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ARTICLE

Relationship between factor VIII levels and bleeding for rFVIII-SingleChain in severe hemophilia A: A repeated time-to-event analysis

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Abstract

Publications on the exposure-effect relationships of factor concentrates for hemophilia treatment are limited, whereas such analyses give insight on treatment efficacy. Our objective was to examine the relationship between the dose, factor VIII (FVIII) levels and bleeding for rFVIII-SingleChain (lonoctocog alfa, Afstyla). Data from persons with severe hemophilia A on rFVIII-SingleChain prophylaxis from three clinical trials were combined. The published rFVIII-SingleChain population pharmacokinetic (PK) model was evaluated and expanded. The probability of bleeding was described with a parametric repeated time-to-event (RTTE) model. Data included 2080 bleeds, 2545 chromogenic stage assay, and 3052 one-stage assay FVIII levels from 241 persons (median age 19 years) followed for median 1090 days. The majority of the bleeds occurred in joints (65%) and the main bleeding reason was trauma (44%). The probability of bleeding decreased during follow-up and a FVIII level of 8.9 IU/dL (95% confidence interval: 6.9–10.9) decreased the bleeding hazard by 50% compared to a situation without FVIII in plasma. Variability in bleeding hazard between persons with similar FVIII levels was large, and the pre-study annual bleeding rate explained part of this variability. When a FVIII trough level of 1 or 3 IU/dL is targeted during prophylaxis, simulations predicted two (90% prediction interval [PI]: 0–17) or one (90% PI: 0–11) bleeds per year, respectively. In conclusion, the developed PK-RTTE model adequately described the relationship between dose, FVIII levels and bleeds for rFVIII-SingleChain. The obtained estimates were in agreement with those published for the FVIII concentrates BAY 81-8973 (octocog alfa) and BAY 94-9027 (damoctocog alfa pegol), indicating similar efficacy to reduce bleeding.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Publications on the exposure bleeding relation of the available factor concentrates for hemophilia treatment are sparse, whereas such analyses give insight on the efficacy of treatment.

WHAT QUESTION DID THIS STUDY ADDRESS?

In this study, the relation between the dose, factor VIII (FVIII) levels and bleeding for the FVIII concentrate rFVIII-SingleChain was evaluated in a large population of 241 persons with severe hemophilia A using a repeated time-to-event model.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The bleeding hazard declined during the study and no time-dependency between successive bleeds was observed. A FVIII level of 8.9 IU/dL decreased the bleeding hazard by 50% compared to a situation without FVIII. Persons with a higher annual bleeding rate pre-study had a higher bleeding hazard during the study. Annual bleeding rates were simulated when FVIII trough levels of 1, 3, 5, 10, and 20 IU/dL are maintained during prophylaxis.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The potency of rFVIII-SingleChain was similar to previously described potencies of the FVIII concentrates BAY 81-8973 and BAY 94-9027. This indicates that differences in annual bleeding rates for FVIII concentrates are driven by differences in achieved factor levels (pharmacokinetics) and similar target FVIII levels can be used.

INTRODUCTION

Persons with severe, and sometimes moderate, hemophilia A are prophylactically treated with factor VIII (FVIII) concentrates to prevent spontaneous bleeding and bleeding after minor trauma short term, and hemophilic arthropathy and invalidity long term. Several FVIII concentrates with varying pharmacokinetic (PK) properties are available for prophylactic treatment.[1] Lonoctocog alfa (Afstyla), in this report referred to as rFVIII-SingleChain, is a B-domain truncated recombinant FVIII concentrate, that was developed to have a higher binding affinity with von Willebrand factor (VWF), to prolongate elimination half-life in comparison to standard half-life recombinant FVIII concentrates.[2]

Traditionally, factor concentrates are dosed based on bodyweight. However, a large interindividual variability (IIV) in PKs of factor concentrates is observed.[3,4] To overcome this variability and optimize dosing regimens, it is recommended to perform PK-guided dosing.[5–8] When applied, population PK models are used to derive individual PK parameters, with which an adequate dosing regimen for an individual person is calculated. These dosing regimens are generally calculated to maintain coagulation factor levels above a certain trough target level, for instance 1 IU/dL and increasingly greater than 3 IU/dL.[9,10] Nonetheless, these factor level targets should be individually set according to bleeding tendency, weekly physical activities, presence of target joints, and other characteristics or circumstances, aiming for true personalization of treatment.[11–14] Data show that it is probably impossible to identify one factor trough target level for all persons with hemophilia, without excessive over- or underdosing of many. Not only do these individual differences and requirements make the selection of a target value difficult. Additionally, PK-pharmacodynamic (PD) relations between factor concentrates may vary and, accordingly, influence the relationship between factor levels and bleeding. Therefore, both PK and PD properties of factor concentrates should be considered when comparisons are made.

Together, this underlines the urgency to describe the relationships between factor levels and bleeding in persons with hemophilia for available factor concentrates in more detail. To date, publications on the exposure-effect relation of the available factor concentrates are sparse. Recently, a study was published in which factor concentrate exposure was associated with bleeding response in persons with severe hemophilia A treated with the FVIII concentrate octocog alfa (BAY 81-8973), using a repeated time-to-event (RTTE) model.[15] Furthermore, one abstract described a similar analysis for damoctocog alfa pegol (BAY 94-9027).[16] Performing these analyses for other

factor concentrates enables comparison of factor concentrates in terms of efficacy to reduce bleeding. Therefore, our objective was to construct a PK-RTTE model for rFVIII-SingleChain in order to relate dose, FVIII levels, and observed bleeding events, in this manner, describing the exposure-effect relation of rFVIII-SingleChain.

METHODS

Participants and data

Data from three clinical trials that evaluated the efficacy, safety, and PKs of rFVIII-SingleChain (Afstyla; CSL Behring GmbH), were used to examine the relationship between factor levels and bleeding of rFVIII-SingleChain in persons with severe hemophilia A. All persons with data on both PK and bleeding were analyzed for this report. Importantly, persons treated on demand, receiving treatment with factor concentrate in the event of bleeding or surgery, were excluded from our analysis.

The first clinical trial was a phase I–III study (CSL627_1001), which included persons greater than or equal to 12 years with severe hemophilia A (endogenous FVIII <1 IU/dL).[17] Participants were treated with 20–50 IU/kg every second day or two to three times per week, based on choice of the investigator. A second trial included children less than 12 years with severe hemophilia A (CSL627_3002). The children received 15–50 IU/kg rFVIII-SingleChain every second day or two to three times a week, at the investigator's discretion based on historical dosing and PK data.[18] The third clinical trial was a phase III open-label extension study which included persons with severe hemophilia A of all ages receiving rFVIII-SingleChain prophylaxis (CSL627_3001).[19] In this trial, the rFVIII-SingleChain dose was determined by the investigator based on individual PK characteristics, previous FVIII treatment, and bleeding phenotype. Persons included in multiple clinical studies were linked and analyzed as one person. The trials are registered on clinicaltrials.gov under #NCT01486927, #NCT02093897, and #NCT02172950. Ethical approval, approval by the relevant national authorities, and informed consent for every participant were obtained prior to enrollment.

FVIII levels were measured with chromogenic (CSA) and one-stage (OSA) assays. These analyses were conducted in CSL Behring's Central Laboratory in Marburg, Germany. The CSA was performed with the Coamatic test kit (Chromogenix) and used the Behring Coagulation System (Siemens Healthcare Diagnostics). The OSA was performed with the Pathromtin SL reagents. The lower limit of quantification (LLOQ) was 0.1 IU/dL for the OSA and 1 IU/dL for the CSA. VWF antigen (VWF:Ag) was determined with a commercially available test kit from Siemens Healthcare Diagnostics using the Behring Coagulation System (Siemens Healthcare Diagnostics).

Population PK model

The population PK model for rFVIII-SingleChain published by Zhang et al.[20] was used as starting point. This model was developed based on the FVIII levels from study CSL627_1001 and part of study CSL627_3002 measured by CSA, as the CSA is recommended for rFVIII-SingleChain.[21] The published population PK model is a two-compartment model with first-order elimination, including the following covariates: bodyweight on central volume of distribution, bodyweight on clearance (CL), and VWF:Ag on CL. During analysis, FVIII levels below the quantification limit were set to half of the LLOQ, as minimization problems occurred with the Laplacian estimation method when the M3 method was applied.[22] Initially, we evaluated if the published population PK model described the FVIII levels in our larger dataset adequately. As several FVIII levels before the first rFVIII-SingleChain dose were greater than 1 IU/dL, the model was extended with estimation of both an endogenous FVIII level and a residual FVIII level from a previous (unknown) dose. For persons with a predose level less than or equal to 1 IU/dL only the endogenous FVIII level was estimated. This endogenous FVIII level remained constant over time, whereas the residual FVIII level decayed by assuming first-order elimination based on the individually estimated elimination rate constant.

Second, as one-stage assay data were also available and no population PK model has been published in which one-stage assay data for rFVIII-SingleChain are included, the constructed model was expanded to also include the description of the one-stage assay FVIII levels. A correlation factor describing the difference between OSA and CSA FVIII levels was estimated. The residual error model was re-evaluated and separate residual errors were estimated for OSA and CSA data. Furthermore, inclusion of IIV on the residual error was examined.

Finally, inclusion of the covariates was re-evaluated. For persons with no VWF:Ag data, VWF:Ag was predicted using the age of the person. For this reason, a linear model between VWF:Ag and age was developed based on data from the persons where VWF:Ag was reported (Figure S1).

Repeated time-to-event model

The probability of bleeding was evaluated with an RTTE model. For this analysis, all reported bleeds in the described

clinical studies were used. An RTTE model describes the occurrence of repeated events, such as bleeds over time simultaneously with available predictors of events, such as FVIII levels, using parametric survival analysis.[15,23]

An RTTE model uses a hazard function to describe the instantaneous rate at which a bleed occurs. We evaluated exponential, Gompertz and Weibull hazard functions to describe the distribution of time to bleeding. IIV on the overall bleeding hazard was explored. At the end of the individual observation period, right censoring was applied. When the exact timing of bleeding was unknown and only the day of the bleed was documented, bleeds were interval-censored over that day.

The relationship between FVIII levels and the bleeding hazard was evaluated with linear, exponential, and maximum inhibitory (I_{max}) models. FVIII levels measured by CSA were used, as the CSA is recommended for rFVIII-SingleChain.[21] Furthermore, it was assessed if successive bleeds are dependent on time since the latest bleed using a Markov dependence hazard function in which the time was described as the time since previous bleed.[15]

The association between characteristics and the bleeding hazard was evaluated using a full fixed effects covariate approach.[24] Covariates were selected based on clinical interest and scientific plausibility. When high correlation (correlation coefficient >0.5) between covariates was present, one of these covariates was excluded. This resulted in inclusion of the following covariates: bodyweight, VWF:Ag, region, presence of hemophilic arthropathy, and pre-study annual bleeding rate. Continuous covariates without zero values were included using power models centered around the median covariate value, whereas the continuous covariates including zero values were included using a linear model centered around the median covariate value. Categorical covariates were modeled using index variables in which 0 or 1 was used to switch the effect of the category off or on. Inferences on the association between the characteristics and the bleeding hazard were made based on the magnitude and the precision of the effect size based on 1000 bootstrap samples.

Model development and evaluation

Models were developed using nonlinear mixed effects modeling in NONMEM (version 7.4.1; Icon Development Solutions).[25] The models were evaluated based on the scientific plausibility of the parameter estimates, the precision of the parameters, and the objective function value (OFV). The Akaike information criterion was used to compare and select non-nested models. The precision of the parameters was described by the 95% confidence interval (CI) based on 500 bootstrap samples. Furthermore, the adequacy of the RTTE model to describe the data was examined by a Kernel-based visual hazard comparison and by comparison of the observed and model-predicted Kaplan Meier curves.[26] For nested models, a drop of 6.64 in the OFV was considered significant ($p < 0.01$, 1 degree of freedom, Chi-square distribution). For data management, model management, and visualization, R version 4.1.1, Pirana version 2.9.9, and PsN version 5.2.6 were used.[27,28]

More details can be found in the Supplementary Materials.

RESULTS

Participants and data

Data from three clinical studies were combined. These studies included 74, 210, and 81 persons with hemophilia, respectively (Table 1). From studies CSL627_1001 and CSL627_3002, 124 persons enrolled to the CSL627_3001 extension study, resulting in a total of 241 unique participants. At baseline, the median age of the participants was 19 years (range: 1–58 years) and the median bodyweight was 60 kg (range: 10–120 kg). For the population PK model, 3052 FVIII levels measured by OSA and 2545 levels measured by CSA were available. Figure S2 depicts the difference between the FVIII levels measured by OSA and CSA. Of these FVIII levels, 6.9% were below the limit of quantification. A total of 2080 bleeds were recorded during the median observation period of 1090 days (range: 3–1909 days). The majority of these bleeds occurred in joints (65%), were spontaneous (43%) or trauma-related (44%), and treated with factor concentrate in 91% of cases.

Population PK model

The previously published population PK model based on CSA FVIII levels showed adequate predictive performance, but was improved by inclusion of estimation of the endogenous FVIII level and residual FVIII level of a previous dose ($p < 0.01$). Furthermore, IIV on residual error was added ($p < 0.01$). Additionally, the model was expanded to describe FVIII levels measured by the OSA and CSA. FVIII levels measured by OSA were estimated to be a factor 0.53 (95% CI: 0.51–0.54) lower than CSA FVIII levels. Subsequently, the effect of covariates on the PK parameters were re-estimated. In Table 2, the final estimates of the population PK model are presented. The visual predictive check in Figure S3 demonstrates adequate model performance.

TABLE 1 Participant characteristics, laboratory measurements, and bleeding events.

	Study number			
	CSL627_1001	CSL627_3001	CSL627_3002	Total[a]
Participant characteristics				
Participants (*n*)	74	210	81	241[a]
Follow-up time (days; median, range)	188 (53–749)	1001 (34–1429)	169 (29–345)	1090 (29–1910)
Age (years; median, range)	27 (12–58)	20 (2–58)	6 (1–11)	19 (1–58)
Bodyweight (kg; median, range)	69 (37–112)	61 (12–120)	25 (10–88)	60 (10–120)
Body mass index (kg/m^2; median, range)	22 (15–37)	21 (12–39)[b]	17 (12–30)	21 (12–39)[b]
VWF:Ag (IU/dL; median, range)	118 (58–296)	–[b]	84 (43–154)[b]	113 (43–296)[b]
Endogenous FVIII level (IU/dL; median, range)	<1 (100)	<1 (100)	<1 (100)	<1 (100)
Region (*n*, %)				
Europe	29 (39)	112 (53)	46 (57)	135 (56)
Japan	9 (12)	6 (3)	0 (0)	9 (4)
United States	10 (14)	20 (10)	4 (5)	22 (9)
Rest of the World[c]	26 (35)	72 (34)	31 (38)	75 (31)
Pharmacokinetic data				
FVIII dose per week (IU/kg; median, range)	82.6 (42.3–173.0)	92.9 (38.1–229.0)	84.6 (19.6–309.0)	91.6 (19.6–246.0)
FVIII levels OSA (*n*)	1358	1475	219	3052
FVIII levels CSA (*n*)	1282	1050	213	2545
FVIII levels per patient OSA (median, range)	13 (10–53)	5 (1–51)	6 (1–12)	10 (1–86)
FVIII levels per patient CSA (median, range)	13 (10–26)	3 (1–53)	6 (4–6)	8 (1–71)
Bleeding data				
Bleeds total (*n*, %)	166	1650	264	2080
Treated bleeds	150 (90)	1533 (93)	220 (83)	1903 (91)
Patients without bleeding (*n*, %)	26 (35)	39 (19)	17 (21)	40 (17)
No. bleeds per patient (median, range)	1 (0–11)	3 (0–153)	2 (0–21)	4 (0–153)
Pre-study ABR (median, range)	13 (0–96)	9.5 (0–168)	4 (0–74)	8 (0–168)
Presence of arthropathy (*n*, %)	47 (63)	101 (48)	10 (12)	112 (46)
ABR during study (median, range)	2.7 (0–41.4)	1.3 (0–48.5)	4.5 (0–28.9)	1.6 (0–48.5)
ABR treated bleeds during study (median, range)	1.7 (0–41.4)	1.2 (0–42.6)	3.6 (0–22.4)	1.5 (0–40.0)
Location and type of bleeds (*n*, %)				
Joint	127 (77)	1084 (66)	135 (51)	1346 (65)
Mucosal	3 (2)	44 (3)	17 (6)	64 (3)
Muscle	15 (9)	221 (14)	35 (13)	271 (13)
Other	21 (13)	297 (18)	77 (29)	395 (19)
Unknown	0 (0)	4 (0)	0 (0)	4 (0)
Cause of bleed (*n*, %)				
Spontaneous	106 (64)	684 (41)	94 (36)	884 (43)
Trauma-related	43 (26)	758 (46)	116 (44)	917 (44)
Post-surgery	0 (0)	10 (1)	0 (0)	10 (0)
Unknown	17 (10)	198 (12)	54 (20)	269 (13)

TABLE 1 (Continued)

Abbreviations: ABR, annual bleeding rate; CSA, chromogenic assay; FVIII, factor VIII; OSA, one-stage assay; VWF:Ag, von Willebrand factor antigen.

[a]Patients that continued after study CSL627_1001, CSL627_3002 in the extension study CSL627_3001 ($n = 124$) were analyzed as one patient.

[b]Body mass index was missing for two patients, VWF was not determined in study CSL627_3001 and missing in 70 patients in CSL627_3002. For patients that enrolled in CSL627_3001 from one of the other studies, the VWF level determined in the earlier study was used, leading to a missing VWF level for 154 patients in the total dataset.

[c]Rest of the world included: Australia, Canada, Lebanon, Malaysia, Philippines, Russian Federation, South Africa, and Ukraine.

The developed population PK model was used to estimate the individual FVIII over time curves. The median FVIII level at which bleeding occurred was 4.8 IU/dL (interquartile range [IQR]: 1.3–14.4 IU/dL). More specifically, during study CSL627_1001, CSL627_3001, and CSL627_3002 the median FVIII levels at which bleeding occurred were 6.7 (IQR: 1.4–17.4), 5.5 (IQR: 1.5–15.3), and 2.0 (IQR: 0.7–5.9) IU/dL, respectively.

Repeated time-to-event model

The time to bleeding was best described by a Weibull distribution and demonstrated a declining bleeding hazard over time. The relation between FVIII levels and the bleeding hazard was best described by an I_{max} model (dOFV = −429), validating a reduced bleeding hazard at increasing FVIII levels. Successive bleeds were not found to be dependent, as addition of a Markov element in the bleeding hazard did not statistically significantly improve the model. The bleeding hazard was described by Equation 1.

$$h_i(t) = \lambda\,\gamma\,(\lambda\,t)^{(\gamma-1)}\left(1 - \frac{\mathrm{FVIII}_i\,(t)}{\mathrm{FVIII}_i\,(t) + \mathrm{IC}_{50}}\right)e^{\eta i} \quad (1)$$

in which $h_i(t)$ describes the individual bleeding hazard at time t, λ the scale of the Weibull distribution, γ the shape of the Weibull distribution, $\mathrm{FVIII}_i\,(t)$ the FVIII level at time t, IC_{50} the FVIII level at which 50% of the inhibitory effect on the bleeding hazard occurs, and η_i is a random effect describing the IIV in bleeding hazard, with mean 0 and estimated variance ω^2.

The parameters of the RTTE model are described in Table 3. The scale ($6.37\,\mathrm{year}^{-1}$) describes the bleeding hazard at a FVIII level of 0 IU/dL in the beginning of the follow-up period, whereas the shape (0.78) describes a declining bleeding hazard over time. The IC_{50} value depicts the potency of FVIII to lower the bleeding hazard, and demonstrates that a constant FVIII level of 8.9 IU/dL lowers the bleeding hazard by 50% compared to a situation with no FVIII present. A visual representation of the estimates of the RTTE model can be found in Figure 1. After 1 year follow-up, a bleeding hazard of 3.3 bleeds per year is estimated when no FVIII is present in the plasma. When a person has a constant FVIII level of 1 IU/dL, a bleeding hazard of 3.0 bleeds per year is estimated. This indicates that at a constant FVIII level of 1 IU/dL, a median annualized bleeding rate of 3.0 (95% CI: 2.0–4.1) would be expected. At a constant FVIII level of 20 IU/dL, a median annualized bleeding rate of 1.0 (95% CI: 0.6–1.6) would be observed. Figure 2 presents the relationship among FVIII levels, the bleeding hazard, and the probability to be bleed-free for two illustrative individual persons from the dataset. Moreover, the model described the data adequately as demonstrated by the overlapping simulated and observed Kaplan Meier curves (Figure S4) and the Kernel-based visual hazard comparison (Figure S5).[26]

The effect of covariates on the bleeding hazard was evaluated with a full covariate model. During this analysis, a pre-study annual bleeding rate greater than 100 ($n = 11$) was regarded as clinically improbable as a person would experience a bleed almost every third day (data described in Figure S6). Therefore, a separate slope was estimated for a pre-study annual bleeding rate less than or equal to 100 and greater than 100. The slope of less than or equal to 100 bleeds was regarded to reflect the effect of pre-study annual bleeding rate on the bleeding hazard. Results demonstrated that subjects with a higher pre-study annual bleeding rate (≤100) experienced a higher bleeding hazard during study (Figure 3). Alterations in bodyweight, VWF, presence of arthropathy, and treatment region (Europe, United States, Japan, or Rest of the world) did not have a clinically important effect on the bleeding hazard.

Simulations

The relationship between FVIII levels and bleeding in the previously discussed Figure 1b is based on constant FVIII levels and depicts this relationship for a median person. Simulations were performed to evaluate the effect of fluctuating FVIII levels during prophylactic treatment on the annual bleeding rate and to examine the effect of IIV in the bleeding hazard. A virtual set of 1000 persons with a bodyweight of 70 kg with typical PK parameters was used. Administration of 1500, 5500, 9500, 19,500, and 39,500 IU every 72 h resulted in maintaining FVIII trough levels of 1, 3, 5, 10, and 20 IU/dL, respectively.

TABLE 2 Final estimates of the expanded population pharmacokinetic model for rFVIII-SingleChain.

Parameter	Estimate	95% CI
PK: Structural model		
Clearance (CL, dL/h)	2.08	1.99–2.18
Central volume of distribution (V1, dL)	34.4	33.3–35.4
Intercompartmental clearance (Q, dL/h)	1.53	0.96–2.43
Peripheral volume of distribution (V2, dL)	2.40	1.71–3.27
Endogenous FVIII level (IU/dL)	0.27	0.23–0.31
Residual FVIII level previous dose (IU/dL)[a]	1.41	0.69–2.57
OSA instead of CSA[b]	0.53	0.51–0.54
PK: Covariates		
Bodyweight on V1	0.84	0.79–0.89
Bodyweight on CL	0.70	0.61–0.79
VWF on CL	−0.65	−0.83 to −0.49
PK: Interindividual variability (IIV)		
Clearance (CV%)	25.1	19.5–30.2
Central volume of distribution (CV%)	15.5	13.2–17.6
Endogenous FVIII level (CV%)	165.6	136.7–204.8
Residual FVIII level previous dose (CV%)	596.6	229.8–2208.4
Residual error (CV%)	36.8	32.2–41.1
PK: Residual variability		
Proportional error CSA (CV%)	7.17	4.8–9.5
Scale proportional error OSA (CV%)	41.6	36.6–47.6
Exponent proportional error OSA	0.73	0.70–0.76
Correlation proportional error OSA CSA	0.0298	0.019–0.0419
Additive error CSA (IU/dL)	0.55	0.41–0.77
Proportional error multiple measurements same sample OSA	13.3	11.5–15.9
Proportional error multiple measurements same sample CSA	21.4	18.8–24.1

Abbreviations: CI, confidence-interval from bootstrap ($n = 500$); CSA, chromogenic assay; CV, coefficient of variation calculated as $\sqrt{e^{\omega^2} - 1}$; OSA, one-stage assay; PK, pharmacokinetic; VWF, von Willebrand factor antigen; WT, bodyweight.Typical values are obtained for a patient with a bodyweight of 70 kg and Von Willebrand factor level antigen of 113%. Typical PK parameters are described as follows: CL = 2.08 * (WT/68)**0.70 * (VWF/113)**−0.65 and V1 = 34.4 * (WT/68)**0.84. The error structure for FVIII levels measured by OSA is described by $Y = \text{IPRED} + (\text{IPRED}^{0.73} * \text{EPS(prop)} + \text{IPRED} * \text{EPS(multiple)}) * \text{EXP(ETA(residual error))}$. The error structure for FVIII levels measured by CSA is described by: $Y = \text{IPRED} + ((\text{IPRED} * \text{EPS(prop)} + \text{IPRED} * \text{EPS(multiple)}) + \text{EPS(additive)}) * \text{EXP(ETA(residual error))}$.

[a]Estimated for subjects with endogenous FVIII level >1 IU/dL.

[b]FVIII level measured by OSA = FVIII level measured by CSA * 0.53. η-shrinkage varied between 10% (IIV V1) and 55% (IIV residual FVIII level) and ε-shrinkage was around 2.8%.

TABLE 3 Final estimates of RTTE model.

Parameter	Estimate	RSE (%) [shrinkage]
Scale, λ ($year^{-1}$)	6.37	14.3
IC_{50} (IU/dL)	8.90	11.7
Shape, γ	0.78	2.2
IIV hazard (CV%)	330.8	6.7 [10.6]

Abbreviations: CV, coefficient of variation calculated as $\sqrt{e^{\omega^2} - 1}$; IC_{50}, Factor VIII level resulting in 50% of reduction of the bleeding hazard without FVIII; Scale, the scale of the bleeding hazard at a factor VIII (FVIII) level of 0 IU/dL; IIV, interindividual variability; RSE, relative standard error; Shape, the shape factor indicates a decreasing bleeding hazard over time described by a Weibull distribution.

In Figure 4, the simulated annual bleeding rates when maintaining FVIII trough levels during prophylactic treatment are presented, including the percentage of patients that experienced zero bleeds. This figure illustrates that a median person experiences two bleeds per year (90% PI: 0–17 bleeds per year) when a trough FVIII level of 1 IU/dL is maintained during prophylaxis. The median annual bleeding rate is lowered to 1.0 bleeds when a trough level of 3, 5, or 10 IU/dL is maintained. Importantly, the 80% PI decreases at a maintained FVIII trough level of 10 IU/dL, indicating few to no bleeds in most patients when this FVIII level is maintained.

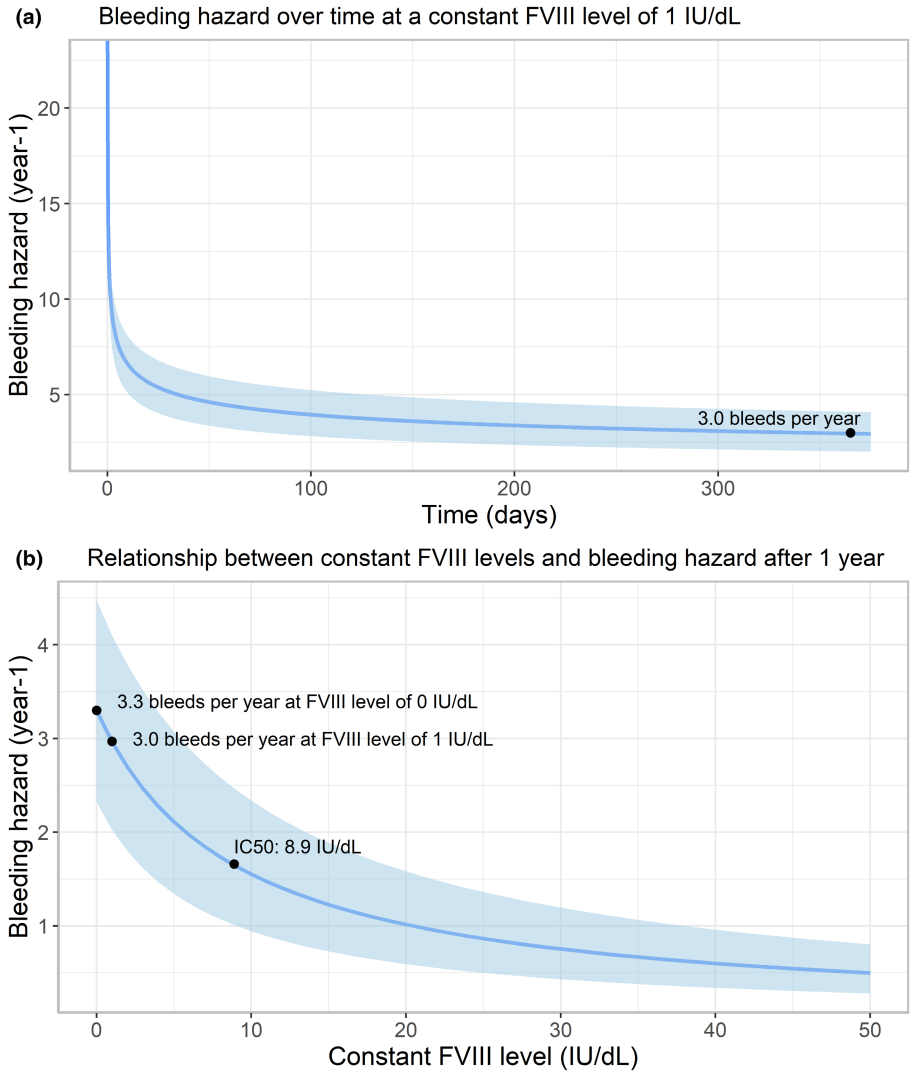

FIGURE 1 Visual representation of the final estimates of the repeated-time to event (RTTE) model. (a) Bleeding hazard over time is presented, in which the solid line depicts the estimated bleeding hazard over time for a constant factor VIII (FVIII) level of 1 IU/dL, and the shaded area the 95% confidence interval. The bleeding hazard is described by a Weibull function, which demonstrates a decreasing bleeding hazard over time. After 1 year follow-up, the estimated bleeding hazard is three bleeds per year, which corresponds to an annual bleeding rate of three bleeds when a patient has a constant FVIII level of 1 IU/dL. (b) The relation between constant FVIII levels and bleeding hazard after 1 year follow-up in the study. The IC_{50} demonstrates that at a constant FVIII level of 8.9 IU/dL the bleeding hazard is lowered by 50% compared to when no FVIII is present.

DISCUSSION

To our knowledge, we present the first study quantifying the exposure-effect relation of rFVIII-SingleChain. An RTTE model was developed that described the relation among the dose, FVIII levels, and bleeding for rFVIII-SingleChain. The bleeding hazard declined during the study and bleeding did not affect timing of a subsequent bleed. A constant FVIII level of 8.9 IU/dL decreased the bleeding hazard by 50%. Furthermore, annual bleeding rates when targeting FVIII trough levels of 1, 3, 5, 10, and 20 IU/dL during prophylactic treatment were simulated, resulting in a median of two bleeds per year (90% PI: 0–17) when an FVIII trough level of 1 IU/dL is maintained, a median of one bleed per year (90% PI: 0–11) when an FVIII level of 3 IU/dL is maintained, and a median of one bleed per year (90% PI: 0–8) when an FVIII level of 5 IU/dL is maintained.

Explanation findings

The previously published population PK model was optimized and expanded to also enable description of OSA FVIII levels. FVIII levels measured by OSA were estimated to be a factor 0.53 lower than FVIII levels

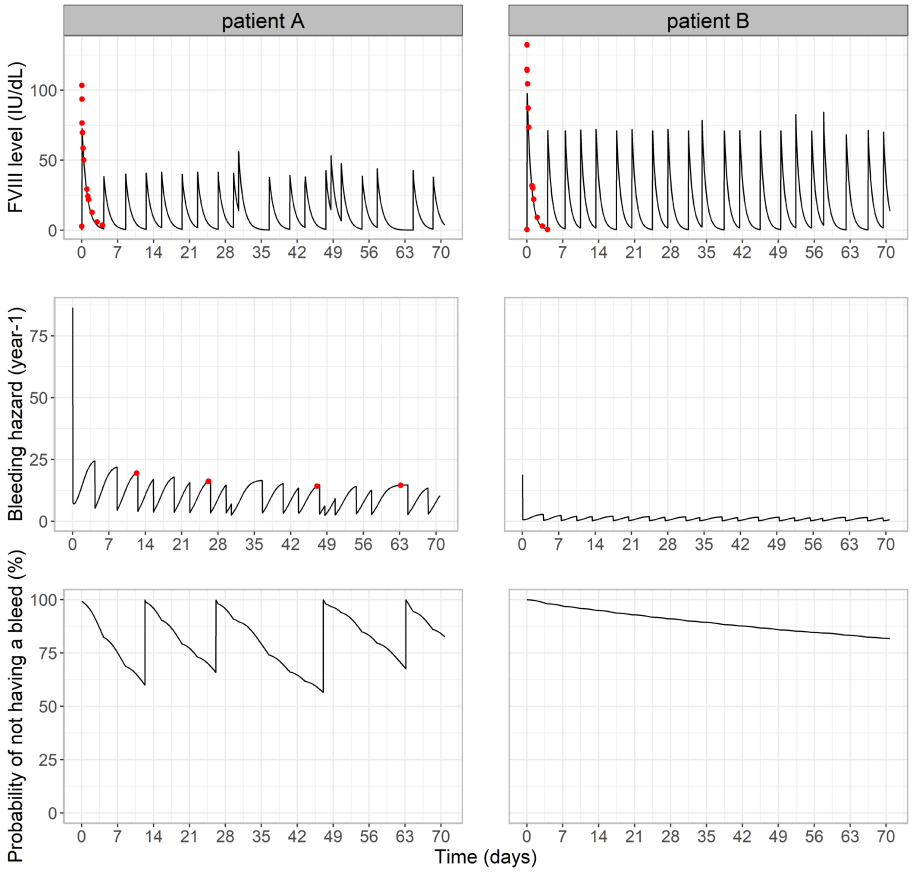

FIGURE 2 Factor VIII (FVIII) levels, bleeding hazard, and probability of not having a bleed for two illustrative patients from the dataset. Patient A (38 kg, 13 years old, administrated 1000 IU two times per week) experienced 4.5 bleeds per year during the study, whereas patient B (60 kg, 19 years, treated with 2000 IU two times per week) experienced zero bleeds per year. The top panels describe the model predicted FVIII levels based on PK observations (red dots). The middle panels show the model predicted bleeding hazard including bleeding observations (red dots). These plots demonstrate an inverse correlation between bleeding hazard and FVIII levels. The bottom panels show that the survival probability (probability of not having a bleed) declines faster with a higher bleeding hazard and returns to 100% after a bleed occurs. PK, pharmacokinetic.

measured by CSA, which corresponds to OSA and CSA differences of around 50% described in earlier studies.[29] Importantly, FVIII levels of rFVIII-SingleChain measured by OSA underestimate the FVIII activity, therefore the CSA is recommended for rFVIIII-SingleChain monitoring.[21]

The observed decreasing bleeding hazard in the RTTE model suggests a higher bleeding risk at the start of the study than after a year of follow-up. This may be explained by the fact that participants were treated differently before the study. Unfortunately, no information was available on specifications of previous treatment and previous treatment intensity. However, from clinical trial publications we derived that, respectively, 60% and 17% of participants in CSL627_1001 and CSL627_3002 were treated on demand before study inclusion.[17,18] Possibly at study initiation, a higher bleeding hazard was still present due to less effective prior treatment.

In our analysis, we did not observe a time-dependency between successive bleeds. This demonstrates that the probability of a successive bleed is not increased or decreased directly after a bleed in this dataset. In clinical practice, on one hand, this means that persons are not more careful after a bleed occurred preventing a subsequent bleed. On the other hand, occurrence of a bleed does not increase the risk on a successive bleed, which contrasts with clinical experience. In agreement with our results, a similar study examining another FVIII concentrate did not observe a time-dependency between successive bleeds.[15] Importantly, reporting bias could have influenced this observation, as some recurrent bleeds might not have been reported as they were regarded as the same bleed.

During covariate analysis, a relationship between the pre-study annual bleeding rate and the bleeding hazard during study was observed. This observation may reflect the individual bleeding tendency which remains similar for an individual before and during the study, also when

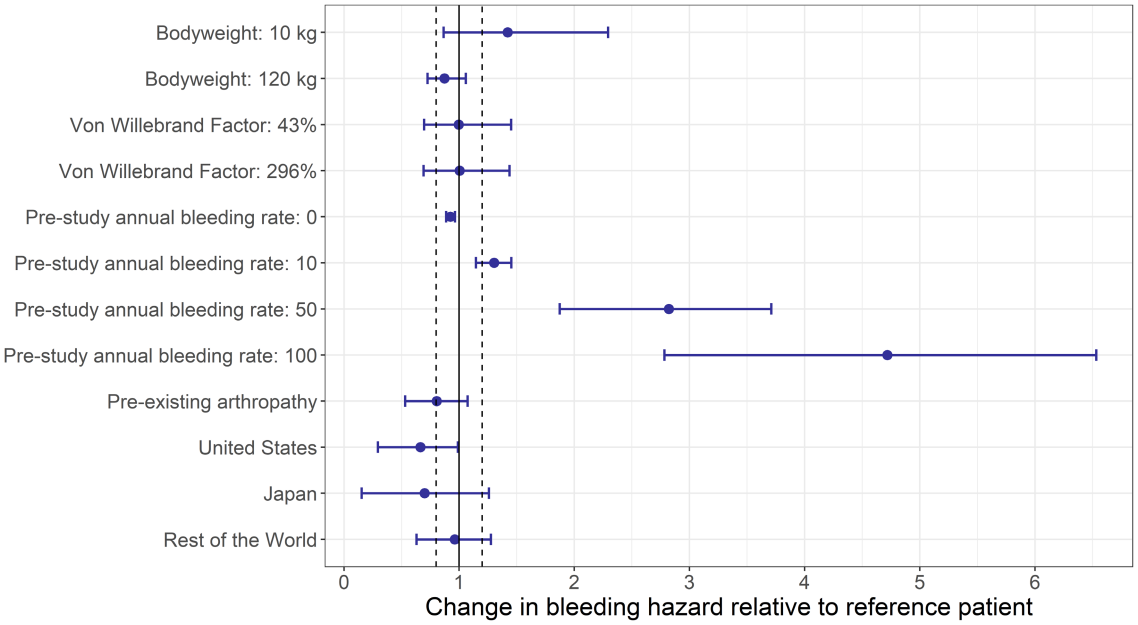

FIGURE 3 Covariate effects on the bleeding hazard. The change in bleeding hazard of various values of covariates on the bleeding hazard compared to the reference subject are presented with a point estimate and 95% confidence interval. The minimal, maximal covariate values are shown, as well as arbitrary selected values for illustration. The reference subject is 60 kg, has VWF level of 113%, a pre-study annual bleeding rate of eight, has no pre-existing hemophilic arthropathy and was treated in Europe. An effect greater than ±20% was regarded as clinically important (dotted lines). This plot demonstrates that alterations in pre-study annual bleeding rate has a clinically important effect on the bleeding hazard, whereas this is not the case for the other examined covariates. VWF, von Willebrand factor.

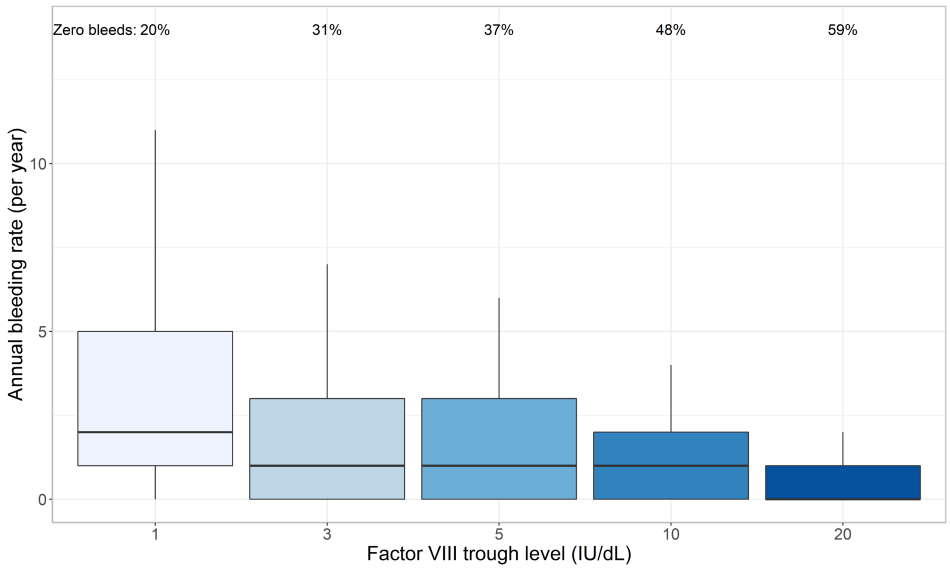

FIGURE 4 Simulated annualized bleeding rates when various factor VIII (FVIII) trough levels are maintained during prophylaxis. Virtual patients ($n = 1000$) of 70 kg were assumed to have typical PK parameters. Dosing with 21, 79, 136, 279, and 564 IU/kg every 72 h resulted in maintaining FVIII trough levels of 1, 3, 5, 10, and 20 IU/dL, respectively. Whiskers of the boxplot range until the 80% prediction interval. The percentage of persons with zero bleeds is presented on top. When an FVIII trough level of 3, 5 or 10 IU/dL is maintained during prophylaxis a median annualized bleeding rate of 1.0 is observed. However, the percentage of persons with zero bleeds decreases with higher targeted FVIII trough levels. Importantly, these simulations evaluate the effect of fluctuating FVIII levels during prophylaxis, in comparison to Figure 1, which presents results for constant FVIII levels. PK, pharmacokinetic.

the treatment is changed. Another study also observed a relationship between the pre-study annual bleeding rate and the bleeding hazard, strengthening this observation.[15] During this analysis, we excluded annual bleeding rate greater than 100, as, in our opinion, these high values do not reflect clinical practice. Unexpectedly, we did not observe a relationship between the presence of hemophilic arthropathy and the bleeding hazard.[30] However, there was some correlation between the pre-study annual bleeding rate and the presence of arthropathy (Figure S7). Both covariates were introduced in the full covariate model at the same time, indicating that presence of hemophilic arthropathy did not have an additional effect besides what was described by the pre-study annual bleeding rate.

Comparison to other studies

Abrantes et al. examined the relation between FVIII levels and bleeding for the FVIII concentrate BAY 81-8973 (octocog alfa) in severe hemophilia using an RTTE model.[15] Their study observed a bleeding hazard of 3.0 $year^{-1}$ without the presence of FVIII after 1 year follow-up and an IC_{50} value of 10.2 IU/dL. Another study of Jentsch et al., evaluated the relation between FVIII levels and bleeding for the FVIII concentrate BAY 94-9027 (damoctocog alfa pegol), resulting in a IC_{50} value of 8.15 IU/dL.[16] The obtained estimates for BAY 81-8973 and BAY 94-9027 are similar to the estimates observed for rFVIII-SingleChain, as we obtained a bleeding hazard of 3.3 $year^{-1}$ without FVIII after 1 year follow-up and an IC_{50} of 8.9 IU/dL (95% CI: 6.9–10.9).

Study strengths and limitations

Strengths of the study include the size of the dataset, as 2080 bleeds from 241 persons with hemophilia A were analyzed. Furthermore, the development of the RTTE model enabled evaluation of the occurrence of the bleeding over time together with the time-varying FVIII levels. This method is superior over a standard regression approach.[16]

Limitations of the study include the fact that bleeds were mostly self-reported by participants. Therefore, some persons might have under- or over-reported the actual number of bleeds. Moreover, almost half of the participants had pre-existing arthropathy and a high number of bleeds occurred spontaneously (43%). Therefore, our population also included persons with damaged joints, which should be taken into account when interpreting the data. Unfortunately, we did not have data on some interesting covariates, such as: type of treatment before study inclusion, number of target joints, age of prophylaxis start, and sports participation, whereas these factors are known to impact the bleeding hazard.

CONCLUSIONS, CLINICAL IMPLICATIONS, AND FUTURE RESEARCH

In conclusion, the developed population PK and RTTE model described the relation among dose, FVIII levels, and bleeds for rFVIII-SingleChain adequately. The simulations performed give insights into which FVIII trough levels may be needed to prevent bleeding in clinical practice. However, the IIV in the bleeding hazard (coefficient of variation: 331%) was notably high, demonstrating a highly variable bleeding tendency for persons with severe hemophilia at similar FVIII levels. This indicates that tailoring the dose based on both the individual PK and the individual bleeding risk can have additional value over using solely individual PK parameters, as demonstrated by Abrantes et al.[31] Interestingly, the developed RTTE model for rFVIII-SingleChain can be used to support dose tailoring for an individual based on both individual PK and the individual bleeding risk. Herewith, the idea that FVIII targets should be set based on bleeding tendency can be implemented objectively. Although external validation of the RTTE model with independent data is recommended first.

The observed IC_{50} value for rFVIII-SingleChain was similar to IC_{50} values of BAY 81-8973, BAY 94-9027 in literature. These almost identical estimates suggest a similar exposure-effect relation for FVIII concentrates. Therefore, differences in annual bleeding rates for FVIII concentrates are driven by differences in achieved factor levels (PK) and similar target FVIII levels can be used. In future studies, the exposure-effect relation of other factor concentrates should be evaluated, as, for instance, factor IX extended half-life concentrates might have different exposure-effect relations.

AUTHOR CONTRIBUTIONS

L.H.B. wrote the manuscript. L.H.B., S.J., M.O.K., M.H.C., and R.A.A.M. designed the research. All authors performed the research. L.H.B., S.J., M.O.K., and R.A.A.M. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

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

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