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Full Length Article

Factor IX antibodies and tolerance in hemophilia B in the Nordic countries – The impact of *F9* variants and complications

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ABSTRACT

Introduction: The development of inhibitory antibodies (inhibitors) in persons with hemophilia B (PwHB) causes significant morbidity. Data on the impact of the *F9* variant and immune tolerance induction (ITI) outcome are limited.

The aim of this study was to investigate the presence of neutralizing and non-neutralizing antibodies (NNA) in severe hemophilia B (HB) and to evaluate ITI outcome and complications in relation to the pathogenic *F9* variant.

Materials and methods: Persons with severe HB in the Nordic countries were enrolled and information on *F9* variants, inhibitors, ITI and complications were collected. Analyses of anti-FIX antibodies with a fluorescence-immunoassay (xFLI) and an ELISA method were conducted.

Results: Seventy-nine PwHB were enrolled. Null variants were seen in 33 (42 %) PwHB and 12 (15 %) had a current or former inhibitor. Eleven (92 %) of the inhibitor patients had experienced allergic manifestations and three (25 %) nephrotic syndrome. Of 10 PwHB with at least one ITI attempt, eight (80 %) were considered tolerant at enrolment. Immunosuppression was included in seven of eight successful or partially successful attempts. Five PwHB had at least one ITI failure before a successful or partially successful ITI. No NNA could be identified.

Conclusion: A high proportion of severe *F9* gene defects among persons with severe HB in the Nordic countries may explain the observed relatively high prevalence of inhibitors. ITI success was independent of the *F9* variant and attained despite allergic manifestations and previous ITI failures. Inclusion of immunosuppression tentatively enhances the chances of ITI success. No NNA were observed.

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1. Introduction

Hemophilia B (HB) is a rare bleeding disorder occurring in 1 in 30,000 males [1]. The recommended treatment for persons with HB (PwHB) with a severe bleeding phenotype is prophylactic replacement therapy with the deficient factor IX (FIX) protein [2]. A serious complication to the treatment is the development of neutralizing antibodies (inhibitors) against FIX, which can result in the loss of function of infused concentrates. Inhibitors are reported more commonly in individuals with genetic null variants [2–6], i.e. no antigen is being produced, and most often occur before 20 exposures of factor treatment [2,3,7]. Inhibitor development can be complicated further by allergic reactions to replacement therapy, as well as by nephrotic syndrome.

The experience of immune tolerance induction (ITI) to eradicate the inhibitors in PwHB is limited and there is no established consensus on the management of these patients [2]. Different regimens with varied dosing and frequencies of FIX concentrates with or without the addition of immunosuppressive agents have been reported [3,8–12] but the study cohorts are small. Consequently, clinical management is often extrapolated from regimens and studies based on persons with the more common bleeding disorder hemophilia A (HA), i.e. deficiency of coagulation factor VIII (FVIII). However, phenotype and management of inhibitors differ between HA and HB. First, the incidence of inhibitors overall in patients with HB is often reported to be <5 % [2] and is thus much lower than in those with HA. In addition, inhibitors to FIX are mainly observed in patients with the severe form of the disease, i.e. a FIX activity <0.01 IU/mL. In HA, inhibitors are also seen, yet not as frequently, in the non-severe forms [2]. Furthermore, anaphylaxis and nephrotic syndrome are rare in HA, and ITI success rates seem to differ from HB. ITI success rates of 70–80 % are usually reported for HA, compared to only 30–35 % in HB [2,7]. As a conclusion, experience and treatment regimens used for inhibitors in HA cannot be extrapolated easily to manage individuals with HB.

In addition to inhibitory antibodies, the presence and clinical significance of non-neutralizing (non-inhibitory) antibodies (NNA) in HA have been studied and discussed over the years. In a recent meta-analysis, the pooled prevalence of NNA towards FVIII in HA was 25 % [13], and it has been suggested that NNA may predict the development of inhibitors and enhance the clearance of the administered factor concentrates [14–16]. Data on NNA in PwHB are sparse. Boylan et al. [17] assessed the relationship between anti-FIX antibody profiles and inhibitor formation with a fluorescence-based immunoassay (FLI) and found one or more classes of anti-FIX antibodies in 40 % of patient samples which tested negative by the Nijmegen-Bethesda assay. Further studies are, however, warranted to fully appreciate the value of monitoring NNA in routine clinical practice.

The aim of this study was to investigate the presence of neutralizing and non-neutralizing antibodies in patients with severe HB in the Nordic countries and to evaluate ITI outcome and complications in relation to the pathogenic *F9* variant.

2. Methods

2.1. Study design and study population

The B-NORD study is an observational multicenter study conducted in Denmark, Finland, Norway and Sweden and has been described previously [18]. Individuals of all ages with severe congenital HB were enrolled between the years 2017 and 2020. Information on inhibitors, ITI, allergic reactions and nephrotic syndrome was collected. The criteria used for ITI success were at the discretion of the treating physician and included a negative inhibitor titer and the possibility of using replacement therapy. A normal recovery and/or half-life of FIX concentrates were also reported, but not in a systematic manner. The treating physician reported whether the patient was considered tolerant or not at enrollment, but no consensus criteria on tolerance were used.

A positive inhibitor titer was defined according to the cut-off level for inhibitor detection at the local center. The Nijmegen-modified Bethesda assay, described previously [17,19], was performed at the local laboratory and the cut-off levels were 0.4 or 0.5 BU/mL (Bethesda units). The Malmö inhibitor assay was used previously to estimate inhibitors and expressed the inhibitor activity in plasma as the number of units of FIX inactivated by 1 mL of patient plasma [20]. One Malmö inhibitor unit (MIU) corresponds to about 3 BU.

The study was approved by the Regional Ethical Board in Lund, Sweden (Dnr 2016/1089) and by the independent ethics committees in each country. Written informed consent was collected from the study subject or his legally acceptable representative in accordance with the Declaration of Helsinki.

2.2. Variant analysis of F9

Variant analyses from PwHB in Sweden and Finland were performed at the genetic laboratory in association with the hemophilia treatment center (HTC) in Malmö, Sweden. Variant analyses from Norway were performed at the HTC in Oslo, Norway. No variant data were available for the patients from Denmark.

The promoter region of the F9 gene and all eight exons with the flanking intron regions were amplified by polymerase chain reaction (PCR) using primers described by Green et al. [21], modified with M13 tails. Variants were identified by Sanger sequencing as described by Mårtensson et al. [22]. Large deletions and duplications were determined by Multiplex Ligation-dependent Probe amplification (MLPA) using P207-F9 (MRC Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol. All reports were classified uniformly according to the recommendations of the Human Genome Variation Society (HGVS). The variants were interpreted for clinical significance according to the American College of Medical Genetics and Genomics (ACMG) guidelines applicable in 2021, using the VarSome's ACMG implementation [23] with an automated scoring and a manual review and adjustment of specific criteria. The FIX Gene Variant Database [24,25] was used for comparison.

2.3. Anti-FIX assays for the detection of non-neutralizing antibodies

Two different assays were used to investigate the presence of NNA: one Multi-Analyte Profiling (Luminex xMAP) based fluorescence immunoassay (xFLI) and one enzyme-linked immunosorbent assay (ELISA). Results from the Nijmegen-Bethesda assays were used for comparison to distinguish inhibitors from NNA.

2.3.1. Anti-FIX Luminex xMAP-based fluorescence immunoassay — xFLI method

FIX (nonacog alfa, BeneFIX) was coated to MagPlex microspheres. Citrated plasma samples were diluted in phosphate buffer saline (PBS, Hyclone) supplemented with 0.05 % Tween-20 (PBST, Merck) and 0.1 % ovalbumin (Sigma) (PBST-O), added to wells containing FIX-coupled microspheres and incubated for 2 h, washed with PBST, and incubated with R-phycoerythrin-labeled goat anti-human IgG (Jackson ImmunoResearch, Ely; Cambridgeshire, UK). Readings in a MagPix instrument (Luminex, Corporation, Austin Texas, US) were recorded as median fluorescence intensity (MFI). A general cut-off for positivity was determined from the mean + 3 SDs in healthy individuals (n = 26). The inter-assay CV was 12.2 % for the high and 14.3 % for the low positive control.

2.3.2. Anti-FIX immunological assay — ELISA method

An in-house ELISA was used, in which FIX (nonacog alfa, BeneFIX) was coated overnight. Plasma samples were diluted 50-fold in a Tris-blocking buffer supplemented with 1 mM $CaCl_2$ and incubated for 2 h. The secondary antibody was horseradish-peroxidase conjugated polyclonal rabbit anti-human IgG (Agilent, Santa Clara, CA, US). Absorbance was measured in a microplate reader (Tecan Infinite 200, Männedorf,

Switzerland). The cut-off for each test run was determined by analyzing normal plasma samples (n = 10–12) per test run and given as the mean + 3 SDs. The inter-assay CV was >50 % for the positive control.

2.4. Statistical analysis

Descriptive statistics were used. Continuous variables were described using medians and first-to-third quartiles (Q1-Q3). Categorical data were reported as numbers and percentages. Comparisons of two independent groups of continuous, non-normally distributed variables were performed using the Mann-Whitney *U* test. For binary or categorical data, the Chi-square test or Fisher's exact test was used. A *p*-value of <0.05 was considered to be statistically significant. Statistical analyses were performed using IBM SPSS Statistics 25.

3. Results

3.1. Patient characteristics

Out of 108 persons with severe HB registered at the study centers, 79 (73 %), median age 30 years (Q1-Q3 19–53), were enrolled in the B-NORD study [18]. Patient characteristics are presented in Table 1.

Out of the 79 enrolled PwHB, 12 (15 %) were reported to have current or former inhibitors, all registered at the HTCs in Sweden (Table 1). Two of the inhibitor patients were brothers and two were related more distantly. The age at start of prophylaxis did not differ between PwHB with and without inhibitors, median ages of 2.7 (Q1-Q3 1.0–29) and 3.0 years (Q1-Q3 1.0–16), respectively. The median age at inhibitor detection was 2.0 years (Q1-Q3 1.0–8.0) and in all reported cases occurred before 20 exposure days (missing data n = 5). Eight (67 %) of the 12 patients with inhibitors were considered tolerant at study enrollment by their treating physician and were treated with prophylactic FIX replacement therapy, median dose 6638 IU/kg/year (Q1-Q3 4141–10,115). Four of these tolerant PwHB were on plasma-derived and four on recombinant standard half-life products (SHL). The corresponding consumption for those without inhibitor history was significant lower with a median dose of 3406 IU/kg/year (Q1-Q3 2178–4583) ($p = 0.005$). The four remaining patients with inhibitors had either ongoing ITI, prophylactic treatment with rFVIIa only or were on investigational study drugs (two patients).

Table 1
Study cohort characteristics.

	Inhibitor patients n = 12	Non-inhibitor patients n = 67
Enrollment country (%)		
Denmark	–	9 (13)
Finland	–	9 (13)
Norway	–	15 (22)
Sweden	12 (100)	34 (51)
Age at enrollment, years, median (Q_1-Q_3)	26 (18–42)	31 (19–54)
Age at diagnosis, years, median (Q_1-Q_3)	0 (0–0)	0 (0–1)
Family history of hemophilia (%)†	7 (58)	30 (45)
CVAD, current or previous (%)	5 (42)	12 (18)
BMI, kg/m^2, median (Q_1-Q_3)	23 (19–29)	25 (22–28)
Current treatment (%)		
On-demand FIX-replacement	–	2 (3.0)
Prophylaxis FIX-replacement	8 (67)	65 (97)
Bypass-therapy	2 (17)	–
Non-factor replacement	2 (17)	-
Age at 1st joint bleed, years, median (Q1-Q3) ‡	1.5 (0.71–3.2)	2.1(1.0–4.4)
Age at start of prophylaxis, years, median (Q_1-Q_3) §	1.4 (1–25)	3.3 (1–16)
Previous joint surgery (%)	4 (33)	23 (34)¶
Age at inhibitor detection, median (Q_1-Q_3)	2.0 (1.0–8.0)	NA
Allergic manifestation (%)	11 (92)	1 (1.5)
Nephrotic syndrome (%)	3 (25)	–
HIV positive (%)	1 (8.3)	3 (4.5)
Unknown/not tested	2 (17)	14 (21)
HCV status (%)		
Never infected (Ab-/PCR-)	7 (58)	30 (45)
HCV positive (Ab+/PCR+)	–	4 (6.0)
Recovered infection (Ab+/PCR-)	3 (25)	24 (36)
Unknown/not tested	2 (17)	9 (13)

Numbers (%) or median (Q1, first quartile - Q3, third quartile). BMI, body mass index. CVAD, central venous access device. HCV, hepatitis C virus. HIV, human immunodeficiency virus. NA, not applicable.
The number of patients (n) is noted if it deviates from the total number: †n = 11 (inhibitor), n = 65 (non-inhibitor), ‡n = 9 (inhibitor), n = 48 (non-inhibitor), §n = 11 (inhibitor), n = 60 (non-inhibitor), ¶n = 65.

3.2. F9 variants and comparison to the EAHAD FIX Gene Variant Database

The *F9* variant was identified in 64 patients (81 %). In total, 42 different variants were found (Table 2). Thirty of the variants had been reported previously in the FIX Gene Variant Database. All but one of the *F9* variants identified were classified as 'pathogenic' according to the ACMG classifying system. The remaining variant (c.253-12_253-3delTATTCTTTAT) was classified as 'likely pathogenic'. Null variants defined as nonsense variants, frameshift outside poly-A runs, large structure deletions, and splice-site mutations involving conserved nucleotides were seen in 33 patients (42 %), nine of whom had an inhibitor history. The distribution of variants is presented in Fig. 1 and demonstrates a higher occurrence of large structure deletions of 10 % in the B-NORD cohort (persons with unknown variants are excluded from the calculation), compared to 4.8 % in the FIX Gene Variant Database. Table 3 shows the genetic variants divided by country. As shown in Fig. 2, the frequency of inhibitor development by variant effect was 71 % (5/7) for large structure changes, 17 % (1/6) for frameshift, 15 % (3/20) for nonsense and 12 % (3/26) for missense variants. No PwHB with splice or in-frame variants had an inhibitor history.

Out of the 12 inhibitor patients, nine had a null *F9* variant. Interestingly, two brothers in the study had the *F9* variant c.316G > A and both had developed inhibitors despite the fact that this variant is reported 74 times in the FIX Gene Variant Database without any previously reported inhibitor cases. Six patients had the large structure deletion g.(?_139530767)_(139562071_?)del, and all but one developed inhibitors. The one patient with this large structure deletion but no inhibitors started prophylaxis at the age of 19 years and has since been on prophylaxis with SHL FIX for >40 years.

3.3. Immune tolerance induction

At study enrollment, all but one of the PwHB with inhibitors either were on ongoing ITI or had completed at least one attempt. Detailed information on all 22 ITI attempts performed over the years in the 11 patients is presented in Table 4 and Fig. 3. All but one of the ITI attempts were based on daily administration of factor products with doses of 60–250 IU/kg. No difference could be seen in dosing between successful or non-successful ITI attempts. Out of the 22 attempts, one was ongoing at study start, four (19 %) of the completed attempts were considered successful by the treating physician, four (19 %) were considered partially successful and 13 (62 %) were considered unsuccessful. The shortest time to a successful ITI was 3 months. In total, 10 patients had finished at least one ITI attempt, and eight (80 %) of these were considered tolerant at enrollment. All four patients with partially successful ITI attempts were thus considered tolerant by their treating physician at the time of enrollment in the study and were treated with FIX prophylaxis. However, the definitions used of partial success, normal recovery and half-life differed between the cases. Two of the PwHB considered partially tolerized had a low-titer inhibitor, but were treated successfully with FIX products, and two patients had a negative inhibitor titer, yet without a normal recovery or half-life.

As shown in Table 4, the *F9* variants in the four PwHB having a

Table 2
Genetic variants in the FIX gene found in the B-NORD cohort. No. of inhibitor patients specified in parenthesis.

Variant type	Variant effect	Domain	Coding DNA†	Protein‡	No. (with inhibitors)	No. in the FIX Variant Database* (with inhibitors)
Substitution	Missense	Protease	c.1304G > A	p.(Cys435Tyr)	3	18
			c.1145G > A	p.(Cys382Tyr)	1	8
			c.1237G > A	pGly413Arg	1	7
			c.1052G > A	p.(Gly351Asp)	1	3
			c.1058 T > G	p.(Val353Gly)	1	3
			c.1295G > T	p.(Gly432Val)	1	2
			c.799C > T	p.(His267Tyr)	1	2
			c.1025C > A	p.(Thr342Lys)	1	2
			c.1289G > T	p.(Ser430Ile)	1	1
			c.1069G > C	p.(Gly357Arg)	1 (1)	–
			c.893G > C	p.(Arg298Pro)	1	–
			c.982A > T	p.(Asn328Tyr)	1	–
			c.998C > T	p.(Pro333Leu)	1	–
		EGF1	c.316G > A	p.(Gly106Ser)	2 (2)	74
			c.316G > T	p.(Gly106Cys)	1	2
		EGF2	c.464G > C	p.(Cys155Ser)	1	3
			c.400 T > A	p.(Cys134Ser)	1	–
		Pro-Peptide	c.127C > T	p.(Arg43Trp)	2	65
		Linker	c.533G > T	p.(Cys178Phe)	2	3
		Gla	c.251C > G	p.(Thr84Arg)	1	1
		Act-Peptide	c.676C > T	p.(Arg226Trp)	1	44
	Nonsense	Protease	c.880C > T	p.(Arg294*)	5 (1)	70 (4)
			c.1135C > T	p.(Arg379*)	4	65
			c.892C > T	p.(Arg298*)	2	63 (1)
			c.719G > A	p.(Trp240*)	2 (2)	7 (1)
			c.709C > T	p.(Gln237*)	1	4 (1)
			c.1305 T > A	p.(Cys435*)	1	–
		EGF2	c.484C > T	p.(Arg162*)	3	22
		Linker	c.535G > T	p.(Gly179*)	1	1
		Gla	c.223C > T	p.(Arg75*)	1	73 (8)
	Splice¶	N/A	c.392-1G > C	N/A	1	4
Deletion	Frameshift	Protease	c.969_975del	p.(Pro324Cysfs*2)	1 (1)	–
			c.815delG	p.(Gly272Valfs*25)	1	–
			c.1295delG	p.(Gly432Valfs*6)	1	–
		Gla	c.229delG	p.(Val77Phefs*27)	1	1
			c.161_162del	p.(Glu54Valfs*7)	1	–
		Act-Peptide	c.668delA	p.(Asp223Alafs*22)	1	1
	Large Structure Change (>50 bp)§		g.(?_139530767)_(139562071_?)del	p.0	6 (5)	60 (21)
			g.(?_139530767)_(139551238_139560770)del	p.0	1	2
	Splice¶	N/A	c.253-12_253-3delTATTCTTTAT	N/A	1	1
	In-frame	Protease	c.689_691delGAG	p.(Gly230del)	1	7
Duplication	In-frame	EGF1	c.353_358dup	p.(Cys119_Pro120insArgCys)	2	0
No variant found					3	
Missing data					12	

No., number of patients. *Accessed on 2021-03-05.:†NM_000133.3. ‡NP_000124.1, ¶NG_0079994.1 §NC_000023.11.

successful ITI included one large structure deletion, one frameshift deletion, one nonsense substitution and one missense substitution. The *F9* variants in the four patients with partially successful ITIs, but later tolerant after additional factor IX treatment, included one large structure deletion, two nonsense substitutions and one missense substitution. Finally, the two patients not tolerant at enrollment carried a large deletion and a missense substitution, respectively. In summary, no correlation between ITI outcome and type of underlying *F9* variant was seen in our cohort.

Two of the PwHB with a successful ITI had high-responding inhibitors. However, all of the successful attempts started with a titer <5 BU/mL. The inhibitor titer at the start of ITI was overall low with a median value of 2.1 BU/mL (Q1-Q3 0.93–12). The corresponding figures for 'successful', 'partially successful' and 'not successful' were 0 BU/mL (Q1-Q3 0–2.0), 1.5 BU/mL (Q1-Q3 0.53–11) and 5.7 BU/mL (Q1-Q3 1.2–18), respectively ($p = 0.18$).

The ITI regimens are provided in Table 4. Immunosuppression was included in three of the four successful ITIs and in all of the partially successful attempts. Six (46 %) of the failures included immunosuppression. Among the four successful attempts, one was considered tolerant after the first ITI attempt, one after the second, one after the third and one after the sixth ITI attempt. Recombinant factor products were used in two (50 %) of the successful, one (25 %) of the partially successful and in one (8 %) of the unsuccessful attempts.

3.4. Allergic reactions and nephrotic syndrome

Eleven (92 %) of the PwHB and inhibitors were reported to have experienced allergic manifestations towards FIX compared to only one (1.5 %) of the PwHB without inhibitors (Table 1). In five (42 %) inhibitor patients, the allergic reaction was reported as anaphylaxis. All of these patients had a high-titer inhibitor. In four of these patients, the *F9* variants were null variants (two large deletions, one frameshift deletion, one nonsense substitution) and in one case a missense substitution. The

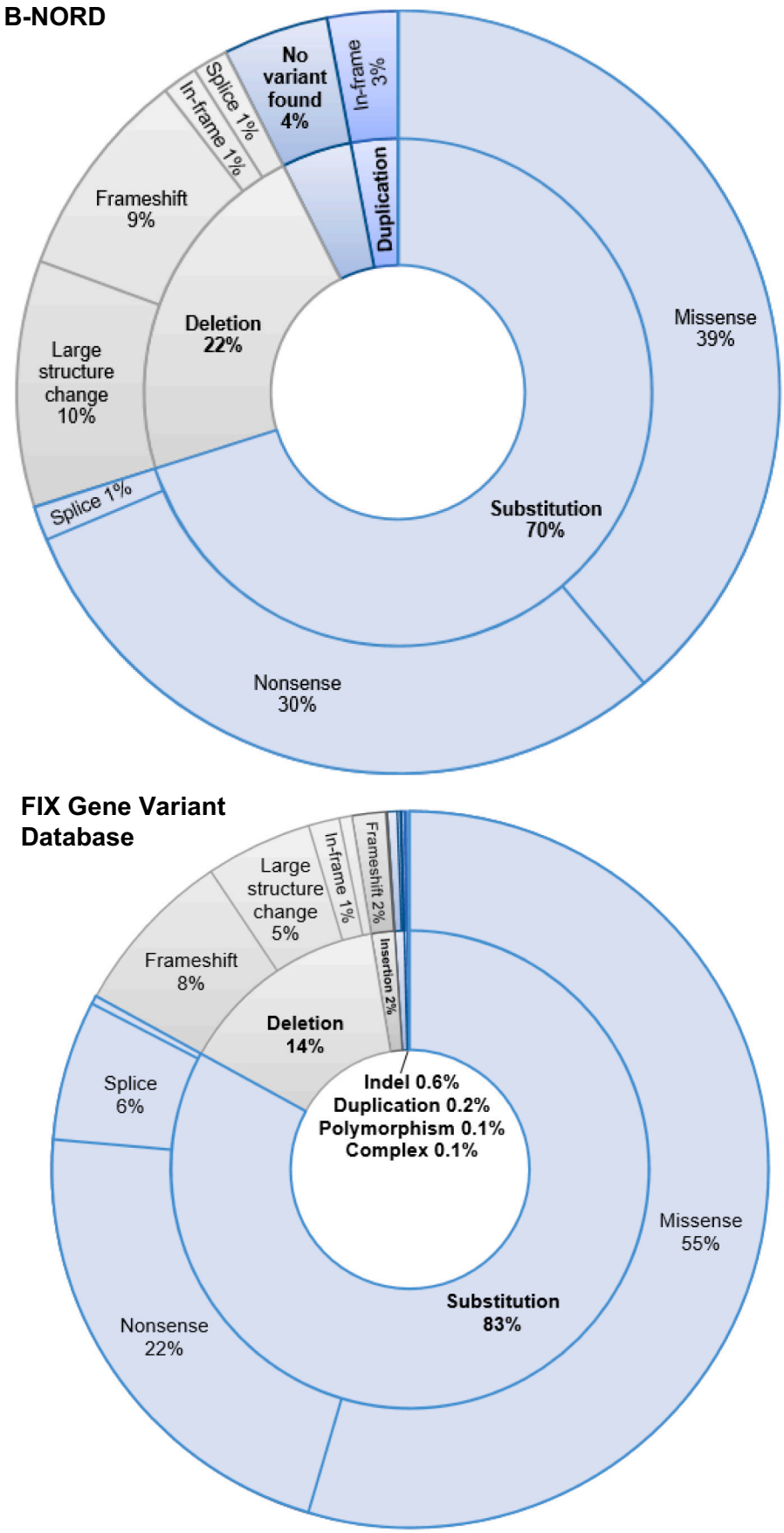

Fig. 1. Genetic variants. Genetic variants by variant type (inner circle) and variant effect (outer circle) in the B-NORD cohort and in severe hemophilia B in the FIX Gene Variant Database. For comparison, missing data is excluded from the B-NORD cohort.

Table 3
Genetic variants divided by country. No. of inhibitor patients specified in parenthesis.

Country	Variant effect No. (with inhibitors)							
	Missense	Nonsense	Large structure change	Frameshift	In-frame	Splice	No variant found	Missing data
Sweden	16 (3)	14 (3)	7 (5)	5 (1)	-	2	-	2
Norway	7	4	-	1	-	-	3	-
Finland	3	2	-	-	3	-	-	1
Denmark	-	-	-	-	-	-	-	9

No., number of patients.

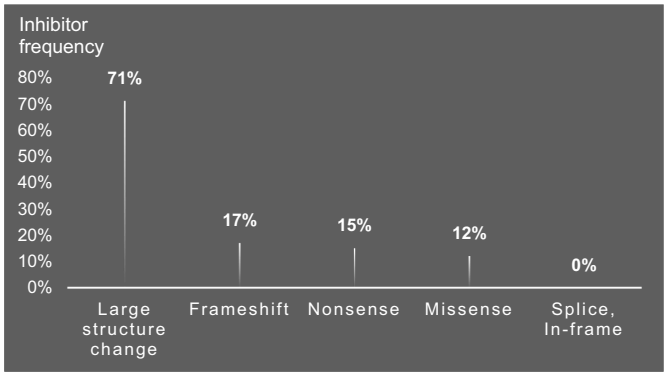

Fig. 2. Frequency of inhibitor development by gene variant effect.

remaining seven inhibitor patients reporting allergic manifestations, but no anaphylaxis, all experienced skin rash with/without additional symptoms. In six patients, the allergic reactions occurred after inhibitor detection, in three cases before and in two patients the onset of allergic reaction in relation to inhibitor development was not reported. The one patient with allergic symptoms in the absence of inhibitor history carried a nonsense substitution (FIX: c.892C > T; p.Arg298*), which is reported 63 times in the FIX Gene Variant Database, one of these with an inhibitor.

Nephrotic syndrome was reported in three (25 %) of the 12 inhibitor patients and in none of the PwHB without inhibitors (Table 1). In all three cases, the nephrotic syndrome was diagnosed after inhibitor detection, and in two cases, the nephrotic syndrome was diagnosed during ITI and contributed to the interruption of the ITI. Two of the PwHB and nephrotic syndrome were not considered tolerant at study enrollment. The genetic *F9* variants in association with nephrotic syndrome include one large structure deletion, one nonsense substitution and one missense substitution (Table 4).

3.5. Non-neutralizing antibodies

Samples from 53 (67 %) of the PwHB were collected and analyzed using the ELISA method and in 48 cases also with the xFLI assay (Table 5).

Samples from all 12 patients with a history of inhibitors were tested with ELISA and 10 of them also with the xFLI assay. The only two samples with a positive Bethesda titer (3 BU/mL and 0.4 BU/mL, respectively) were also positive in both immunoassays. No consistent findings for NNA were obtained in any of the remaining samples. In two cases, however, both negative in the xFLI assay, results were initially positive in the ELISA assay. On retesting, the ELISA assay was negative in one and borderline positive (4.2 SDs above mean) in the other case.

Among the samples from non-inhibitor patients, no consistent findings of NNA were observed. In four cases, the outcome of the two assays was initially discrepant, with the ELISA assay positive in three cases and the xFLI assay positive in one sample. In none of the cases, could retesting confirm the presence of NNA. Altogether, the concordance between the two immunoassays was 87.5 %.

4. Discussion

This Nordic study of persons with severe HB reveals a relatively high proportion of severe *F9* gene defects and a high prevalence of inhibitors. Our study also illustrates the unpredictable challenges, but also possibilities, in the management of PwHB and inhibitors.

A prevalence of 15 % of persons with a history of inhibitors in our Nordic HB population is relatively high compared to many other published reports. Our cohort was, however, restricted to severe HB patients and the inhibitor figure is consistent with the Swedish data previously reported [26], and not dissimilar from that reported recently for the severe subgroup of PwHB in the PedNet Registry [7]. Admittedly, not all persons with severe HB registered at the HTCs were enrolled in our study. The inhibitor prevalence would, however, still be at least 11 %, if the entire severe HB population was included, indicating that inhibitor development in the severe HB population is a significant problem. Importantly, the prevalence of severe gene defects, i.e. large deletions and nonsense variants, is also relatively high, which we believe to be the main explanation for the observed prevalence of inhibitors. The variant distribution in the B-NORD cohort is otherwise largely in agreement with the FIX Variant Database (Fig. 1).

Out of 11 patients having at least one ITI attempt, only one patient had ITI ongoing at study enrollment, with a duration of 2 months. Eight of the remaining 10 patients were considered tolerant at enrollment. This makes a total success rate of 80 % and indicates that tolerance may be achievable for the majority of PwHB and inhibitors. Interestingly, four (40 %) of these patients were considered only partially tolerized after their final ITI, but tolerant with additional long-term FIX replacement. This indicates that tolerance may be achieved with continuous exposure of the deficient factor for bleed prevention. Importantly, the criteria for ITI success and tolerance were determined by the individual physician in our study and the lack of well-defined established definitions of ITI success, and tolerance in HB complicates the comparisons of the outcome of various ITI attempts as well as the evaluation on treatment duration and when tapering of the dose is suitable.

Five patients had at least one ITI failure before an attempt leading to success or partial tolerance, which indicates, in line with recently published data [27], that ITI success can be attained despite previous ITI failures and that more than one ITI attempt can be considered in PwHB. We could not identify any favorable or unfavorable *F9* variant on the ITI outcome and no difference in outcome for plasma-derived or recombinant products. In this context, it is important to highlight that no extended half-life (EHL) products were used. In seven out of eight (88 %) successful or partially successful ITI attempts, immunosuppression was included in the regimen. In three of these attempts a combination of rituximab, intravenous immunoglobulin (IVIG), dexamethasone and mycophenolate, in line with the Beutel protocol [8], was used and in four cases a combination of cyclophosphamide and IVIG. In two of these latter cases, corticosteroids were used in addition. Only one case of ITI success was achieved without immunosuppression. This was in a patient with a missense substitution in the *F9* gene and a low-responding inhibitor and the treating physician reported doubting the clinical

Table 4
Detailed data on immune tolerance induction attempts.

ID	Genetic variant	Age at inhibitor detection (years)	Tolerant at enrollment†	Peak titer (BU)	Allergic symptoms	Nephrotic syndrome	ITI attempt	Age at ITI (years)	Titer at start of ITI (BU)	ITI regimen§	ITI success‡ (time to success or termination)
1	c.-29-?_1386+?del	9	N	129	Y	N	1.	14	129	**PD non-monoclonal antibody purified SHL** (*NanoFIX*)(68 IU/kg once daily) On-going, duration 2 months at study enrollment	On-going
2	c.-29-?_1386+?del	1	Y	2.7	Y	N	1.	3	2.4	**PD non-monoclonal antibody purified SHL** (*Nanotiv)* (88 IU/kg twice daily) ***IVIG, Dexamethasone/ Betamethasone, Mycophenolate***	N (57 months)
							2.	14	1.7	***Recombinant SHL*** (*BeneFIX*) (91 IU/kg twice daily) ***Rituximab, IVIG, Dexamethasone, Mycophenolate***	PT (42 months)
3	c.-29-?_1386+?del	1	N	61	Y	Y	1.	0.5	MD	**PD non-monoclonal antibody purified SHL** (*Nanotiv)* (100 IU/kg daily)	N (1 day)**
							2.	2	MD	**PD non-monoclonal antibody purified SHL** (*Nanotiv)* (80 IU/kg daily) ***IVIG***	N (35 months) ***
4	c.-29-?_1386+?del	1	Y	28	Y	N	1.	1	*1.2**	**PD non-monoclonal antibody purified SHL** (*Nanotiv)* (100–200 IU/kg 2–3 times per week)	N (3 months)
							2.	2	*9.0**	**PD monoclonal antibody purified SHL** (*Mononine*) (35 IU/kg 3 times per week, after 20 months increased dose to 105 IU/kg daily)	N (25 months)
							3.	19	<0.4	***Recombinant SHL*** (*BeneFIX*) (65 IU/kg twice daily, tapering of the dose after 1 month) ***Rituximab, IVIG, Dexamethasone, Mycophenolate*** Simultaneous implantation of venous access catheter	Y (6 months)
5	c.719G > A	2	Y	2.2	Y	Y	1.	1	1.0	***Recombinant SHL*** (*BeneFIX*) (60 IU/kg daily)	N (36 months) ***
							2.	4	<0.4	***Recombinant SHL*** (*BeneFIX*) (86 IU/kg twice daily) ***Rituximab, IVIG, Dexamethasone, Mycophenolate***	Y (3 months)
6	c.719G > A	16	Y	*>300**	Y	N	1.	53	*1.2**	**PD monoclonal antibody purified SHL** (*Mononine*) (35 IU/kg 4 times daily, after 15 days tapering of the dose) ***Cyclophosphamide, Hydrocortisone, IVIG***	PT¶1 (40 days)
7	c.316G > A	2	N	40	Y	Y	1.	2	1.8*	**PD monoclonal antibody purified SHL** (*Mononine*) (110 IU/kg daily) ***IVIG, Cyclophosphamide***	N (15 months)
8	c.316G > A	2	Y	1.9	N	N	1.	4	0.3*	**PD monoclonal antibody purified SHL** (*Mononine*) (93 IU/kg daily) ***IVIG, Cyclophosphamide***	PT (MD)

(*continued on next page*)

Table 4 (*continued*)

ID	Genetic variant	Age at inhibitor detection (years)	Tolerant at enrollment†	Peak titer (BU)	Allergic symptoms	Nephrotic syndrome	ITI attempt	Age at ITI (years)	Titer at start of ITI (BU)	ITI regimen§	ITI success‡ (time to success or termination)
9	c.880C > T	15	Y	*>300**	Y	N	1.	37	*21**	**PD non-monoclonal antibody purified SHL** (*Preconativ)* (total dose 69,000 IU during 10 days ¤) ***Plasmapheresis, Cyclophosphamide*** Simultaneous surgery of elbow	N (10 days)
							2.	39	*14**	**PD non-monoclonal antibody purified SHL** (*Preconativ)* (31 IU/kg/ dose 3–4 times daily) ***Plasmapheresis, Cyclophosphamide, IVIG*** Simultaneous extraction of eight teeth	PT (15 days)¶2
10	c.1069G > C	5	Y	0.9	Y	N	1.	1	<0.5	**PD non-monoclonal antibody purified SHL** (*NanoFIX)* (71 IU/kg once daily)	Y (4 months)
11	c.969_975del	5	Y	*>300**	Y	N	1.	10	*0.9**	**PD non-monoclonal antibody purified SHL** (*Preconativ*) (total dose 24,500 IU during 9 days¤) **IVIG** Simultaneous straightening treatment of knee	N (9 days)
							2.	10	*150**	**PD non-monoclonal antibody purified SHL** (*Preconativ)* (total dose 30,000 IU during 9 days¤) ***Plasmapheresis, IVIG*** Simultaneous straightening treatment of knee	N (9 days)
							3.	11	*6**	**PD non-monoclonal antibody purified SHL** (*Preconativ)* (one dose of 227 IU/kg, hereafter 45 IU/kg three times daily) ***Cyclophosphamide, IVIG*** Simultaneous surgery of knee	N (8 days)
							4.	11	*18**	**PD non-monoclonal antibody purified SHL** (*Preconativ)* (total dose 71,000 IU during 8 days¤) ***Plasmapheresis, IVIG, Cyclophosphamide, Hydrocortisone*** Simultaneous treatment of larger bleed	N (8 days)
							5.	12	*5.7**	**PD non-monoclonal antibody purified SHL** (*Preconativ)* (total dose 76,000 IU during 11 days ¤) ***Plasmapheresis, IVIG, Cyclophosphamide, Hydrocortisone*** Simultaneous surgery of knee, extraction of teeth and injection therapy of elbow	N (11 days)
							6.	13	*2.7**	**PD non-monoclonal antibody purified SHL** (*Preconativ)* (one dose of 125 IU/kg, hereafter 33 IU/kg 2–6 times daily, day 14 tapering of the dose)	Y (3 months)

(*continued on next page*)

Table 4 (*continued*)

ID	Genetic variant	Age at inhibitor detection (years)	Tolerant at enrollment†	Peak titer (BU)	Allergic symptoms	Nephrotic syndrome	ITI attempt	Age at ITI (years)	Titer at start of ITI (BU)	ITI regimen§	ITI success‡ (time to success or termination)
										IVIG, Cyclophosphamide, Hydrocortisone Simultaneous straightening treatment of knee	

Y, yes. N, no. BU, Bethesda units. PT, partial tolerance. PD, plasma-derived FIX. SHL, standard half-life. IVIG, intravenous immunoglobulin. MD, missing data. *Inhibitor titer measured and reported in MIU, Malmö inhibitor units, and recalculated to Bethesda units by multiplying by a factor of three. **Termination due to anaphylaxis. ***Termination due to nephrotic syndrome. §In case of changed doses, the most intensive regimen is presented. †Considered tolerant by the treating physician. ‡Assessed ITI success by the treating physician. ¤No data on dose/kg and frequency could be collected from the medical journals. ¶1After 40 days considered partially tolerant and transition to every other day prophylaxis. ¶2Termination of ITI after 15 days, considered partial tolerant since treatable with FIX-concentrate.

Fig. 3. Schematic illustration of immune tolerance induction attempts (ITI) in eleven persons with hemophilia B and inhibitors. Each line illustrates the ITI experience of one patient and each circle represents an ITI attempt. *The treating physician reported whether the patient was considered tolerant or not at enrolment in the study.

Table 5
Anti-FIX ELISA and xFLI results.

		ELISA			
		Neg	Pos	MD	Total
xFLI	Neg	40	5	0	45
	Pos	1	2†	0	3
	MD	4	1	26	31
	Total	45	8	26	79

MD, missing data.
† Both samples were positive in Bethesda (3 BU/mL and 0.4 BU/mL).

relevance of the inhibitor. A successful use of immunosuppression is in concordance to several previous reports of ITI in HB [8,9,27–33] and our study further supports this approach; adding immunosuppression as a first-line treatment should be considered in these patients. Interestingly, one PwHB had five ITI failures before he became tolerant after the sixth attempt. This attempt was mainly distinguished from the previous attempts by a longer duration of 3 months, indicating that treatment should not be terminated too early. The shortest time to a successful ITI among our patients was 3 months.

All but one of the persons with inhibitors (92 %) had experienced allergic manifestations towards FIX. This figure is high compared to that of 60 % reported by the ISTH-SSC International FIX Inhibitor registry [3], or that of 41 % in the recent B-NATURAL study [27]. This may be due to the underlying *F9* genetic profile in our cohort. Five patients had experienced anaphylaxis; at enrollment, three of these were considered tolerant. Different desensitization protocols have previously been described [34,35] in attempts to overcome the allergic reactions to FIX. Seven of the patients with inhibitors and allergic reactions in our study

underwent some kind of desensitization therapy, four of these with a reported successful or partly successful outcome. Desensitization regimens have however not been the focus of the B-NORD study and therefore no further details can be provided. Accordingly, allergic reactions to FIX complicate an ITI but they are not a definite predictor of failure. The same reasoning applies to the development of nephrotic syndrome. Out of the three PwHB who developed nephrotic syndrome, one was considered tolerant at enrollment. In our, as well as in other published cohorts, however, the combination of a high-titer inhibitor together with the occurrence of both anaphylaxis and nephrotic syndrome seems to be associated with a poor prognosis for achieving tolerance. Importantly, although anaphylaxis and nephrotic syndrome predominantly occurred in patients with null variants, they were also seen in one patient with a missense substitution.

The median factor consumption of 6638 IU/kg/year for the tolerized inhibitor patients in our cohort is significantly higher than that reported for the non-inhibitor patients and well above 4000 IU/kg/year, the level of high-dose prophylaxis, defined by the WFH [2]. This raises the question as to whether the high consumption reported may actually indicate an unfavorable pharmacokinetic profile due to non-neutralizing and/or small amounts of neutralizing antibodies not detectable with the Nijmegen-Bethesda method. However, we did not find any evidence for this when using both the ELISA and xFLI anti-FIX methods. The concordance obtained between the ELISA and xFLI assays was high, but we observed some discrepancies, mainly explained by a lack of reproducibility of the ELISA assay in the low-titer range. The cut-off used in each ELISA test-run is variable, since it is dependent on the normal samples run in each test. The high coefficient of variation (CV) (>50 %) for the positive control in the ELISA assay reflects this issue and indicates the need for further validation of this assay or replacement with the xFLI assay.

4.1. Strengths and limitations

Besides the relatively low number of inhibitor patients, which is a concern in all studies of PwHB, the retrospective study design with the extraction of data from medical records brings further limitations. A key limitation is also the lack of consistent criteria for ITI success in HB. The strengths of the study include the still relatively large study population of PwHB with carefully defined *F9* variants genotyped enrolled at HTCs with a close collaboration and the common Nordic treatment guidelines [36]. In addition, we have evaluated the presence of all types of antibodies using both the Nijmegen-Bethesda assay and two different immunoassays.

5. Conclusions

Our study reveals a high proportion of severe *F9* gene defects among persons with severe HB in the Nordic countries and a relatively high frequency of inhibitors, but no evidence of NNA. Our data also indicate that ITI success can be attained in PwHB despite previous ITI failures independent of the type of *F9* variant and that the addition of immunosuppression to the regimen may enhance the chances of success. Furthermore, our study supports the findings that allergic reactions as well as the development of nephrotic syndrome complicate the clinical management, but do not necessarily correlate with specific *F9* null variants.

Disclosures

KK has received research grants from CSL Behring, Stockholm, Sweden. FB has received honoraria as a member of an advisory board and/or speaker from Sobi, Shire/Takeda, Novo Nordisk, Bayer, Roche, UniQure, Octapharma, BioMarin and Pfizer. MB was supported by funds from Stockholm County Council. EF has received honorarium as a speaker for Shire, Roche, Sobi and Takeda. PAH has acted as a paid consultant to Bayer, Shire, Novo Nordisk, Octapharma, CSL Behring, Pfizer and Sobi including lectures. RL has been a member of advisory boards for Sobi, CSL Behring, Takeda, BioMarin, Novo Nordisk, Pfizer, Roche and Bayer. VN has received research grant from CSL Behring, Stockholm, Sweden. SR has received research grants from the Childhood Cancer Foundation, PedNet and Stockholm County Council, is a member of steering committee for Roche and investigator in clinical trials promoted by Roche, Novo Nordisk and Sobi. KS has received speaker fees from Octapharma, Sobi, Shire and Novo Nordisk and has been scientific advisory board member for Novo Nordisk, Sobi and BioMarin. NGA has served as a speaker and/or on advisory boards for Bayer, CSL Behring, Octapharma and Sobi. EB has received research grants and paid consultancy from CSL Behring, Stockholm, Sweden. JA has received research grants from Sobi, CSL Behring, Takeda/Shire, and Bayer and speaker's fee and consultant's fee for Octapharma, NovoNordisk, Pfizer, Bayer, Sobi, CSL Behring, Takeda/Shire, BioMarin, Uniqure, and Spark Therapeutics. MM stated that she had no interests, which might be perceived as posing a conflict or bias.

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CRediT authorship contribution statement

JA, EB and KK designed the research study. KK analyzed the data. KK, JA and NGA interpreted the data and drafted the paper. KS and MM designed the NNA methods and drafted these parts of the paper. KK, JA, NGA, FB, MB, EF, PAH, RL, VN and SR enrolled patients and collected the clinical data. All authors reviewed the manuscript critically and read and approved the final version of the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: KK has received research grants from CSL Behring, Stockholm, Sweden.

FB has received honoraria as a member of an advisory board and/or speaker from Sobi, Shire/Takeda, Novo Nordisk, Bayer, Roche, UniQure, Octapharma, BioMarin and Pfizer.

MB was supported by funds from Stockholm County Council.

EF has received honorarium as a speaker for Shire, Roche, Sobi and Takeda.

PAH has acted as a paid consultant to Bayer, Shire, Novo Nordisk, Octapharma, CSL Behring, Pfizer and Sobi including lectures.

RL has been a member of advisory boards for Sobi, CSL Behring, Takeda, BioMarin, Novo Nordisk, Pfizer, Roche and Bayer.

VN has received research grant from CSL Behring, Stockholm, Sweden.

SR has received research grants from the Childhood Cancer Foundation, PedNet and Stockholm County Council, is a member of steering committee for Roche and investigator in clinical trials promoted by Roche, Novo Nordisk and Sobi.

KS has received speaker fees from Octapharma, Sobi, Shire and Novo Nordisk and has been scientific advisory board member for Novo Nordisk, Sobi and BioMarin.

NGA has served as a speaker and/or on advisory boards for Bayer, CSL Behring, Octapharma and Sobi.

EB has received research grants and paid consultancy from CSL Behring, Stockholm, Sweden.

JA has received research grants from Sobi, CSL Behring, Takeda/Shire, and Bayer and speaker's fee and consultant's fee for Octapharma, NovoNordisk, Pfizer, Bayer, Sobi, CSL Behring, Takeda/Shire, BioMarin, Uniqure, and Spark Therapeutics.

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References

[1] F. Peyvandi, I. Garagiola, G. Young, The past and future of haemophilia: diagnosis, treatments, and its complications, Lancet 388 (2016) 187–197, https://doi.org/10.1016/S0140-6736(15)01123-X.
[2] A. Srivastava, E. Santagostino, A. Dougall, S. Kitchen, M. Sutherland, S.W. Pipe, M. Carcao, J. Mahlangu, M.V. Ragni, J. Windyga, A. Llinás, N.J. Goddard, R. Mohan, P.M. Poonnoose, B.M. Feldman, S.Z. Lewis, H.M. van den Berg, G. F. Pierce, WFH guidelines for the management of hemophilia, 3rd edition, Haemophilia 26 (2020) 1–158, https://doi.org/10.1111/hae.14046.
[3] M. Chitlur, I. Warrier, M. Rajpurkar, J.M. Lusher, Inhibitors in factor IX deficiency a report of the ISTH-SSC international FIX inhibitor registry (1997–2006), Haemophilia 15 (2009) 1027–1031, https://doi.org/10.1111/j.1365-2516.2009.02039.x.
[4] C.P. Radic, L.C. Rossetti, M.M. Abelleyro, M. Candela, R.P. Bianco, M.de T. Pinto, I. B. Larripa, A. Goodeve, C.D.De Brasi, Assessment of the F9 genotype-specific FIX inhibitor risks and characterisation of 10 novel severe F9 defects in the first molecular series of Argentinian patients with haemophilia B, Thromb. Haemost. 109 (2013) 24–33, https://doi.org/10.1160/TH12-05-0302.
[5] G. Dolan, G. Benson, A. Duffy, C. Hermans, V. Jiménez-Yuste, T. Lambert, R. Ljung, M. Morfini, S.Zupančić Šalek, Haemophilia B: where are we now and what does the future hold? Blood Rev. 32 (2018) 52–60, https://doi.org/10.1016/j.blre.2017.08.007.
[6] D. Belvini, R. Salviato, P. Radossi, F. Pierobon, P. Mori, G. Castaldo, G. Tagariello, Molecular genotyping of the Italian cohort of patients with hemophilia B, Haematologica 90 (2005) 635–642.
[7] C. Male, N.G. Andersson, A. Rafowicz, R. Liesner, K. Kurnik, K. Fischer, H. Platokouki, E. Santagostino, H. Chambost, B. Nolan, C. Königs, G. Kenet, R. Ljung, M. van den Berg, Inhibitor incidence in an unselected cohort of previously untreated patients with severe haemophilia B: a PedNet study, Haematologica 106 (2021) 123–129, https://doi.org/10.3324/haematol.2019.239160.
[8] K. Beutel, H. Hauch, J. Rischewski, U. Kordes, J. Schneppenheim, R. Schneppenheim, ITI with high-dose FIX and combined immunosuppressive therapy in a patient with severe haemophilia B and inhibitor, Hamostaseologie 29 (2009) 155–157, https://doi.org/10.1055/s-0037-1617018.
[9] D. Klarmann, I. Martinez Saguer, M.B. Funk, R. Knoefler, N. Von Hentig, C. Heller, W. Kreuz, Immune tolerance induction with mycophenolate-mofetil in two children with haemophilia B and inhibitor, Haemophilia 14 (2008) 44–49, https://doi.org/10.1111/j.1365-2516.2007.01584.x.
[10] C. Freiburghaus, E. Berntorp, M. Ekman, M. Gunnarsson, B.M. Kjellberg, I. M. Nilsson, Tolerance induction using the Malmö treatment model 1982–1995, Haemophilia 5 (1999) 32–39, https://doi.org/10.1046/J.1365-2516.1999.00195.X.
[11] R. Ljung, G. Auerswald, G. Benson, G. Dolan, A. Duffy, C. Hermans, V. Jiménez-Yuste, T. Lambert, M. Morfini, S. Zupančić-Šalek, E. Santagostino, Inhibitors in haemophilia a and B: management of bleeds, inhibitor eradication and strategies for difficult-to-treat patients, Eur. J. Haematol. 102 (2019) 111–122, https://doi.org/10.1111/EJH.13193/FORMAT/PDF.
[12] D.M. DiMichele, B.L. Kroner, S. Adair, J. Addiego, V. Anderson, J. Barbosa, P. Blatt, P. Bockenstedt, V. Castle, P. Chenaille, J. Cohen, E. Czapek, J. Davis, G. Davignon, P. De Alarcon, R. Dubowy, J. DuCore, M. Dugdale, B. Ewenstein, J. Fahner, T. J. Gribble, D. Gnarra, R. Gruppo, N. Hakami, M. Hanna, W. Hanna, P. Haut, G. Heggie, A. Homans, J. Hutter, C. Johnson, M. Kajani, M. Karpatkin, C. Kasper, J. Katz, C. Kessler, N. Key, M. Koerper, B. Konkle, R. Kosinski, J. Lazerson, A. Lightsey, T. Loew, J. Lusher, W. MacLaughlin, M. Manco-Johnson, C. Manno, K. McRedmond, P. Phatak, J. Powell, M. Ragni, C. Rosenfield, J. Sanders, P. Santiago-Borrero, P. Saidi, R. Seeler, S. Seremetis, J. Sexauer, S. Travis, L. Valdez, E. Warner, G. White, D. Barrand, J. Drown, J. Herst, M. Inwood, D. Lillicrap, G. Rivard, S. Rubin, H. Stranczyniski, J. Teitel, The North American Immune Tolerance Registry: practices, outcomes, outcome predictors, Thromb. Haemost. 87 (2002) 52–57, https://doi.org/10.1055/s-0037-1612943.
[13] A. Abdi, M.R. Bordbar, S. Hassan, F.R. Rosendaal, J.G. Van Der Bom, J. Voorberg, K. Fijnvandraat, S.C. Gouw, Prevalence and incidence of non-neutralizing antibodies in congenital hemophilia a—a systematic review and meta-analysis, Front. Immunol. 11 (2020) 563, https://doi.org/10.3389/FIMMU.2020.00563.
[14] A. Cannavò, C. Valsecchi, I. Garagiola, R. Palla, P.M. Mannucci, F.R. Rosendaal, F. Peyvandi, Nonneutralizing antibodies against factor VIII and risk of inhibitor development in severe hemophilia A, Blood 129 (2017) 1245–1250, https://doi.org/10.1182/BLOOD-2016-06-720086.
[15] C.J. Hofbauer, S. Kepa, M. Schemper, P. Quehenberger, S. Reitter-Pfoertner, C. Mannhalter, B.M. Reipert, I. Pabinger, FVIII-binding IgG modulates FVIII half-life in patients with severe and moderate hemophilia a without inhibitors, Blood 128 (2016) 293–296, https://doi.org/10.1182/blood-2015-10-675512.
[16] B. Reipert, B. Gangadharan, C. Hofbauer, M. van den Berg, H. Schweiger, J. Bowen, J. Blatny, K. Fijnvandraat, E. Mullins, J. Klintman, C. Male, C. McGuinn, S. Meeks, V. Radulescu, M. Ragni, M. Recht, A. Shapiro, J. Staber, H. Yaish, E. Santagostino, D. Brown, The prospective hemophilia inhibitor PUP study reveals distinct antibody signatures prior to FVIII inhibitor development, Blood Adv. 4 (2020) 5785–5796, https://doi.org/10.1182/BLOODADVANCES.2020002731.
[17] B. Boylan, A. Rice, A. Neff, M. Manco-Johnson, C. Kempton, C. Miller, Survey of the anti-factor IX immunoglobulin profiles in patients with hemophilia B using a fluorescence-based immunoassay, J. Thromb. Haemost. 14 (2016) 1931–1940, https://doi.org/10.1111/JTH.13438.
[18] K. Kihlberg, F. Baghaei, M. Bruzelius, E. Funding, P. Andre, S. Ranta, Mehdi Osooli, Erik Berntorp, J. Astermark, Treatment outcomes in persons with severe haemophilia B in the Nordic region: the B-NORD study, Haemophilia 27 (2021) 366–374, https://doi.org/10.1111/hae.14299.
[19] C. Miller, S. Platt, A. Rice, F. Kelly, J. Soucie, Validation of Nijmegen-Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance, J. Thromb. Haemost. 10 (2012) 1055–1061, https://doi.org/10.1111/J.1538-7836.2012.04705.X.
[20] I.M. Nilsson, U. Hedner, Immunosuppressive treatment in haemophiliacs with inhibitors to factor VIII and factor IX, Scand. J. Haematol. 16 (1976) 369–382, https://doi.org/10.1111/j.1600-0609.1976.tb00330.x.
[21] P.M. Green, D.R. Bentley, R.S. Mibashan1, I.M. Nilsson2, F. Giannelli, Molecular pathology of haemophilia B, EMBO J. 8 (1989) 1067–1072.
[22] A. Mårtensson, A. Letelier, C. Halldén, R. Ljung, Mutation analysis of Swedish haemophilia B families - high frequency of unique mutations, Haemophilia 22 (2016) 440–445, https://doi.org/10.1111/hae.12854.
[23] C. Kopanos, V. Tsiolkas, A. Kouris, C.E. Chapple, M.A. Aguilera, R. Meyer, A. Massouras, VarSome: the human genomic variant search engine, Bioinformatics 35 (2019) 1978–1980.
[24] P.M. Rallapalli, G. Kemball-Cook, E.G. Tuddenham, K. Gomez, S.J. Perkins, An interactive mutation database for human coagulation factor IX provides novel insights into the phenotypes and genetics of hemophilia B, J. Thromb. Haemost. 11 (2013) 1329–1340, https://doi.org/10.1111/jth.12276.
[25] Factor IX Gene Variant Database. http://www.factorix.org/, (n.d.). http://www.factorix.org/ (accessed March 5, 2021).
[26] K. Knobe, E. Sjörin, L. Tengborn, P. Petrini, R. Ljung, Inhibitors in the Swedish population with severe haemophilia A and B: a 20-year survey, Acta Paediatr. 91 (2002) 910–914, https://doi.org/10.1080/080352502760148621.
[27] J. Astermark, K. Holstein, Y.L. Abajas, S. Kearney, S.E. Croteau, R. Liesner, E. Funding, C.L. Kempton, S. Acharya, S. Lethagen, P. LeBeau, J. Bowen, E. Berntorp, A.D. Shapiro, The B-Natural study—the outcome of immune tolerance induction therapy in patients with severe haemophilia B, Haemophilia 27 (2021) 802–813, https://doi.org/10.1111/hae.14357.
[28] E. Berntorp, J. Astermark, E. Carlborg, Immune tolerance induction and the treatment of hemophilia.Malmö protocol update, Haematologica 85 (2000) 48–51.
[29] C. Barnes, A. Davis, J. Furmedge, B. Egan, L. Donnan, P. Monagle, Induction of immune tolerance using rituximab in a child with severe haemophilia B with inhibitors and anaphylaxis to factor IX, Haemophilia 16 (2010) 840–841, https://doi.org/10.1111/j.1365-2516.2007.01446.x.
[30] D.C.A. Cross, H.M. van den Berg, Cyclosporin a can achieve immune tolerance in a patient with severe haemophilia B and refractory inhibitors, Haemophilia 13 (2007) 111–114, https://doi.org/10.1111/j.1365-2516.2006.01411.x.
[31] K. Holstein, R. Schneppenheim, J. Schrum, C. Bokemeyer, F. Langer, Successful second ITI with factor IX and combined immunosuppressive therapy: a patient with severe haemophilia B and recurrence of a factor IX inhibitor, Hamostaseologie 34 (2014) S5–S8, https://doi.org/10.5482/HAMO-14-01-0010.
[32] R. Kobayashi, H. Sano, D. Suzuki, K. Kishimoto, K. Yasuda, R. Honjo, M. Hirose, S. Fujita, S. Abe, K. Kobayashi, Successful treatment of immune tolerance induction with rituximab in a patient with severe hemophilia B and inhibitor, Blood Coagul. Fibrinolysis 26 (2015) 580–582, https://doi.org/10.1097/MBC.0000000000000288.
[33] J. Kuhn, C. Noda, G.V. Massey, Successful multi-modal immune tolerance induction for factor IX deficiency with inhibitors and allergic reactions, Haemophilia 24 (2018) 133–136, https://doi.org/10.1111/hae.13457.
[34] A. Bon, M. Morfini, A. Dini, F. Mori, S. Barni, S. Gianluca, M. De Martino, E. Novembre, Desensitization and immune tolerance induction in children with severe factor IX deficiency; inhibitors and adverse reactions to replacement therapy: a case-report and literature review, Ital. J. Pediatr. 41 (2015), https://doi.org/10.1186/S13052-015-0116-8.
[35] J.R. Greenmyer, C.J. Grindeland, N.L. Kobrinsky, Eradication of factor IX neutralizing and anaphylactic inhibitors in a patient with severe haemophilia B using cyclophosphamide immune suppression and factor IX desensitization, Haemophilia 26 (2020) e51–e54, https://doi.org/10.1111/HAE.13926.
[36] Nordic Hemophilia Council, Nord. Hemoph. Counc. (n.d.). http://nordhemophilia.org/ (accessed January 26, 2022).