

Meeting Report: Ninth and Tenth Workshops of the European Paediatric Network for Haemophilia Management (PedNet)

K. KURNIK * and A. E. THOMAS† ON BEHALF OF THE EUROPEAN PAEDIATRIC NETWORK FOR HAEMOPHILIA MANAGEMENT (PEDNET)¹

*Dr von Haunersches Kinderspital, University of Munich, Munich, Germany; and †Royal Hospital for Sick Children, Edinburgh, UK

Summary. This meeting report presents an overview of the discussions at the ninth and tenth workshops of the European Paediatric Network for Haemophilia Management (PedNet) that occurred in 2005 and 2006. Among numerous topics, a major theme

of these workshops was the formation of inhibitors to replacement factor.

Keywords: factor IX, factor VIII, haemophilia A, haemophilia B

Introduction

The European Paediatric Network for Haemophilia Management (PedNet) is a collaborative group of 23 paediatricians from 16 countries across Western Europe. This report summarizes discussions at the ninth PedNet workshop in Juan les Pins, France, 22–24 September 2005, and the 10th PedNet workshop in Amsterdam, the Netherlands, 28–30 September 2006.

Inhibitor formation in children with haemophilia

Major discussion points of both workshops were predisposing factors to the development of factor VIII inhibitory antibodies and management of patients with an inhibitor.

Inhibitor development and the neonatal immune system

Guest speaker in 2005, Sebastian Lacroix-Desmazes of INSERM in Paris, France, outlined an immunol-

ogist's view of inhibitor development in young children. Although favouring tolerance over activation [1], the neonatal immune system is capable of mounting responses to protein vaccines from 6 weeks of age. Therefore, an immune response to early infusion of FVIII is not unexpected because: (i) the absence of FVIII prevents tolerization in neonates with severe haemophilia; (ii) autoreactive antibodies to FVIII have been identified even in healthy individuals [2]; (iii) the natural occurrence of anti-idiotypic antibodies can modulate the immune system in addition to blocking FVIII inhibitors [3]; (iv) anti-FVIII and anti-idiotypic antibodies, but not antigen, are present in the haemophilic fetus; (v) FVIII recognition during pregnancy might lead to a maternal autoimmune prone status and (vi) maternal IgG might imprint the neonatal immune repertoire via transplacental idiotype regulation [4].

Genetic factors impacting inhibitor development

Jan Astermark from the Malmö University Hospital in Malmö, Sweden, lectured on potential genetic predispositions for inhibitor development beyond those related to the FVIII gene mutation. Ethnicity and a close genetic relationship have been identified as risk factors for inhibitor formation [5–7]. Sibling data from the Malmö International Brother Study (MIBS) showed that the type of FVIII gene mutation is a risk factor for inhibitor formation, but other

¹Full PedNet member list found in the Appendix.

Correspondence: Dr Karin Kurnik, Dr v. Haunersches Kinderspital, University of Munich, Lindwurmstr. 4, D-80337 Munich, Germany. Tel.: 0049 89 51 60 28 11/2853; fax: 0049 89 51 60 44 53; e-mail: karin.kurnik@med.uni-muenchen.de

Accepted after revision 24 April 2007

genetic and environmental factors also contribute significantly to the risk [7].

The interleukin genes are involved in antibody-mediated immune responses. A MIBS analysis identified a promotor region polymorphism of the IL-10 gene that was associated with an elevated risk for inhibitor development (odds ratio 4.4). IL-10 plays a role in antibody formation in Systemic Lupus Erythematosus, an autoimmune disease characterized by B-cell hyperactivity. Also, in African-Americans, polymorphisms in the IL-10 promoter, though different from the inhibitor-associated polymorphism identified in the MIBS study, have been associated with high antibody production.

Age at first exposure and choice of therapeutic regimen

Carmen Escuriola Ettingshausen from the Johann-Wolfgang-Goethe University Hospital in Frankfurt, Germany, presented inhibitor research from the Frankfurt treatment centre and an ongoing study of the German Society of Thrombosis and Haemostasis Research (GTH). In a cohort of prospectively followed previously untreated patients (PUPs) from Frankfurt with severe haemophilia A, no correlation was found between age at first exposure and inhibitor formation. Of 23 patients who had received primary prophylaxis, only one transient inhibitor was detected compared to 18 of 43 (42%) patients who had received secondary prophylaxis or on-demand therapy. This suggests that early initiation of prophylaxis may protect against inhibitor formation. Similarly, a GTH study analysis of exogenous risk factors in PUPs showed no correlation between inhibitor formation and age at first exposure, and primary prophylaxis had an apparent protective effect on inhibitor formation compared to on-demand treatment.

Elena Santagostino from the A. Bianchi Bonomi Haemophilia and Thrombosis Centre in Milan, Italy, presented data from an Italian case-controlled study that evaluated various potential risk factors for inhibitor development in children [8]. In this study, patients who started prophylaxis at an early age had a significantly smaller risk of developing inhibitors than patients started on prophylaxis when older, even after adjustment for known genetic factors and age at first exposure. The study also found no statistically significant differences between the inhibitor group and non-inhibitor controls with respect to prenatal and perinatal events such as amniocentesis or villocentesis and premature or caesarean birth, FVIII

infusions given during a period of infection or vaccination, surgical procedures, CNS bleeding, duration of breast-feeding and patient age at treatment initiation. Family history of inhibitor and null gene mutations were again associated with an increased risk of inhibitor development.

Choice of factor concentrate

Controversy surrounds whether or not choice of a recombinant FVIII (rFVIII) or plasma-derived FVIII (pdFVIII) has an impact on inhibitor formation. Guest speaker in 2006 Gil White, of the Blood Center of Wisconsin, Milwaukee, WI, USA, reviewed the literature regarding potential differences between rFVIII and pdFVIII. Peptide antigen fragments are displayed by antigen-presenting cells (APCs) to T cells. Unlike B-cell-mediated immune mechanisms, the T-cell process should recognize identical protein sequences similarly regardless of three-dimensional structural differences. Mouse data suggest that pdFVIII and rFVIII are immunologically indistinguishable [9]. Moreover, there is no real difference observed in the rates of inhibitor development between pdFVIII and rFVIII in previously treated patients (PTPs), the ideal population for assessing immunogenicity [10], arguing in favour of them being the same immunologically.

Hervé Chambost, of the University Children Hospital in Marseilles, France, presented the results of recent studies conducted in France that evaluated inhibitor risk associated with FVIII concentrate. Goudemand *et al.* [11], using a combination of retrospective and prospective data, showed a higher relative risk (2.4; $P = 0.049$) for inhibitor development in PUPs treated with rFVIII ($n = 86$) compared with pdFVIII ($n = 62$). Though not comparative, other unpublished French retrospective studies support a low inhibitor rate with pdFVIII. The FranceCoag Network database, with a cohort of more than 220 PUPs, should also contribute important information. The French data are all based on a single von Willebrand factor (VWF)-containing pdFVIII produced by LFB, and so may not be broadly applicable. Importantly, these findings will need to be confirmed in prospective comparative cohorts or randomized-controlled clinical trials, in which inhibitor testing is performed identically in both cohorts.

Angela Thomas, of the Royal Hospital for Sick Children, Edinburgh, Scotland, presented inhibitor data from a retrospective cohort study by the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) Paediatric Working Party. The study included 348 children with severe haemophilia A and over 40 exposure days (EDs) [12]. The cumulative

inhibitor incidence was 20% over a median treatment period of 21 months, and half the cases were high-titre inhibitors. Univariate analyses showed a higher incidence of inhibitors with rFVIII products than with pdFVIII products. The pdFVIII group represented high and intermediate purity concentrates, some containing VWF and others not. However, the differences between products were not statistically significant in the multivariate analysis where only genetic mutation of the FVIII gene was shown to be significant

Escuriola Ettingshausen presented the results of a study to evaluate the use of a single rFVIII product (Kogenate™ Bayer) in young children with severe haemophilia [13]. The study population ($n = 61$) consisted of PUPs and minimally treated patients (MTPs), who had received ≤ 4 previous treatments. In this study, nine of 60 (15%) evaluable patients developed an inhibitor [14]. Genetic profiling indicated that the study population was at comparable risk for inhibitor development as the general severe haemophilia A population [15].

The CANAL Study

Marijke van den Berg of the Van Creveldkliniek in Utrecht, the Netherlands, presented data from the retrospective CANAL study, which collected data on 376 PUPs with severe haemophilia A (FVIII:C $< 2\%$). A multivariate analysis using data from 316 patients treated with known FVIII products for their first 50 EDs showed no statistical difference in the risk of clinically relevant inhibitor development between patients treated with rFVIII and pdFVIII products [16]. Moreover, there was no evidence linking VWF content in FVIII products with the risk of inhibitor formation. The study also found that switching between FVIII products did not put patients at an increased risk for inhibitor formation on the new product.

Immune tolerance induction

There is also controversy surrounding the efficacy of FVIII products for immune tolerance induction (ITI). Manuel Carcao from the Hospital for Sick Children, Toronto, Canada, presented the findings of a retrospective study of ITI in 32 patients with haemophilia [17]. Twenty-three patients received daily FVIII infusions (mean 98 IU kg^{-1}) and nine patients received FVIII at 50 IU kg^{-1} three times per week. Overall, ITI was successful in 79.3% (23 of 29) of patients and there was no statistically significant difference in the success rates between patients who

received ITI treatment exclusively with rFVIII and those who received a pdFVIII.

Treatment strategies

The PedNet group discussed potential treatment strategies that might reduce the risk of inhibitor development and reached the following recommendations based on the group's collective experience. Additional studies are necessary to address the relationships between particular treatment strategies and inhibitor formation fully.

1. High-dose levels either due to frequent infusion or use of whole vials should be avoided during the first 20 exposures. Dose should be determined according to patient body weight rather than utilization of a complete product vial per treatment.
2. Surgical procedures should be avoided for the first 20 EDs where possible.
3. Consideration whether after first exposure, continuation of factor administration with prophylaxis might reduce inhibitor development.
4. Initiation of prophylaxis at an early age may be considered due to the possible reduced risk of inhibitor formation.

Bleeding in neonates with haemophilia

Why do newborns with haemophilia rarely bleed?

Wolfgang Muntean of the Medical University of Graz, Austria, presented research on the biochemistry of neonatal coagulation. Despite lower levels of prothrombin complex and clotting factors such as XI and XII compared to adults, newborns do not exhibit an increased tendency to bruise or bleed [18]. With low levels of tissue factor, cord blood clots faster and achieves higher thrombin levels compared to adult blood, a finding that may be related to low levels of circulating anticoagulant proteins such as antithrombin, tissue factor pathway inhibitor, activated protein C and protein S in neonates [19]. As there is often minimal difference in thrombin generation between normal neonates and those with haemophilia [20], it is possible that the low anticoagulant levels may be the biochemical basis for the lower than expected incidence of bruising and bleeding in neonates with haemophilia [21].

Intracranial haemorrhage

Rolf Ljung of Lund University, Malmö, Sweden, reviewed published studies reporting the frequency

of intracranial haemorrhage (ICH), which suggest that in the absence of prophylaxis treatment approximately one in 20 neonates with haemophilia develop a clinical ICH. The incidence in older children is at least as high. Therefore, ICH is surprisingly frequent and appears to be overlooked as a problem, particularly in the postneonatal period.

Adolescents with haemophilia

A new book discussed by author Martti Siimes, of the Hospital for Children and Adolescents, University of Helsinki, Finland, and co-authored by Veikko Aalberg and Pia Petrini, seeks to improve the treatment of adolescents with haemophilia [22]. Normally, boys shift their primary emotional focus from their mothers as a child, towards their fathers during puberty, and to their peers postpuberty, but the potential overprotectiveness of mothers whose sons have haemophilia may prolong the child–mother relationship. It is recommended that adolescent patients visit the treatment centre without their mothers for routine care. Furthermore, doctors can help adolescent development proceed normally by providing patients with haemophilia-positive feedback on their development, emphasizing that pubescent changes are proceeding properly, and encouraging communication between doctor and patient.

Current status of gene therapy in haemophilia

Guest speaker in 2006, Thierry VandenDriessche of the Institute for Biotechnology, University of Leuven, Belgium, reviewed advances in gene therapy for haemophilia [23], which has recently focussed on development of improved viral vectors to increase transfection efficacy and reduce immunogenicity. New AAV vectors of serotypes AAV8 and AAV9 can deliver to muscle or liver and, in the case of AAV9, to cardiac cells [24]. Using a platelet-specific promoter, FVIII can be released only in the time and place it is required, which is at the bleeding site, keeping FVIII masked from the immune system when not needed [25].

PedNet Haemophilia Registry

The PedNet Haemophilia Registry was initiated to improve the understanding of disease pathophysiology, current clinical management of children with haemophilia, and the safety and efficacy of treatment strategies [26,27]. Baseline data are registered on all children born in 2000 or later. The Registry is being coordinated by the Van

Creveldekliniek in Utrecht, with Marijke van den Berg serving as principal investigator. As of September 2006, 15 PedNet treatment centres have entered baseline data on over 250 patients. The RODIN study, which aims to identify factors that induce inhibitors, is the first research project based on Registry data [28].

Acknowledgements

Drs Andrea Trawinski and Barry Lubarsky for editorial assistance, and Prof. Ljung for critical review. PedNet is supported by funding from Bayer HealthCare Pharmaceuticals, Hematology/Cardiology (Leverkusen, Germany).

Appendix

The members of PedNet do not represent their respective countries or any national organization.

2005–06 Members of the PedNet: Sophie Aronis-Vournas, Athens, Greece*†; Günter Auerswald, Bremen, Germany; Marijke van den Berg, Utrecht, the Netherlands*†; Elio Boeri, Genoa, Italy; Hervé Chambost, Marseille, France*†; Niels Claussen, Aarhus, Denmark†; Ségolène Donadel-Claeysens, Toulouse, France*†; Christine van Geet, Leuven, Belgium†¹; Anders Glomstein, Oslo, Norway; Frank Hill, Birmingham, UK*†; Rainer Kobelt, Bern, Switzerland*†; Wolfhart Kreuz, Frankfurt, Germany²; Karin Kurnik, Munich, Germany*†; Ri Liesner, London, UK*†; Rolf Ljung, Malmö, Sweden*†; Wolfgang Muntean, Graz, Austria*†; Rosario Perez Garrido, Seville, Spain*†; Pia Petrini, Stockholm, Sweden†; Anne Rafowicz, Paris, France*†; Lino Rosado, Lisbon, Portugal; Martii Siimes, Helsinki, Finland*†; Owen Smith, Dublin, Ireland³; Angela E. Thomas, Edinburgh, UK*†.

*Present at the ninth workshop; †present at the 10th workshop; ¹represented at the ninth workshop by Veerle Labarque; ²represented at the ninth and 10th workshops by Carmen Escuriola Ettingshausen; ³represented at the ninth workshop by Beatrice Nolan.

References

- 1 Madoiwa S, Yamauchi T, Hakamata Y *et al.* Induction of immune tolerance by neonatal intravenous injection of human factor VIII in murine hemophilia A. *J Thromb Haemost* 2004; 2: 754–62.
- 2 Moreau A, Lacroix-Desmazes S, Stieltjes N *et al.* Antibodies to the FVIII light chain that neutralize FVIII procoagulant activity are present in plasma of non-responder patients with severe hemophilia A and in

- normal polyclonal human IgG. *Blood* 2000; **95**: 3435–41.
- 3 Sultan Y, Kazatchkine MD, Maisonneuve P, Nydegger UE. Anti-idiotypic suppression of autoantibodies to factor VIII (antihaemophilic factor) by high-dose intravenous gammaglobulin. *Lancet* 1984; **2**: 765–8.
 - 4 Kohler PF, Dubois RS, Merrill DA, Bowes WA. Prevention of chronic neonatal hepatitis B virus infection with antibody to the hepatitis B surface antigen. *N Engl J Med* 1974; **291**: 1378–80.
 - 5 Scharrer I, Bray GL, Neutzling O. Incidence of inhibitors in haemophilia A patients—a review of recent studies of recombinant and plasma-derived factor VIII concentrates. *Haemophilia* 1999; **5**: 145–54.
 - 6 Gill JC. The role of genetics in inhibitor formation. *Thromb Haemost* 1999; **82**: 500–4.
 - 7 Astermark J, Berntorp E, White GC, Kroner BL. The Malmo International Brother Study (MIBS): further support for genetic predisposition to inhibitor development in hemophilia patients. *Haemophilia* 2001; **7**: 267–72.
 - 8 Santagostino E, Mancuso ME, Rocino A *et al*. Environmental risk factors for inhibitor development in children with haemophilia A: a case-control study. *Br J Haematol* 2005; **130**: 422–7.
 - 9 Pittman DD, Alderman EM, Tomkinson KN *et al*. Biochemical, immunological, and in vivo functional characterization of B-domain-deleted factor VIII. *Blood* 1993; **81**: 2925–35.
 - 10 White GC, DiMichele D, Mertens K *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 Haemost* 1999; **81**: 462.
 - 11 Goudemand J, Rothschild C, Demiguel V *et al*. Influence of the type of factor VIII concentrate on the incidence of factor VIII inhibitors in previously untreated patients with severe hemophilia A. *Blood* 2006; **107**: 46–51.
 - 12 Chalmers EA, Brown SA, Keeling D *et al*. Early factor VIII exposure and subsequent inhibitor development in children with severe haemophilia A. *Haemophilia* 2007; **13**: 149–55.
 - 13 Kreuz W, Gill JC, Rothschild C *et al*. Full-length sucrose-formulated recombinant factor VIII for treatment of previously untreated or minimally treated young children with severe haemophilia A: results of an international clinical investigation. *Thromb Haemost* 2005; **93**: 457–67.
 - 14 Lusher JM. First and second generation recombinant factor VIII concentrates in previously untreated patients: recovery, safety, efficacy, and inhibitor development. *Semin Thromb Hemost* 2002; **28**: 273–6.
 - 15 Oldenburg J, Ivaskevicius V, Schroder J, Muller CR, Ganguly A. Genetic background and inhibitors in previously untreated or minimally treated young patients with severe haemophilia A treated with sucrose-formulated recombinant factor VIII. *Thromb Haemost* 2006; **95**: 903–5.
 - 16 Gouw SC, van der Bom JG, Auerswald G *et al*. Recombinant versus plasma-derived factor VIII products and the development of inhibitors in previously untreated patients with severe hemophilia A: the CANAL cohort study. *Blood* 2007; doi: 10.1182/blood-2006-11-056317.
 - 17 Barnes C, Rivard GE, Poon MC *et al*. Canadian multi-institutional survey of immune tolerance therapy (ITT) – experience with the use of recombinant factor VIII for ITT. *Haemophilia* 2006; **12**: 1–6.
 - 18 Muntean W, Leschnik B, Baier K, Cvirn G, Gallistl S. In vivo thrombin generation in neonates. *J Thromb Haemost* 2004; **2**: 2071–2.
 - 19 Andrew M, Paes B, Milner R *et al*. Development of the human coagulation system in the full-term infant. *Blood* 1987; **70**: 165–72.
 - 20 Fritsch P, Cvirn G, Cimenti C *et al*. Thrombin generation in factor VIII-depleted neonatal plasma: nearly normal because of physiologically low antithrombin and tissue factor pathway inhibitor. *J Thromb Haemost* 2006; **4**: 1071–7.
 - 21 Chalmers EA. Haemophilia and the newborn. *Blood Rev* 2004; **18**: 85–92.
 - 22 Siimes MA, Aalberg V, Petrini P. *Boys with Haemophilia: Physical and Psychosocial Development in Adolescence*. Helsinki, Finland: Nemo Publishers, 2006.
 - 23 Lillicrap D, VandenDriessche T, High K. Cellular and genetic therapies for haemophilia. *Haemophilia* 2006; **12** (Suppl. 3): 36–41.
 - 24 VandenDriessche T, Thorrez L, Acosta-Sanchez A *et al*. Efficacy and safety of adeno-associated viral vectors based on serotype 8 and 9 versus lentiviral vectors for hemophilia B gene therapy. *J Thromb Haemost* 2006; **5**: 16–24.
 - 25 Shi Q, Wilcox DA, Fahs SA *et al*. Factor VIII ectopically targeted to platelets is therapeutic in hemophilia A with high-titer inhibitory antibodies. *J Clin Invest* 2006; **116**: 1974–82.
 - 26 PedNet website. Available at: <http://www.pednet.nl> (accessed on 22 November 2006).
 - 27 Donadel-Claeyssens S. Current co-ordinated activities of the PEDNET (European Paediatric Network for Haemophilia Management). *Haemophilia* 2006; **12**: 124–7.
 - 28 PedNet RODIN Study. Available at: <http://www.juliuscentrum.nl/pednet/rodin.htm> (accessed on 22 November 2006).