Received: 27 August 2018 | Revised: 5 March 2019 | Accepted: 8 March 2019

DOI: 10.1111/hae.13747

ORIGINAL ARTICLE

Clinical haemophilia

WILEY Haemophilia

Recombinant factor VIII products and inhibitor development in previously untreated patients with severe haemophilia A: Combined analysis of three studies

Peter Volkers[1] | Kay-Martin Hanschmann[1] | Thierry Calvez[2] | Hervé Chambost[3] | Peter W. Collins[4] | Virginie Demiguel[5] | Daniel P. Hart[6] | Charles R.M. Hay[7] | Jenny Goudemand[8] | Rolf Ljung[9] | Ben P. Palmer[10] | Elena Santagostino[11] | Ella M. van Hardeveld[12] | Marijke van den Berg[12] | Brigitte Keller-Stanislawski[1]

[1]Paul-Ehrlich-Institut, Langen, Germany

[2]INSERM, Institut Pierre Louis d'Épidémiologie et de Santé publique, Sorbonne Universités, Paris, France

[3]Service d'Hématologie Oncologie Pédiatrique, La Timone, APHM and Aix Marseille Université, Marseille, France

[4]The University Department of Haematology, University Hospital of Wales Hospital, Cardiff, UK

[5]Santé publique France, French National Public Health Agency, Saint-Maurice, France

[6]Barts and The London School of Medicine & Dentistry, The Royal London Hospital Haemophilia Centre, QMUL, London, UK

[7]Department of Haematology, Manchester Royal Infirmary, Manchester University, Manchester, UK

[8]Service d'Hématologie et de Transfusion, Faculté de Médecine, Centre Hospitalier Universitaire de Lille, Université Lille 2, Lille, France

[9]Department of Clinical Sciences–Paediatrics, Lund University, Lund, Sweden

[10]The United Kingdom National Haemophilia Database, Manchester, UK

[11]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy

[12]PedNet Haemophilia Research Foundation, Baarn, The Netherlands

Correspondence
Brigitte Keller-Stanislawski, Paul-Ehrlich-Institut, Langen, Germany.
Email: Brigitte.Keller-Stanislawski@pei.de

Funding information
The present study was funded by the Paul-Ehrlich-Institut.

Introduction: Standard treatment of congenital haemophilia A is based on replacement therapy with coagulation factor VIII (FVIII) products. A major complication of FVIII therapy is the occurrence of IgG alloantibodies (inhibitors) that neutralize FVIII activity.

Aim: The aim of the analysis was estimating the risk of high-titre inhibitor associated with the second-generation full-length product compared to third-generation full-length product and other recombinant FVIII (rFVIII).

Methods: We conducted a combined analysis of individual patient data from three large studies in previously untreated patients (PUPs) with severe haemophilia A.

Results: A total of 1109 PUPs were treated from 1993 to 2013 including 787 PUPs treated from 2004 onwards (primary analysis cohort). A total of 322 patients (29.0%) developed an inhibitor, of which 192 (17.3%) a high-titre inhibitor. In the primary analysis set, 29.9% of patients developed an inhibitor and 17.2% a high-titre inhibitor. The combined analysis indicated a lower risk of high-titre inhibitor development for the third-generation rFVIII product compared to the second-generation rFVIII product (primary analysis: adjusted hazard ratio (HR) = 0.72, 95% CI: 0.49 to 1.06). Adjusted HR for all inhibitor development was significantly lower for the third-generation product compared to the second-generation product.

Conclusion: The trend of an increased risk of inhibitor development in PUPs for one recombinant product illustrates that extrapolation from one recombinant factor VIII product to other products might not be justified.

KEYWORDS
antibodies, blood coagulation disorders, blood coagulation factor inhibitors, factor VIII, haemophilia A, neutralizing, recombinant factor VIII products

1 | INTRODUCTION

Inhibitor development is the most serious and challenging complication in the treatment of patients with haemophilia A. Inhibitor development in particular high-titre inhibitors (≥5 Bethesda Units, BU) is associated with a reduction of efficacy of haemostatic therapy with FVIII and increased morbidity (arthropathies and life-threatening intracranial and visceral bleedings).

The current view is that the risk of inhibitor development is determined by the interplay between genetic and environmental factors such as factor VIII mutations, ethnicity, and high-dose intensive FVIII treatment.[1-3,5,6] Cumulative FVIII exposure is a well-defined determinant of FVIII inhibitor development, as the large majority of inhibitor cases occur during the first 50 exposure days (ED).

There is an ongoing debate whether certain brands of factor VIII may be associated with an increased risk of inhibitor development in previously untreated patients (PUPs) with severe haemophilia A.[7-10] The results of the Research of Determinants of Inhibitor Development (PedNet study group),[9] the FranceCoag Network[11] and the UK Haemophilia Centre Doctors' Organisation (UKHCDO)[12] study groups consistently indicated an increased risk for inhibitor development following treatment with the second-generation full-length recombinant factor VIII product (rFVIII).

Based on a common analysis model, we conducted a combined analysis of these three observational studies. The aim was to put observations from single studies into a broader context and provide more robust comparisons not only between second- and third-generation full-length rFVIII products but also with other rFVIII products which was requested as a response to the PedNet study group publication.[13] Another goal of the analysis was to further investigate whether potential changes in treatment practices or in the immunogenicity of any rFVIII product during the time period of the three individual studies contributed to the results.

2 | MATERIAL AND METHODS

2.1 | Data sets and study objectives

The study protocol was accepted in May 2015 by the Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines Agency (EMA).[14]

The available study data consist of PUPs starting treatment with rFVIII products between 2000 and 2012 from the PedNet registry, PUPs first treated with rFVIII products between 2000 and 2013 and covered by the UKHCDO study, and PUPs first treated with rFVIII products between 1993 and 2013 and covered by the FranceCoag study.

The primary objective of the patient-based combined analysis was to evaluate the risk of high-titre inhibitor development in PUPs with severe haemophilia A (factor VIII level < 1 IU/dL) associated with the second-generation full-length product compared to third-generation full-length product and other rFVIII products from the year 2004 onwards when both second- and third-generation full-length rFVIII products were marketed in parallel in the European Union. Secondary objectives were the frequency of patients developing high-titre inhibitors in the whole study period of the three cohorts as well as the evaluation of the risk of any inhibitor development.

The definition of positive inhibitor titre, as well as high-titre inhibitor, was taken from the individual studies. Only those positive inhibitor results with a second positive value and/or documentation of bypass therapy were considered. High-titre inhibitor was defined as at least 5 Bethesda Units (≥5 BU).

2.2 | Products

Products included in the combined analysis were first (Recombinate, product D)-, second (Kogenate Bayer, product A)- and third (Advate, product B)-generation full-length and second (ReFacto, product C)- and third (ReFacto FS, product E)-generation B-domain-deleted recombinant factor VIII products. Products B, C, D and E are issued from Chinese Hamster Ovary (CHO) cell lines and product A from Baby Hamster Kidney (BHK) cell lines. The first-generation full-length recombinant Kogenate produced from BHK cell lines has not been included in the combined analysis because the number of patients treated with this product was too small to provide reliable results.

2.3 | Data conventions

UK Haemophilia Centre Doctors' Organisation patients born until 1 January 2010, who started treatment between 2000 and 2010 inclusive [2000, 2010] according to the UKHCDO data set and were evaluated also by the PedNet study group, were excluded from the analysis in order to avoid duplicates. UKHCDO patients treated from 2010 onwards were included. Unlike the original publications dealing with the PedNet data set and the FranceCoag database, calendar time was

used to measure time-to-event data, as this was the only information available from the UKHCDO database.

2.4 | Statistical analysis

Demographic and baseline data were described by statistical characteristics, stratified by study and product as well as overall, including the frequency of subjects with missing values. The primary analysis was a COX regression model with time from first treatment until occurrence of a high-titre inhibitor as outcome (dependent) variable, factor concentrate as independent variable and age at first exposure, ethnicity, family history of haemophilia, family history of inhibitors, treatment intensity at first treatment, and genotype classification as covariates. Multiple imputation was applied to account for missing data in the covariates.[15] For the analysis, patients were censored at the time of the last documented application of the initial rFVIII product in case of no inhibitor development, or development of an inhibitor not classified as 'high-titre inhibitor' (whichever was first). This approach was chosen in accordance with the data available from the three studies.

Using appropriate contrasts, the hazard ratios (HRs) relative to second-generation full-length product (A), and corresponding 95% confidence intervals (CIs), were calculated for each of the rFVIII products. The 'classic' 2-stage approach for meta-analyses with study as a fixed factor was the primary analysis according to protocol; results of a 2-stage approach with study as a random factor, as well as results from a 1-stage analysis approach, were provided as additional analyses.

In addition to the analysis including all patients starting initial treatment with rFVIII products from 2004 onwards, several analyses were performed to assess the robustness of the results:

- Unadjusted analyses (if applicable)
- Repeating the analysis for different time windows
- Investigating the impact of the time scale
- Investigating interactions between covariates and treatment

Other time-to-event endpoints were analysed similarly.

SAS version 9.4 was used for the analysis.

2.5 | Data protection

Data submission, handling and storage were in accordance with the European Union data protection law. Only relevant and anoymized data were analyzed.

3 | RESULTS

3.1 | Data sets

From the 1232 patients (522 PedNet, 303 FranceCoag and 407 UKHCDO) with information on exposure to rFVIII, a total of 123 patients were excluded from the analysis: two patients were not considered PUPs; 26 patients did not start on a rFVIII product, 17 patients started treatment on first-generation Kogenate (for which the total number was too low for a meaningful analysis) and 78 patients reported by the UKHCDO who were born between 1 January 2000 and 1 January 2010, started treatment in [2000, 2010] and were also reported in the PedNet database. From the FranceCoag database, only data from patients not evaluated by the PedNet study group were available.

The data set available for the analysis consisted of 1109 patients, 487 from the PedNet registry, 293 from the FranceCoag and 329 from the UKHCDO data set. The majority of patients were either treated by second (n = 405)- or third (n = 387)-generation full-length recombinant products (Table 1). The primary analysis set (patients starting treatment from 2004 onwards, excluding UKHCDO patients evaluated by the PedNet study group) consists of 787 patients (Table 1). The exposure per product of the whole study population differs over time (Figure 1).

3.2 | Patient characteristics

With the exception of age at first exposure (whole study as well as primary analysis population) and ethnicity (primary analysis population), no significant differences concerning patient characteristics were observed between products (Table 2; Appendix, Table S1).

3.3 | Risk factors for inhibitor development

Risk factors for the development of high-titre inhibitors were assessed on the whole data set as well as for the primary analysis population. Regardless of the analysis population, the development of high-titre and

TABLE 1 Study population (whole data set and primary analysis set)

	Study			
Data set	PedNet	FranceCoag	UKHCDO	Total
Whole data set				
Product				
A	184	111	110	405
B	163	97	127	387
C	76	27	40	143
D	61	48	9	118
E	3	10	43	56
Total	487	293	329	1109
Primary analysis set				
Product				
A	134	80	64	278
B	163	97	127	387
C	32	5	8	45
D	11	9	1	21
E	3	10	43	56
Total	343	201	243	787

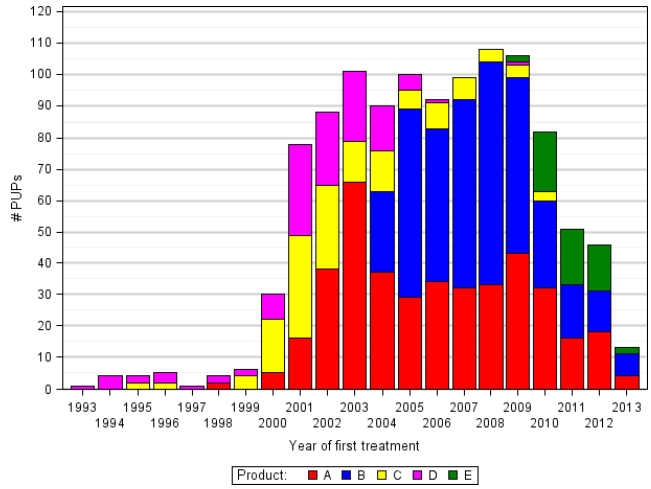

FIGURE 1 Whole data set by year of first treatment and product

any inhibitor, respectively, was associated with high-risk F8 gene mutation, family history of inhibitor, intensive treatment at start of treatment and ethnicity (sometimes borderline) (Table 3; Appendix, Table S2).

3.4 | Inhibitor development

Concerning the primary analysis set, 61/278 (21.9%) PUPs treated with product A developed a high-titre inhibitor (any inhibitor: 102/278 [36.7%]; low-titre inhibitor: 41/278 [14.7%]). A total of 58/387 (15.0%) PUPs treated with product B developed a high-titre inhibitor (any inhibitor: 101/387 [26.1%]; low-titre inhibitor: 43/387 [11.1%]). Eight out of 45 (17.8%) patients treated with product C developed a high-titre inhibitor (any inhibitor: 11/45 [24.4%]; low-titre inhibitor: 3/45 [6.7%]) and three out of 21 (14.3%) patients treated with product D developed a high-titre inhibitor (no other inhibitor reported). Concerning product E, 5/56 (8.9%) patients developed a high-titre inhibitor (any inhibitor: 18/56 [32.1%]; low-titre inhibitor: 13/56 [23.2%]). In the whole data set, the number of patients developing high-titre inhibitors was as follows: 89/405 (22.0%) for product A, 58/387 (15.0%) for product B, 22/143 (15.4%) for product C, 18/118 (15.3%) for product D and 5/56 (8.9%) for product E (Table 4).

In the primary analysis set, the adjusted HR for comparing the risk of high-titre inhibitor development of the third-generation full-length product (B) relative to the second-generation full-length product (A) was HR = 0.72, 95% CI: 0.49-1.06 (2-stage approach, identical for fixed and random effects model); the corresponding HR in the whole data set was HR = 0.67, 95% CI: 0.46-0.96 (fixed effects, 2-stage approach) and HR = 0.67, 95% CI: 0.43-1.02 (random effects, 2-stage approach) (Table 5). Based on the point estimates, the risk of high-titre inhibitor development for products D and E was lower when compared to product A. However, none of these analyses reached statistical significance (Table 5).

A statistical assessment of heterogeneity in the context of the 2-stage model did not indicate heterogeneity between studies.

TABLE 2 Patient characteristics of the whole data set

	Product					
Parameter	A (n = 405) (%)	B (n = 387) (%)	C (n = 143) (%)	D (n = 118) (%)	E (n = 56) (%)	*P*-value[a]
Ethnicity						
Caucasian	345 (85.2)	313 (80.9)	121 (84.6)	99 (83.9)	50 (89.3)	0.356
Other	60 (14.8)	74 (19.1)	22 (15.4)	19 (16.1)	6 (10.7)	
F8 gene defect[b]						
Low	127 (31.4)	122 (31.5)	38 (26.6)	33 (28.0)	14 (25.0)	0.874
High	250 (61.7)	240 (62.0)	86 (60.1)	80 (67.8)	32 (57.1)	
Family history of haemophilia						
No	218 (53.8)	175 (45.2)	73 (51.0)	66 (55.9)	28 (50.0)	0.118
Yes	181 (44.7)	211 (54.5)	67 (46.9)	51 (43.2)	28 (50.0)	
Family history of inhibitor						
No	336 (83.0)	322 (83.2)	116 (81.1)	95 (80.5)	49 (87.5)	0.552
Yes	39 (9.6)	34 (8.8)	16 (11.2)	11 (9.3)	2 (3.6)	
Age at first exposure						
<6 mo	84 (20.7)	115 (29.7)	50 (35.0)	35 (29.7)	11 (19.6)	0.004
6-<12 mo	155 (38.3)	142 (36.7)	46 (32.2)	50 (42.4)	26 (46.4)	
≥12 mo	166 (41.0)	130 (33.6)	47 (32.9)	33 (28.0)	19 (33.9)	
Intensive treatment at start						
No	353 (87.2)	329 (85.0)	123 (86.0)	106 (89.8)	49 (87.5)	0.733
Yes	49 (12.1)	55 (14.2)	18 (12.6)	12 (10.2)	7 (12.5)	

[a]CHM test stratified for study.

[b]Low-risk genotypes include those with small deletions and insertions, missense mutation, splice site mutation; high-risk genotype includes those with large deletions, nonsense mutations and intron 1 and 22 inversions.

TABLE 3 Risk of high-titre inhibitor development based on patient characteristics in the whole data set

	High-titre inhibitor n/N (%)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Ethnicity			
Caucasian	146/928 (15.7)	0.61 (0.44-0.85)	0.64 (0.45-0.92)
Other	46/181 (25.4)	1.0	1.0
F8 gene defect*			
Low	20/334 (6.0)	0.22 (0.14-0.34)	0.22 (0.14-0.36)
High	162/688 (23.5)	1.0	1.0
Family history of haemophilia			
No	101/560 (18.0)	1.12 (0.84-1.49)	1.54 (1.07-2.22)
Yes	90/538 (16.7)	1.0	1.0
Family history of inhibitor			
No	149/918 (16.2)	0.42 (0.29-0.60)	0.36 (0.23-0.56)
Yes	36/102 (35.3)	1.0	1.0
Age at first exposure			
<6 mo	59/295 (20.0)	1.0	1.0
6-<12 mo	70/419 (16.7)	0.88 (0.62-1.24)	1.13 (0.78-1.66)
≥12 mo	63/395 (15.9)	0.79 (0.55-1.12)	1.11 (0.74-1.68)
Intensive treatment at start			
No	143/960 (14.9)	0.35 (0.25-0.48)	0.37 (0.25-0.54)
Yes	47/141 (33.3)	1.0	1.0
Start of treatment			
Before 2004	57/322 (17.7)	0.93 (0.66-1.33)	0.82 (0.56-1.19)
2004-2007	66/381 (17.3)	0.94 (0.67-1.33)	0.85 (0.59-1.23)
After 2007	69/406 (17.0)	1.0	1.0

*Low-risk genotypes include those with small deletions and insertions, missense mutation, splice site mutation; high-risk genotype includes those with large deletions, nonsense mutations and intron 1 and 22 inversions.

The risk (relative to product A) for developing low-titre inhibitors and any inhibitor, respectively, is summarized in Tables 6 and 7.

3.5 | Sensitivity analyses

Sensitivity analyses for high-titre inhibitor with different time windows revealed results consistent with those reported above (Appendix, Table S3).

As only calendar time information was available for UKHCDO patients, the analyses were restricted to the PedNet & FranceCoag data only. With the exception of product D, the estimated HRs compared to product A based on exposure days are generally slightly lower than those based on calendar time, also in most cases the use of EDs as time scale increases the precision of the HR estimates (Appendix, Table S4).

Based on the data of all patients with complete covariate information, possible interactions between rFVIII products and the various covariates were included into a COX regression model stratified for study and period (<2004, 2004-2007, >2007). No statistically significant interaction between product and any of the risk factors was seen (Appendix, Table S5).

4 | DISCUSSION

We conducted a combined analysis of inhibitor development in a total of 1109 PUPs with severe haemophilia A from PedNet, FranceCoag and UKHCDO studies and independently confirmed the data of the original studies. Overall, 322/1109 PUPs developed an inhibitor (29.0%) and 192 PUPs (17.3%) developed a high-titre inhibitor. Thirty-seven per cent (37%) of PUPs treated with product A developed an inhibitor and 22% of patients developed a high-titre inhibitor. The frequency of inhibitor development was comparable for the primary analysis from 2004 onwards when products A and B were marketed in parallel and the whole study period. A total of 26% of PUPs treated with product B developed an inhibitor and 15% experienced a high-titre inhibitor. The combined analysis indicated a lower frequency of high-titre and all inhibitor development for the third-generation full-length product B compared to the second-generation full-length product A which was of borderline statistical significance in case of high-titre development, but statistically significant in case of all inhibitor development. With the exception of product E regarding any inhibitor

TABLE 4 Inhibitor development

Data set	Product	Exposure (y)	High-titre inhibitors (%)	Low-titre inhibitors (%)	Any inhibitor (%)
Primary analysis set	A	414.4	61/278 (21.9)	41/278 (14.7)	102/278 (36.7)
	B	744.3	58/387 (15.0)	43/387 (11.1)	101/387 (26.1)
	C	70.3	8/45 (17.8)	3/45 (6.7)	11/45 (24.4)
	D	15.4	3/21 (14.3)	0/21 (0.0)	3/21 (14.3)
	E	64.2	5/56 (8.9)	13/56 (23.2)	18/56 (32.1)
Whole data set	A	700.0	89/405 (22.0)	59/405 (14.6)	148/405 (36.5)
	B	744.3	58/387 (15.0)	43/387 (11.1)	101/387 (26.1)
	C	359.5	22/143 (15.4)	9/143 (6.3)	31/143 (21.7)
	D	172.3	18/118 (15.3)	6/118 (5.1)	24/118 (20.3)
	E	64.2	5/56 (8.9)	13/56 (23.2)	18/56 (32.1)

TABLE 5 High-titre inhibitor development (adjusted for covariates)

			Primary analysis set		Whole data set	
Product	Method		HR relative to product A	95% CI	HR[a] relative to product A	95% CI
Product B	2-stage	Fixed	0.72	0.49-1.06	0.67*	0.46-0.96
		Random	0.72	0.49-1.06	0.67	0.43-1.02
	1-stage	Fixed	0.70	0.48-1.02	0.66*	0.46-0.95
		Random	0.69[b]	0.48-1.00	0.65*	0.46-0.94
Product C	2-stage	Fixed	1.14	0.51-2.52	0.64	0.38-1.08
		Random	1.44	0.35-5.98	0.64	0.38-1.08
	1-stage	Fixed	0.89	0.41-1.93	0.63	0.37-1.06
		Random	0.88	0.41-1.91	0.65	0.39-1.09
Product D	2-stage	Fixed	0.69	0.21-2.30	0.69	0.38-1.26
		Random	0.69	0.21-2.30	0.69	0.38-1.26
	1-stage	Fixed	0.71	0.22-2.31	0.70	0.39-1.24
		Random	0.70	0.21-2.28	0.67	0.38-1.18
Product E	2-stage	Fixed	0.83	0.30-2.30	0.70	0.25-1.94
		Random	0.83	0.30-2.30	0.70	0.25-1.94
	1-stage	Fixed	0.73	0.28-1.89	0.76	0.29-2.02
		Random	0.66	0.26-1.70	0.59	0.23-1.53

[a]stratified for period (start of treatment: <2003, [2004, 2007], >2007).
*$P < 0.05$.

development, similar trends were also seen for the other rFVIII products included in this analysis compared to product A, although the results were not significant. A range of complementary analyses showed consistent results with regard to the comparison of second- and third-generation full-length recombinant FVIII products.

Taking into account, the whole observation period for each study and stratifying the analysis for calendar period of first treatment did not relevantly change the results. Thus, there is no evidence of a bias by changing medical practice (eg, recognition of the effect of intensive treatment on inhibitor development) during the study periods of the three observational studies, in particular for high-titre inhibitors.

The strength of the analysis is the large number of PUPs from three robustly populated observational studies with a long follow-up period of patients in the individual studies (up to 75 exposure days in the PedNet and FranceCoag and a median follow-up of 45 months in the UKHCDO data set). A long follow-up is warranted to ensure that PUPs who developed their inhibitor late are also included in the analysis. We found no major baseline imbalances of patient characteristics among studies and recombinant FVIII products as well as no heterogeneity of the results among the three studies.

TABLE 6 Development of any inhibitor (adjusted for covariates)

Product	Method		Primary analysis set		Whole data set	
			HR relative to product A	95% CI	HR[a] relative to product A	95% CI
Product B	2-stage	Fixed	0.71*	0.53-0.95	0.68*	0.52-0.91
		Random	0.71*	0.53-0.95	0.68*	0.52-0.91
	1-stage	Fixed	0.71*	0.53-0.94	0.68*	0.51-0.90
		Random	0.70*	0.53-0.93	0.68*	0.51-0.89
Product C	2-stage	Fixed	0.89	0.46-1.73	0.56*	0.37-0.87
		Random	1.02	0.39-2.62	0.55*	0.34-0.90
	1-stage	Fixed	0.83	0.43-1.59	0.57*	0.37-0.87
		Random	0.81	0.43-1.55	0.58*	0.38-0.89
Product D	2-stage	Fixed	0.46	0.14-1.47	0.64	0.39-1.04
		Random	0.46	0.14-1.47	0.64	0.39-1.04
	1-stage	Fixed	0.47	0.15-1.50	0.60*	0.37-0.98
		Random	0.46	0.15-1.48	0.57*	0.35-0.91
Product E	2-stage	Fixed	1.67	0.88-3.18	1.34	0.71-2.53
		Random	1.67	0.88-3.18	1.34	0.71-2.53
	1-stage	Fixed	1.41	0.82-2.43	1.31	0.75-2.29
		Random	1.37	0.80-2.36	1.17	0.69-1.99

[a]Stratified per period (start of treatment <2003, [2004, 2007], >2007).
*$P < 0.05$.

TABLE 7 Low-titre inhibitor development (adjusted for covariates)

Product	Method		Primary analysis set		Whole data set	
			HR relative to product A	95% CI	HR[a] relative to product A	95% CI
Product B	2-stage	Fixed	0.67	0.42-1.06	0.69	0.43-1.10
		Random	0.68	0.40-1.16	0.72	0.39-1.31
	1-stage	Fixed	0.72	0.47-1.12	0.71	0.46-1.10
		Random	0.71	0.46-1.10	0.70	0.45-1.08
Product C	2-stage	Fixed	0.83	0.24-2.95	0.58	0.27-1.26
		Random	0.83	0.24-2.95	0.36	0.07-1.83
	1-stage	Fixed	0.66	0.20-2.19	0.45*	0.21-0.95
		Random	0.63	0.19-2.07	0.44*	0.21-0.94
Product D	2-stage	Fixed	—	—	0.54	0.22-1.34
		Random	—	—	0.54	0.22-1.34
	1-stage	Fixed	—	—	0.41	0.16-1.04
		Random	—	—	0.38*	0.16-0.94
Product E	2-stage	Fixed	3.05[b]	1.24-7.49	2.83*	1.15-6.97
		Random	3.05[b]	1.24-7.49	2.83*	1.15-6.97
	1-stage	Fixed	2.16[b]	1.09-4.27	1.78	0.87-2.02
		Random	2.17[b]	1.11-4.25	1.73	0.88-3.39

[a]Stratified for period (start of treatment: <2003, [2004, 2007], >2007).
*$P < 0.05$.

We included PUPs and excluded minimally treated patients. Calendar time was considered as exposure unit in the Cox regression model instead of exposure days which were available for all patients in the PedNet and FranceCoag but not in the UKHCDO data set. This might have led to an underestimation of the risk associated with product A as demonstrated in the sensitivity analyses using data from the FranceCoag and PedNet data set with treatment exposure based on EDs.

The combined analysis is based on studies which included all eligible patients from two countries (FranceCoag and UKHCDO) and all additional patients from a substantial number of centres in Europe, Israel and Canada (PedNet). Thus, there is no indication for an enrolment bias. In order to minimize a prescription bias that means that patients, for example with a F8 mutation known to be associated with a higher risk for inhibitor development may preferentially be treated with a certain product, we used a Cox regression analysis accounting for identified risk factors for inhibitor development, which are consistent with other study results.[2,16-21]

Nevertheless, covariates used to adjust for potential confounding were limited to the parameters shared by all three individual studies. Therefore, the results could not be adjusted for additional potentially important covariates such as treatment centre or history of surgery. This could potentially lead to less effective control of confounding in the combined analysis. These potential limitations were also reflected in the statement of the Pharmacovigilance Risk Assessment Committee of the European Medicines Agency and were apparently one of the reasons for the conclusions of the committee.[22] Also, frequency of inhibitor testing was performed at the discretion of the treating physician. Considering the positive correlation between testing frequency and increased detection of low-titre inhibitors, we might have underestimated the incidence of transient and low-titre inhibitors.[23,24] Since no central retesting of inhibitors with a standard Bethesda assay was performed, we focused on high-titre inhibitors as a primary outcome of the combined analysis. In contrast to low-titre inhibitors, high-titre inhibitors cannot be overcome with higher doses of FVIII. Due to their clinical presentation, it is highly unlikely that we have missed high-titre inhibitors.

No potential mechanism has been identified which might explain our results for product A. Differences in peptide sequence, glycosylation profile[25] and biological activity[26] among products have been discussed; however, the clinical impact of these findings remains unclear. Also, no significant changes of biophysical and biochemical characteristics and no significant changes in post-translational modifications, aggregation profile, specific activity or excipients of product A over time have been identified.[27]

For orphan diseases such as haemophilia A, the sample size of prelicensure clinical trials is usually small and not powered to identify uncommon and rare adverse reactions. In combination with the usually uncontrolled study design, such trials do not allow the identification of subtle, but potentially meaningful differences in the safety profile of individual products. Our study showed that it is feasible to combine raw data of different studies/registries thereby increasing the sample size. However, common data elements are a prerequisite. In addition, considering the limited sample size of prelicensure studies, postmarketing surveillance of factor VIII products in large, well-characterized and unselected cohorts is deemed to be important to further characterize the safety profile of individual products.[28] Our combined analysis demonstrates that large cohorts are needed to investigate subtle, but potentially meaningful differences among various products.

5 | CONCLUSION

While the role of FVIII products on inhibitor development in PUPs is considered large, the actual impact of any specific product remains to be a matter of additional investigations. International collaborations are needed to further explore the mechanism of inhibitor formation. The result of this combined analysis of three large cohort studies indicates that the risk of inhibitor development should be evaluated individually for each medicinal product and that extrapolation of the risk of inhibitor development from one recombinant factor VIII product to other recombinant products might not be justified.

6 | DISCLAIMER

The views expressed in this article are the personal views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of the agencies or organizations with which the authors are affiliated.

ACKNOWLEDGEMENT

The authors thank the Pharmacovigilance Risk Assessment Committee and the European Medicines Agency for critical review of the study protocol of the combined analysis and Doris Oberle/Paul-Ehrlich-Institut for the independent validation of the data migration. All authors designed the study protocol; Peter Volkers, Kay-Martin Hanschmann and Brigitte Keller-Stanislawski performed the combined analysis and wrote the manuscript. Thierry Calvez, Hervé Chambost, Peter Collins, Virginie Demiguel, Daniel Hart, Charles Hay, Jenny Goudemand, Rolf Ljung, Ben Palmer, Elena Santagostino, Ella M. van Hardeveld and H. Marijke van den Berg provided data and/or commented upon manuscript.

DISCLOSURES

RL has during the last 5 years received speaker's fee or consultancy fee (advisory board or data safety Monitoring Committees) from Shire/Baxter, Bayer, Novo Nordisk, Pfizer, CSL Behring and Octapharma, however, not related to this paper. ES has acted as a member of advisory boards and/or speaker bureau for Bayer, Shire, Novo Nordisk, SOBI, Bioverativ, Pfizer, CSL Behring, Roche, Grifols, Kedrion and Octapharma. PWC has received

research support from CSL Behring and acted as a paid consultant to Bayer, CSL Behring, Shire and Novo Nordisk. DPH has received research funding from Shire, Octapharma and Bayer. He also has received honoraria for speaking, or advisory commitments from Pfizer, Shire, Bayer, Novo Nordisk, Biotest, Biomarin, Roche and SOBI. TC has received support for attending scientific meetings from Baxalta-Shire and LFB and honoraria (consultant on an advisory board) from Roche (none of these relates to the present study). HC has received support for attending scientific meetings and honoraria (speaker fees/consultant on advisory boards) from Baxalta-Shire, Bayer, CSL Behring, LFB, Novo Nordisk, Octapharma, Pfizer, Roche and Sobi; has been an investigator in studies sponsored by Baxalta-Shire, Bayer, Biogen/Bioverativ, CSL Behring, LFB and Octapharma; and has received research support from CSL Behring, LFB, Novo Nordisk, Octapharma and SOBI (none of these relates to the present study). CRH acted as an advisor to Pfizer and has been a member of their speaker bureau. He has advised or has received speaker honoraria from Sobi, Biotest, Novo Nordisk and Shire.

ORCID

Charles R.M. Hay https://orcid.org/0000-0002-0162-6828

Brigitte Keller-Stanislawski https://orcid.org/0000-0001-7559-3363

REFERENCES

1. Osooli M, Berntorp E. Inhibitors in haemophilia: what have we learned from registries? A systematic review. *J Intern Med*. 2015;277(1):1-15.
2. Gouw SC, van den Berg HM, Oldenburg J, et al. F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. *Blood*. 2012;119(12):2922-2934.
3. Astermark J, Donfield SM, Gomperts ED, et al. The polygenic nature of inhibitors in hemophilia A: results from the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort. *Blood*. 2013;121(8):1446-1454.
4. Gorski MM, Blighe K, Lotta LA, et al. Whole-exome sequencing to identify genetic risk variants underlying inhibitor development in severe hemophilia A patients. *Blood*. 2016;127(23):2924–2933.
5. 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(6):1256-1265.
6. Astermark J, Berntorp E, White GC, Kroner BL. The Malmö International Brother Study (MIBS): further support for genetic predisposition to inhibitor development in hemophilia patients. *Haemophilia*. 2001;7(3):267-272.
7. Vézina C, Carcao M, Infante-Rivard C, et al. Incidence and risk factors for inhibitor development in previously untreated severe haemophilia A patients born between 2005 and 2010. *Haemophilia*. 2014;20(6):771-776.
8. 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(1):46-51.
9. 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(3):231-239.
10. Peyvandi F, Mannucci PM, Garagiola I, et al. A randomized trial of factor VIII and neutralizing antibodies in hemophilia A. *N Engl J Med*. 2016;374(21):2054-2064.
11. 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(23):3398-3408.
12. 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 haemophilia A, 2000–2011. *Blood*. 2014;124(23):3389-3397.
13. Kessler CM, Iorio A. The Rodin (Research Of Determinants of Inhibitor Development among PUPs with haemophilia) study: the clinical conundrum from the perspective of haemophilia treaters. *Haemophilia*. 2013;19(3):351-354.
14. Pharmacovigilance Risk Assessment Committee (PRAC): Inhibitor development in previously untreated patients with severe haemophilia A treated with recombinant factor VIII products, 13 May 2016, EMA/PRAC/332348/2016, www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500206411. Accessed January 10, 2019.
15. White IR, Royston P. Imputing missing covariate values for the Cox model. *Stat Med*. 2009;28(15):1982-1998.
16. Oldenburg J, Schroder J, Brackmann HH, Muller-Reible C, Schwaab R, Tuddenham E. Environmental and genetic factors influencing inhibitor development. *Semin Hematol*. 2004;41(1 Suppl 1):82-88.
17. Gouw SC, van der Bom JG, van den Berg HM. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study. *Blood*. 2007;109(11):4648-4654.
18. Gouw SC, Van den Berg HM, Le Cessie S, van der Bom JG. Treatment characteristics and the risk of inhibitor development: a multicenter cohort study among previously untreated patients with severe hemophilia A. *J Thromb Haemost*. 2007;5(7):1383-1390.
19. Maclean PS, Richards M, Williams M, et al. Treatment related factors and inhibitor development in children with severe haemophilia A. *Haemophilia*. 2011;17(2):282-287.
20. Ragni MV, Ojeifo O, Feng J, et al. Risk factors for inhibitor formation in haemophilia: a prevalent case-control study. *Haemophilia*. 2009;15(5):1074-1082.
21. 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(5):958-967.
22. Pharmacovigilance Risk Assessment Committee (PRAC): Inhibitor development in previously untreated patients with severe haemophilia A treated with recombinant factor VIII products: EMA/PRAC/332348/2016, 13 May 2016. http://www.ema.europa.eu/docs/en_GB/document_library/Report/2016/05/WC500206411.pdf. Accessed January 10, 2019 .
23. 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: 2013 update. *Semin Thromb Hemost*. 2013;39(7):752-766.
24. Berg H, Hashemi SM, Fischer K, et al. Increased inhibitor incidence in severe haemophilia A since 1990 attributable to more low titre inhibitors. *Thromb Haemost*. 2016;115(4):729-737.
25. Preston R, Rawley O, Gleeson EM, O'Donnell JS. Elucidating the role of carbohydrate determinants in regulating hemostasis: insights and opportunities. *Blood*. 2013;121(19):3801-3810.
26. Pahl S, Pavlova A, Driesen J, Muller J, Potzsch B, Oldenburg J. In vitro characterization of recombinant factor VIII concentrates

reveals significant differences in protein content, activity and thrombin activation profile. *Haemophilia*. 2013;19(3):392-398.

27. Kogenate Bayer and Helixate Nexgen Article-20-procedure-PRAC assessment report: Scientific conclusions and grounds for variation to the terms of the marketing authorisations. Annex IV. http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Kogenate_Bayer_Helixate_NexGen_20/European_Commission_final_decision/WC500164885.pdf. Accessed January 10, 2019 .
28. Keipert C, van den Berg HM, Keller-Stanislawski B, Hilger A. Haemophilia registries to complement clinical trial data: a pious hope or an urgent necessity?: Reflections on a possible way forward. *Haemophilia*. 2016;22(5):647-650.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Volkers P, Hanschmann K-M, Calvez T, et al. Recombinant factor VIII products and inhibitor development in previously untreated patients with severe haemophilia A: Combined analysis of three studies. *Haemophilia*. 2019;25:398–407. https://doi.org/10.1111/hae.13747