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IN FOCUS

Timing and severity of inhibitor development in recombinant versus plasma-derived factor VIII concentrates: a SIPPET analysis

F. PEYVANDI,*† A. CANNAVÒ,* I. GARAGIOLA,* R. PALLA,* P. M. MANNUCCI,*† and F. R. ROSENDAAL‡ FOR THE SIPPET STUDY GROUP[1]

**Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and Luigi Villa Foundation, Milan, Italy; †Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy; and ‡Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands*

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Essentials

- Recombinant factor VIII (rFVIII) was contrasted with plasma-derived FVIII (pdFVIII).
- In previously untreated patients with hemophilia A, rFVIII led to more inhibitors than pdFVIII.
- Inhibitors with rFVIII developed earlier, and the peak rate was higher than with pdFVIII.
- Inhibitors with rFVIII were more severe (higher titre) than with pdFVIII.

Summary. *Background:* The development of neutralizing antibodies (inhibitors) against factor VIII (FVIII) is the most severe complication in the early phases of treatment of severe hemophilia A. Recently, a randomized trial, the Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET) demonstrated a 2-fold higher risk of inhibitor development in children treated with recombinant FVIII (rFVIII) products than with plasma-derived FVIII (pdFVIII) during the first 50 exposure days (EDs). *Objective/Methods:* In this post-hoc SIPPET analysis we evaluated the rate of inhibitor incidence over time by every 5 EDs (from 0 to 50 EDs) in patients treated with different classes of FVIII product, made possible by a frequent testing regime. *Results:* The highest rate of inhibitor development occurred in the first 10 EDs, with a large contrast between rFVIII and pdFVIII during the first 5 EDs: hazard ratio 3.14 (95% confidence interval [CI], 1.01–9.74) for all inhibitors and 4.19 (95% CI, 1.18–14.8) for high-titer inhibitors. For patients treated with pdFVIII, the peak of inhibitor development occurred later (6–10 EDs) and lasted for a shorter time. *Conclusion:* These results emphasize the high immunologic vulnerability of patients during the earliest exposure to FVIII concentrates, with the strongest response to recombinant FVIII products.

Keywords: antibodies; factor VIII; hemophilia A; inhibitor; plasma.

Introduction

The development of alloantibodies (inhibitors) neutralizing factor VIII (FVIII) coagulant activity represents the main complication of treatment of hemophilia A. It occurs in approximately one-third of previously untreated patients (PUPs) with severe disease and causes substantial morbidity with a major increase in cost of treatment [1–3]. Inhibitor development has a multi-causal etiology [4–7], and it usually occurs in young children during the first 20–30 exposure days (EDs) to FVIII replacement [8–11]. Recently, the randomized SIPPET trial demonstrated that in these early stages of treatment the risk of inhibitor development was nearly 2-fold higher in patients treated with recombinant FVIII products (rFVIII) than in those treated with

Correspondence: Flora Peyvandi, Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Department of Pathophysiology and Transplantation, University of Milan, Via Pace, 9-20122, Milan, Italy
Tel.: +39 25 503 5414
E-mail: flora.peyvandi@unimi.it

[1]See Appendix for full list of contributors

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plasma-derived FVIII (pdFVIII), with the majority of inhibitors occurring during the first 20 EDs [12].

There are few data available on the time course of inhibitor development after FVIII exposure [8–11] because of the absence of studies with frequent serial inhibitor measurements. In the SIPPET study, inhibitor monitoring was performed at strict, frequent and standardized time-intervals in all patients [12]. This strict monitoring schedule allowed us to perform an analysis to investigate the risk of inhibitor development in the early risk period to assess whether there was a difference in the time course of inhibitor development between patients treated with the classes of pdFVIII or rFVIII products. In addition, we assessed the severity of inhibitors for the two product classes.

Study design

This is a *post hoc* analysis performed in the frame of the SIPPET randomized trial [12]. Two hundred and fifty-one male patients with severe hemophilia A aged < 6 years, negative for inhibitor measurement, never exposed to FVIII concentrates, and not or minimally treated (less than 5 EDs) with blood components (whole blood, fresh frozen plasma, packed red cells, platelets or cryoprecipitate) were included [12]. Patients were randomized to treatment with a single brand of a rFVIII or pdFVIII product containing von Willebrand factor and were followed for the first 50 EDs, or until 3 years after enrolment or study termination or inhibitor development, whichever occurred first. Thirty-five patients who had not reached 50 EDs at the time of study termination were censored, and of those 25 had less than 20 EDs. Data were also collected on self-reported family history of hemophilia and inhibitors, age at first treatment, country site, previous exposure to blood components and FVIII gene mutations (for more details, see [12]).

Methods and timing of inhibitor testing were as previously reported [12,13]. Briefly, patients who received on-demand treatment underwent inhibitor testing every 3–4 EDs during the first 20 infusions, then every 10 EDs or every 3 months. Patients who received prophylaxis underwent inhibitor testing every 2 weeks. Additional testing was carried out at the time of clinical suspicion of inhibitor and for all patients after study completion. In case of inhibitor occurrence, levels were confirmed on a second sample within 14 days after the first positivity, and then monthly for 6 months.

Kaplan–Meier survival analyses were performed to estimate cumulative incidences for all and high-titer inhibitors by FVIII treatment class, and incidence rates were compared with Cox regression survival analyses taking into account as covariates FVIII gene mutations, self-reported family history of hemophilia or inhibitor, age at first treatment (in months), treatment modality, ethnicity, country and treatment center. To study the risk over time, we calculated the proportion developing an inhibitor during intervals of 5 EDs, accounting for those having an event or being censored during an interval, as by a standard life-table technique. Confidence intervals were derived from the binomial distribution according to Wilson [14]. Adjustments in multivariate Cox models were in a bivariate manner, as well as with full models. Confidence intervals were derived from this model. Statistical analyses were performed within SPSS, version 23.0 (IBM Corp., Armonk, NY, USA).

Results and discussion

Seventy-six of 251 patients developed an inhibitor, 50 of which were high titer. All inhibitors occurred before 39 EDs and 90% (68/76) within 20 EDs. All high-titer inhibitors occurred before 34 EDs and 90% (45/50) within 16 EDs for patients in both arms. The cumulative incidence of all inhibitors during the first 50 EDs was 35.4% (95% confidence interval [CI], 28.9–41.9), and for high-titer inhibitors it was 23.3% (95% CI, 17.6–29.0) [12].

When the rate of inhibitor development was investigated over time, in the group of patients randomized to a rFVIII product, inhibitors occurred earlier than with pdFVIII, whereas the rate per short time-interval remained high until 20 EDs; for patients treated with a pdFVIII product the peak was lower, occurred later and lasted for a shorter time than with rFVIII. Table 1 shows the development of inhibitors over short time-intervals of 5 EDs for each of the FVIII product classes. In detail, 16 of the 76 (21%) inhibitors developed during the first 5 EDs; that is, 12 of the 47 (26%) patients treated with rFVIII for an incidence of 9.5%, and four of 29 (14%) inhibitors in patients treated with pdVIII for an incidence of 3.2%. In patients treated with rFVIII, all 12 inhibitors that occurred early (0–5 EDs) were high titer (in patients treated with pdFVIII, three of four (75%) were high titer). The hazard ratio (HR) was particularly high during this early exposure period, both for all (HR, 3.14; 95% CI, 1.01–9.74) and high-titer inhibitors (HR, 4.19; 95% CI, 1.18–14.84) (Table 2), showing a 3- to 4-fold increased risk of inhibitor for rFVIII products in the earliest phase of treatment, with an absolute difference in inhibitor development of 6.3%. During the interval of 6–10 EDs the absolute difference in inhibitor development was 5%, and the relative rate attenuated (HR, 1.42; 95% CI, 0.72–2.80). After this early treatment period, the difference between the two classes remained but became less pronounced. After bivariate adjustment for putative confounders, the risk of inhibitor development during the first 5 EDs did not change (Table 2), nor in a full model adjusted for age, mutation, center, family history and previous exposure to blood products (all inhibitors, HR 3.30, 95% CI 0.87–12.52; high-titer inhibitors, HR 4.77, 95% CI 1.01–22.50).

Severity of the immunogenic effect was studied by using different cut-off values, in Bethesda units, for (high-titer) inhibitors (Table 3). Whereas the hazard ratio was

Table 1 Risk of inhibitor incidence over time. (A) Shows the risk of all inhibitors in relation to the class of FVIII products (recombinant or plasma derived). (B) Shows the risk of high-titer inhibitors

	Plasma-derived FVIII			Recombinant FVIII		
ED	Number at risk*	Number of inhibitors	Incidence % (95% CI)	Number at risk*	Number of inhibitors	Incidence % (95% CI)
(A) All inhibitors						
0–5	125	4	3.2 (1.3–7.9)	126	12	9.5 (5.5–15.9)
6–10	112	15	13.4 (8.3–20.9)	103	19	18.4 (12.1–27.0)
11–15	92	3	3.3 (1.1–9.2)	76	6	7.9 (3.7–16.2)
16–20	84	4	4.8 (1.9–11.6)	65	5	7.7 (3.3–16.8)
21–25	76	0	0.0 (0–4.8)	56	2	3.6 (1.0–12.1)
26–30	65	2	3.1 (0.8–10.5)	52	0	0.0 (0–6.9)
31–35	58	1	1.7 (0.3–9.1)	48	2	4.2 (1.2–14.0)
36–40	55	0	0.0 (0–6.5)	43	1	2.3 (0.4–12.1)
(B) High-titer inhibitors						
0–5	125	3	2.4 (0.8–6.8)	126	12	9.5 (5.5–15.9)
6–10	112	12	10.7 (6.2–17.8)	103	11	10.7 (6.1–18.1)
11–15	92	2	2.2 (0.6–7.6)	76	4	5.3 (2.1–12.8)
16–20	84	2	2.4 (0.7–8.3)	65	2	3.1 (0.8–10.5)
21–25	76	0	0.0 (0–4.8)	56	1	1.8 (0.3–9.4)
26–30	65	0	0.0 (0–5.6)	52	0	0.0 (0–6.9)
31–35	58	1	1.7 (0.3–9.1)	48	0	0.0 (0–7.4)
36–40	55	0	0.0 (0–6.5)	43	0	0.0 (0–8.2)

*Patients at risk at the beginning of the time interval. CI, confidence interval.

Table 2 Cox regression for inhibitor development by class of FVIII products during 0–5 exposure days with plasma-derived FVIII as the reference category

Adjustment variable	All inhibitors	High-titer inhibitors
None	3.14 (1.01–9.74)	4.19 (1.18–14.84)
Age	3.19 (1.03–9.91)	4.23 (1.19–15.02)
Mutation	2.93 (0.93–9.20)	3.90 (1.09–13.99)
Country	3.16 (1.02–9.80)	4.22 (1.19–14.97)
Ethnicity	3.22 (1.04–10.0)	4.30 (1.21–15.23)
Family history of hemophilia	2.95 (0.94–9.28)	3.91 (1.09–14.04)
Family history of inhibitor	2.98 (0.96–9.24)	3.97 (1.12–14.08)
Previous exposure to blood components	3.12 (1.01–9.68)	4.17 (1.18–14.79)
Treatment regimen	3.14 (1.01–9.76)	4.15 (1.17–14.75
Treatment center	3.39 (1.08–10.62)	4.49 (1.26–16.10)

Hazard ratio compares the incidence of inhibitor development among patients treated with recombinant products and those treated with plasma-derived products.

Table 3 Hazard ratio for inhibitors for rFVIII vs. pdFVIII with increasing Bethesda units cut-offs

Cut-off (BU)	HR	95% CI
0.6	1.87	1.17–2.96
2	1.67	1.02–2.75
4	1.71	1.00–2.94
8	2.12	1.16–3.89
12	2.26	1.19–4.29
16	2.71	1.29–5.66
20	2.75	1.27–5.98
30	3.05	1.28–7.27
40	3.16	1.24–8.01
50	4.74	1.54–14.08
100	8.87	2.04–38.57

Hazard ratios (HRs) with 95% confidence intervals (95% CIs) are given for rFVIII vs. pdFVIII for increasing cut-off values (Bethesda units [BU]), with levels exceeding these values as the outcome.

1.87 (95% CI, 1.17–2.96) for values exceeding 0.6 BU (which captures all inhibitors, ranging in peak levels from 0.7 to 1850 BU), the hazard steadily increased for more severe inhibitors, with hazard ratios of 4.74 (95% CI, 1.54–14.08) for titers exceeding 50 BU, and 8.87 (95% CI, 2.04–38.87) for titers over 100 BU.

The development of anti-FVIII neutralizing antibodies is the most serious complication of FVIII replacement therapy [1–3]. The class of FVIII products and its role in the occurrence of this complication have been discussed and analyzed in different observational studies and meta-analyses [8–11,15,16], as well as in the frame of a single randomized clinical trial [12]. SIPPET showed a higher immunogenicity of rFVIII products than of pdFVIII containing von Willebrand factor during the first 50 EDs, and confirmed that most inhibitors develop within the first 20 EDs, as previously suggested in several observational studies [8–11]. In the latter, lack of data stemming from protocolized and centralized inhibitor monitoring did not allow time-dependent analyses. SIPPET, by means of uniquely frequent follow-up monitoring and centralized inhibitor measurement, allowed a precise assessment of the timing of the FVIII antibody development. Whereas we confirmed that almost 90% of inhibitors occurred during the first 20 EDs for both treatment arms, the immune response to rFVIII occurred earlier, had a higher peak incidence, and lasted longer

than in patients treated with pdFVIII. During the first 5 EDs, patients treated with recombinant products had a 3–4-fold higher risk of inhibitor development, including high-titer inhibitors, than patients treated with pdFVIII.

When we investigated the severity of the inhibitor, the risk conferred by rFVIII relative to pdFVIII increased steadily over increasing cut-off BU levels, demonstrating that the difference in immunogenicity between rFVIII and pdFVIII is quantitative, as well temporal and qualitative. The strong immunogenic effect of rFVIII was also shown in a previous analysis, where we reported that patients with a low genetic risk of inhibitors (as a result of missense mutations where some defective protein is secreted) have a low risk of inhibitors when treated with pdFVIII, whereas with rFVIII this protection disappears [17].

A limitation of the current analysis is that it was based on a relatively small sample, hemophilia being a rare disorder. Nevertheless, the pattern of an earlier, longer lasting risk period was clear. A major strength is that the analysis was performed in a randomized study, in which confounding by extraneous factors is avoided, as was evident from the absence of an effect of adjustment. A detailed discussion of strengths and weaknesses of SIPPET can be found elsewhere [18].

Different mechanisms may play a role in the fast and strong immune reaction to recombinant FVIII products. It is well established that post-translational modifications (e.g. glycosylation) occur with these products [19,20], and that a fraction of rFVIII is unable to bind to von Willebrand factor, potentially decreasing FVIII recognition by antigen presenting cells [21]. Furthermore, pdFVIII products may contain immunomodulating human proteins, which may play a role in inducing greater FVIII tolerance [22,23]. However, it remains to be elucidated why the immunogenicity of rFVIII also lasts longer than with pdFVIII, which may be related to environmental stimuli. The fast recognition of rFVIII products by the immune system in the first few days of replacement therapy, and the much stronger response than with pdFVIII in relation to inhibitor titers, corroborates the notion that their high inhibitor risk is a result of an increased immunogenicity.

Addendum

F. Peyvandi designed the study and wrote the paper; A. Cannavò analyzed the data and contributed to writing the paper; R. Palla and I. Garagiola collected data and were involved in the analysis; P. M. Mannucci and F. R. Rosendaal supervised the study and critically revised the paper. All authors approved the final version of the manuscript before submission.

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Disclosure of Conflict of Interests

F. Peyvandi has received honoraria or consultation fees from Freeline, Kedrion Biopharma, LFB, and Octapharma, and speaker fees for participating at educational meetings organized by Ablynx, Bayer, Grifols, Novo Nordisk, and Sobi; she is also a member of the advisory board of Ablynx and of F. Hoffmann-La Roche. R. Palla has received travel support from Pfizer and Grifols. P. M. Mannucci has received speaker fees from Alexion, Baxalta/Shire, Bayer, CSL Behring, Grifols, Kedrion, LFB, and Novo Nordisk and he is a member of the following advisory boards: Bayer and Kedrion. The other authors state that they have no conflict of interest.

Appendix

SIPPET Study Group

A. El-Beshlawy, M. Elalfy, Cairo, Egypt; V. Ramanan, Pune, India; P. Eshghi, Tehran, Iran; S. Hanagavadi, Davangere, India; R. Varadarajan, Chennai, India; M. Karimi, Shiraz, Iran; M. V. Manglani, Mumbai, India; C. Ross, Bangalore, India; G. Young, Los Angeles, USA; T. Seth, New Delhi, S. Apte, Pune, India; D. M. Nayak, Karnataka, India; E. Santagostino, M. Elisa Mancuso, Milan, Italy; A. C. Sandoval Gonzalez, Monterrey, Mexico; J. N. Mahlangu, Johannesburg, South Africa; S. Bonanad Boix, Valencia, Spain; M. Cerqueira, Rio de Janeiro, Brazil; N. P. Ewing, Duarte, USA; C. Male, Vienna, Austria; T. Owaidah, Riyadh, Saudi Arabia; V. Soto Arellano, Fargo, USA; N. L. Kobrinsky, Jackson, USA; S. Majumdar, R. Perez Garrido, Sevilla, Spain; A. Sachdeva, New Delhi, India; M. Simpson, Chicago, USA; M. Thomas, Kerala, India; E. Zanon, Padova, Italy; B. Antmen, Adana, Turkey; K. Kavakl, Izmir, Turkey; M. J. Manco-Johnson, Aurora, USA; M. Martinez, Buenos Aires, Argentina; E. Marzouka, Santiago, Chile; M. G. Mazzucconi, Rome, Italy; D. Neme, Buenos Aires, Argentina; A. Palomo Bravo, Malaga, Spain; R. Paredes Aguilera, México City, Mexico; A. Prezotti, Vitoria, Brazil; K. Schmitt, Linz, Austria; B. M. Wicklund, Kansas City, USA; B. Zulfikar, Istanbul, Turkey.

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