is a need for careful monitoring of all parameters. The need for close collaboration between the clinical laboratories and the physicians has been clearly emphasised during the meeting: linking of the results of FVIII inhibitor tests to clinical signs of each patient appears crucial to determine the best therapeutic approach.

- *Some patients with low titre FVIII inhibitors show an increased FVIII consumption. Should the clinical relevance of borderline/low titre and intermittent FVIII inhibitors be further investigated and how should clinical studies and their follow-up be designed to obtain meaningful data?*

Generally there is a lack of knowledge of the clinical relevance of transient and low titre inhibitors. It is not known whether these might become clinically relevant in a PTP later in life. This indicates the importance of prolonged follow-up of patients. However, such long-term data are much more difficult to collect, but data are essential for pharmacovigilance assessment and registries. Another confounder of data relates to the fact that FVIII inhibitor testing is most often carried out in local laboratories only rather than a central laboratory. It was noted that the CDC is developing central laboratory testing for surveillance (see session 3) in the USA.

In this context, consensus on the following points was reached:

- Monitoring of inhibitor development should be started at the onset of treatment in order to obtain solid baseline data.
- Regular laboratory measurements of inhibitors should be implemented in clinical studies. In addition, clinical signs suggesting inhibitor development (see previous question) should be defined and used to trigger investigations between the regular laboratory visits.

- Routine testing for inhibitors should be carried out on a regular basis. In particular, testing should be frequent in the early phases of substitution. However, it was acknowledged that frequent sampling might prove difficult in young children.
- In order to disclose product related immunogenicity, all inhibitors should be documented.
- Linking clinical and laboratory findings is very important.

SESSION 5 CLINICAL DATA – STUDY DESIGN

This session was divided into 5 sub-sessions in order to address all identified issues related to clinical trials and study design in relation to the risk of inhibitor development.

5.1 REGULATORY REQUIREMENTS ON CLINICAL STUDY DESIGN EU/USA

Session 5.1, chaired by J. Ingerslev, addressed the regulatory requirements on clinical study design in EU and USA.

Presentations

A. Hilger presented the EU requirements as outlined in the current CHMP note for guidance on clinical investigation of plasma-derived and recombinant FVIII products. Prior to marketing authorisation, a pharmacokinetic trial should be performed in at least 12 immunocompetent subjects suffering from severe haemophilia. Efficacy, safety and immunogenicity data are required from 50 PTPs (≥ 150 ED, aged > 12 years) with residual FVIII levels ≤ 2%. Immunogenicity data should be based on follow-up over a period of ≥ 50 ED or 6 months. The inhibitor titre should be monitored at baseline and every 3 months using the Nijmegen modification of the Bethesda assay. There is no formal requirement for a PUP study. However, treatment of PUPs should be documented and should not be initiated before data on 50 ED from 20 PTPs included in the clinical trials are available. As outlined in the concept paper for the revision of the guideline, the EU requirements will be revised in line with the outcome of this workshop. Proposals for revision also include dosage regimen, risk management plan, definition of patient characteristics, the paediatric population, and improved design of post marketing studies.

J. Lozier presented the outcome of the 2003 FDA workshop on FVIII inhibitors and the current considerations for clinical study design in USA. At the workshop, the FDA encouraged manufacturers of recombinant products to put data from product development and clinical trials into the public domain. In addition, FDA intended to advance the discussion of inhibitor definition, risk, measurement and regulatory concerns in a public forum. The current FDA approach is to request efficacy and safety in PTPs (defined as >150 ED) rather than in PUPs, and a PK comparative study vs. equivalent recombinant product. Eighty PTPs should be studied for 50 ED to be able to permit the detection of one inhibitor while ruling out by statistical analysis a true inhibitor rate of >5.6%. Paediatric studies are preferred over PUP studies and are required as a phase IV commitment although the protocol should be approved prior to licensure.

Unresolved issues for the FDA were highlighted such as the inhibitor threshold of <1.0 BU, post-marketing surveillance, alternative statistical approaches (Bayesian) and inhibitors in PUP studies.

Discussion and Conclusions

The approach for regulatory requirements for pre-licensing studies clearly differs between Europe and the USA. In Europe it is considered impractical to perform pre-licensing studies with sufficient statistical power to reliably exclude abnormal immunogenicity of a new product. Therefore a pre-licensing study carefully assessing inhibitor occurrence in a limited PTP number is combined with mandatory post-licensing studies and pharmacovigilance. In the USA, the acceptability criteria of "ruling out of a true inhibitor rate of >5.6%" for a new product has been chosen based on observations

made in Canada during the switch from plasma derived concentrates to one recombinant FVIII product.

Several participants highlighted the need for harmonization of the requirements between EU and USA taking account of the limited size of the patient population available.

The experts addressed two questions: the first on the most relevant patient population to be studied for inhibitor development and the second on the use of MTPs.

- *The current CHMP guidance recommends that PTPs rather than PUPs should be used to study product related immunogenicity. Since children may respond differently from adults, children under the age of 6 should also be studied regardless of prior treatment. There is no requirement for specifically studying PUPs. However, the core SPC recommends inclusion of information on the incidence of inhibitors in PUPs and PTPs as addressed in the core SPC section 4.8 Undesirable effects.*

  Is the general philosophy of this approach still valid?

  In daily practice, the clinically most significant and relevant inhibitors occur in PUPs. An important issue in the treatment of PUPs is the choice between plasma-derived FVIII or recombinant FVIII product and the weighing of the potential risks of transmission of infectious agents vs. the risk of inhibitor development. Therefore, some of the haemophilia treaters participating in the meeting strongly recommended the investigation of inhibitor development in PUPs treated with plasma-derived FVIII compared to recombinant FVIII product as the highest priority. To study this aspect in an evidence-based approach, a randomised controlled clinical trial was suggested (for possible trial design see session 2). Experts recognised that in principle a randomised controlled trial is the most reliable study design, but highlighted difficulties to use this design (small patient population, which product to use, dose regimen design (prophylaxis vs. on demand)). It was noted that in some EU countries randomised controlled trials comparing plasma-derived FVIII to recombinant FVIII products are not feasible as only recombinant FVIII products are used.

  At the time of marketing authorisation, the most important question is whether a new plasma-derived FVIII or recombinant FVIII product reveals an abnormal immunogenic profile compared to products already on the market. Since PTPs are considered at low risk for inhibitor development, they should be included in immunogenicity investigations of products because excessive inhibitor formation in PTPs would indicate increased immunogenicity of a product.

  Previous experience with the occurrence of two clusters of inhibitors in PTPs treated with two modified plasma-derived FVIII products showed no abnormal product immunogenicity during the clinical pre-licensing phase, while the first inhibitors occurred post approval (detected 18 months after market introduction for one of the products; most patients had more than 100 ED). Based on this experience and considering the small patient population, it was concluded that full immunogenicity data could not be collected pre-authorisation. It was proposed to require limited data prior to authorisation and to strictly follow-up patients post-authorisation.

  In conclusion, some agreement was reached that inhibitor development in PTPs remains the main focus in pre-authorisation studies. The opinion of experts on the utility of clinical studies in the paediatric population (e.g. PUPs or PTPs, plasma-derived vs. recombinant FVIII) seemed to be divided.

- *Should minimally treated patients (MTPs) be reported together with PUPs?*

  As the population of MTPs is not well defined, participants unanimously agreed not to combine data on MTPs with data on PUPs.