Journal of Thrombosis and Haemostasis, **8**: 1256–1265 DOI: 10.1111/j.1538-7836.2010.03823.x

ORIGINAL ARTICLE

Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: a systematic review

A. IORIO,* S. HALIMEH,† S. HOLZHAUER,‡ N. GOLDENBERG,§ E. MARCHESINI,* M. MARCUCCI,* G. YOUNG,¶ C. BIDLINGMAIER,‡‡ L. R. BRANDAO,§§ C. E. ETTINGSHAUSEN,¶¶ A. GRINGERI,** G. KENET,*** R. KNÖFLER,††† W. KREUZ,¶¶ K. KURNIK,‡‡ D. MANNER,†† E. SANTAGOSTINO,** P. M. MANNUCCI** and U. NOWAK-GÖTTL††

**Internal and Vascular Medicine &Hemophilia Centre, University of Perugia, Perugia, Italy; †Medical Thrombosis and Hemophilia Treatment/ Blood Transfusion Center Duisburg, Duisburg; ‡Department of Pediatric Hematology/Oncology, Charite, Berlin, Germany; §Department of Pediatrics, Hematology/Oncology/BMT, University of Colorado and The Children's Hospital, Denver, CO, USA; ¶Division of Hematology/ Oncology, Children's Hospital, Los Angeles, CA; **Department of Medicine and Medical Specialties, Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre, IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milan, Italy; ††Department of Pediatric Hematology/ Oncology, University Hospital Münster, Münster; ‡‡Department of Pediatrics, University Hospital Munich, Munich, Germany; §§Department of Pediatric Hematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada; ¶¶Department of Pediatric Hematology/Oncology, University Hospital Frankfurt, Frankfurt, Germany; ***The Israel National Hemophilia Centre, Sheba Medical Centre, Tel-Hashomer, Israel; and †††Department of Pediatric Hematology/Oncology, University Hospital Dresden, Dresden, Germany*

To cite this article: Iorio A, Halimeh S, Holzhauer S, Goldenberg N, Marchesini E, Marcucci M, Young G, Bidlingmaier C, Brandao LR, Ettingshausen CE, Gringeri A, Kenet G, Knöfler R, Kreuz W, Kurnik K, Manner D, Santagostino E, Mannucci PM, Nowak-Göttl U. 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.

Summary. *Background*: Different rates of inhibitor development after either plasma-derived (pdFVIII) or recombinant (rFVIII) FVIII have been suggested. However, conflicting results are reported in the literature. *Objectives*: To systematically review the incidence rates of inhibitor development in previously untreated patients (PUPs) with hemophilia A treated with either pdFVIII or rFVIII and to explore the influence of both study and patient characteristics. *Methods:* Summary incidence rates (95% confidence interval) from all included studies for both pdFVIII and rFVIII results were recalculated and pooled. Sensitivity analysis was used to investigate the effect of study design, severity of disease and inhibitor characteristics. Meta-regression and analysis-of-variance were used to investigate the effect of covariates (testing frequency, follow-up duration and intensity of treatment). *Results:* Two thousand and ninety-four patients (1167 treated with pdFVIII, 927 with rFVIII; median age, 9.6 months) from 24 studies were investigated and 420 patients were observed to develop inhibitors. Pooled incidence rate was 14.3% (10.4–19.4) for pdFVIII and 27.4% (23.6–31.5) for rFVIII; high responding inhibitor incidence rate was 9.3% (6.2–13.7) for pdFVIII and 17.4% (14.2–21.2) for rFVIII. In the multi-way ANOVA study design, study period, testing frequency and median follow-up explained most of the variability, while the source of concentrate lost statistical significance. It was not possible to analyse the effect of intensity of treatment or trigger events such as surgery, and to completely exclude multiple reports of the same patient or changes of concentrate. *Conclusions:* These findings underscore the need for randomized controlled trials to address whether or not the risk of inhibitor in PUPs with hemophilia A differs between rFVIII and pdFVIII.

Keywords: factor VIII concentrates, hemophilia A, inhibitor development, previously untreated patients, systematic review.

Correspondence: Alfonso Iorio, Internal and Vascular Medicine & Hemophilia Centre, University of Perugia, Perugia, Italy.
Tel.: +39 5 578 2309; fax: +39 075 578 2309.
E-mail: iorioa@unipg.it

Received 18 December 2009, accepted 17 February 2010

Introduction

Hemophilia A (HA) is an X-linked genetic hemorrhagic disorder resulting from a deficiency of blood coagulation factor VIII (FVIII). In recent years, the treatment of patients with severe HA has dramatically improved owing to (i) the availability of FVIII concentrates that are manufactured with improved viral safety measures, (ii) better techniques for performing venipunctures during home treatment, and (iii) the widespread adoption of prophylaxis to prevent bleeding episodes [1–5]. The development of inhibitors, that is alloantibodies that inhibit the procoagulant function of FVIII, is currently the most challenging complication of treatment in

persons with HA, resulting in increased morbidity and significant economic burden [6]. Although several factors are known to influence the risk of inhibitor development (type and severity of HA, nature of gene defect, ethnicity, intensive factor exposure at the time of surgery, and prophylactic or on-demand treatment regimens), the source of FVIII employed for replacement therapy may also have an effect on inhibitor development. The first indication of the influence of FVIII source on the risk of inhibitor development originated from two pioneering studies [7,8] and a systematic review [9], which highlighted that in HA patients treated exclusively with recombinant FVIII (rFVIII) the cumulative incidence of inhibitors was more than 2-fold higher than in those patients treated exclusively with plasma-derived FVIII (pdFVIII). Despite similar findings being subsequently obtained in two retrospective cohort studies by Goudemand *et al*. [10] and Chalmers *et al*. [11], another cohort study by Gouw *et al*. [12] found no significant difference in the risk of developing inhibitors between patients receiving FVIII from these two different sources.

In addition to these patient studies, *in vitro* data support the contention that von Willebrand factor, the carrier protein of FVIII contained in large amounts in most pdFVIII products but not in rFVIII, reduces the immunogenicity of FVIII by preventing its entry into professional antigen-presenting cells [13] and thus favouring a cytokine microenvironment less favourable to the development of inhibitory alloantibodies [14,15]. Overall, though the available experimental and clinical data suggest the reduced immunogenicity of pdFVIII concentrates, clinical equipoise (lack of unequivocal evidence in favour of either source of FVIII) exists. A multicenter, multinational clinical trial randomizing previously untreated patients to pdFVIII vs. rFVIII is under way [16]. With this as background, we carried out a systematic review of the available clinical studies, specifically aimed to re-evaluate the rate of inhibitor development in previously untreated HA patients that were treated with either pdFVIII or rFVIII, and to explore for the first time the role of study and patient characteristics in the incidence of inhibitor development.

Materials and methods

This systematic review was performed in accordance with MOOSE (Meta-analysis Of Observational Studies in Epidemiology [17] and STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines [18].

Inclusion criteria

Studies Prospective and retrospective studies with at least 10 HA patients treated with any type of FVIII product, in which data on inhibitor development were available, were evaluated. Study characteristics and quality were evaluated using the Newcastle-Ottawa Scale [19].

Patients All included HA patients had to be previously untreated patients (PUPs), defined by no prior exposure to FVIII concentrates. Patients with previous exposure to blood components other than plasma derivatives (i.e. red blood cells or platelets) or with < 5 exposure days (ED) to fresh frozen plasma were also considered as PUPs [20]. Patients with all severities of HA were included. HA was defined as severe if the plasma factor VIII:C level was $< 1\%$, moderate if it was between 1% and 5%, and mild if it was between 5% and 40%. If the definitions for the severity of HA in the included studies differed from our criteria and if individual patient data were available, patients were reclassified in accordance with our own definitions. Patients were analysed in three groups: all patients (all FVIII:C levels and all degrees of severity), severe HA (FVIII:C $< 1\%$) and severe plus moderate HA (FVIII:C $\leq 5\%$).

Inhibitors Patients with all types of inhibitors were included in the analysis. A high titre or high responding inhibitor was defined by an inhibitor titre ≥ 5 Bethesda units. Classification of inhibitors as high responding was based on source reports. If the definition for high responding inhibitors in any of the included studies differed from the above and if individual patient data were available, patients were reclassified in accordance with our definition. Inhibitors were defined as transient when they spontaneously disappeared within 6 months without the need to change the treatment regimen. Inhibitors had to be objectively confirmed and laboratory methods and cut-offs reported or provided by the authors upon request.

Search strategy and data extraction

A systematic search of electronic databases (Medline, EMBASE, OVID, Web of Science, The Cochrane Library) for studies published from 1970 to March 2009 was conducted. To avoid counting duplicating patients that were included in more than one report, patient recruitment periods and catchment areas were evaluated and authors were contacted for clarification if needed. If any of the required data could not be found in the published report, the corresponding author was contacted to provide the missing data of interest. More details are given in the Supporting Information.

Statistical analysis

Single study analysis The incidence rate of inhibitor development was estimated by the number of new inhibitor cases reported during the observation period divided by the number of HA patients that were initially reported as inhibitor-free and thus at risk of developing an inhibitor, without taking into account the observation time length. The incidence rate of inhibitor development for each included study was recalculated for the whole study cohort and relevant subgroups and expressed as rate per 100 patients.

Pooled analysis of single arm studies (pooled incidence rates) The recalculated rates of inhibitor development for each included study were pooled with the statistical method of Laird and Mosteller [21], assuming fixed or random effect, as appropriate. Studies reporting data on two cohorts of patients treated with different FVIII concentrates (i.e. rFVIII vs. pdFVIII) were considered as providing data on two unrelated cohorts. The pooled rates were calculated by grouping the studies according to the kind of treatment (pdFVIII or rFVIII). Pooled rates in the two groups were indirectly compared by evaluating inter-treatment group heterogeneity according to the Cochran Q statistic.

Sensitivity analysis For sensitivity analysis, calculations were repeated where possible for: (i) prospective studies, (ii) patients with severe FVIII deficiency, (iii) moderate plus severe FVIII-deficient patients, (iv) high responding inhibitors, (v) non-transient inhibitors, (vi) high or low purity pdFVIII concentrates, and (vii) single or multiple concentrate usage. For the purposes of the sensitivity analysis, we classified the concentrates as high or low purity according to the authors' definitions. Very high and intermediate purity concentrates were classified as high and low purity, respectively. We classified the products defined as highly purified but containing a high level of von Willebrand factor as low purity. Multiple concentrate usage reflected cohorts of patients that were treated with different products, but not switching from one product to another.

Meta-regression Meta-regression was performed for all studies included in the pooled analysis, using as predictors: (i) the study period, (ii) the frequency of inhibitor testing, (iii) the median duration of follow up, and (iv) intensity of treatment.

Analysis of variance The effects of study and patient characteristics on inhibitor rate were explored by ANOVA. The ANOVA was performed on severe plus moderate patients. Weights inversely proportional to the estimated sampling variance were used. One-way ANOVA was initially performed to separately evaluate the continuous variables, including study period, test frequency and median follow-up duration, treatment intensity, and also categorical variables, including source of FVIII (i.e. pdFVIII or rFVIII), type of study (i.e. retrospective or prospective), proportion of patients developing high responding inhibitors, proportion of patients developing non-transient inhibitors, concentrate purity, and single concentrate usage. Finally, a multi-way ANOVA model was obtained that included all the variables found to be statistically significant ($P < 0.05$) using one-way analysis plus their interactions using a forward stepwise approach. Coding details for the variables are provided in the Supporting Information.

Pooled analysis of studies describing parallel cohorts treated with pdFVIII or rFVIII concentrates (pooled relative risk) A formal meta-analysis was performed on the study subset that has directly compared parallel cohorts (concurrent or not concurrent) of patients treated with pdFVIII or rFVIII. Summary relative risk and 95% confidence intervals were calculated from the effect estimates of the individual studies weighted by standard error using a fixed or a random-effect model as appropriate. Additional details are provided in the Supporting Information.

STATA (STATA version 9.2, Statacorp, College Station, TX, USA), StatsDirect (version 2.6.6 update, StatsDirect Ltd, Altrincham, UK: http://www.statsdirect.com), and Comprehensive Meta-Analysis (version 2.2.046, Biostat, Englewood, NJ, USA) were used for statistical analyses.

Results

Study characteristics

A total of 224 potentially relevant references were screened, and 24 studies were ultimately included in the analyses, of which three were in abstract form. Figure 1 depicts the flow chart of the study selection process. Details of the studies included in the analysis are provided in the Supporting Information.

Patient characteristics

Two thousand and ninety-four HA PUPs were included in the analysis (1167 on pdFVIII and 927 on rFVIII), of whom 887 were in the severe HA group and 1965 were in the severe plus moderate group. Forty-one patients were mild and the severity of 88 patients was not classified. The total number of patients that developed inhibitors was 420, of which 160 (13.7%) were treated with pdFVIII and 260 (28.0%) were treated with rFVIII. High responding inhibitors were found in 252/1864 patients (101/1022 [9.8%] for pdFVIII and 151/842 [17.9%] for rFVIII). Non-transient inhibitors were 175/1117 (77/643 [12.0%] for pdFVIII and 98/474 [20.7%] for rFVIII). Eight references reported peak inhibitor titres and the overall weighted median peak inhibitor titre was 5.09 Bethesda units (range 0.7–272), while the median peak inhibitor titre was 79.0 Bethesda units (range 0.70 to ≥ 79) for pdFVIII and 4.09 Bethesda units (range 3–272) for rFVIII, using the number of inhibitor patients in the study as weight.

Pooled analysis of single arm studies

Inhibitor development incidence rates were pooled for pdFVIII- and rFVIII-treated patients from 24 references, for a total of 16 pdFVIII and 16 rFVIII arms (Table 1 and Fig. 2). In patients treated with rFVIII, the inhibitor development rate was significantly higher than in those patients treated with pdFVIII (27.4% vs. 14.3%, Cochran Q = 11.7, $P < 0.001$). Because significant heterogeneity was observed, the random effect method was used, while no significant publication bias was found. Further details are provided in the Supporting information.

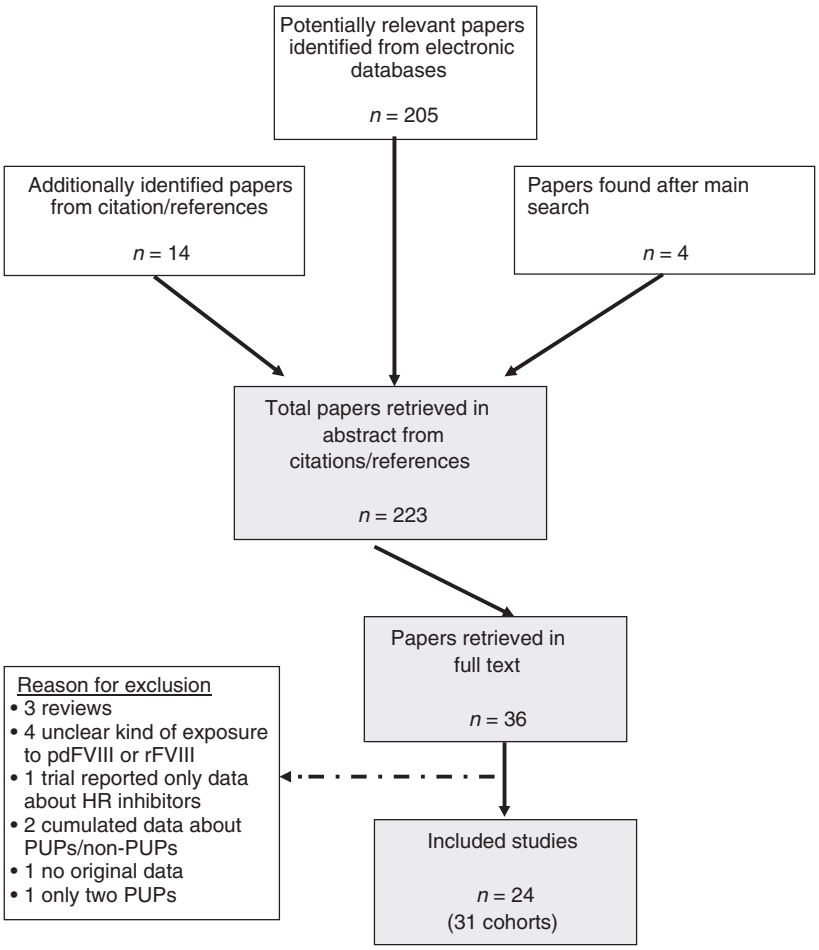

Fig. 1. Flow chart of results of the search strategy and reasons for exclusion.

Sensitivity analysis

Disease severity The event rate was significantly higher when the analysis was limited to patients with severe HA (pdFVIII = 15.9%, 95% confidence interval 10.5–23.3; rFVIII = 34.5%, 95% confidence interval 29.3–40.1; Cochrane Q = 14.2; $P < 0.001$) or to moderate plus severe patients (pdFVIII = 15.4%, 95% confidence interval 11.1–21.0; rFVIII = 28.5%, 95% confidence interval 25.1–32.2; Cochrane Q = 13.6; $P < 0.001$). Inhibitor development rate increased in relation to HA severity, both in pdFVIII- and rFVIII-treated patients, although more evidently in rFVIII-treated patients.

Inhibitor characteristics The incidence of inhibitor development was significantly higher in rFVIII vs. pdFVIII for high responding but not for non-transient inhibitors (Table 1).

Purity of pdFVIII concentrates Most of the studies classified the pdFVIII concentrates used as low, intermediate or high purity. Only a minority of the reports distinguished the concentrates on the basis of their von Willebrand factor content. The inhibitor incidence rate was 10.2% (95% confidence interval 6.5–15.6) for high purity and 15.9% (95% confidence interval 11.4–21.6) for low purity (Cochrane Q = 3.0; $P = 0.221$).

Multiple FVIII concentrate use Inhibitor incidence rates were recalculated after exclusion of studies reporting the use of multiple concentrates in the observed cohort. The inhibitor incidence rate was 25.2% (95% confidence interval 20.6–30.4) for rFVIII and 8.5% (95% confidence interval 5.7–12.4) for pdFVIII (Cochrane Q = 25.6; $P < 0.001$). Within those patients receiving pdFVIII concentrates, the inhibitor incidence rate was 8.1% (95% confidence interval 1.5–34.4) for high and 8.2% (95% confidence interval 5.3–12.5) for low purity (Cochrane Q = 0.01; $P = 0.988$). The inhibitor incidence rate in cohorts using single concentrates was lower in both rFVIII- and pdFVIII-treated patients as compared with cohorts treated with multiple concentrates. The difference was of borderline statistical significance for rFVIII and significant for pdFVIII.

Meta-regression

Inspection of meta-regression plots of inhibitor rate vs. study period showed a trend for higher inhibitor detection rate in

Table 1 Inhibitor development rates (per cent) in relation to factor VIII source

	Main analysis		
	Plasma-derived FVIII	Recombinant FVIII	*P* value (pdFVIII vs. rFVIII)
	Event rate (95% CI)	Event rate (95% CI)	(Cohran Q)
All studies	14.3 (10.4–19.4)	27.4 (23.6–31.5)	< 0.001
	Sensitivity analyses		
	Event rate (95% CI) [number of studies]	Event rate (95% CI) [number of studies]	(Cohran Q)
Prospective studies			
All patients	9.1 (5.6–14.4) [9]	23.7 (18.5–29.7) [10]	< 0.001
Severe HA, HR only	6.0 (1.1–27.7) [2]	19.4 (9.0–36.9) [1]	0.195*
HR inhibitors			
All patients	9.3 (6.2–13.7) [13]	17.4 (14.2–21.2) [13]	0.004
Severe HA	9.0 (4.0–19.2) [5]	18.2 (13.9–23.5) [3]	0.009
Non-transient inhibitors			
All patients	11.8 (6.9–19.6) [8]	19.8 (15.3–25.3) [10]	0.076**
Severe HA	16.3 (0.8–30.1 [3]	25.8 (13.5–43.7) [1]	0.317***

HA, hemophilia A; HR, high responding inhibitors; CI, confidence interval. The recalculated power of the subanalyses was *42.2%, **94.5% and ***20.6%. The pooled percentages given in the table are slightly different from the raw percentages given in the results section due to the use of weights.

more recent study periods (Fig. 3). Similarly, a positive association was found between inhibitor development rate and inhibitor testing frequency (i.e. higher inhibitor development rates were found in those studies with more frequent testing) (Fig. 3). No apparent trend was observed when investigating the effect of duration of follow-up. Insufficient data were available to test the effect of the intensity of treatment.

Analysis of variance

Details about univariate and bivariate analyses are provided in the Supporting Information. The final multi-way ANOVA model was built by including source of concentrate, study design, testing frequency, study period and median follow-up duration plus the interactions of each of the three modifier variables with either source of concentrate or study design. The model was overall significant (adjusted $R^2 = 0.80$, $P = 0.01$), and explained as much as twice the amount of variability in inhibitor development rate as compared with the univariate model that included concentrate source alone (adjusted $R^2 = 0.40$, $P < 0.001$). None of the variables was associated with a significant *P* value, highlighting that there was no clear independent predictor of inhibitor development rate.

Pooled analysis of studies involving parallel cohorts treated with pdFVIII or rFVIII concentrates

In six studies (1259 patients), data on high or low titre inhibitors in patients treated with rFVIII or pdFVIII were reported. No significant heterogeneity was found between studies. Statistically significant associations of either high- or low-titre inhibitors were demonstrated for rFVIII vs. pdFVIII use, with summary relative risk of 1.7 (95% confidence interval 1.3–2.7, $P < 0.001$; Cochran Q chi-squared = 1.97, $P = 0.853$) for high responding inhibitors and 2.0 (95% confidence interval 1.5–2.6, $P < 0.001$; Cochrane Q chi-squared = 3.03, $P = 0.695$) for all inhibitors. Forest plots revealed that HA patients that were initially treated with rFVIII had an increased risk of developing an inhibitor (Fig. 4).

Discussion

This systematic review demonstrates that rFVIII concentrates are associated with a higher incidence rate of inhibitor development than pdFVIII concentrates, both in the frame of an overall analysis and in the analysis of studies reporting parallel cohorts. However, this effect became progressively less important when all inhibitors, high responding inhibitors or non-transient inhibitors were analysed. In the sensitivity and multivariate analysis, retrospective studies overestimate the inhibitor development rate, and inhibitor testing frequency, study period and follow-up duration appeared to explain a significant proportion of the variability observed in inhibitor detection rate between pdFVIII and rFVIII products. Purity of pdFVIII concentrates did not affect inhibitor rate, but adequate data were not available to enable assessment of the influence of FVIII treatment intensity.

The novelty of this systematic review relies on the inclusion of 13 additional trials (including 1468 additional patients) beyond those included in the systematic review by Wight and Paisley [9]. Furthermore, while that review aimed to estimate the cumulative incidence and prevalence of inhibitors in various HA populations, this review was specifically focused on estimating the effect of the source of FVIII. Finally, Wight and Paisley provided a statistical evaluation of the sources of

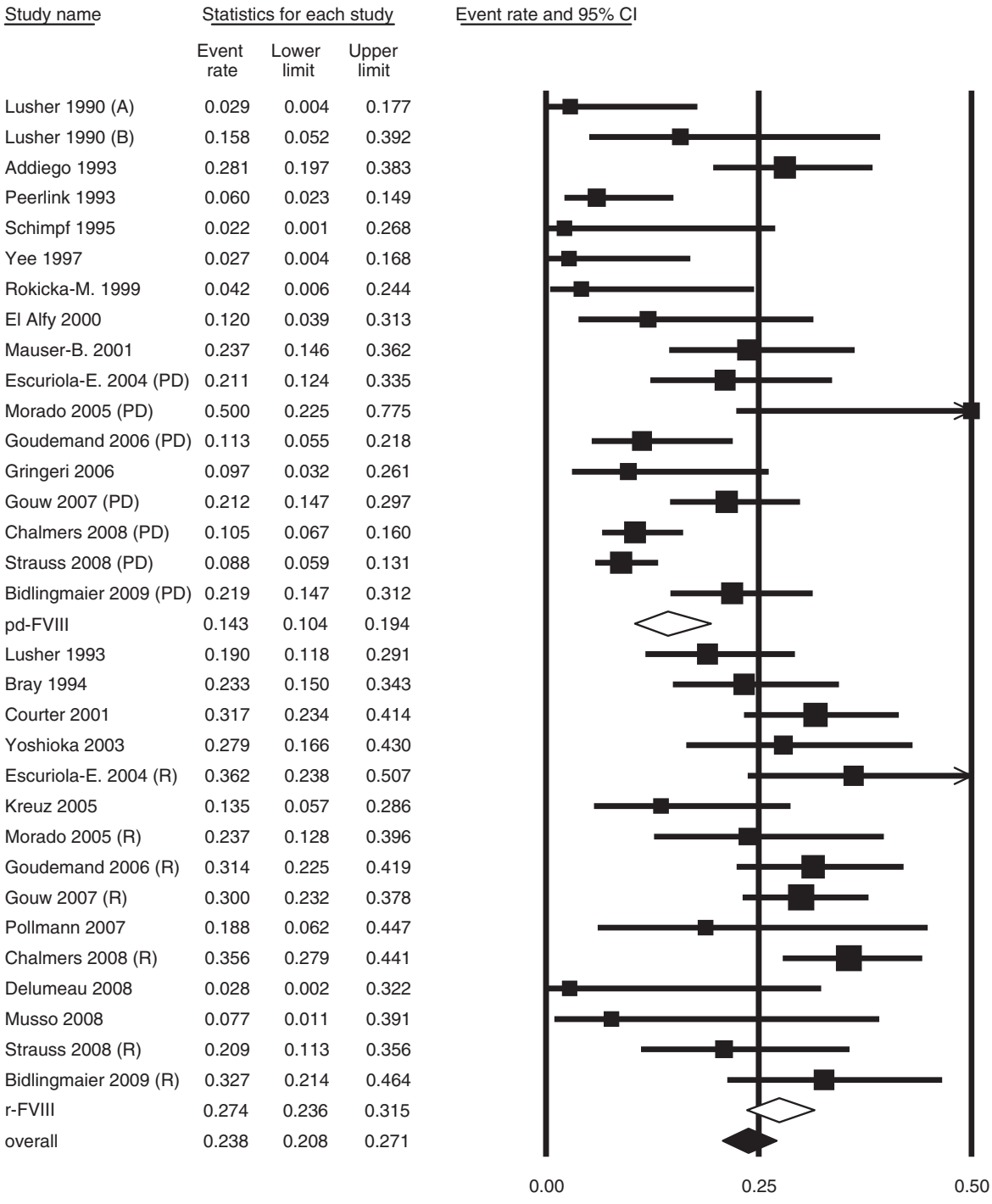

Fig. 2. Incidence rate and 95% confidence interval of all inhibitors in PUPs with HA. Study author and year of publication are indicated on the *y*-axis and the incidence rate (0–50%) on the *x*-axis. Studies ordered by pdFVIII and rFVIII usage and by year of publication. The pooled estimate is based on random effects, and shown for subgroups and for the whole population. (R) or (PD) are indicated in brackets when only data of the relevant subgroup of patients are considered.

variability only as secondary objective, whereas this was performed extensively in this analysis. When compared with Calvez *et al*. [22], who analysed four studies on the inhibitor development rate in pdFVIII- or rFVIII-treated patients, we not only considered two additional studies but also performed a formal pooled analysis, evaluating heterogeneity and adjusting for partial overlap of the included patients. Finally, this is the first formal demonstration that variables such as frequency of inhibitor testing, the recency of study period and the duration of follow-up can, to a large extent, explain the differences between pdFVIII and rFVIII, because the difference in the incidence of inhibitor development diminished to

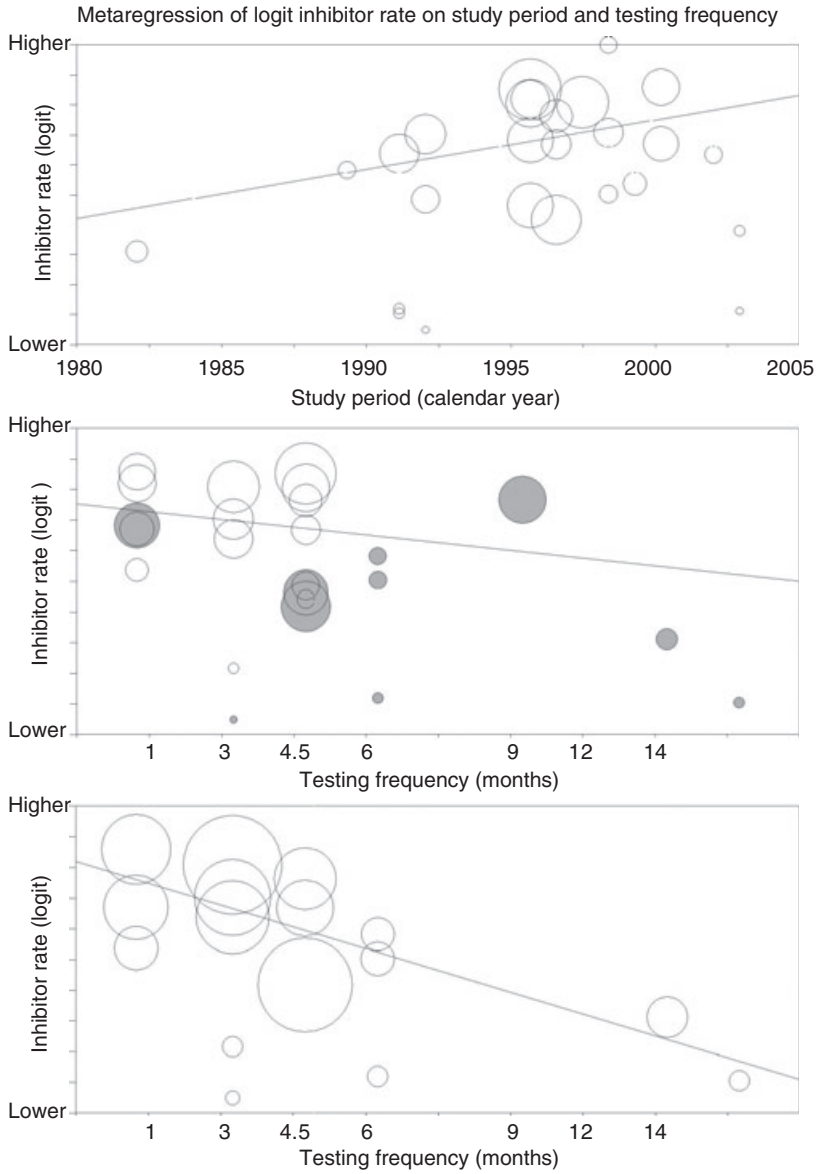

Fig. 3. The metaregression plots for study period (top) and testing frequency (middle and bottom) are shown. The *y*-axis shows the logit of the incidence rate of inhibitors, from 0 to −4. The incidence rate increases from the axis origin upward. Each bubble in the plot represents a single study, and the bubble diameter is inversely proportional to the variance of the study, that is to the influence of the study on the slope of the regression line. The top insert shows the effect of study period, from 1975 to 2005. The middle insert shows the effect of testing frequency, from every month to every 14 months. White bubbles indicate cohorts of patients treated with rFVIII, gray bubbles those treated with pdFVIII. The bottom insert represents the effect of testing frequency when only prospective studies are considered.

the point of complete disappearance when transient inhibitors were excluded. This observation might be the consequence of lower statistical power that arises due to the smaller sample sizes of the studies from which data on transient inhibitors were derived. However, it could also mean that in more recent studies performed with rFVIII, the use of more frequent and regular inhibitor testing led to more frequent detection of transient, and less clinically relevant, inhibitors. This view is strengthened by the observation that the median inhibitor peak titre was higher for pdFVIII than for rFVIII products, indicating that although there were fewer inhibitors diagnosed in pdFVIII-treated patients these were more clinically relevant.

Our study has some limitations. The major limit is that systematic reviews and meta-analyses of observational trials entail several kinds of bias. In the specific context of FVIII inhibitors [9,22], the included studies differed methodologically, particularly pertaining to the presence or absence of survival analyses, variability of inhibitor testing intervals and inclusion or exclusion of other known risk factors for inhibitor development. With these considerations in mind, significant efforts were made to control for bias in this analysis. First, the

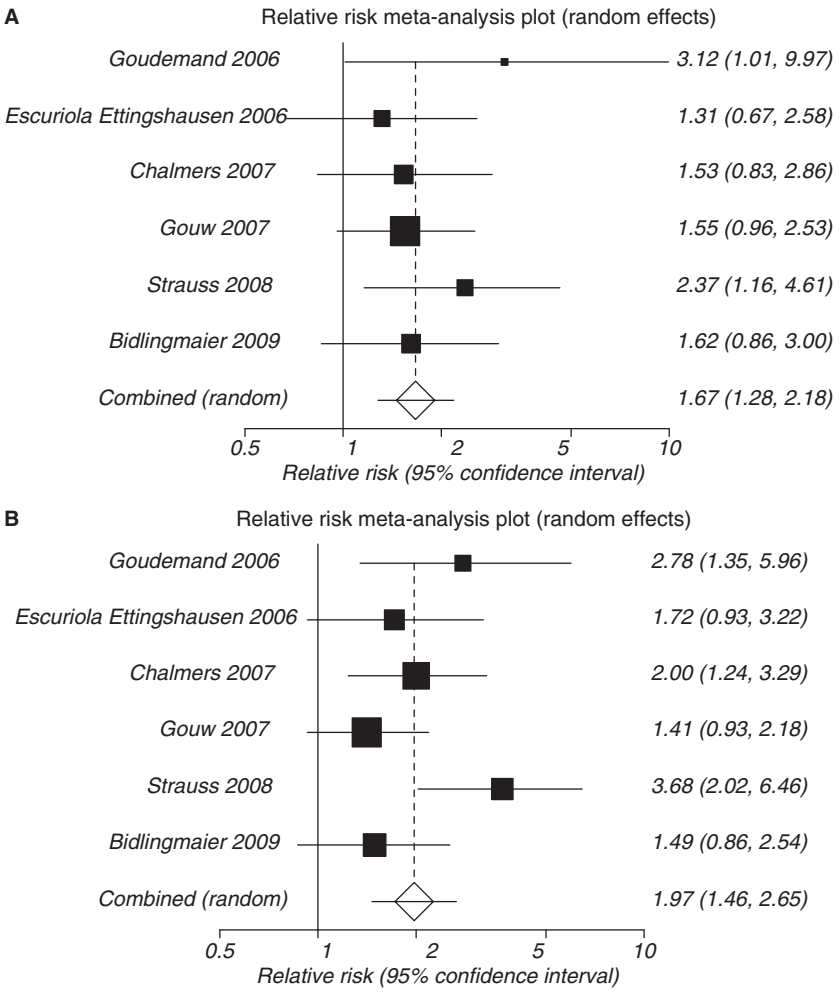

Fig. 4. Risk ratios and 95% confidence interval for parallel cohort studies investigating the influence of FVIII source on the onset of high responding inhibitors (Insert A) or all inhibitors (Insert B) in PUPs with HA. The study author and year of publication are indicated on the *y*-axis. Studies are ordered in descending order by year of publication.

literature search was as comprehensive as possible, including also studies published as abstracts, and the occurrence of publication bias was formally investigated. A trade-off for the completeness of study inclusion was the choice to perform the main analysis on patients with all degrees of HA severity, inclusive of 41 cases with mild and 108 with undefined severity. However, no difference was found when analysing the subset of studies reporting data on severe and severe-moderate patients.

Second, it should also be pointed out that the analysis is based on the inhibitor incidence rate (i.e. the number of detected inhibitors among the patients at risk at the beginning of the observation period). Even though inhibitor development is an event that most frequently occurs early in the patient's history, most of the patients were followed-up for a sufficient time span. Despite this, a better approach would have been to calculate the cumulative incidence in a survival analysis setting. Unfortunately, such a survival analysis would require patient level data that were only available from a small subset of inhibitor-positive patients. However, it must be emphasized that 10 studies including 1364 patients observed their patients for longer than 50 EDs, and that we also tried to adjust the analysis for the median duration of follow-up in the included studies, which was indeed a relevant covariate in our ANOVA model.

Third, the STROBE guidance [18] was used to systematically appraise the studies included in this review to identify and manage, as far as possible, any likely sources of bias. Fourth, single arm cohort data were only pooled and set aside using the Cochran Q test to evaluate whether or not data heterogeneity was partitioned by grouping pdFVIII- and rFVIII-treated cohorts, without performing a formal meta-analysis except for a secondary subset of highly selected studies. Fifth, multiple reporting of the same data was carefully corrected whenever possible by requesting authors to provide missing data or to clarify such issues. Accordingly, it was possible to exclude this major source of heterogeneity, at least in the subset of studies included in the secondary analysis of parallel cohort trials, so that the homogeneity achieved allowed us to combine the six cohort studies by means of the fixed effects model.

An additional limitation is the inability to completely rule out that the patients that were included may have switched

from one source of FVIII product to the other during the observation period. Although patient cross-over was excluded from the subset of studies that were fully meta-analysed, such individuals may have been retained in some of the earlier single arm cohorts. Finally, other likely causes of variability that might explain the difference in the inhibitor development rate could be the uneven distribution of uncontrolled risk factors for inhibitor development, such as the intensity and modality of treatment (i.e. prophylaxis vs. on-demand) and the occurrence of trigger events (i.e. surgery). These details were insufficiently reported in the frame of the studies that were included in the present analysis. This could be a major flaw of this systematic review, particularly in light of the likely relevance of this issue recently reported in a prospective controlled observation [23].

Notwithstanding the aforementioned limitations, the results of this systematic review highlight that, at this time, it is not possible to either prove or disprove the hypothesis that there is a higher risk of inhibitor development associated with the use of rFVIII products when compared with pdFVIII concentrates. Our findings underscore the importance of uniform and systematic collection of inhibitor data with respect to FVIII source in observational studies, and warrant evaluation of this issue in a randomized controlled clinical trial before adjusting clinical practise [16]. In the meantime, patients/families should continue to be counselled regarding potential risks and benefits of available sources of FVIII, specifically addressing the issue of their safety regarding the transmission of known or unknown pathogens, prior to selecting the most appropriate choice of factor for their specific situation.

Addendum

P. M. Mannucci is the guarantor. N. Goldenberg and G. Young defined the relevant outcome measures to be extracted and analysed. S. Halimeh and E. Marchesini performed the literature search, selected studies and extracted data. S. Holzhauer, A. Iorio, M. Marcucci and U. Nowak-Göttl were responsible for statistical calculations. A. Iorio, P. M. Mannucci and U. Nowak-Göttl were responsible for drafting the manuscript. All the authors had full access to the data and took part in the design of the study, discussion of results and in revising the draft manuscript.

Acknowledgements

We thank K. Berger, S. Bergs and D. Kunkel for epidemiologic and technical support. We thank S. Gouw, M. vandenBerg and L. Chalmers for reclassification of the CANAL study patients. The study was partly supported by unrestricted grants from the 'Förderverein Schlaganfall und Thrombosen im Kindesalter e.V.' and received financial support as part of the EUHASS project. The EUHASS project is supported by the European Union in the frame of the Public Health Program.

Disclosure of Conflict of Interests

A. Gringeri, A. Iorio, P. M. Mannucci and E. Santagostino received fees as speakers at several meetings organized by the manufacturers of products employed in the treatment of hemophilia. A. Iorio received fees for sitting on advisory boards of Wyeth and Baxter and received unrestricted research funds from Wyeth and Grifols. All the other authors state that they have no financial or personal relationships that inappropriately influence their role in this work. No funds for the content of this manuscript, or for the preparation of the manuscript, were received by any of the authors.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Additional details about Methods, Included studies Chracteristics and Analysis Results.
Table S1. Characteristics of included studies.
Table S2. List of topics included in the extraction sheet.
Fig. S1. Meta-analysis of single arm studies (pooled incidence rates).
Fig. S2. Meta-analysis of double arm studies (pooled rate ratio): HR inhibitors.
Fig. S3. L'Abbe plot.
Fig. S4. Meta-analysis of double arm studies (pooled rate ratio): low responder inhibitors.
Fig. S5. L'Abbe plot.
Fig. S6. Meta-analysis of double arm studies (pooled rate ratio): all inhibitors.
Fig. S7. L'Abbe plot.
Appendix S1. Details about unpublished studies included in the analysis.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

1 Nilsson IM, Berntorp E, Lofqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *J Intern Med* 1992; **232**: 25–32.
2 Astermark J, Petrini P, Tengborn L, Schulman S, Ljung R, Berntorp E. Primary prophylaxis in severe haemophilia should be started at an early age but can be individualized. *Br J Haematol* 1999; **105**: 1109–13.
3 Fischer K, van der Bom JG, Mauser-Bunschoten EP, Roosendaal G, Prejs R, Grobbee DE, van den Berg HM. Changes in treatment strategies for severe haemophilia over the last 3 decades: effects on clotting factor consumption and arthropathy. *Haemophilia* 2001; **7**: 446–52.
4 Ljung R. Paediatric care of the child with haemophilia. *Haemophilia* 2002; **8**: 178–82.
5 Blanchette VS, Manco-Johnson M, Santagostino E, Ljung R. Optimizing factor prophylaxis for the haemophilia population: where do we stand? *Haemophilia* 2004; **10**: 97–104.

6 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.
7 Guerois C, Laurian Y, Rothschild C, Parquet-Gernez A, Duclos AM, Negrier C, Vicariot M, Fimbel B, Fressinaud E, Fiks-Sigaud M. Incidence of factor VIII inhibitor development in severe hemophilia A patients treated only with one brand of highly purified plasma-derived concentrate. *Thromb Haemost* 1995; **73**: 215–8.
8 Peerlinck K, Rosendaal FR, Vermylen J. Incidence of inhibitor development in a group of young hemophilia A patients treated exclusively with lyophilized cryoprecipitate. *Blood* 1993; **81**: 3332–5.
9 Wight J, Paisley S. The epidemiology of inhibitors in haemophilia A: a systematic review. *Haemophilia* 2003; **9**: 418–35.
10 Goudemand J, Rothschild C, Demiguel V, Vinciguerrat C, Lambert T, Chambost H, Borel-Derlon A, Claeyssens S, Laurian Y, Calvez T. 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.
11 Chalmers EA, Brown SA, Keeling D, Liesner R, Richards M, Stirling D, Thomas A, Vidler V, Williams MD, Young D. Early factor VIII exposure and subsequent inhibitor development in children with severe haemophilia A. *Haemophilia* 2007; **13**: 149–55.
12 Gouw SC, van der Bom JG, Auerswald G, Ettinghausen CE, Tedgard U, van den Berg HM. 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; **109**: 4693–7.
13 Dasgupta S, Repesse Y, Bayry J, Navarrete AM, Wootla B, Delignat S, Irinopoulou T, Kamate C, Saint-Remy JM, Jacquemin M, Lenting PJ, Borel-Derlon A, Kaveri SV, Lacroix-Desmazes S. VWF protects FVIII from endocytosis by dendritic cells and subsequent presentation to immune effectors. *Blood* 2007; **109**: 610–2.
14 Waters B, Qadura M, Burnett E, Chegeni R, Labelle A, Thompson P, Hough C, Lillicrap D. Anti-CD3 prevents factor VIII inhibitor development in hemophilia A mice by a regulatory CD4 + CD25 +-dependent mechanism and by shifting cytokine production to favor a Th1 response. *Blood* 2009; **113**: 193–203.
15 Qadura M, Waters B, Burnett E, Chegeni R, Bradshaw S, Hough C, Othman M, Lillicrap D. Recombinant and plasma-derived factor VIII products induce distinct splenic cytokine microenvironments in hemophilia A mice. *Blood* 2009; **114**: 871–80.
16 Mannucci P, Gringeri A, Peyvandi F, Santagostino E. Factor VIII products and inhibitor development: the SIPPET study (survey of inhibitors in plasma-product exposed toddlers). *Haemophilia* 2009; **13**: 65–8.
17 Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB, for the Meta-analysis Of Observational Studies in Epidemiology Group. Meta-analysis of observational studies in epidemiology: a proposal for reporting. *JAMA* 2000; **283**: 2008–12.
18 von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med* 2007; **147**: 573–7.
19 Wells G, Shea B, O'Connel D, Robertson J, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the Quality of Nonrandomized Studies in meta-analysis. 2009;
20 Emea. Report of expert meeting on factor VIII products and inhibitor development. 2007; DOC Ref:EMEA/CHMP/BPWP/12835/2006:
21 Laird NM, Mosteller F. Some statistical methods for combining experimental results. *Int J Technol Assess Health Care* 1990; **6**: 5–30.
22 Calvez T, Laurian Y, Goudemand J. Inhibitor incidence with recombinant vs. plasma-derived FVIII in previously untreated patients with severe hemophilia A: homogeneous results from four published observational studies. *J Thromb Haemost* 2008; **6**: 390–2.
23 Kurnik K, Bidlingmaier C, Engl W, Chehadeh H, Reipert B, Auerswald G. New early prophylaxis regimen that avoids immunological danger signals can reduce FVIII inhibitor development. *Haemophilia* 2010; **16**: 256–62.