Product type and the risk of inhibitor development in nonsevere haemophilia A patients: a case–control study

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A complete list of the members of the INSIGHT Study Group appears in the 'Appendix'.

Summary

Inhibitor development is a major complication of treatment with factor VIII concentrates in nonsevere haemophilia A. It has been suggested that plasma-derived factor VIII (FVIII) concentrates elicit fewer inhibitors than recombinant FVIII concentrates, but studies in severe haemophilia A patients have shown conflicting results. We designed a case–control study to investigate the clinical and genetic risk factors for inhibitor development in nonsevere haemophilia A patients. We investigated whether the type of FVIII concentrate was associated with inhibitor development in nonsevere haemophilia A patients. This nested case–control study includes 75 inhibitor patients and 223 controls, from a source population of the INSIGHT study, including all nonsevere haemophilia A patients (FVIII:C 2–40%) that were treated with FVIII concentrates in 33 European and one Australian centre. Cases and controls were matched for date of birth and cumulative number of exposure days (CED) to FVIII concentrate. A conditional logistic regression model was used to calculate unadjusted and adjusted odds ratios. No increased risk for inhibitor development was found for any type of FVIII concentrate; either when comparing recombinant FVIII concentrates to plasma-derived FVIII concentrates (adjusted odds ratio 0·96, 95% confidence interval (CI) 0·36–2·52) or for specific types of FVIII concentrates.

Keywords: haemophilia, antibodies, factor VIII, risk factors.

Haemophilia A (HA) is a X-linked inherited bleeding disorder resulting from a deficiency of clotting factor VIII. The disease is categorised based on the residual factor VIII activity level. In general, patients with mild or moderate (nonsevere) disease only experience bleeding complications after trauma or surgery. Factor VIII (FVIII) concentrates are administered to prevent or treat bleeding, but patients can develop anti-FVIII antibodies (inhibitors), which is a major complication of this treatment. These antibodies render the replacement therapy with FVIII concentrates ineffective and result in increased morbidity and mortality (Hay et al., 1998; Darby et al., 2004; Eckhardt et al., 2015).

We need to identify risk factors for the development of inhibitors in HA patients to enable the identification of high risk patients, and to design patient-tailored treatment preventing the development of inhibitors.

Genetic risk factors for inhibitor development in HA patients that have been identified and studied are the *F8* genotype and polymorphisms in several immunoregulatory genes (Astermark et al., 2006a, 2006b, 2007,b, 2007; Gouw & Van Den Berg, 2009). Several treatment-related risk factors for inhibitors have been studied, mostly in severe HA patients, such as age and treatment intensity at first exposure, the reason for treatment (e.g. surgery), and the dose of FVIII.

Additionally, the type of FVIII concentrate used for treatment is one of the most debated risk factors. After the introduction of recombinant FVIII concentrates (recFVIII), it has been suggested that plasma-derived factor VIII concentrates (pdFVIII) elicit fewer inhibitors than recombinant FVIII concentrates. It is recognised that inhibitor screening practice intensified after introduction of recFVIII in the early exposure days (EDs) of severe HA, leading to greater detection of any anti-FVIII antibody activity.

One of the hypotheses for plasma-derived FVIII concentrates being less immunogenic is based on the presence of varying amounts of von Willebrand factor in pdFVIII concentrates, depending on the specific brand and manufacturing process. *In vitro* studies have shown that the von Willebrand factor (VWF) which is present in pdFVIII potentially masks inhibitor epitopes on the FVIII protein (Delignat et al., 2012). Other *in vitro* studies have demonstrated that VWF protects FVIII from being endocytosed by human dendritic cells and subsequently being presented to FVIII-specific T cells (Dasgupta et al., 2007; Kaveri et al., 2007).

However, numerous clinical studies and systematic reviews have yielded conflicting results, with the majority of the studies only including severe HA patients, and studies focusing on nonsevere haemophilia are scarce (Wight & Paisley, 2003; Gouw et al., 2007, 2013, 2013; Iorio et al., 2010; Franchini et al., 2012).

Recently studies in severe HA patients showed that second-generation recombinant FVIII concentrates were associated with a higher risk for inhibitor development (Gouw et al., 2013; Collins et al., 2014; Calvez et al., 2014).

In addition, in 2016 the SIPPET study was published, a multicentre open label randomised study in nonsevere HA patients, comparing plasma-derived and recombinant FVIII concentrates. The results of the SIPPET study showed an association between recombinant FVIII concentrates and the risk of inhibitor development in severe HA patients.

These results have perpetuated the debate about the type of FVIII concentrate being a risk factor for inhibitor development. In the search for risk factors for inhibitor development, nonsevere HA has been a neglected area of research. Moreover, studies have shown several differences and similarities between severe and nonsevere patients with regard to inhibitor development, e.g. in treatment, underlying biology and genetics (Fijnvandraat et al., 2003; d'Oiron et al., 2008; Peerlinck & Jacquemin, 2010; Castaman & Fijnvandraat, 2014).

In this nested case–control study we analysed the association of the type of FVIII concentrate and inhibitor development in nonsevere HA patients.

Methods

Patients

We conducted a case–control study, nested in a cohort of 2709 consecutive nonsevere HA patients (FVIII:C 2–40%), who received at least one exposure to FVIII concentrate in 33 European centres and one Australian centre between 1 January 1980 and 1 January 2011. The institutional review boards of all participating centres approved the study and have indicated that signed informed consent was not required.

All patients from the source population were followed-up from birth until death, emigration, loss to follow-up, or the end of the study. For each centre, we individually decided if data was available and reliable up until start of inclusion. For further information, we refer to previously published papers of the INSIGHT study, specifically the first paper published on the case–control study. (Eckhardt et al., 2013; Eckhardt et al., 2015; van Velzen et al., 2015; van Velzen et al., 2016, 2017, 2017).

Nonsevere HA patients who developed a clinically relevant inhibitor during follow-up were identified as case patients. One to four control patients (nonsevere HA patients without inhibitor development at the time of data collection) were matched by date of birth and cumulative number of exposure days (CEDs) to FVIII concentrates to each case (van Velzen et al.).

The cases and matched controls that received >75 CEDs were excluded from this primary analysis. Unfortunately, there were not enough patients, even in this large cohort, with this number of CEDs. Due to the low numbers, the uncertainty of the estimates would have been too large and we thus had to restrict our analyses to the exposure period in which we could produce reliable estimates, i.e. the period before 75 CEDs.

Data collection

Baseline clinical data were collected for the complete INSIGHT cohort, including the FVIII:C baseline level, cumulative number of EDs, *F8* genotype, ethnicity, family history of haemophilia A and inhibitor development. *F8* genotype was categorised into three categories (low risk mutation, high risk mutation, unknown) based on the HAMSTERS and CHAMP databases (Center for Disease Control & Prevention. CHAMP: CDC Haemophilia A Mutation Project. http:// www.cdc.gov/ncbddd/hemophilia/champs.html; Green *et al.*, 2008). We chose these two references because they are a better random sample, compared to our own INSIGHT database in which there is a stronger selection bias due to the nature of the study and this specific determinant. If for a mutation listed in the CHAMP *F8* mutation list and/or in the HAMSTERS database there was a reported history of inhibitor development, this mutation was classified as high risk mutation, and if there was no reported inhibitor development, the mutation was classified as low risk mutation.

For the cases and controls, detailed clinical data of every FVIII exposure day were collected until inhibitor development in cases, and up to the same number of EDs in controls, including the calendar date of every exposure day (of each patient), type, dose and mode of administration of FVIII product, mode and reason for treatment.

Outcome

The primary outcome was clinically relevant inhibitor development, defined as having at least two consecutive positive Bethesda inhibitor assay titres of ≥1·0 Bethesda Units (BU) per ml. Patients with inhibitor titres between 0·6 and 1·0 BU/ml had to fulfil one of the following two criteria to be classified as having a clinically relevant inhibitor: i) a decrease in endogenous FVIII plasma level to at least 50% of the baseline level, or ii) a reduced half-life of <6 h after FVIII concentrate administration. Patients who were not tested for inhibitors during the follow-up period and who had no clinical features of inhibitor development (e.g. increased bleeding tendency) were classified as negative for inhibitors.

Determinants

Factor VIII concentrates. For every exposure day of each patient, we collected information on the type of FVIII concentrate administrated. Patients were classified into categories representing the most frequently used type of FVIII concentrate. This was defined by the type of FVIII concentrate that was used for at least 50% of the EDs. If the type of concentrate was unknown for more than 50% of the EDs in a patient, we classified this patient into the category 'unknown'. This was also done for the first and the last 10 EDs of every patient.

For the sensitivity analysis of recombinant FVIII concentrate compared to plasma-derived FVIII concentrate, we defined the most frequently used type of FVIII concentrate as the concentrate used for at least 80% of the EDs with one type of concentrate. For the majority of the patients in our cohort, mainly one type of concentrate was used.

Firstly, we grouped all plasma-derived FVIII concentrates together and compared them to all recombinant FVIII concentrates grouped together. Secondly, we analysed whether the amount of von Willebrand factor antigen present in a FVIII product was associated with the risk of inhibitor development. We compared FVIII products containing no von Willebrand factor (all recombinant FVIII products), to products containing <0·01 International Units (IU) of von Willebrand factor antigen per IU of FVIII antigen ('low VWF') and products containing ≥0·01 IU of von Willebrand factor per IU of FVIII antigen ('high VWF'). This classification was based on the classification used in the RODIN study (Gouw *et al.*, 2013).

Thirdly, the different generations of recombinant FVIII products (first-generation recFVIII, second-generation recFVIII and third-generation recFVIII) were compared to all plasma-derived products.

For all cases, the last FVIII infusion was defined as the last one administered before inhibitor detection (the first positive Bethesda inhibitor test), and for controls the last factor infusion was the last CED that was included, based on the number of EDs of the matched case.

Dose. To study the dose as a determinant, we calculated the mean dose FVIII concentrate in International Units (IU) per kilogram bodyweight (IU/kg) of all EDs. In the majority of the patients, only the total administered dose of FVIII concentrate was available for each ED. To calculate the dose in IU/ kg/ED we imputed the weight of the patients on that specific ED using age-weight statistics (for adults) and growth curves (for children). The mean dose of all EDs for each patient was calculated and this was classified into 3 categories: 0–25 IU/ kg, 25–45 and >45 IU/kg per ED (van Velzen *et al.*, 2017).

Peak treatment. We defined three categories of peak treatment moments:

1. at least three consecutive EDs to FVIII concentrate within a maximum of five calendar days,
2. at least five consecutive EDs within a maximum of 5–10 calendar days
3. at least 10 consecutive EDs within a maximum of 14 calendar days.

To adjust for peak treatment moments in the analyses, we classified all patients into the following categories:

4. patient has never had a peak treatment moment during follow-up
5. patient has had at least one peak treatment moment with three consecutive EDs during follow-up

6. patient has had at least one peak treatment moment with five consecutive EDs

7. patient has had at least one peak treatment moment with 10 consecutive Eds.

Surgery. We collected data on the reason for treatment on every exposure day of each patient. To adjust for surgery, we classified patients into the following categories:

8. did this patient ever had a surgical intervention during follow-up? (yes/no)

9. did this patient have surgery in the last six months before end of follow-up? (yes/no)

For further details on all the determinants described above, please see *Methods* section in this paper on the INSIGHT case–control study.

Missing data

The missing calendar dates of EDs were unconditionally imputed with the middle value between the dates before and after the missing dates (<0·5%).

If the reason for treatment was missing (5·5%) for EDs one calendar day before or after an ED for which the reason for treatment was known, the missing value was replaced with the reason for treatment of that ED. In all other cases, missing values were unconditionally imputed with 'trauma' as the reason for treatment, since the assumption was made that this is the most probable reason for treatment in this patient group when reason of treatment was missing.

In the majority of the patients, only the total administered dose of FVIII concentrate was available for each ED. To calculate the dose in IU/kg/ED, we imputed the weight of the patients on that specific ED, using age-weight statistics (for adults) and growth curves (for children) (Centraal Bureau voor de Statistiek; Royal College of Paediatrics & Child Health; Australasian Paediatric Endocrine Group; McDowell *et al.*, 2008; Australian Bureau of Statistics, 2012; Destatis Statistisches Bundesamt, 2013; Food & Agriculture Organization of the United Nations, 2015). The mean dose of all EDs for each patient was calculated and this was classified into three categories: 0–25 IU/kg, 25–45 and >45 IU/kg per ED. Missing values of the FVIII dose (14%) were replaced with a median dose calculated with all EDs with that specific treatment indication in that specific treatment centre.

When the type of FVIII concentrate was missing (10%) for an exposure day and this ED was one of several subsequent EDs for one specific reason for treatment, the missing value was replaced with the type of FVIII concentrate that was reported for the other EDs.

Data analyses

To analyse the association between the type of FVIII concentrate and inhibitor development, we used conditional logistic regression methods. This method accounts for the matching of cases and controls, and analysis is performed using the matching groups (i.e. one case and 1–4 controls).

Crude as well as adjusted odds ratios (aORs) are presented. We adjusted for determinants that could have possibly confounded the associations studied, independent of their statistical significance in univariate analyses. The predefined confounders we adjusted for in the analysis are: endogenous FVIII level, ethnicity, *F8* genotype, positive family history for inhibitors, age at first ED and at last ED, calendar date, reason for treatment at first exposure, surgery, dose and peak treatment moment.

Results

Patient characteristics

In total, 7832 EDs for 298 patients were included in this case–control study. Figure 1 shows an overview of the patient inclusion.

The median age at first exposure was 23 years (interquartile range (IQR) 5–44) and the median baseline (endogenous) FVIII level was 8 IU/dL (IQR 4–14). The 75 cases (inhibitor patients) developed an inhibitor after a median of 25 ED (IQR 12–40) and the median inhibitor peak titre was 7 BU/ml (IQR 2–26). Baseline characteristics for cases and controls are shown in Table I (van Velzen *et al.*).

Plasma-derived versus recombinant factor VIII

In total, 179 patients were mainly treated with plasma-derived FVIII concentrates, and in 39 of these patients an inhibitor occurred during follow-up, compared to 36 patients of the 119 patients mainly treated with recombinant FVIII concentrates. All crude and adjusted relative risks for inhibitor development are displayed in Table II.

The risk of inhibitor development after treatment with recombinant FVIII products was not significantly increased compared to treatment with plasma-derived products in this study, whether analysed for all ED aOR 0·96, 95% confidence interval (CI) 0·36–2·52) or the first 10 EDs (aOR 0·84, CI 0·31–2·31) or last 10 EDs (aOR 1·27, CI 0·51–3·19).

Plasma-derived FVIII concentrates: von Willebrand factor-content

The majority of the patients (94, 53%) treated with plasma-derived FVIII concentrates received plasma-derived concentrates with a high von Willebrand factor content.

Compared to FVIII products containing no von Willebrand factor, the risk for inhibitor development was similar for FVIII products with a low von Willebrand content (aOR 1·69, CI 0·38–7·45) and for those with a high von Willebrand content (aOR 1·11, CI 0·38–3·33). This did not substantially change when the von Willebrand content of the type of
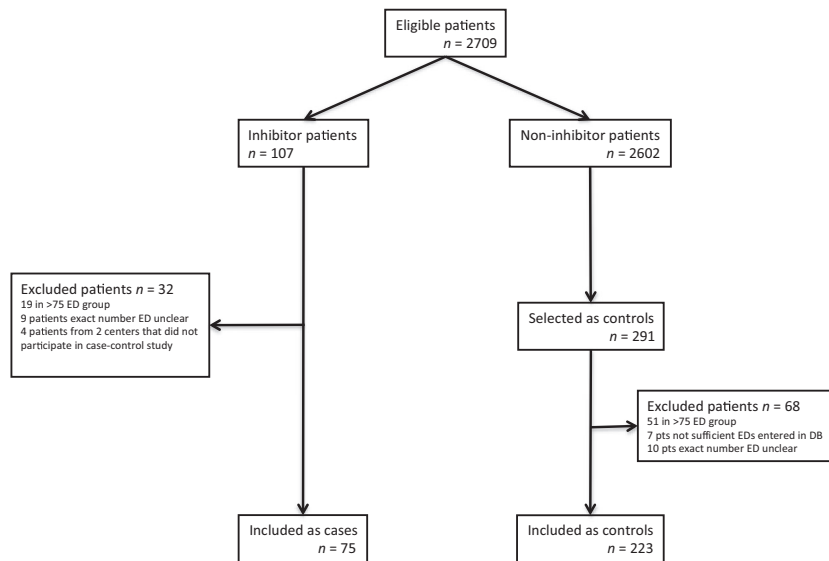
Fig 1. Inclusion of patients for case–control study from INSIGHT cohort.

Table I. Baseline characteristics.

Characteristics	All patients (*n* = 298) Median (IQR) Number (%)	Cases (*n* = 75) Median (IQR) Number (%)	Controls (*n* = 223) Median (IQR) Number (%)
Baseline FVIII level, IU/l	8 (4–14)	9 (5–14)	8 (4–14)
Age at first ED, years	23 (5–44)	26 (6–51)	21 (5–42)
Age at last ED, years	30 (13–54)	37 (14–61)	29 (13–52)
Number of ED's	20 (10–37)	25 (12–40)	18 (9–36)
Caucasian ethnicity	285 (96)	71 (95)	214 (96)
Family history of inhibitors			
Yes	13 (4·4)	8 (11)	5 (2)
No	195 (65·4)	43 (57)	152 (68)
Unknown	90 (30·2)	24 (32)	60 (30)
F8 genotype			
High risk mutation	93 (31)	41 (55)	52 (23)
Low risk mutation	91 (31)	12 (16)	79 (35)
Unknown	114 (38)	22 (29)	92 (41)

Values are medians (interquartile ranges).

concentrate used mainly for the first and last 10 EDs was analysed.

Recombinant FVIII concentrates: first, second and third generation concentrates

There was no difference in the risk for inhibitor development when comparing first-generation recombinant FVIII concentrates, second-generation concentrates and third-generation concentrates to plasma-derived FVIII concentrates, as shown in Table IIC.

Sensitivity analyses

The result of the sensitivity analysis (please see *Methods* section) regarding the use of plasma-derived FVIII versus recombinant FVIII was comparable to the primary analysis (unadjusted OR 1·84, CI 0·94–3·62 and aOR 1·19, CI 0·42–3·36).

Discussion

In this nested case–control study we investigated the association of the type of FVIII concentrate with inhibitor development among 295 nonsevere HA patients. We did not find an increased risk for inhibitor development for any type of FVIII concentrate – either when comparing recombinant FVIII concentrates to plasma-derived FVIII concentrates, or for specific types of FVIII concentrates.

However, the results of the recently published SIPPET study in severe HA showed different results. This was a multicentre open label randomised study, including 251 patients with a median follow-up of 22 EDs. After adjustment for confounders, recFVIII was associated with a 70–90% higher incidence on inhibitor development in severe HA patients (Peyvandi *et al.*, 2016). In comparing the different types of recFVIII to pdFVIII concentrates, several studies have found an increased risk for second generation

Table II. Type of concentrate and inhibitor development.

A) All plasma-derived versus all recombinant concentrates

Characteristics	No. pts	Crude OR (95% CI)	Number of events	Adjusted OR (95% CI)*
Type of FVIII concentrate				
All EDs				
Plasma -derived (ref.)	179	1	39	1
Recombinant	119	1·70 (0·91–3·18)	36	0·96 (0·36–2·52)
First 10 EDs				
Plasma -derived (ref.)	190	1	42	1
Recombinant	108	1·78 (0·89–3·47)	33	0·84 (0·31–2·31)
Last 10 EDs				
Plasma -derived (ref.)	128	1	37	1
Recombinant	170	1·64 (0·87–3·09)	38	1·27 (0·51–3·19)

B) Plasma-derived FVIII concentrates; different von Willebrand factor (VWF) content

Characteristics	No. pts	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)*	P-value
All EDs					
No VWF	119	1		1	
Low VWF	42	0·77 (0·32–1·84)	0·56	1·69 (0·38–7·45)	0·49
High VWF	94	0·56 (0·26–1·22)	0·15	1·12 (0·37–3·33)	0·84
Other/Unknown	43	0·43 (0·16–1·18)	0·11	0·60 (0·15–2·50)	0·49
First 10 EDs					
No VWF	108	1		1	
Low VWF	37	0·43 (015–1·22)		0·60 (0·12–2·98)	
High VWF	106	0·62 (0·28–1·37)		1·30 (0·43–3·93)	
Other/Unknown	47	0·61 (0·25–1·51)		1·88 (0·45–7·74)	
Last 10 EDs					
No VWF	128	1		1	
Low VWF	42	0·71 (0·29–1·69)		0·52 (0·12–2·25)	
High VWF	89	0·66 (0·31–1·45)		1·08 (0·36–3·24)	
Other/Unknown	39	0·36 (0·12–1·11)		0·61 (0·14–2·76)	

C) Recombinant FVIII concentrates; first, second and third generation

Characteristics	No. pts	Crude OR (95% CI)	Adjusted OR (95% CI)*
All EDs			
Plasma-derived	179	1	1
Generation recombinant FVIII concentrate			
First generation	52	1·38 (0·62–3·11)	0·91 (0·28–2·98)
Second generation	45	2·40 (1·10–5·27)	1·05 (0·28–3·84)
Third generation	7	0·72 (0·09–7·51)	0·17 (0·01–2·87)
Other/Unknown	15	1·18 (0·30–4·67)	1·42 (0·19–10·37)
First 10 EDs			
Plasma-derived	190	1	1
First generation	54	1·53 (0·67–3·52)	0·74 (0·23–2·39)
Second generation	31	2·74 (1·09–6·87)	1·60 (0·39–6·44)
Third generation	4	1·58 (0·15–16·44)	0·44 (0·02–13·35)
Other/Unknown	19	0·98 (0·28–3·39)	0·19 (0·02–1·56)

recFVIII concentrate (Gouw et al., 2013; Calvez et al., 2014; Collins et al., 2014).

The difference between these results in severe HA and our findings might be caused by the differences in study design (the SIPPET study was a randomised trial) but also by differences in immunological and treatment characteristics between severe and nonsevere HA. Firstly, severe HA patients have no measurable FVIII activity, which in many

Table II. (*Continued*)

C) Recombinant FVIII concentrates; first, second and third generation

Characteristics	No. pts	Crude OR (95% CI)	Adjusted OR (95% CI)*
Last 10 EDs			
Plasma-derived	171	1	1
First generation	49	1·17 (0·51–2·73)	0·79 (0·24–2·64)
Second generation	54	2·29 (1·03–5·13)	1·87 (0·55–6·42)
Third generation	11	0·86 (0·16–4·77)	0·14 (0·01–2·36)
Other/Unknown	13	1·21 (0·30–4·87)	1·00 (0·07–14·12)

Table A, B and C: Values are medians (interquartile ranges); Number of patients in each group based on cut-off of >50% of type of concentrate. *Adjusted for confounders: endogenous FVIII level, ethnicity, *F8* genotype, positive family history for inhibitors, age at first ED and at last ED, calendar date, reason for treatment at first exposure, surgery, dose, peak treatment.

is caused by an absence of extracellular FVIII protein. However, nonsevere patients produce endogenous FVIII that often differs only by a single amino-acid from the wild type (infused) FVIII protein. This may result in a different immunological response in severe HA patients compared to nonsevere. Secondly, the standard treatment for severe HA patients is prophylactic treatment, starting at a young age, and due to the prophylactic regimen these patients receive the first 15–20 EDs in a rather brief time period. Most nonsevere HA patients do not need prophylactic treatment and only receive FVIII to control bleeding after trauma or surgery. Therefore, nonsevere HA patients mostly receive their first treatment at a later age and the administration of FVIII concentrates is almost always on demand with the appearance of some amount of tissue damage. The combined action of risk factors may contribute to different results of risk factor analysis in inhibitor development in nonsevere HA. Also, the older age of first exposure may suggest that our population phenotype was a phenotype with lower bleeding, and this may have contributed to the lack of finding a difference.

Until now it has not been clarified which phase in treatment is the most important with regard to inhibitor development. The first exposures with FVIII concentrate could be the most important, when the immune system of a patient processes the FVIII concentrate for the first time. On the other hand, the last exposures to FVIII, promptly before inhibitor development, could be the moment the immune system becomes disrupted. Therefore, we analysed the first and last period of treatment (first and last 10 EDs), but we did not find a difference in inhibitor risk for one type of concentrate there either.

One of the unique strengths of this study is the case–control study design and the way patients were matched for the CEDs. We were able to include a large number of patients and collect detailed data on every ED for all these patients. Due to the detailed data collection, we were able to adjust for all putative confounders.

Thirdly, the duration of the observation period of the study was extensive; we were therefore able to include sufficient patients that were treated with plasma-derived FVIII concentrates. Recent studies often include a small number of patients treated with plasma-derived FVIII concentrates due to a shorter observation period. This causes an uneven distribution of patients over the different product groups.

In our study, there is heterogeneity of FVIII concentrates and of each class of FVIII concentrates, because we collected data from multiple centres in order to be able to study this rare disease. There is a great number of patients who were treated with several different products, and we have therefore chosen the most frequently used and the last product used, as shown in Table IIA–C.

Even though this is a large study for this specific patient group, the number of patients in some of the groups for different types of concentrates was still small. Therefore, our analysis for the subtypes of FVIII concentrates may be underpowered, increasing the chance of a type II error (the analysis not showing a difference in risk for inhibitor development is a false negative finding).

Due to the long observation period and the retrospective character of this study, there was missing data in different variables. Due to the missing data, there was a need for data imputation which may have influenced the outcome of our analyses.

Recently, several studies on the type of FVIII concentrate and the risk for inhibitor development in severe HA have been published, all showing an increased risk for second-generation recombinant FVIII concentrates (Gouw *et al.*, 2013; Calvez *et al.*, 2014; Collins *et al.*, 2014). The univariate analysis in our study did show an increased risk for inhibitor development for second-generation FVIII concentrates, but after adjustment for confounders, this finding did not reach statistical significance. Again, this may be caused by the fact that the total number of patients treated with a second-generation FVIII concentrate is small.

To conclude, in this nested case–control study including nonsevere HA patients, the type of FVIII concentrate was not associated with the development of inhibitors. These findings suggest that inhibitor development in nonsevere HA patients may be dependent on different determinants than inhibitor development in severe HA patients.

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Author contributions

A.S.V. collected, interpreted, cleaned, checked and analysed the data, and wrote the manuscript; C.L.E. collected and interpreted the data and reviewed, edited and approved the final version of the manuscript; J.G.B. designed the study, supervised the interpretation and statistical analysis of the data, and reviewed and approved the final version of the manuscript; K.F. designed and supervised the study, wrote the protocol, and wrote and edited the manuscript; and the other authors collected the data or supervised data collection, and reviewed and approved the final version of the manuscript.

Conflict of interest

A.S.V. has given lectures at educational symposiums organised by Novo Nordisk, Baxter and Pfizer and has received unrestricted research funding from CSL Behring. C.L.E. has received an unrestricted grant from The Netherlands Organisation for Health Research and Development (ZonMW) and has given lectures at educational symposiums organised by Novo Nordisk and Baxter. G.C. participated in Advisory boards for Bayer, Shire, Sobi, Pfizer, CSL Behring, Novo Nordisk, Kedrion. J.G.B. has received payment for consultancy meetings with Bayer and Wyeth, has received grants from Bayer Schering Pharma, Baxter, CSL Behring, Novo Nordisk and Wyeth and has received payment for lectures from Bayer. K.F. is a member of the European Hemophilia Treatment and Standardization Board sponsored by Baxter, has received unrestricted research grants from CSL Behring, Pfizer and Bayer, and has given lectures at educational symposiums organised by Pfizer, Baxter and Bayer. The remaining authors declare no competing financial interests.

Appendix

The investigators and institutions participating in the INSIGHT study are as follows. *Steering Committee* – K. Fijnvandraat (principal investigator and chair), M. Peters, P.W. Kamphuisen, Academic Medical Center, Amsterdam, the Netherlands; J.G. van der Bom, Leiden University Medical Center and Sanquin Research, Leiden, the Netherlands; K. Peerlinck, University of Leuven, Leuven, Belgium; J. Oldenburg, University Clinic Bonn, Bonn, Germany; C.R.M. Hay, Manchester Royal Infirmary, Manchester, United Kingdom; E. Santagostino, IRCCS Maggiore Hospital Foundation and University of Milan, Milan, Italy; J. Astermark, Skåne University Hospital Malmö, Malmö, Sweden.

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