ORIGINAL ARTICLE

A Randomized Trial of Factor VIII and Neutralizing Antibodies in Hemophilia A

F. Peyvandi, P.M. Mannucci, I. Garagiola, A. El-Beshlawy, M. Elalfy, V. Ramanan, P. Eshghi, S. Hanagavadi, R. Varadarajan, M. Karimi, M.V. Manglani, C. Ross, G. Young, T. Seth, S. Apte, D.M. Nayak, E. Santagostino, M.E. Mancuso, A.C. Sandoval Gonzalez, J.N. Mahlangu, S. Bonanad Boix, M. Cerqueira, N.P. Ewing, C. Male, T. Owaidah, V. Soto Arellano, N.L. Kobrinsky, S. Majumdar, R. Perez Garrido, A. Sachdeva, M. Simpson, M. Thomas, E. Zanon, B. Antmen, K. Kavakli, M.J. Manco-Johnson, M. Martinez, E. Marzouka, M.G. Mazzucconi, D. Neme, A. Palomo Bravo, R. Paredes Aguilera, A. Prezotti, K. Schmitt, B.M. Wicklund, B. Zulfikar, and F.R. Rosendaal

ABSTRACT

BACKGROUND

The development of neutralizing anti–factor VIII alloantibodies (inhibitors) in patients with severe hemophilia A may depend on the concentrate used for replacement therapy.

METHODS

We conducted a randomized trial to assess the incidence of factor VIII inhibitors among patients treated with plasma-derived factor VIII containing von Willebrand factor or recombinant factor VIII. Patients who met the eligibility criteria (male sex, age <6 years, severe hemophilia A, and no previous treatment with any factor VIII concentrate or only minimal treatment with blood components) were included from 42 sites.

RESULTS

Of 303 patients screened, 264 underwent randomization and 251 were analyzed. Inhibitors developed in 76 patients, 50 of whom had high-titer inhibitors (≥5 Bethesda units). Inhibitors developed in 29 of the 125 patients treated with plasma-derived factor VIII (20 patients had high-titer inhibitors) and in 47 of the 126 patients treated with recombinant factor VIII (30 patients had high-titer inhibitors). The cumulative incidence of all inhibitors was 26.8% (95% confidence interval [CI], 18.4 to 35.2) with plasma-derived factor VIII and 44.5% (95% CI, 34.7 to 54.3) with recombinant factor VIII; the cumulative incidence of high-titer inhibitors was 18.6% (95% CI, 11.2 to 26.0) and 28.4% (95% CI, 19.6 to 37.2), respectively. In Cox regression models for the primary end point of all inhibitors, recombinant factor VIII was associated with an 87% higher incidence than plasma-derived factor VIII (hazard ratio, 1.87; 95% CI, 1.17 to 2.96). This association did not change in multivariable analysis. For high-titer inhibitors, the hazard ratio was 1.69 (95% CI, 0.96 to 2.98). When the analysis was restricted to recombinant factor VIII products other than second-generation full-length recombinant factor VIII, effect estimates remained similar for all inhibitors (hazard ratio, 1.98; 95% CI, 0.99 to 3.97) and high-titer inhibitors (hazard ratio, 2.59; 95% CI, 1.11 to 6.00).

CONCLUSIONS

Patients treated with plasma-derived factor VIII containing von Willebrand factor had a lower incidence of inhibitors than those treated with recombinant factor VIII. (Funded by the Angelo Bianchi Bonomi Foundation and others; ClinicalTrials.gov number, NCT01064284; EudraCT number, 2009-011186-88.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Peyvandi at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Ca' Granda Maggiore Policlinico Hospital Foundation, and Department of Pathophysiology and Transplantation, University of Milan, Via Pace 9, 20122 Milan, Italy, or at flora.peyvandi@unimi.it.

Drs. Peyvandi and Mannucci contributed equally to this article.

N Engl J Med 2016;374:2054-64.
DOI: 10.1056/NEJMoa1516437

Hemophilia A is an inherited bleeding disorder characterized by plasma deficiency of coagulation factor VIII.[1,2] A major complication in 30% of patients is the occurrence of alloantibodies (inhibitors) that inactivate factor VIII activity and may nullify replacement therapy.[3-6] Risk factors include unmodifiable patient-related factors such as residual plasma factor VIII concentration and gene mutation.[7-9] Putative treatment-related risk factors are early replacement therapy and the source of factor VIII (i.e., human plasma or recombinant DNA technology).[3,8,10-13] Experimental studies have shown that plasma-derived factor VIII in complex with the chaperone protein von Willebrand factor, which masks critical factor VIII epitopes, has reduced immunogenicity.[14,15] Alternative explanations may be the presence of immunomodulating proteins in plasma-derived factor VIII and post-translational modifications present in recombinant products, different from those in plasma-derived factor VIII. Observational studies involving humans have been inconclusive, possibly owing to confounding by indication — that is, product choices based on perceived inhibitor risk as well as heterogeneity in study design.[16-18]

We carried out an investigator-initiated, multicenter, randomized, open-label clinical trial, named Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET), in which previously untreated or minimally treated patients with severe hemophilia A were assigned to receive infusions of either plasma-derived factor VIII containing von Willebrand factor or recombinant factor VIII with no von Willebrand factor. We assessed the incidence of all inhibitors and high-titer inhibitors in the two groups.

Methods

Trial Design and Enrollment Criteria

From January 14, 2010, through December 1, 2014, we screened 303 boys at 42 sites in 14 countries (see the Supplementary Appendix, available with the full text of this article at NEJM.org) after informed consent was obtained from the parent or legal guardian. The trial was approved by the institutional review board at each site. A data and safety monitoring board evaluated adverse events and the interim analysis. Site investigators made all decisions about clinical management. Data were collected with the use of electronic forms; data accuracy and integrity were ensured by the contract research organization Sintesi Research (Milan) and checked extensively before database freeze and statistical analysis. The first, second, and last authors had full access to all the data and vouch for the completeness and accuracy of the data and data analysis and for the fidelity of the trial to the protocol (available at NEJM.org).

Eligibility criteria were male sex, an age of younger than 6 years at screening, severe hemophilia A (factor VIII coagulant activity <1 IU per deciliter, confirmed at the Milan central laboratory by means of a one-stage functional assay),[19] no previous treatment with any factor VIII concentrate, no treatment or minimal treatment (<5 times) with blood components (whole blood, fresh-frozen plasma, packed red cells, platelets, or cryoprecipitate), no treatment with investigational drugs, and a negative test for factor VIII inhibitors at the central laboratory.[20]

Investigational Factor VIII Products

Factor VIII products included Food and Drug Administration–approved and European Medicines Agency–approved commercial brands that were purchased for the trial. In each country, one locally licensed plasma-derived factor VIII concentrate and one recombinant factor VIII concentrate were chosen without any role of the sponsors, and patients were randomly assigned to one of the two products. The recombinant products, all produced from hamster-cell cultures, were Recombinate (Baxalta), Kogenate FS (Bayer AG), Advate (Baxalta), and ReFacto AF (Pfizer). The plasma-derived products were Alphanate and Fanhdi (Grifols), Emoclot (Kedrion Biopharma), and Factane (LFB). The ratio of von Willebrand factor to factor VIII was on average close to 1 for Alphanate and Fanhdi[21,22] and close to 0.5 for Emoclot and Factane.[23,24] Grifols, Kedrion Biopharma, and LFB provided unrestricted grants to the primary funder (the Angelo Bianchi Bonomi Foundation) but had no role in the trial design; protocol preparation; patient recruitment; data collection, handling, analysis, and interpretation; or the writing of this report.

Randomization and Follow-up

Patients were randomly assigned in a 1:1 ratio to receive either plasma-derived factor VIII or recombinant factor VIII. Randomization was performed with a block size of 2 per center by means of

sealed envelopes that were prepared by Sintesi Research according to factor VIII class. For each patient, a single product belonging to the class assigned per randomization was allocated. Patients who underwent randomization were followed for 50 consecutive exposure days or 3 years or until inhibitor development was confirmed at the central Milan laboratory, whichever occurred first. After being diagnosed as having an inhibitor, patients were followed for 6 months. An exposure day was defined as a calendar day with one or more infusions of factor VIII.

OUTCOME MEASURES AND ADVERSE EVENTS

The primary outcome was the development of an inhibitor with a titer of at least 0.4 Bethesda units by the Bethesda assay with the Nijmegen modification.[20] High-titer inhibitors were a secondary outcome, defined by peak levels of at least 5 Bethesda units during 6 months of observation. Transient inhibitors were those that disappeared spontaneously within 6 months without immunotolerance treatment. Patients who received on-demand treatment underwent inhibitor testing every 3 to 4 exposure days during the first 20 infusions, then every 10 exposure days or every 3 months, whichever came first, and at each annual visit. Patients who received prophylaxis underwent inhibitor testing every 2 weeks. Patients were also tested when an inhibitor was clinically suspected, and one central test was performed in all patients at trial completion. Positive tests were repeated twice, both in the local laboratory and the central laboratory within 14 days after first being found positive, then monthly for 6 months. Mutation analysis of the factor VIII gene[25-27] was performed at the central laboratory. Adverse events were reported by the site investigators according to severity and the likelihood of being related to the trial treatment.

SAMPLE-SIZE CALCULATION

The planned sample size was based on an expected frequency of inhibitors of 25% among patients treated with recombinant factor VIII and 12.5% among those treated with plasma-derived factor VIII, with a type I error of 0.05 and type II error of 0.20. A total of 270 patients was required; under the assumption that 10% of the patients would drop out, the sample size was set at 300. However, dropouts were included in the time-dependent analysis, so follow-up durations differed among patients.

TRIAL TERMINATION

By December 2014, consent to participate had been obtained from the parent or legal guardian of 303 patients. Enrollment was ended because of the announcement by the World Federation of Hemophilia (September 2014) that it may be prudent, when other safe clotting-factor concentrates are available, to consider not using Kogenate FS or Helixate NexGen (CSL Behring) for patients with newly diagnosed severe hemophilia A who have not previously received treatment, owing to an increased risk of inhibitor development.[28] Given the logistics of the trial, with specific brands per country and specific ethical approval, the steering committee approved termination of the trial on May 7, 2015.

STATISTICAL ANALYSIS

Descriptive data are presented as means and standard deviations, medians and ranges, or percentages. A survival analysis was conducted with the number of exposure days as the time variable. Cumulative incidences, overall and according to treatment group, were estimated with the use of the Kaplan–Meier method. Confidence intervals were based on asymptotic standard errors of the survival function. The incidence of inhibitors in the two treatment groups was compared by means of Cox regression models; this was the primary analysis, with the hazard ratio as the primary effect measure. Confidence intervals for the hazard ratios were constructed with standard errors derived from the model. Proportionality of the hazards was inspected by visualization of log–log plots, which showed no gross violations.

Putative confounders were examined for their association with the treatment group. Potential confounding variables defined a priori were age; country; gene mutations (null allele [i.e., inversions, large deletions, and frameshift and nonsense mutations]); race or ethnic group; family history of hemophilia or of inhibitors; intensive treatment (defined as ≥5 consecutive daily doses of ≥50 IU per kilogram of body weight); previous minimal treatment with blood components; and an on-demand treatment regimen, defined as replacement therapy when a hemorrhage occurred, as compared with standard prophylaxis (three times weekly) or modified prophylaxis (once weekly). Four variables were closely linked: country, self-reported race or ethnic group, treatment regimen, and type of product within the class. The variable country was used in the extended model

as a proxy adjustment variable for these four. Age was included as a continuous variable (months of age at first treatment), country was recoded from 14 to 5 categories that accounted for more than 80% of patients, and race or ethnic group was coded in 4 categories.

Recent reports[28-30] have shown a higher risk of inhibitor development with second-generation full-length recombinant factor VIII products than with other recombinant factor VIII products. We assessed the effect of the other recombinant products versus plasma-derived factor VIII products while maintaining a randomized comparison by means of an analysis restricted to the countries where this recombinant factor VIII had not been used.

RESULTS

BASELINE CHARACTERISTICS AND INHIBITOR INCIDENCE

A total of 264 patients were randomly assigned to receive plasma-derived factor VIII or recombinant factor VIII, of whom 13 were excluded for

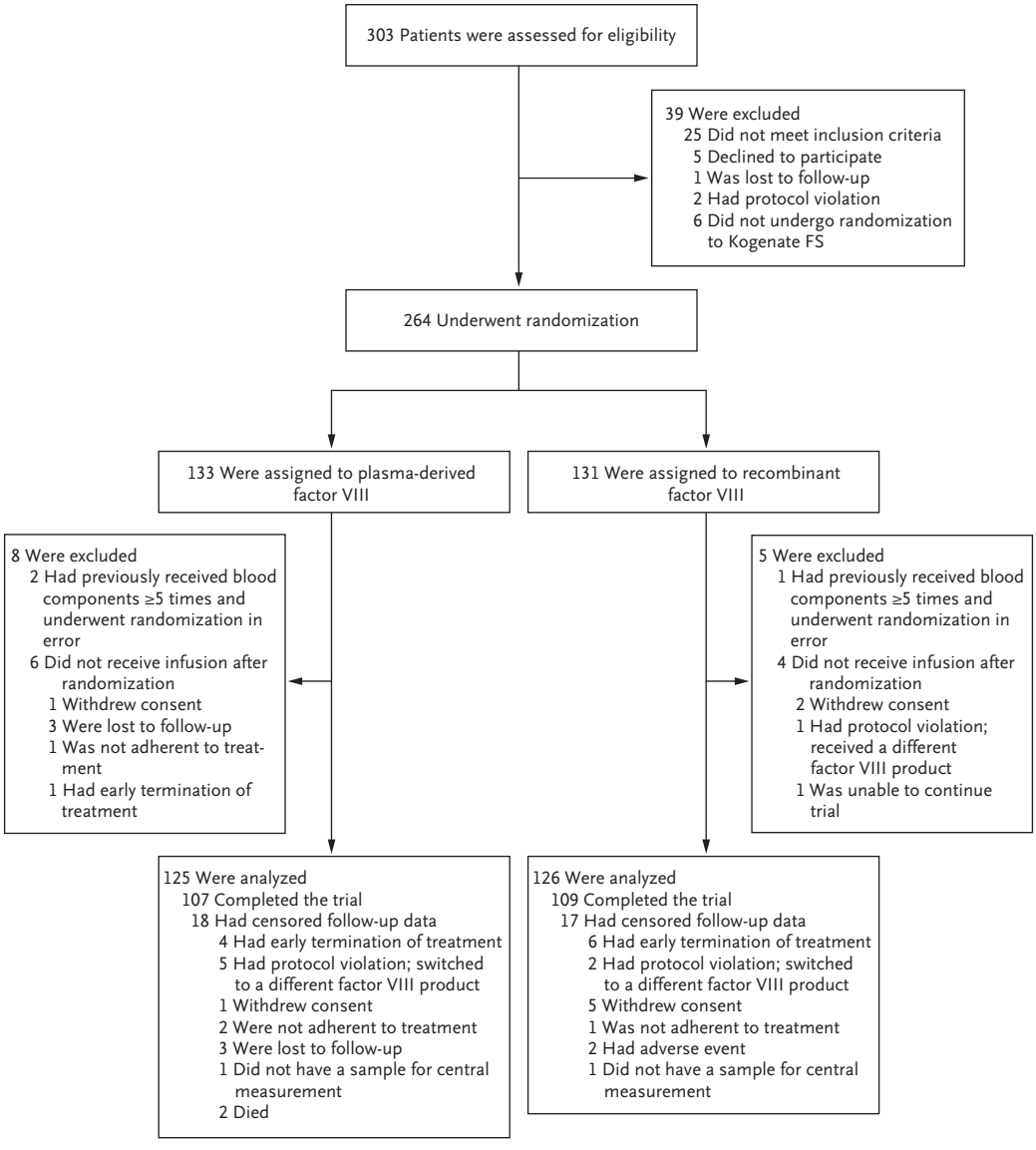

Figure 1. Screening, Randomization, and Follow-up.

Table 1. Baseline Characteristics of the Randomly Assigned Patients.*

Characteristic	Plasma-Derived Factor VIII (N=125)	Recombinant Factor VIII (N=126)
Country — no. (%)		
India	40 (32.0)	43 (34.1)
Egypt	38 (30.4)	37 (29.4)
Iran	14 (11.2)	18 (14.3)
United States	9 (7.2)	9 (7.1)
Italy	5 (4.0)	4 (3.2)
Other†	19 (15.2)	15 (11.9)
Age at first treatment — mo		
Median (range)	15.0 (0–67)	16.0 (0–75)
Mean	19.1±14.3	21.3±16.3
Type of mutation — no./total no. (%)‡		
Intron 22 inversion	57/117 (48.7)	53/118 (44.9)
Intron 1 inversion	5/117 (4.3)	1/118 (0.8)
Nonsense	14/117 (12.0)	20/118 (16.9)
Large deletion	8/117 (6.8)	8/118 (6.8)
Frameshift	17/117 (14.5)	14/118 (11.9)
Missense	10/117 (8.5)	12/118 (10.2)
Splice site	3/117 (2.6)	9/118 (7.6)
Only polymorphisms	3/117 (2.6)	0/118
No mutation	0/117	1/118 (0.8)
Mutation status — no./total no. (%)‡		
Non-null mutation	16/117 (13.7)	21/117 (17.9)
Null mutation	101/117 (86.3)	96/117 (82.1)
Family history of hemophilia — no./total no. (%)		
Yes	59/124 (47.6)	52/122 (42.6)
No	65/124 (52.4)	70/122 (57.4)
Family history of inhibitor development — no./total no. (%)		
Yes	13/113 (11.5)	12/119 (10.1)
No	100/113 (88.5)	107/119 (89.9)
Race or ethnic group — no. (%)§		
White	39 (31.2)	45 (35.7)
Black	5 (4.0)	2 (1.6)
Asian	41 (32.8)	43 (34.1)
Other	40 (32.0)	36 (28.6)
Previous treatment — no. (%)		
Yes¶	56 (44.8)	53 (42.1)
No	69 (55.2)	73 (57.9)
Treatment regimen — no. (%)‖		
On-demand	61 (48.8)	56 (44.4)
Standard prophylaxis	21 (16.8)	19 (15.1)
Modified prophylaxis	43 (34.4)	51 (40.5)

Table 1. (Continued.)

Characteristic	Plasma-Derived Factor VIII (N=125)	Recombinant Factor VIII (N=126)
Brand of concentrate — no. (%)		
Alphanate	9 (7.2)	
Emoclot	61 (48.8)	
Factane	43 (34.4)	
Fanhdi	12 (9.6)	
Advate		13 (10.3)
Kogenate FS		61 (48.4)
Recombinate		45 (35.7)
ReFacto AF		7 (5.6)

* Plus–minus values are means ±SD.
† Each of the other countries had fewer than nine patients in the combined groups.
‡ There were no cells or DNA available for eight patients in each group.
§ Race or ethnic group was self-reported.
¶ A total of 59 patients had received cryoprecipitate (29 in the group assigned to plasma-derived factor VIII and 30 in the group assigned to recombinant factor VIII), and 50 had received fresh-frozen plasma, packed red cells, platelets, or combinations thereof (27 in the group assigned to plasma-derived factor VIII and 23 in the group assigned to recombinant factor VIII).
‖ An on-demand treatment regimen was defined as on-demand replacement therapy when a hemorrhage occurred. Standard prophylaxis was defined as administration of treatment three times weekly. Modified prophylaxis was defined as administration of treatment once weekly.

various reasons (Fig. 1). Therefore, 251 patients received between 1 and 50 infusions of plasma-derived factor VIII or recombinant factor VIII, 216 (86%) completed the trial according to the protocol, and 35 had censored follow-up data (25 dropped out and 10 had early termination of treatment) (Fig. 1). Among the 251 patients analyzed, 21 of the 175 in whom an inhibitor did not develop had 20 or fewer exposure days; these patients were equally distributed between the two groups (Table S1 in the Supplementary Appendix).

Of the 251 patients who underwent randomization and received treatment, 126 were assigned to recombinant factor VIII and 125 to plasma-derived factor VIII. Baseline characteristics were evenly distributed between the two groups and did not differ significantly (Table 1). The frequency of surgery and intensity of treatment during follow-up did not differ significantly between the two groups. Inhibitors were detected in 76 patients, 50 of whom had high-titer inhibitors (Table 2). The cumulative incidence of all inhibitors was 35.4% (95% confidence interval [CI], 28.9 to 41.9) and of high-titer inhibitors was 23.3% (95% CI, 17.6 to 29.0). All inhibitors occurred before 39 exposure days (range, 2 to 38); all high-titer inhibitors occurred before 34 exposure days (range, 2 to 33), with a median time of 7 or 8 days (Table 2).

When putative risk factors were analyzed for their association with inhibitor risk, only null mutations were associated with an increased incidence of inhibitors (hazard ratio, 1.81; 95% CI, 0.83 to 3.95). There were no clear associations with age, family history, race or ethnic group, or previous exposure to blood components.

INHIBITORS ACCORDING TO PRODUCT CLASS

Inhibitors developed in 29 of the 125 patients treated with plasma-derived factor VIII (23.2%); 20 patients (16.0%) had high-titer inhibitors (Table 2). Inhibitors developed in 47 of the 126 patients treated with recombinant factor VIII (37.3%); 30 patients (23.8%) had high-titer inhibitors. The cumulative incidence of all inhibitors was 26.8% (95% CI, 18.4 to 35.2) with plasma-derived factor VIII and 44.5% (95% CI, 34.7 to 54.3) with recombinant factor VIII; the cumulative incidence of high-titer inhibitors was 18.6% (95% CI, 11.2 to 26.0) and 28.4% (95% CI, 19.6 to 37.2),

Table 2. Characteristics of the Patients in Whom Inhibitors Developed.*

Characteristic	Plasma-Derived Factor VIII	Recombinant Factor VIII
Type of inhibitor — no./total no. (%)		
All	29/125 (23)	47/126 (37)
Transient†	7/27 (26)	12/44 (27)
Persistent†	20/27 (74)	32/44 (73)
High titer	20/125 (16)	30/126 (24)
Transient†	3/18 (17)	2/27 (7)
Persistent†	15/18 (83)	25/27 (93)
Low titer	9/125 (7)	17/126 (13)
Transient	4/9 (44)	10/17 (59)
Persistent	5/9 (56)	7/17 (41)
Time of development — exposure days		
All		
Mean	11.2	10.9
Median (range)	8 (3–33)	8 (2–38)
High titer		
Mean	9.8	8.1
Median (range)	8 (3–33)	7 (2–21)
Low titer		
Mean	14.4	15.9
Median (range)	12 (4–29)	11 (7–38)
Peak titer — Bethesda units		
All		
Mean	62.2	124.5
Median (range)	12 (0.8–1100)	16.3 (0.7–1850)
High titer		
Mean	88.9	193.7
Median (range)	17.5 (6–1100)	113.5 (10–1850)
Low titer		
Mean	3.1	2.4
Median (range)	4 (0.8–5)	2 (0.7–5)

* High-titer inhibitors were defined by peak levels of at least 5 Bethesda units. Low-titer inhibitors were defined by levels of 0.4 to less than 5 Bethesda units. Transient inhibitors were those that disappeared spontaneously within 6 months without immunotolerance treatment. Inhibitors developed in 76 patients; during the 6 months of observation after inhibitor development, 19 patients (25%) stopped treatment, 49 (65%) continued with the trial product on demand or as prophylaxis, and 8 (10%) received factor VIII–bypassing agents for bleeding.

† Data at 6-month follow-up were missing for two patients assigned to plasma-derived factor VIII and three patients assigned to recombinant factor VIII.

respectively. Figure 2 shows Kaplan–Meier curves for all inhibitors and high-titer inhibitors.

Of the 264 patients who underwent randomization, 13 could not be analyzed because they had received at least five previous blood-component treatments (3 patients) or because they had dropped out before any treatment was given (10 patients). They did not differ significantly from the other patients in relevant variables. When we performed sensitivity analyses to study the effect of the follow-up data that were censored for 35 patients owing to early trial termination or dropout, hazard ratios did not change materially for the two extreme situations in which an inhibitor developed in all patients with censored data or all remained inhibitor-free for 50 exposure days.

In Cox regression models, the rate of inhibitor development was 87% higher with recombinant factor VIII than with plasma-derived factor VIII (hazard ratio, 1.87; 95% CI, 1.17 to 2.96). For high-titer inhibitors, the hazard ratio was 1.69 (95% CI, 0.96 to 2.98). The inhibitor was persistent in 73% of the patients in whom inhibitors developed and 89% of those with high-titer inhibitors; these percentages did not differ significantly between treatment groups (Table 2).

ADJUSTED ANALYSES

In analyses including putative confounding variables, hazard ratios did not deviate materially from the unadjusted hazard ratio (Table S2 in the Supplementary Appendix). We applied two multivariable models: in the first, including age, mutation, country, and previous exposure to blood components, the hazard ratio was 1.95 (95% CI, 1.21 to 3.15). In the model adjusted for age, mutation, country, and family history, the hazard ratio was 1.88 (95% CI, 1.16 to 3.04). For high-titer inhibitors, a model including age, mutation, previous exposure, and country yielded a hazard ratio of 1.73 (95% CI, 0.97 to 3.10); in the second multivariable model, the hazard ratio was 1.64 (95% CI, 0.91 to 2.95).

Analyses including interaction terms between country and treatment group did not change the results (data not shown). To assess whether the overall results could have been derived from one specific country, we performed sensitivity analyses, which showed no deviations from the overall estimate (Fig. 3). We also assessed whether previous exposure to blood products (in 109 patients equally distributed between the two groups) differentially affected inhibitor formation relative to treatment product class and found no evidence for this (P=0.39 for interaction).

To maintain the randomized comparison of

product class while excluding second-generation full-length recombinant factor VIII, we excluded countries in which this recombinant factor VIII was used. This analysis included 131 patients (66 patients treated with plasma-derived factor VIII and 65 treated with recombinant factor VIII), 44 of whom had inhibitor development. In Cox regression models, the hazard ratio was 1.98 for all inhibitors (95% CI, 0.99 to 3.97) and 2.59 (95% CI, 1.11 to 6.00) for high-titer inhibitors; these ratios did not change materially after adjustment for putative confounding variables (Table S3 in the Supplementary Appendix).

Two deaths occurred during the trial (one due to intraabdominal bleeding and one to a motor vehicle accident); both were in the group assigned to plasma-derived factor VIII. Severe nonfatal adverse events were nine episodes of intracranial bleeding (five intracerebral, three subdural, and one epidural) and two episodes of gastrointestinal bleeding. Five of the severe nonfatal adverse events occurred in the group assigned to plasma-derived factor VIII and six in the group assigned to recombinant factor VIII. (For more on severe adverse events, see Table S4 in the Supplementary Appendix.)

DISCUSSION

In this randomized, controlled trial involving 251 patients with severe hemophilia A who were previously untreated or minimally exposed to blood components, replacement products belonging to the class of plasma-derived factor VIII containing von Willebrand factor were associated with a lower incidence of inhibitors than those produced by recombinant DNA technology. These findings may have clinical relevance because the development of factor VIII alloantibodies is currently the major therapeutic complication in hemophilia A.[4-6,31,32]

Recombinant factor VIII products had nearly twice the rate of inhibitor development as plasma-derived products (hazard ratio, 1.87), in line with the trial hypothesis. A similarly increased hazard ratio (1.69) with a slightly wider confidence interval was observed for the secondary outcome of high-titer inhibitors. This estimate was not significant by conventional standards, probably owing to a small sample size.

We postulated a 25% incidence of inhibitors with recombinant factor VIII and 12.5% with plasma-derived factor VIII but found an incidence of 37.3% and 23.2%, respectively. This difference from our conservative estimate based on previous observational studies is probably due in part to the regularly scheduled testing for inhibitors, which exceeded testing frequency in routine care. Moreover, our findings are similar to those of

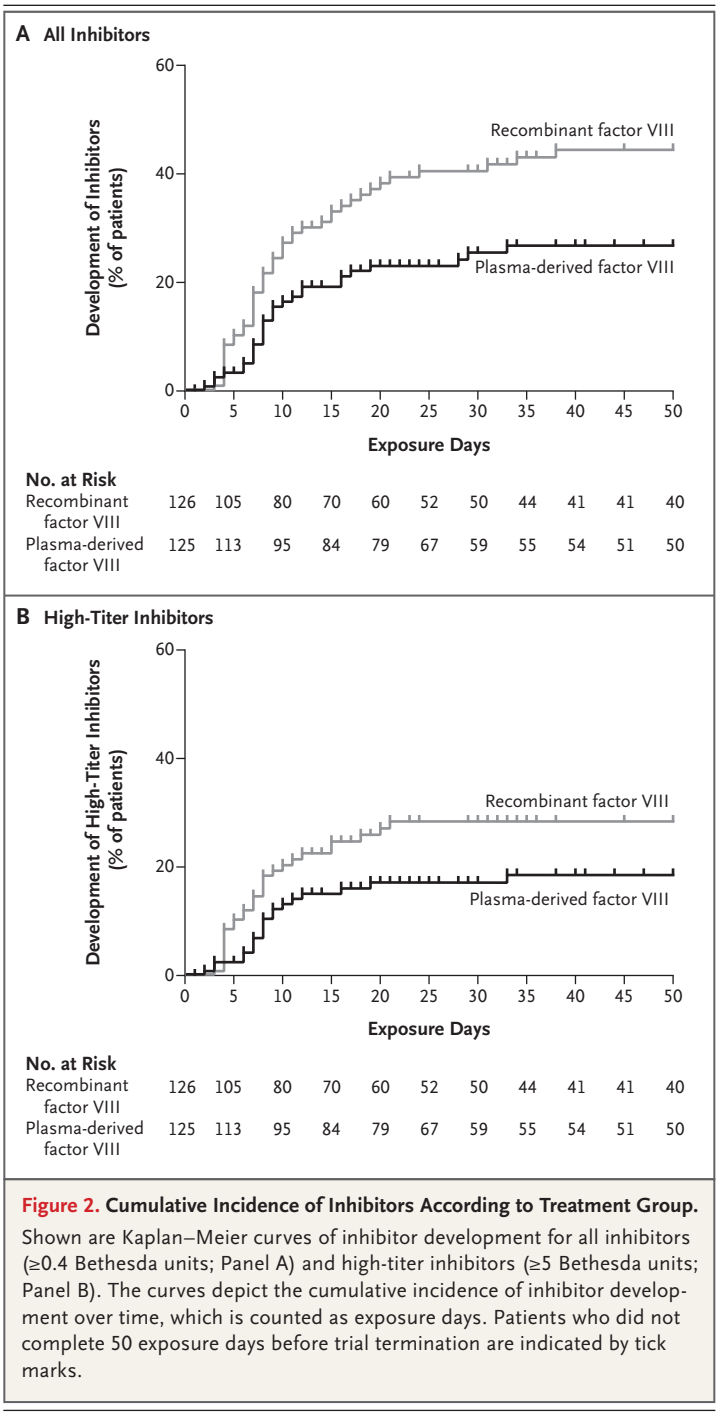

Figure 2. Cumulative Incidence of Inhibitors According to Treatment Group.
Shown are Kaplan–Meier curves of inhibitor development for all inhibitors (≥0.4 Bethesda units; Panel A) and high-titer inhibitors (≥5 Bethesda units; Panel B). The curves depict the cumulative incidence of inhibitor development over time, which is counted as exposure days. Patients who did not complete 50 exposure days before trial termination are indicated by tick marks.

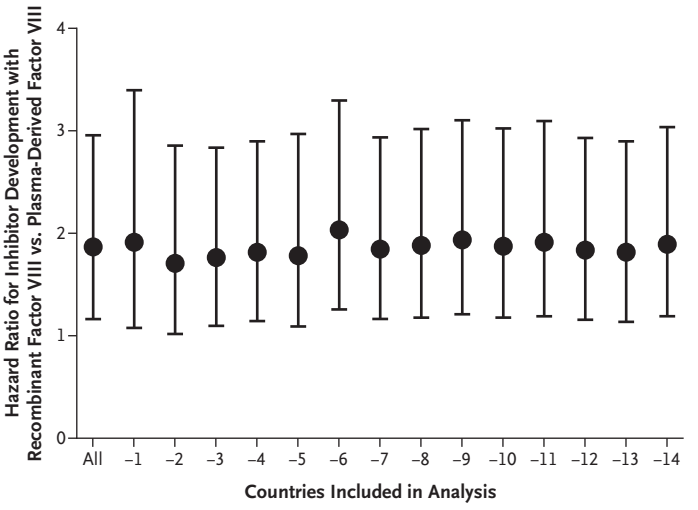

Figure 3. Sensitivity Analyses for Inhibitor Development with Recombinant Factor VIII, as Compared with Plasma-Derived Factor VIII.

In each subsequent analysis, 1 of the 14 countries (numbered 1 to 14) was left out of the analysis, which every time included the other 13 countries. I bars represent 95% confidence intervals.

two large systematic reviews,[16,17] one of which showed an overall incidence of 23.8%, with individual studies reporting frequencies ranging from 3 to 50%.[16] In a recent analysis of studies published since 2009, the overall incidence based on individual patient data was 27% at 20 exposure days.[18]

Observational studies and meta-analyses thereof on the immunogenicity of plasma-derived factor VIII versus recombinant factor VIII have been suggestive of an increased rate with the latter but remained inconclusive, probably owing to differences in patients' risk profiles for inhibitor development.[8,10-13,16-18,28] Moreover, the studies included in the meta-analyses differed in terms of design, enrollment criteria, definition of hemophilia A, sample size, method of inhibitor detection, and intervals of follow-up testing.[16-18] Randomized trials that would remove this confounding by indication were required to answer the question more definitively.

Our trial was specifically designed to compare the immunogenicity of factor VIII products.[33] As a result of randomization, the main risk factors for inhibitor development were evenly distributed between the two factor VIII classes, and our results did not change materially after adjustment for putative confounders.

Several recent reports have shown an increased incidence of inhibitors with second-generation full-length recombinant factor VIII products (60% higher than with other recombinant factor VIII products).[28-30] In an analysis restricted to countries that had no patient randomly assigned to the second-generation product Kogenate FS, the risk of inhibitor development with other recombinant factor VIII products was still nearly twice as high as the risk with plasma-derived factor VIII.

We used only one plasma-derived factor VIII and one recombinant factor VIII per country, for reasons of practicality. Therefore, commercial brands within a class, country-specific treatment regimens, and race or ethnic group could not be analyzed as separate covariates. Because patients were randomly assigned within their center to either plasma-derived factor VIII or recombinant factor VIII, this does not affect the comparison between the product classes, and adjustment for country had no effect on the between-class comparison.

Except for type of mutation, we found no clear association between inhibitor risk and previously reported factors such as race or ethnic group, intensity of treatment, and age at first treatment. The trial was not powered to assess these factors, for which the evidence is equivocal. Randomization with a fixed block size theoretically informs physicians about the treatment assignment of a subsequent patient, but the small number of patients per center included over a 5-year period renders bias unlikely.

A limitation of this trial is that it was terminated prematurely. However, because the observed incidence of inhibitors was higher than anticipated, the study power was higher than originally expected, at 83%. The majority of patients (86%) reached a prespecified end point; of the 35 patients who did not, only 10 had shortened follow-up owing to trial termination (median, 25 exposure days). For practical reasons, the trial was unblinded. The objective measurement of inhibitor development makes it unlikely that investigators could have influenced the results.

In summary, we found that early replacement therapy with plasma-derived factor VIII was associated with a lower incidence of inhibitor development than was therapy with recombinant factor VIII. The finding that native factor VIII products from human plasma are less immunogenic than those engineered by recombinant DNA technology in animal cell lines has the potential

to affect treatment strategies and open new investigations to better understand the mechanisms of the immunogenicity of various factor VIII preparations.

Supported by the nonprofit Angelo Bianchi Bonomi Foundation and the Italian Ministry of Health (Progetti Finalizzati and Agenzia Italiana del Farmaco). Grifols, Kedrion Biopharma, and LFB provided unrestricted grants to the Angelo Bianchi Bonomi Foundation.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Alessandro Gringeri, M.D., who had a major role in the early planning of this trial; the members of the steering committee, who helped with critical advice during the preparation of the protocol; and particularly the chair and members of the data and safety monitoring board, who assisted us in monitoring the safety aspects of the trial. We acknowledge Prasad Mathew, M.D. (Bayer HealthCare), who was initially a member of the steering committee; Roberta Palla, Ph.D., and Marzia Menegatti, Ph.D. (both from the Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan), who helped in the preparation of the manuscript; and Dr. Luigi Flaminio Ghilardini (Università degli Studi di Milano, Milan) for his help with figures and tables.

APPENDIX

The authors' full names and academic degrees are as follows: Flora Peyvandi, M.D., Ph.D., Pier M. Mannucci, M.D., Isabella Garagiola, Ph.D., Amal El-Beshlawy, M.D., Mohsen Elalfy, M.D., Vijay Ramanan, M.D., Peyman Eshghi, M.D., Suresh Hanagavadi, M.D., Ramabadran Varadarajan, M.D., Mehran Karimi, M.D., Mamta V. Manglani, M.D., Cecil Ross, M.D., Guy Young, M.D., Tulika Seth, M.D., Shashikant Apte, M.D., Dinesh M. Nayak, D.N.B., Elena Santagostino, M.D., Maria Elisa Mancuso, M.D., Adriana C. Sandoval Gonzalez, M.D., Johnny N. Mahlangu, M.Med., Santiago Bonanad Boix, M.D., Monica Cerqueira, M.D., Nadia P. Ewing, M.D., Christoph Male, M.D., Tarek Owaidah, F.R.C.P.A., Veronica Soto Arellano, M.D., Nathan L. Kobrinsky, M.D., Suvankar Majumdar, M.B., Ch.B., Rosario Perez Garrido, M.D., Anupam Sachdeva, M.D., Mindy Simpson, M.D., Mathew Thomas, M.D., Ezio Zanon, M.D., Bulent Antmen, M.D., Kaan Kavakli, M.D., Marilyn J. Manco-Johnson, M.D., Monica Martinez, M.D., Esperanza Marzouka, M.D., Maria G. Mazzucconi, A.P., Daniela Neme, M.D., Angeles Palomo Bravo, M.D., Rogelio Paredes Aguilera, M.D., Alessandra Prezotti, M.D., Klaus Schmitt, M.D., Brian M. Wicklund, M.D., Bulent Zulfikar, M.D., and Frits R. Rosendaal, M.D., Ph.D.

The authors' affiliations are as follows: the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Ca' Granda Ospedale Maggiore Policlinico (F.P., P.M.M., E.S., M.E.M.), and Department of Pathophysiology and Transplantation, Università degli Studi di Milano (F.P., P.M.M., I.G.), Milan, Clinica Medica II, Azienda Ospedaliera di Padova, Centro Emofilia, Padua (E.Z.), and Ematologia, Unità Operativa Diagnostica Speciale e Terapia delle Malattie dell'Emostasi e della Trombosi, Università Sapienza, Policlinico Umberto I, Rome (M.G.M.) — all in Italy; the Pediatric Hematology Department, Cairo University Pediatric Hospital (A.E.-B.), and Department of Pediatrics, Faculty of Medicine, Ain Shams University (M.E.), Cairo; Jehangir Clinical Development Center, Department of Hematology, Jehangir Hospital Premises (V.R.), and Sahyadri Speciality Hospital (S.A.), Pune, Jagadguru Jayadeva Murugarajendra Medical College, Davangere (S.H.), Center for Blood Disorders, Chennai (R.V.), Lokmanya Tilak Municipal Medical College and General Hospital, Mumbai (M.V.M.); St. John's Medical College Hospital, Bangalore (C.R.), All India Institute of Medical Sciences, Department of Hematology (T.S.), and Pediatric Hematology Oncology and Bone Marrow Transplantation, Institute for Child Health, Sir Ganga Ram Hospital (A.S.), New Delhi, Melaka-Manipal Medical College, Manipal University, Manipal (D.M.N.), and Kerala Institute of Medical Science, Trivandrum (M.T.) — all in India; the Congenital Pediatric Hematologic Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran (P.E.), and Hematology Research Center, Shiraz University of Medical Sciences, Shiraz (M.K.) — both in Iran; Children's Hospital Los Angeles, Los Angeles (G.Y.), and City of Hope National Medical Center, Duarte (N.P.E.) — both in California; Hospital de Especialidades Unidad Médica de Alta Especialidad, Instituto Mexicano del Seguro Social, Monterrey (A.C.S.G.), and Instituto Nacional de Pediatria, Mexico City (R.P.A.) — both in Mexico; Faculty of Health Sciences, School of Pathology, University of the Witwatersrand, National Health Laboratory Service and Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg (J.N.M.); Hospital Universitario La Fe, Unidad Coagulopatias Congenitas, Valencia (S.B.B.), Hospital Universitario Virgen del Rocío, Unidad de Hemofilia, Seville (R.P.G.), and Hospital Regional Universitario Carlos Haya, Malaga (A.P.B.) — all in Spain; Centro de Pesquisa Clinica Hemorio–Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti, Rio de Janeiro (M.C.), and Centro de Hematologia e Hemoterapia do Espírito Santo, Vitoria (A.P.) — both in Brazil; Medizinische Universität Wien, Department of Pediatrics, Vienna (C.M.), and Department of Pediatric and Adolescent Medicine, Kepler University Clinic, Linz (K.S.) — both in Austria; King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia (T.O.); Centro de Hemofílicos del Hospital de Niños Dr. Roberto del Río (V.S.A.) and Hospital de Niños Dr. Luis Calvo Mackenna, Centro Hemofílico (E.M.), Santiago, Chile; Sanford Roger Maris Cancer Center, Fargo, ND (N.L.K.); University of Mississippi Medical Center, Division of Pediatric Hematology–Oncology, Jackson (S.M.); Rush Hemophilia and Thrombophilia Center, Rush University Medical Center, Chicago (M.S.); Cukurova Universitesi, Tip Fakultesi Pediatrik Hematoloji Bilim Dali, Adana (B.A.), Ege Universitesi Tip Fakultesi Cocuk Sagligi ve Hastalikari Anabilim Dali, Pediatrik Hematoloji Bilim Dali, Izmir (K.K.), and Istanbul Universitesi Cerrahpasa Tip Fakultesi, Pediatrik Hematoloji Bilim Dali, Istanbul (B.Z.) — all in Turkey; Hemophilia and Thrombosis Center, University of Colorado Denver, Aurora (M.J.M.-J.); Hospital de Niños Sor María Ludovica La Plata, Servicio de Hematología (M.M.), and Fundación de la Hemofilia (D.N.), Buenos Aires; Children's Mercy Hospital, Kansas City, MO (B.M.W.); and the Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands (F.R.R.).

REFERENCES

1. Mannucci PM, Tuddenham EGD. The hemophilias — from royal genes to gene therapy. N Engl J Med 2001;344:1773-9.
2. Plug I, van der Bom JG, Peters M, et al. Mortality and causes of death in patients with hemophilia, 1992-2001: a prospective cohort study. J Thromb Haemost 2006;4:510-6.
3. Gouw SC, van den Berg HM, Fischer K, et al. Intensity of factor VIII treatment and inhibitor development in children with severe hemophilia A: the RODIN study. Blood 2013;121:4046-55.
4. Darby SC, Keeling DM, Spooner RJ, et al. The incidence of factor VIII and factor IX inhibitors in the hemophilia population of the UK and their effect on subsequent mortality, 1977-99. J Thromb Haemost 2004;2:1047-54.
5. Walsh CE, Soucie JM, Miller CH. Impact of inhibitors on hemophilia A mortality in the United States. Am J Hematol 2015;90:400-5.
6. Leissinger C, Cooper DL, Solem CT.

Assessing the impact of age, race, ethnicity and inhibitor status on functional limitations of patients with severe and moderately severe haemophilia A. Haemophilia 2011;17:884-9.
7. Schwaab R, Brackmann HH, Meyer C, et al. Haemophilia A: mutation type determines risk of inhibitor formation. Thromb Haemost 1995;74:1402-6.
8. Gouw SC, van der Bom JG, Marijke van den Berg H. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study. Blood 2007; 109:4648-54.
9. Aledort LM, DiMichele DM. Inhibitors occur more frequently in African-American and Latino haemophiliacs. Haemophilia 1998;4:68.
10. Wight J, Paisley S. The epidemiology of inhibitors in haemophilia A: a systematic review. Haemophilia 2003;9:418-35.
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. Goudemand J, Rothschild C, d'Oiron R, et al. Plasma-derived factor VIII products and inhibitor development in previously untreated boys with severe hemophilia A: report of the FranceCoag Network. J Thromb Haemost 2015;13: Suppl 2:148.
14. Dasgupta S, Repessé Y, Bayry J, et al. VWF protects FVIII from endocytosis by dendritic cells and subsequent presentation to immune effectors. Blood 2007; 109:610-2.
15. Qadura M, Waters B, Burnett E, et al. Recombinant and plasma-derived factor VIII products induce distinct splenic cytokine microenvironments in hemophilia A mice. Blood 2009;114:871-80.
16. Iorio A, Halimeh S, Holzhauer S, et al. Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: a systematic review. J Thromb Haemost 2010;8:1256-65.
17. Franchini M, Coppola A, Rocino A, et al. Systematic review of the role of FVIII concentrates in inhibitor development in previously untreated patients with severe hemophilia A: a 2013 update. Semin Thromb Hemost 2013;39:752-66.
18. Marcucci M, Mancuso ME, Santagostino E, et al. Type and intensity of FVIII exposure on inhibitor development in PUPs with haemophilia A: a patient-level meta-analysis. Thromb Haemost 2015; 113:958-67.
19. Barrowcliffe TW. Laboratory testing and standardisation. Haemophilia 2013; 19:799-804.
20. Verbruggen B, van Heerde W, Novákovà I, Lillicrap D, Giles A. A 4% solution of bovine serum albumin may be used in place of factor VIII:C deficient plasma in the control sample in the Nijmegen Modification of the Bethesda factor VIII:C inhibitor assay. Thromb Haemost 2002;88:362-4.
21. Logan LJ. von Willebrand factor content in Alphanate. Haemophilia 2009;15: 369-71.
22. Hernandez-Navarro F, Quintana M, Jimenez-Yuste V, Alvarez MT, Fernandez-Morata R. Clinical efficacy in bleeding and surgery in von Willebrand patients treated with Fanhdi a highly purified, doubly inactivated FVIII/VWF concentrate. Haemophilia 2008;14:963-7.
23. Peyvandi F, Mannucci PM, Valsecchi C, Pontiggia S, Farina C, Retzios AD. ADAMTS13 content in plasma-derived factor VIII/von Willebrand factor concentrates. Am J Hematol 2013;88:895-8.
24. Samor B, Michalski C, Brandin MP, Andre MH, Chtourou S, Tellier Z. A qualitative and quantitative analysis of von Willebrand factor contained in a very high-purity plasma-derived FVIII concentrate. Vox Sang 2012;103:35-41.
25. Liu Q, Nozari G, Sommer SS. Single-tube polymerase chain reaction for rapid diagnosis of the inversion hotspot of mutation in hemophilia A. Blood 1998;92: 1458-9.
26. Bagnall RD, Waseem N, Green PM, Giannelli F. Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A. Blood 2002;99:168-74.
27. Santacroce R, Acquila M, Belvini D, et al. Identification of 217 unreported mutations in the F8 gene in a group of 1,410 unselected Italian patients with hemophilia A. J Hum Genet 2008;53:275-84.
28. Gouw SC, van der Bom JG, Ljung R, et al. Factor VIII products and inhibitor development in severe hemophilia A. N Engl J Med 2013;368:231-9.
29. Calvez T, Chambost H, Claeyssens-Donadel S, et al. Recombinant factor VIII products and inhibitor development in previously untreated boys with severe hemophilia A. Blood 2014;124:3398-408.
30. Collins PW, Palmer BP, Chalmers EA, et al. Factor VIII brand and the incidence of factor VIII inhibitors in previously untreated UK children with severe hemophilia A, 2000-2011. Blood 2014;124:3389-97.
31. Gringeri A, Mantovani LG, Scalone L, Mannucci PM. Cost of care and quality of life for patients with hemophilia complicated by inhibitors: the COCIS Study Group. Blood 2003;102:2358-63.
32. Guh S, Grosse SD, McAlister S, Kessler CM, Soucie JM. Healthcare expenditures for males with haemophilia and employer-sponsored insurance in the United States, 2008. Haemophilia 2012; 18:268-75.
33. Mannucci PM, Gringeri A, Peyvandi F, Santagostino E. Factor VIII products and inhibitor development: the SIPPET study (Survey of Inhibitors in Plasma-Product Exposed Toddlers). Haemophilia 2007;13: Suppl 5:65-8.