Summary report of the First International Conference on inhibitors in haemophilia A

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Introduction

The First International Conference on Inhibitors in Haemophilia A was held on 4-5 March, 2016. The major theme was the development of factor VIII (FVIII) inhibitors, which occurs in 30-35% of previously untreated children infused with the available commercial products. This meeting was the occasion for a comprehensive discussion on the data that are emerging from recent studies and international registries.

Mechanisms of inhibitor development

Alloantibodies and autoantibodies

Sebastien Lacroix-Desmazes opened this conference with a presentation concerning the mechanism of inhibitor development. The first evidence of the presence of natural anti-FVIII antibodies came in 1992, with the demonstration that heat-treated plasma of 17% of unselected healthy blood donors with otherwise normal levels of circulating FVIII contained antibodies that were able to inhibit the procoagulant activity of FVIII in a functional coagulation assay. It was discovered that such antibodies are natural IgG autoantibodies and/or antibodies directed against epitopes, associated with a so far unidentified allotypic polymorphism of the human FVIII molecule. These findings indicate that the presence of anti-FVIII antibodies is a universal phenomenon, more common than previously thought and that anti-idiotypic antibodies capable of inhibiting the binding of anti-FVIII antibodies to FVIII are produced spontaneously.

Under physiological conditions, there is a steady-state interaction of FVIII with the immune system; at the humoral level, tolerance to FVIII relies on an equilibrium between the recognition of FVIII by naturally occurring, potentially inhibitory anti-FVIII antibodies and their control by neutralising anti-idiotypic antibodies. Neutralising anti-idiotypic antibodies may also regulate the B-cell clones that secrete the FVIII-specific autoantibodies. At the T-cell level, natural FVIII-reactive T cells may be down-regulated by natural regulatory T cells (i.e., CD4+ CD25+ FoxP3+ T cells)

and/or by induced transforming growth factor (TGF-β)-secreting regulatory T cells.

Neutralising antibodies against FVIII remain the major complication of therapy for patients with haemophilia A. In order to understand the evolution of these antibodies better, it was important to generate comprehensive datasets, which included both neutralising and non-neutralising antibodies, their isotypes and IgG subclasses. Literature data revealed significant differences for IgG subclasses of FVIII-binding antibodies among the different study cohorts. IgG4 and IgG1 were the most abundant IgG subclasses in patients with FVIII inhibitors. Strikingly, IgG4 was completely absent in patients with no FVIII inhibitors and in healthy subjects. These findings pointed towards a distinct immune regulatory pathway responsible for the development of FVIII-specific IgG4 associated with FVIII inhibitors. Prompted by these findings, the distinguishing properties among the different populations of FVIII-specific antibodies were investigated. It was hypothesised that the affinity of antibodies would discriminate between the neutralising and non-neutralising antibodies found in different study cohorts. To test this idea, competition-based enzyme-linked immunosorbent assays were designed to assess the apparent affinities for each isotype and IgG subclass of FVIII-specific antibodies without the need for antibody purification. FVIII-specific antibodies, found in patients with FVIII inhibitors, had an up to 100-fold higher apparent affinity than antibodies found in patients without inhibitors and in healthy individuals. FVIII-specific IgG4 found in patients with congenital haemophilia A and FVIII inhibitors expressed the highest affinity of all IgG subclasses. Considering these findings, it was suggested that these antibodies may serve as potential biomarkers for evolving FVIII inhibitor responses in clinical research.

The role of B and T cells and novel therapeutic approaches

David W. Scott presented data on the mechanisms of induction of tolerance to FVIII, focussing on the generation of engineered FVIII-specific human T regulatory cells. The primary immune response is initiated by the internalisation of therapeutically administered FVIII by professional antigen-presenting cells (e.g. dendritic cells) and its subsequent presentation to naïve FVIII-specific CD4+ T cells. Activated CD4+ T cells in turn activate FVIII-specific naïve B cells, which proliferate and differentiate into either plasmocytes (antibody-secreting cells) or FVIII-specific memory B cells. During the secondary immune response, FVIII-specific memory B cells generated during the primary immune response act as antigen-presenting cells and activate FVIII-specific CD4+ T cells. With the help of CD4+ T cells, FVIII-specific memory B cells further differentiate into antibody-secreting cells. In parallel, uptake of FVIII by professional antigen-presenting cells results in activation of T cells that, in turn, activate new FVIII-specific B cells and thus generate additional antibody-secreting cells and memory B cells. Hence, novel therapeutic strategies directed at the elimination of FVIII inhibitors in haemophilia A patients who have developed alloimmunisation to FVIII may be achieved by FVIII-specific targeting of immune effectors, for instance by manipulation of the idiotypic network. Alternatively, immune reactions to therapeutic FVIII may be avoided in previously untreated patients (PUPs) by using structurally modified FVIII, of which the dominant T- and B-cell epitopes and/or structures of FVIII that mediate its internalisation by antigen-presenting cells have been altered. Simultaneously, assessment of the inflammatory state of the patient may help clinicians to reduce the risk of alloimmunisation.

It was suggested that recombinant factor VIII (rFVIII) products are more immunogenic than plasma-derived products (pdFVII). There is biological plausibility that pdFVIII may be less immunogenic than rFVIII. Being extracted from human plasma, these products are more native than FVIII produced by recombinant DNA technology from mammalian cell lines, which causes post-translational modifications in the FVIII molecule. pdFVIII products may be less immunogenic because of their high content of the chaperone protein von Willebrand factor (VWF), which may reduce immunogenicity masking FVIII epitopes and protecting from endocytosis which is mediated by antigen-presenting cells. Moreover, pdFVIII contains human proteins that may have immunomodulatory properties.

Inhibitor data collection systems

United Kingdom, French, Canadian National Registries and European data on previously untreated patients and previously treated patients

A cohort study involving 574 consecutive, previously untreated children with severe haemophilia A, who were born between 2000 and 2010, was designed to evaluate whether the type of FVIII product and switching among products were associated with inhibitor development (RODIN study). In this cohort, recombinant and plasma-derived FVIII products conferred similar risks of inhibitor development, and the content of VWF in the products and switching among products were not associated with the risk of inhibitor development. Second-generation full-length recombinant products were associated with an increased risk, when compared to third-generation products. These results were in agreement with the findings of a similarly designed

study, entitled Concerted Action on Neutralizing Antibodies in Severe Haemophilia A (CANAL) study, in which the risk of inhibitor development was not clearly lower with plasma-derived products than with recombinant products.

Three additional retrospective observational studies from France (FranceCoag), the United Kingdom (UKHCDO) and Canada, presented by *Jenny Goudemand*, substantially confirmed that rFVIII products were associated with an approximately 60% higher inhibitor rate in PUPs. Both the RODIN and FranceCoag cohort studies reported that the cumulative incidence rates of inhibitor development by 75 exposure days (ED) were 32.4% and 40.2% for all inhibitors, and 22.4% and 23.9% for high-titre inhibitors in the RODIN study and in the FranceCoag, respectively.

In addition, *Marijke van den Berg* presented the PedNet Haemophilia Registry, which is a collaborative effort of the European PEDiatric NETwork for haemophilia management. This registry was set up in 2004 by PedNet investigators to promote and facilitate research and healthcare development in children with haemophilia. At the moment, 31 haemophilia treatment centres from 16 countries are collaborating. The aim of the PedNet registry is to include complete cohorts of all newly diagnosed patients born from January 2000 onwards with congenital haemophilia A or B (FVIII/IX≤0.25 IU/dL) who are treated in one of the participating centres. The data collected include many clinical, laboratory and genetic parameters, which makes the registry suitable for a large variety of research questions. Currently 1,531 children are included in the PedNet registry, of whom more than 1,000 have severe haemophilia A or B. The first cohort of patients was born between 2000 and 2010. Inclusion of children born from 2010 onwards (second cohort) is ongoing. Data collected in the PedNet registry (for cohorts I and II) showed that inhibitor development was influenced by many genetic and non-genetic risk factors, and the incidence of inhibitor development following the use of plasma-derived or recombinant products was similar. Moreover the analysis of the data originating from a European Surveillance Registry (EUHASS), presented by *Michael Makris*, showed that there were no class or brand-related differences among the various rFVIII products employed in 68 European centres in PUPs and previously treated patients (PTPs).

Inhibitor development in previously untreated patients

The hypothesis of less inhibitor formation with pdFVIII was supported clinically by a number of observational studies, which found a lower cumulative incidence of inhibitors in PUPs with severe haemophilia A treated exclusively with pdFVIII: 14.5% *vs* 31% for those treated with rFVIII. Although these different inhibitor rates apparently confirmed a higher immunogenicity for rFVIII, the effect of the FVIII source was found to be mainly due to confounders. Hence, meta-analytic findings stemming from observational studies were inconclusive and the performance of randomised trials was necessary.

Results of the SIPPET

On this background, *Frits R. Rosendaal* shared the results of the Study on Inhibitors in Plasma-Product Exposed Toddlers (SIPPET). The SIPPET was an investigator-driven, worldwide, prospective, open-label clinical trial examining 300 PUPs or minimally treated patients with severe haemophilia A and was designed to establish whether or not there was a different incidence of inhibitors between patients randomised to the class of pdFVIII products containing VWF or that of rFVIII not containing VWF. By univariate Cox regression analysis rFVIII was associated with an 87% higher incidence of inhibitors than pdFVIII. For high-titre inhibitors, the rate increased 70%. The associations did not change materially after adjustment for putative confounders: in adjusted models the rate remained elevated by 70-90% for rFVIII *vs* pdFVIII. When the analysis was restricted to sites that had not randomised patients to a second-generation, full-length rFVIII or pdFVIII, the risk associated with other rFVIII concentrates *vs* pdFVIII was still two-fold higher. In conclusion, in this study the class of rFVIII products was associated with a 1.87-fold higher incidence of inhibitors than that of the pdFVIII class. This difference remained even when second-generation, full-length rFVIII concentrates were excluded from the analyses. The results of this randomised study may have implications for the choice of product for the management of PUPs: to treat all PUPs with pdFVIII, to treat first with pdFVIII and then switch to rFVIII, or differentiate low-risk rFVIII and high-risk pdFVIII.

Inhibitor development in previously treated patients

Although common in PUPs, inhibitor development is rare in multiply exposed, well-tolerated patients. In this regard, *Michael Makris* reported that the observed rate of inhibitors in PTPs is approximately 2 per 1,000 patient/years, which makes it difficult to study them and compare rates among different products. He also described a non-evidence-based reluctance to change concentrate because of a greater perceived risk of inhibitor development after product switching. Two reports about outbreaks of inhibitor formation after product switching in the 1990s, two reports from

Canada, one from the United Kingdom, two from Ireland and some others were discussed: the single robust conclusion was that there was no clear signal of increased inhibitor development when switching to or from the currently available factor concentrates.

A recent systematic review considered the studies reporting on PTPs which were included in the Wight and Paisley meta-analysis as well as studies published after 2003, which were identified by a systematic search of MEDLINE, EMBASE, and The Cochrane Library. Studies that investigated the development of inhibitors in PTPs with haemophilia A who were treated with any type of FVIII concentrate and that included at least 25 patients with follow-up were included in the analysis. Thirty-three independent cohorts of PTPs with 4,323 subjects and 43 incident *de novo* inhibitors were found and analysed. This systematic review confirmed a low overall rate of *de novo* inhibitors in PTPs, without any significant effect of putative predictors, including the type of FVIII concentrate. Nevertheless, the proper methodology to address the issue of the comparative immunogenicity of different products and/or associations with switching must be identified. Requisites are to assess the baseline risk, to take into account the attributable risk fraction and to have control groups with the least confounders possible. These conditions can be met in a retrospective fashion, with large, rigorous nested-case control studies embedded in prospective registries, or with large prospective controlled observations. The National Institutes of Health inhibitor study is an example of the former. The EUHASS project is an example of the latter. EUHASS is a pharmacovigilance programme to monitor the safety of treatments for people with inherited bleeding disorders in Europe. Haemophilia treatment centres report adverse events directly to the EUHASS website and regular surveillance reports are produced. It is also recommended that all haemophilia centres and countries planning to switch patients to new FVIII concentrates enrol both switching and non-switching patients in registries, test for inhibitors immediately before the switch and at a minimum of at least 1-2 months after the switch, and formally report their data, either individually or in collaboration.

Clinical significance of low- and high-titre inhibitors

Inhibitors are classified into low or high-responding inhibitors based on a patient's peak inhibitor titre after repeated FVIII exposure. The International Society on Thrombosis and Haemostasis Scientific and Standardisation Committee has recommended that an inhibitor titre of 5 BU differentiates low- from high-responding inhibitors. An antibody titre that is persistently below 5 BU despite repeat challenges with FVIII is considered a low-responding inhibitor. An inhibitor is considered to be high-responding if the assay values have been greater than 5 BU at any time. *Elena Santagostino* reported the cumulative incidence of low-response and high-response inhibitors, adjusted for genetic and non-genetic risk factors, over a 20-year period in the cohort of PUPs with severe haemophilia A from the CANAL study (1990-2000) and PedNet Registry (2000-2009). Patients were consecutively recruited from 31 haemophilia treatment centres in 16 countries and followed until 50 ED or inhibitor development. Inhibitor development was studied in 5-year birth cohorts comparing cumulative incidences. Furthermore, the risk of inhibitor development per 5-year birth cohort was studied using multivariable Cox regression analysis, adjusting for potential genetic and treatment-related confounders. Overall, 926 PUPs were included with a total cumulative inhibitor incidence of 27.5%. The inhibitor incidence increased from 19.5% in 1990-1994 to 30.9% in 2000-2004. The incidence of low-titre inhibitors increased from 3.1% in 1990-1994 to 10.5% in 2005-2009, while the incidence of high-titre inhibitors remained stable over time. In conclusion, the cumulative incidence of inhibitors in PUPs increased significantly between 1990 and 2009, but the incidence of high-titre inhibitors remained stable. The increased inhibitor incidence in severe haemophilia A since 1990 is attributable to the detection of more low-titre inhibitors.

Genetics of inhibitors

How next-generation sequencing can help to understand inhibitor risk better

The risk of developing inhibitors is highest during the first 20 ED. If the patient can be brought through this high-risk period without inhibitor development, the subsequent risk is low. *Johannes Oldenburg* reported that risk factors for inhibitor development could be divided into genetic factors (patient-related) and environmental factors (non-patient-related). Genetic risks include ethnicity, family history, severity of haemophilia, severity of *F8* gene anomalies (i.e., *F8* gene mutations, intron 22 inversion mutation) and immune response genes (i.e., polymorphisms), while environmental factors include treatment regimen, type of concentrate and danger signals. Literature data indicated that minimising danger signals during the first 20 ED may reduce the risk of the formation of inhibitors to FVIII. These results should be confirmed in a large, prospective clinical study. In addition, currently only a few parameters can be built into risk models. Next-generation sequencing may facilitate more complete data assessments and, thus, help to construct more complex risk assessment models.

Treatment of inhibitors

The development of alloantibodies that neutralise FVIII activity renders patients with haemophilia A resistant to FVIII replacement therapy and increases the risk of unmanageable bleeding and of associated morbidity, such as severe arthropathy and disability. Bleeding episodes and surgical interventions in patients with FVIII inhibitors are difficult to control, requiring bypassing agents; the current choices include activated prothrombin complex concentrates (aPCC) or recombinant activated FVII (rFVIIa).

Experience on bypassing therapy in patients with inhibitors. Successes and failures: what else is necessary?

M. Elisa Mancuso reported that both aPCC and rFVIIa have been successfully used to cover major and minor orthopaedic and non-orthopaedic surgical procedures in patients with inhibitors. Moreover, these products have been used to prevent bleeding episodes and reduce bleeding frequency with good results, although not comparable to those of standard prophylaxis in patients without inhibitors. It was demonstrated that aPCC and rFVIIa are equally effective for the treatment of acute bleeds.

It has not so far been demonstrated that there is a significant difference in bleeding frequency between patients with or without inhibitors, but the management of bleeding episodes in the presence of high-titre inhibitors is more problematic. Finally, the risk of thrombotic adverse events is similar and quite low in congenital haemophilia A. The use of these products needs to be optimised and larger cohorts should be studied in order to understand the different haemostatic responses.

The role of non-replacement products (antithrombin, anti-tissue factor pathway inhibitor, bi-specific antibodies)

David Lillicrap underlined that it is important to consider the existence of other strategies to induce haemostasis in patients with FVIII inhibitors: FVIII mimetic therapy (ACE910) and rebalancing haemostasis by anti-thrombin inhibition (fitusiran) and inhibition of tissue factor pathway inhibitor (concizumab). Phase I data indicate that antibody therapy with ACE910 is well tolerated and has a promising efficacy profile in patients with severe haemophilia A. Early data suggest an encouraging reduction in bleeding rates in all patients. ACE910 shows promise as a preventive treatment for haemophilia A, irrespectively of the presence of FVIII inhibitors. Interim results, presented at the American Society of Hematology (ASH) 2015 Annual Meeting, showed that monthly subcutaneous administration of fitusiran achieved potent and dose-dependent lowering of anti-thrombin of up to 88% in patients with haemophilia. This lowering of anti-thrombin was associated with statistically significant increases in thrombin generation and an 85% reduction in estimated median annualised bleeding rates in all evaluable cohorts. The observed bleeding rates were comparable to those reported for prophylactic intravenous infusions of replacement factors in patients with haemophilia. To date, fitusiran has been found to be generally well tolerated: furthermore, there have been no reports of thromboembolic events or clinically significant increases in D-dimer, a biomarker of excessive clot formation. Concizumab showed a favourable safety profile after intravenous or subcutaneous administration of a single dose and non-linear pharmacokinetics were observed due to target-mediated clearance. A concentration-dependent procoagulant effect of concizumab was noted, supporting further study into the potential use of subcutaneous concizumab for the treatment of haemophilia.

Eradication of inhibitors by immune tolerance induction

International data

Charles R.M. Hay spoke about immune tolerance induction (ITI) i.e., the only proven strategy for FVIII inhibitor eradication. No standard ITI regime exists; protocols include the Bonn protocol (high-dose FVIII twice daily), the Malmo protocol and the low-dose van-Creveld protocol. Proposed predictors of success are the following:

- inhibitor titre: historical peak <200 BU, starting titre <10 BU and peak on ITI;
- anti-A2, anti-A1, anti-HC(RAR);
- low-risk *F8* genotype;
- age at start of ITI;
- interval of ≤5 years from inhibitor diagnosis to ITI;
- stopping treatment early or taking breaks in the treatment schedule (missed doses) may interfere with the success of ITI and/or increase the time it takes for the person with inhibitors to achieve tolerance.

Researchers are evaluating whether the type or brand of factor concentrate (intermediate- or high-purity plasma-derived factor concentrates or recombinant products) used in ITI can influence the success of therapy. So far, similar success rates have been obtained with both recombinant and plasma-derived products. With ITI therapy, factor concentrate is given regularly over a period until the immune system is trained to recognise the treatment product without reacting to it. When ITI is successful, inhibitors disappear and the patient's response to factor concentrates returns to normal. The majority of people who undergo ITI will

see an improvement within 12 months, but more difficult cases can require 2 years or longer.

In this context, *Charles R.M. Hay* also presented the results of the ongoing Observational ITI (ObsITI) study that is evaluating ITI in haemophilia A patients with inhibitors and potential predictors of ITI outcome and morbidity; in particular, the influence of VWF in FVIII concentrates. Clinical experience in Germany suggested that ITI may be more successful with FVIII/VWF concentrates using the Bonn protocol (87-91%) compared with some subsequently introduced rFVIII concentrates lacking VWF (success rates 29-54%). Later return to the use of FVIII/VWF concentrates again saw greater success rates of around 80%. ObsITI provides a rare opportunity to prospectively evaluate diverse FVIII products under consistent study conditions. Moreover, ObsITI includes inhibitor patients with poor prognostic risk factors and applies particularly stringent ITI success criteria. Prospective interim ObsITI data were presented for those ITI patients who received a single pdFVIII/VWF concentrate, mainly according to the Bonn protocol. These data are for the largest poor-prognosis group to date to prospectively undergo ITI with a single pdFVIII/VWF concentrate, thus avoiding confounding factors related to product differences. The main objectives were to examine ITI outcomes and the association of prognostic factors with these outcomes. In the interim analysis of the ObsITI study, over 80% of patients had at least one risk factor for poor ITI prognosis at the start of the tolerance induction. Nonetheless, 70.8% achieved complete success, with no relapses, despite exceptionally stringent success criteria. Moreover, 62.9% of patients with ≥1 poor prognostic factor achieved complete success. ITI outcome was significantly associated with inhibitor titre at the start of ITI, number of poor prognostic factors, monthly bleeding rate during ITI and peak inhibitor titre during ITI. In conclusion, treatment with a single pdFVIII/VWF concentrate, mainly according to the Bonn protocol, resulted in a high rate of ITI success in haemophilia A patients with inhibitors and poor prognosis for ITI success. The ongoing ObsITI study will continue to provide valuable prospective reports on ITI in haemophilia A patients with inhibitors.

The Italian experience of immune tolerance induction

Considering the importance of genetic factors, *Giovanni Di Minno* discussed data concerning the relationship between *F8* genotype and ITI outcome in patients with severe haemophilia A and high-responding inhibitors. To investigate this relationship, *F8* mutations were identified in 86 patients recruited as part of the Italian ITI registry (the PROFIT study). ITI outcome was centrally reviewed according to the following definitions: success (undetectable inhibitor and normal FVIII pharmacokinetics); partial success (inhibitor titre <5 BU/mL and/or abnormal FVIII pharmacokinetics); and failure. *F8* mutations known to be associated with a high risk of inhibitor development (large deletions, inversions, nonsense mutations and splice site mutations) were found in 70 patients (81%); among these, the intron 22 inversion was present in 49 patients (57%). In 16 patients (19%) lower-risk *F8* defects (small insertions/deletions and missense mutations) were identified. The latter group of patients showed a significantly higher ITI success rate than those carrying high-risk mutations. On multivariable analysis, the mutation risk class remained a significant predictor of success; other significant predictors were inhibitor titre at the start of ITI and peak titre during ITI. In conclusion, the success of ITI is influenced by *F8* genotype. This knowledge should contribute to the stratification of prognosis and to the clinical choices made regarding ITI for patients with high-responding inhibitors.

Inhibitors in mild haemophilia

The INSIGHT study

The association between *F8* mutation and inhibitor development was also observed in patients with non-severe haemophilia A (FVIII 2-40 IU/dL). In this regard, *Alice S. van Velzen* presented the INSIGHT study. This analysis included 1,112 patients with non-severe haemophilia A from 14 centres in Europe and Australia that had genotyped at least 70% of their patients. Inhibitor risk was calculated as a Kaplan-Meier incidence with cumulative number of ED as the time variable. During 44,800 ED (median, 24 ED per patient; interquartile range [IQR], 7-90), 59 of the 1,112 patients developed an inhibitor, with a cumulative incidence of 5.3% after a median of 28 ED. The inhibitor risk at 50 ED was 6.7% and at 100 ED the risk had further increased to 13.3%. Among a total of 214 different *F8* missense mutations 19 were associated with inhibitor development. These results highlight the importance of *F8* genotyping in non-severe haemophilia A.

Optimal diagnosis and treatment of patients with acquired haemophilia

This Conference also considered acquired haemophilia A which is an autoimmune disease caused by an inhibitory autoantibody to FVIII. The approximate incidence of this condition is 1.48 cases/million per year. The therapeutic aim is two-fold: control of bleeding (of variable intensity at presentation) and eradication of the antibody by immunosuppressive treatment.

Craig Kessler reported that bleeds may be severe and potentially life-threatening in >70% of cases: in the EACH2 Registry 70.3% of the bleeding episodes that

occurred were rated as severe. Most deaths within the first week are due to gastrointestinal and lung bleeding, later deaths result from intracranial and retroperitoneal haemorrhages. Fatal bleeding can occur up to 5 months after the first presentation if the autoantibodies are not eliminated. Diagnostic delays have a significant impact on the interval between onset of bleeding and the start of haemostatic therapy. First-line treatment options for bleeding episodes are rFVIIa and aPCC. Prophylactic use of bypassing agents prior to minor or major invasive procedures is recommended. Acute reduction or removal of the inhibitor to facilitate haemostasis using plasmapheresis or immunoadsorption may be applied under special circumstances. First-line immunosuppressive treatment is represented by corticosteroids ± cyclophosphamide.

Eradication of autoantibodies

In this regard, *Peter Knöbl* reported the results of a German, Austrian, and Swiss registry (GTH-AH study) of acquired haemophilia in order to identify prognostic factors that may guide the choice of immunosuppressive treatment to eradicate the autoantibody contributing to the outcome of the syndrome. Immunosuppressive treatment is associated with frequent adverse events, including infections as a leading cause of death. Predictors of time to remission could help to guide the intensity of immunosuppressive treatment but have not been established. Prognostic factors were analysed in 102 prospectively enrolled patients treated with a uniform immunosuppressive treatment protocol. Partial remission (defined as no active bleeding, FVIII restored to >50 IU/dL, haemostatic treatment stopped for >24 hours) was achieved by 83% of patients after a median of 31 days. Patients with a baseline FVIII <1 IU/dL achieved partial remission less often and later (77%, 43 days) than patients with a baseline level ≥1 IU/dL (89%, 24 days). After adjustment for other baseline characteristics, low FVIII levels remained associated with a lower rate of partial remission. In contrast, the achievement of partial remission in ≤21 days on steroids alone was more common in patients with FVIII ≥1 IU/dL and inhibitor concentration <20 BU/mL. Low FVIII was also associated with a lower rate of complete remission and decreased survival. Thirty percent of the adverse events occurred in relation to the immunosuppressive treatment, including infections, which were the leading cause of death. A high rate of infections and related mortality has been confirmed in the available studies (11%, 4%, and 12% of patients in a United Kingdom surveillance study and in the EACH2 and SACHA registries, respectively), whereas death due to bleeding, compared with historical data, has decreased substantially in the last few years (from 22% to 2.9%).

In conclusion, presenting FVIII levels and inhibitor titre are potentially useful to tailor immunosuppressive treatment in acquired haemophilia A. Hence, this study established clinically useful prognostic factors for remission and survival of patients with acquired haemophilia A. However, it also confirmed that current immunosuppressive treatment regimens often require a very long time to achieve remission and that side effects still cause considerable morbidity and mortality. The challenge for future studies will be to develop immunosuppressive treatment regimens that reduce the burden of side effects, potentially by tailoring their intensity to prognostic baseline characteristics established in the current study.

Cost of inhibitor treatment. A pharmaco-economic perspective

During this meeting, the cost of inhibitor treatment was also considered. *Lorenzo G. Mantovani* focussed on the fact that inhibitors in patients with haemophilia are a complication causing pain and disability, thus impairing quality of life. The Cost of Care - Inhibitors Study (COCIS) group and the Immune Tolerance Economics Retrospective (ITER) study showed that haemophilia complicated by inhibitors required very substantial resources for management in order to provide a satisfactory quality of life. The aim of medical science is to improve the capacity to treat the most severe manifestations of the disease effectively. The expectation is that society will reward this pursuit of new medical knowledge by providing the resources enabling it to be translated into better care for patients.

Patients' needs were identified: improve medical understanding (risk factors and clinical management); facilitate access to diagnosis and treatment; inform and educate.

Finally, specific standards are needed on tailored comprehensive care and all stakeholders must be engaged.

Patients' views and expectations: WFH and EHC initiatives

Mark Brooker from the World Federation of Hemophilia (WFH) stated that, with the exception of lack of access to treatment, inhibitor development is the most significant challenge in haemophilia care. The needs of patients with inhibitors include improved medical understanding which translates into possible prevention and better management for patients. This also means determining good clinical management, for example, how best to treat patients with inhibitors, how to provide prophylaxis for patients with haemophilia with inhibitors, how to ensure haemostasis during surgery, how best to manage acute bleeds and what

new products can do this, etc. The WFH's efforts to address the needs of patients with inhibitors include the promotion and expansion of research, focussing on patients' risk in addition to product risk. The WFH also promotes data collection and international collaboration, encouraging each patient to be in a registry; harmonises definitions; and ensures that there is a way to monitor large groups of patients over the long term. To improve diagnosis and access to treatment products, the WFH runs laboratory training workshops and has produced an online video demonstration of inhibitor assays (coming later this year). Inhibitor testing has been added to International External Quality Assessment Scheme (IEQAS) for monitoring the capacity of centres to make accurate diagnoses. The WFH produces educational resources for patients, families and healthcare providers, including patient booklets, resources for physicians, panel discussions, online demonstration videos on laboratory testing and a dedicated eLearning centre on a new platform, to be launched in July.

In Europe, given the small numbers of people with haemophilia who have inhibitors in each nation, these patients, their families and caregivers are an underserved and isolated subgroup within the European haemophilia population and face significant personal and systemic challenges, including insufficient treatment, lack of information, etc. *Amanda Bok* presented results from two surveys that the European Haemophilia Consortium (EHC) conducted among national member organisations and individual patients/family members/caregivers, respectively, and outlined the main elements of the European Inhibitor Network (EIN) programme, which was built based on the surveys' findings. The EIN is a new multi-faceted, multi-annual programme of the EHC seeking to: improve understanding of this subpopulation's specific needs and how to meet them; build a community of inhibitor patients, families and caregivers to promote mutual support, education and empowerment; provide education and advocacy training to support direct engagement with decision-makers; and work with medical experts towards an agreed framework of treatment and care.

How EMA and FDA are tackling the problem of inhibitor development

International authorities (the European Medicines Agency [EMA] and the American Food and Drug Administration [FDA] represented by *Anneliese Hilger* and *Zuben E. Sauna*, respectively) give guidance when an application for a marketing authorisation for recombinant or human plasma-derived FVIII products is made for use in treatment and prevention of bleeding in patients with haemophilia A. The guidance covers clinical investigations to be conducted before and after marketing authorisation. Guidance is also provided for authorised products when a significant change in the manufacturing process has been made.

Efficacy must be demonstrated in clinical trials, which need to be conducted before marketing authorisation is requested and combined with the commitment to perform post-authorisation investigation(s) to collect additional clinical data and to create a bridge in the long-term between outcomes in clinical trials and outcomes following routine use. When clinically evaluating human plasma-derived or recombinant coagulation factors for the treatment of haemophilia A, the initial trial typically examines the pharmacokinetics of the main active factor. Furthermore, the clinical efficacy of FVIII treatment (e.g. prophylaxis, on demand) should be assessed during a period of a minimum of 50 ED by the patients themselves and the treating physicians.

Safety aspects of FVIII products include viral safety, immunogenicity and other adverse events. The occurrence of neutralising antibodies to FVIII, which is a major complication in haemophilia A treatment, is considered to be a serious adverse event and should be recorded and reported; this requirement should be included in all study protocols.

In general, immunogenicity should be investigated prior to requesting marketing authorisation and substantiated with post-marketing studies. The risk of inhibitor occurrence is higher in patients with severe haemophilia A than in patients with moderate and mild disease; the genotype (high risk: inversions, large deletions or nonsense mutations of the *F8* gene) and ethnic background of the patient are also relevant. In addition, risk may be associated with treatment initiation in PUPs, with switching treatment or with alterations to the antigenicity of a product due to changes in manufacturing processes. PTPs are the most suitable candidates in whom to test the product-related immunogenicity of a FVIII product. The diagnosis of a FVIII inhibitor will be based on clinical observations and be confirmed by FVIII inhibitor testing in the laboratory.

Neutralising antibodies are the most important immunological concern and, therefore, the following aspects and basic principles should be considered:

- inhibitor development should be evaluated in PTPs (>150 ED, suffering from severe haemophilia A with a FVIII level <1%);
- the modified Nijmegen method of the Bethesda assay should be used. Validated testing should be performed in a central laboratory;
- in the case of positive results for an inhibitor, inhibitor retesting using a second separately drawn sample should be performed in a central laboratory for confirmation. The sampling time-points should be recorded and included in the serious adverse events report;

- the defined thresholds are ≥0.6 BU for "a low-titre" inhibitor and >5 BU for a "high-titre" inhibitor;
- preferably, inhibitor testing should be performed when the FVIII level has reached baseline;
- patients' characteristics should be recorded in detail: ethnicity, family history, lifestyle, general health status, infection status, type of *F8* gene mutation, reason for treatment, date of treatment initiation, kind of treatment (on demand, prophylactic, continuous infusion).

Since children may respond differently compared to adults, so a multicentre trial should include at least 50 children allocated to two age cohorts. A minimum of 25 patients should be PTPs aged 6-12 years and at least 25 patients should be <6 years who have undergone >50 ED with previous FVIII products. The clinical trial in children <12 years should not started before safety has been proven for 50 ED each in 20 patients, who are included in the PTP trial of patients ≥12 years. The post-marketing investigation can include PTPs (>150 ED) of any age, provided that a balanced age distribution can be achieved (approximately 60 patients <12 years out of 200 patients). Furthermore, patients <12 years can only be enrolled in the post-marketing investigation when the pre-authorisation study in children <12 years has been completed.

The approval of the indication in PUPs will be based on a clinical trial in a minimum of 50 PUPs evaluated for efficacy and safety during at least 50 ED connected with a post-approval commitment to follow up at least 100 PUPs (50 from the efficacy/safety trial and 50 new patients) for a minimum of 100 ED. The clinical trial in PUPs should commence when data are available from the 20 patients participating in the trial of children <12 years with 50 ED each, including a minimum of 10 patients <6 years, and when pharmacokinetic investigations in children have been performed. A PUP study needs to be conducted for all novel rFVIII products, such as novel genetic constructs or modifications of the FVIII molecule in order to alter its *in vivo* properties (e.g. pharmacokinetics) and for FVIII products manufactured with novel production methods (e.g. a new cell line with which there is limited experience). In the case of pdFVIII products (e.g. manufactured with novel methods), the need for PUP studies will be considered on a case-by-case basis.

All these considerations underline the importance that each PUP should be included in a registry, at least a national one. Moreover, there is a strong need to define a minimum common and relevant dataset and/or to organise the conditions of interoperability between registries in order to collect information on inhibitor incidence in this population rapidly at the international level. This is an urgent task as several innovative concentrates will soon be introduced on the market for which there is limited information available on their long-term immunogenic risk. On the other hand, in this regard, it is important to underline that no randomised clinical trials (except for SIPPET) are available to provide the evidence that is needed: it is not possible to reach definitive conclusions on the incidence of inhibitors with each of the FVIII products, because of differences in the study designs of safety trials. Since it is not known whether new recombinant products are more immunogenic than plasma-derived ones, there is a need for randomised clinical trials to provide definite answers to the questions concerning the immunogenicity of FVIII products.

Disclaimer

This summary is not a full and complete recitation of the conference. It is an attempt to capture, in broad terms, the nature and the scope of the comments. The summary has been prepared in an effort to highlight key elements of the presentations in a concise format, not to replace them. Every effort has been made to avoid mischaracterisation and to present the views provided fairly. Any failure to do so is unintentional.

Images are publicly available from the Speakers' presentations published at the Conference website www.smc-media.eu/inhibitors/ and sourced to the original source when available.

The Authors declare no conflicts of interest.

Arrived: 7 September 2016 - Revision accepted: 29 September 2016
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