

Relationship between factor VIII activity, bleeds and individual characteristics in severe hemophilia A patients

João A. Abrantes,¹ Alexander Solms,² Dirk Garmann,³ Elisabet I. Nielsen,¹ Siv Jönsson¹ and Mats O. Karlsson¹

¹Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden; ²Bayer, Berlin, Germany and ³Bayer, Wuppertal, Germany

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Correspondence: *MATS O. KARLSSON* - mats.karlsson@farmbio.uu.se

Online Supplementary Appendix

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João A. Abrantes, Alexander Solms, Dirk Garmann, Elisabet I. Nielsen, Siv Jönsson and Mats O. Karlsson

Correspondence: Mats O. Karlsson, Department of Pharmaceutical Biosciences, Uppsala University, Box 591, SE-75124, Uppsala, Sweden. e-mail:

mats.karlsson@farmbio.uu.se

Methods

Model development

Model estimation was performed using non-linear mixed effects modelling (also called population modelling) in NONMEM® 7.4.3 (1). This approach uses the data from all individuals to simultaneously estimate the typical parameters (fixed effects) and the stochastic parameters describing variability (random effects) (2).

The model describing the pharmacokinetics (PK) of factor VIII (FVIII) activity, time-to-bleed and bleeding severity, covariates, and the correlations between all components, consisted of three components which were integrated in a step-wise way. Firstly, the population PK model was developed including only PK data. Secondly, the repeated time-to-categorical event (RTTCE) model was developed based on the time-to-bleed data and severity scores. At this stage, the RTTCE component was guided by the individual PK predictions using the individual parameter estimates produced in the previous step, i.e. there was no re-estimation of the PK model. Thirdly, the PK and the RTTCE components of the model were estimated simultaneously. Finally, the fixed effects of the PK and RTTCE components were fixed (i.e. not estimated) and the covariate components were added to the model using a full random effects modelling approach, with estimation of all inter-individual random effects simultaneously.

Repeated time-to-categorical event (RTTCE) modelling

A repeated time-to-categorical event model results from the combination of a parametric survival analysis (RTTE) and a proportional odds model for ordered categorical data (3-6).

1. Bleeding hazard function

The individual bleeding hazard (h_i) was given by:

$$h_i(t) = \lambda \cdot e^{\gamma(t-1)} \cdot \left(1 - \frac{FVIII(t)}{FVIII(t) + IF50}\right) \cdot e^{\eta_i}, \eta_i \sim N(0, \omega^2) \quad (1)$$

where $h_i(t)$ is the i^{th} patient bleeding hazard at time t , λ and γ are the scale and shape factors of the Gompertz distribution, $FVIII(t)$ is the individual PK model-predicted FVIII activity at time t , $IF50$ is the FVIII activity resulting in half-maximum inhibition of the hazard, and η_i is the random effect that describes the difference between the typical and the individual bleeding hazard. η_i is assumed to be normally distributed with mean 0 and an estimated variance ω^2 . Given the exponential model, the bleeding hazard will be log-normally distributed.

The parameters λ and $IF50$ were parameterized in terms of $\lambda_{0.5IU/dL}$ and $\lambda_{20IU/dL}$, which were the parameters estimated. These represent the bleeding hazards when plasma FVIII activity is 0.5 IU/dL and 20 IU/dL, respectively, as follows:

$$\lambda = \frac{\lambda_{0.5IU/dL} \cdot \lambda_{20IU/dL} \cdot (0.5 - 20)}{(\lambda_{0.5IU/dL} \cdot 0.5 - \lambda_{20IU/dL} \cdot 20)} \quad (2)$$

$$IF50 = \frac{(\lambda_{0.5IU/dL} \cdot 0.5 - \lambda_{20IU/dL} \cdot 20)}{(\lambda_{20IU/dL} - \lambda_{0.5IU/dL})} \quad (3)$$

2. Survival function - probability of not having a bleed within a certain time interval

The individual probability of survival (S_i), i.e. the probability of not having a bleed within a certain time interval, is related to the cumulative bleeding hazard in the interval as follows:

$$S_i(t) = e^{-\int_{t_j}^{t_{j+1}} h_i(t) dt} \quad (4)$$

where $S_i(t)$ is the i^{th} patient probability of not having an event within the time interval t_j to t_{j+1} , which are the start and end of the time interval, and $h_i(t)$ the individual bleeding hazard function (eq. 1).

3. Cumulative distribution function - probability of having a bleed within a certain time interval

The individual probability of having a bleed within a certain time interval (F_i) is a function of the survival probability as follows:

$$F_i(t) = 1 - S_i(t) \quad (5)$$

where $F_i(t)$ is the i^{th} patient probability of having a bleed within a certain time interval, and $S_i(t)$ is the probability of not having an event within the same time interval (eq. 4).

4. Proportional odds model for bleeding severity

In addition to describing the time-to-bleed, in the event of a bleed also the likelihood of a bleed to be mild, moderate, or severe was modelled (without any time-varying component). The categorical severity score of the bleeding events (mild, moderate, severe) was described using a proportional odds model, parameterized in terms of estimation of the cumulative probabilities on the logit scale. The logit of the cumulative probability of observing a moderate or severe bleed, or a severe bleed, was given by:

$$\text{logit}_{\text{moderate+severe}} = b_1 + \eta_i, \quad (6)$$

$$\text{logit}_{\text{severe}} = b_1 + b_2 + \eta_i, \quad \eta_i \sim N(0, \omega^2) \quad (7)$$

where b_1 and b_2 are the estimated baseline logits, assuming mild severity as the reference category, and η_i is the random effect that describes the difference between the typical and the individual baseline logit value (increasing values of η_i are associated to increasing severity). η_i is assumed to be normally distributed with mean 0 and an estimated variance ω^2 .

The logits were back-transformed to cumulative probabilities of observing a moderate or severe bleed, or a severe bleed, by:

$$P_{\text{moderate+severe}} = \frac{e^{\text{logit}_{\text{moderate+severe}}}}{1 + e^{\text{logit}_{\text{moderate+severe}}}} \quad (8)$$

$$P_{\text{severe}} = \frac{e^{\text{logit}_{\text{severe}}}}{1 + e^{\text{logit}_{\text{severe}}}} \quad (9)$$

The actual probabilities (P_x) of observing a mild (P_{mild}), moderate (P_{moderate}) or severe (P_{severe}) bleed were given by:

$$P_{\text{mild}} = 1 - P_{\text{moderate+severe}} \quad (10)$$

$$P_{\text{moderate}} = P_{\text{moderate+severe}} - P_{\text{severe}} \quad (11)$$

$$P_{\text{severe}} = (\text{eq. 9}) \quad (12)$$

5. Probability density functions

The likelihood of the observations given the probability density functions were maximized in the estimation. The probability density function of having an event of any severity at time t was given by:

$$f_{i \text{ any severity}}(t) = h_i(t) \cdot S_i(t) \quad (13)$$

where $f_{i \text{ any severity}}(t)$ is the i^{th} patient probability of having an event of any severity at time t , $h_i(t)$ the individual bleeding hazard function (eq. 1) and $S_i(t)$ the survival function (eq. 4).

Thus, by multiplying the probability density function by the probability of observing a mild, moderate or severe bleed, the individual probability density function (f_i) for a bleed of a specific severity score to occur was given by:

$$f_{i \text{ mild}}(t) = h_i(t) \cdot S_i(t) \cdot P_{\text{mild}} \quad (14)$$

$$f_{i \text{ moderate}}(t) = h_i(t) \cdot S_i(t) \cdot P_{\text{moderate}} \quad (15)$$

$$f_{i \text{ severe}}(t) = h_i(t) \cdot S_i(t) \cdot P_{\text{severe}} \quad (16)$$

where $f_{i \text{ mild}}(t)$, $f_{i \text{ moderate}}(t)$ and $f_{i \text{ severe}}(t)$ is the i^{th} patient probability density function for a mild, moderate and severe event at time t , respectively, $h_i(t)$ the individual bleeding hazard function (eq. 1), $S_i(t)$ the survival function (eq. 4), and P_{mild} , P_{moderate} and P_{severe} is the estimated probability of observing a mild, moderate or severe bleed, respectively (eq. 10-12).

Assessment of time-dependency between consecutive bleeds

The inclusion of a time-dependency between consecutive bleeds was tested with a Markov hazard rate accounting for the time since the last bleed (TSB) in the best bleeding hazard model. Two extra parameters (λ_{Markov} and γ_{Markov}) were estimated in an additive exponential term as follows:

$$h_{i \text{ Markov}}(t) = h_i(t) + \lambda_{\text{Markov}} \cdot e^{-\gamma_{\text{Markov}} \cdot \text{TSB}} \quad (17)$$

where $h_{i \text{ Markov}}(t)$ is the i^{th} patient bleeding hazard at time t including the Markov hazard rate, $h_i(t)$ is the i^{th} patient bleeding hazard at time t (eq. 1), λ_{Markov} and γ_{Markov} are the scale and shape factors of the Markov Gompertz distribution, and TSB is the time since the last bleed.

At the start of the study, the time of the last bleed was unknown, and therefore TSB was set to the mean inter-bleed time in the previous 12 months (12 months/number of bleeds in the previous 12 months). For instance, if a patient had two bleeds in the 12 months before the study started, the mean inter-bleed time was 6 months at the start of the study. After the LEOPOLD trial started, TSB was given by the mean inter-bleed time + t , where t represents the time since the start of the trial.

Full random effects modelling

This methodology allows characterizing the extent to which all covariates (i.e. patient and study characteristics) correlate with the PK or RTTCE components of the model, in a single step (7-11). Covariate values were included as observations, and their distributions were modelled as random effects. The covariance elements between the random effects for parameters and covariates were estimated in a full covariance matrix. Coefficients for parameter-covariate relationships were then obtained from parameter-covariate covariances standardized by the covariates variance. All covariate values were log-transformed which corresponds to the use of a power parameter-covariate relationship in case the parameter is log-normally distributed, or a linear relationship with log-transformed covariate values in case the parameter is normally distributed.

Estimation methods and model assessment

The final model was estimated using the Monte Carlo importance sampling assisted by mode a posteriori (IMPMAP) estimation method, with parallel computation. The analysis was assisted by PsN (version 4.8.0) and graphical and statistical analyses were carried out in R (version 3.4.0) and Xpose (version 4.6.0; Uppsala University, Sweden) (12).

Model assessment was based on scientific plausibility, changes in the objective function value ($-2 \cdot \log$ -likelihood), goodness-of-fit plots and precision of the parameter estimates. In particular, the PK component of the model was qualified with stratified prediction-corrected visual predictive checks (VPCs), and the RTTCE component with stratified VPCs of the Kaplan-Meier curves and the kernel-based visual hazard comparison tool (13). Parameter uncertainty was assessed using the standard errors obtained from the NONMEM R matrix following an evaluation step using the Monte-Carlo importance sampling (IMP) estimation method.

Results

The descriptive statistics of study and patient characteristics as well as information on bleeding episodes by age cohort is available in **Table S1**.

Table S1 - Patient characteristics, treatment and bleeding data by age cohort for patients with PK observations enrolled in the bleeding observation period

Study	0-<6 years	6-<12 years	12-<18 years	≥18 years	Total
Patients with PK and bleeding data, <i>n</i> (% of total)	24 (14)	27 (16)	18 (10)	103 (60)	172 (100)
Duration of bleeding observation period, months					
Mean ± SD	6.02 ± 0.483	6.20 ± 0.590	11.1 ± 2.73	12.2 ± 0.221	10.3 ± 2.86
Median [range]	6.09 [3.99-6.48]	6.18 [3.78-7.22]	12.1 [3.08-12.4]	12.2 [11.7-13.1]	12.0 [3.08-13.1]
Individual FVIII dose during treatment period (IU/kg)					
Mean ± SD	38.3 ± 11.3	34.2 ± 9.18	40.7 ± 9.54	38.3 ± 9.23	38.2 ± 9.57
Median [range]	37.5 [21.0-106]	33.9 [19.2-103]	41.6 [7.88-68.8]	37.9 [4.25-199]	37.9 [4.25-199]
Patient characteristics					
Age, years					
Mean ± SD	3.96 ± 1.20	8.93 ± 1.82	14.7 ± 1.45	33.0 ± 10.9	23.2 ± 14.9
Median [range]	4.00 [1.00-5.00]	9.00 [6.00-11.0]	15.0 [12.0-17.0]	31.0 [18.0-61.0]	22.0 [1.00-61.0]
Weight, kg					
Mean ± SD	19.2 ± 5.17	31.9 ± 11.0	64.8 ± 19.2	72.0 ± 15.8	57.6 ± 25.5
Median [range]	18.2 [11.9-39.0]	28.7 [11.0-59.0]	60.0 [45.5-124]	70.3 [39.0-118]	60.0 [11.0-124]
Lean body weight ^a , kg					
Mean ± SD	17.6 ± 3.88	28.4 ± 8.55	52.5 ± 9.79	56.2 ± 8.25	46.0 ± 17.1
Median [range]	17.3 [10.1-30.1]	26.3 [9.25-48.9]	51.0 [39.4-77.0]	56.1 [35.5-79.2]	50.9 [9.25-79.2]
Body mass index ^a , kg·m ⁻²					
Mean ± SD	15.8 ± 2.31	16.8 ± 2.70	22.1 ± 5.55	23.5 ± 4.54	21.3 ± 5.25
Median [range]	15.0 [13.5-24.6]	16.7 [13.0-24.1]	20.0 [16.1-38.3]	23.7 [15.0-33.1]	20.3 [13.0-38.3]
Von Willebrand factor level ^b , %					
Mean ± SD	NA	NA	97.7 ± 24.0	111 ± 38.1	109 ± 36.5
Median [range]	NA	NA	98.0 [58.0-160]	105 [43.0-242]	104 [43.0-242]
Race, <i>n</i> (%)					
White	22 (92)	25 (93)	12 (67)	73 (71)	132 (77)
Black	1 (4.2)	2 (7.4)	1 (5.6)	4 (3.9)	8 (4.7)
Asian	0 (0.0)	0 (0.0)	1 (5.6)	22 (21)	23 (13)
Hispanic	1 (4.2)	0 (0.0)	3 (17)	4 (3.9)	8 (4.7)
Unknown	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)	1 (0.6)
Treatment history, <i>n</i> (%)					
On-demand	1 (4.2)	10 (37)	8 (44)	62 (60)	81 (47)
Prophylaxis	23 (96)	17 (63)	10 (56)	41 (40)	91 (53)

Number of target joints at study start ^c					
Median [range]	1 [1-1]	1 [1-2]	1 [1-3]	2 [1-5]	1 [1-5]
Summary of bleeding episodes					
Total number of bleeds, <i>n</i> (% of total)	46 (7.3)	55 (8.7)	99 (16)	433 (68)	633 (100)
Patients with at least one bleed, <i>n</i> (% of total)	14 (12)	14 (12)	14 (12)	74 (64)	116 (100)
Individual number of bleeds					
Median [range]	1 [0-9]	1 [0-9]	2 [0-26]	2 [0-33]	2 [0-33]
Time to first bleed ^d , days					
Mean ± SD	51.5 ± 40.3	64.5 ± 52.9	104 ± 107	73.3 ± 78.6	73.3 ± 76.8
Median [range]	46.8 [2.97-161]	68.1 [1.17-182]	47.3 [0.958-287]	47.5 [0.606-352]	48.2 [0.606-352]
Bleed type, <i>n</i> (%)					
Spontaneous	10 (22)	14 (25)	43 (43)	321 (74)	388 (61)
Non-spontaneous	36 (78)	41 (75)	56 (57)	112 (26)	245 (39)
Spontaneous bleed location, <i>n</i> (%)					
Joint	2 (4.3)	6 (11)	39 (39)	281 (65)	328 (52)
No joint	44 (96)	49 (89)	60 (61)	152 (35)	305 (48)
Bleed severity, <i>n</i> (%)					
Mild	30 (65)	22 (40)	50 (51)	193 (45)	295 (47)
Moderate	14 (30)	32 (58)	43 (43)	187 (43)	276 (44)
Severe	2 (4.3)	1 (1.8)	6 (6.1)	53 (12)	62 (9.8)
Number of bleeds in the 12 months prior to study start ^e					
Median [range]	1.5 [0-55]	5 [0-49]	12 [0-106]	18 [0-100]	11 [0-106]

n: number; NA: not available; SD: standard deviation; ^aLean body weight and body mass index missing for one patient; ^bVon Willebrand factor levels missing for 55 patients; ^cNumber of target joints missing for 61 patients; ^dTime to first bleed based on patients experiencing at least one bleed; ^eNumber of bleeds in the 12 months prior to study start missing for 3 patients

Kernel-based visual hazard comparison tool

The Kernel-based visual hazard comparison plot is available in **Figure S1**. The mean bleeding hazard predicted by the model follows the trend of the non-parametric kernel bleeding hazard rate, indicating the absence of a major structural model misspecification.

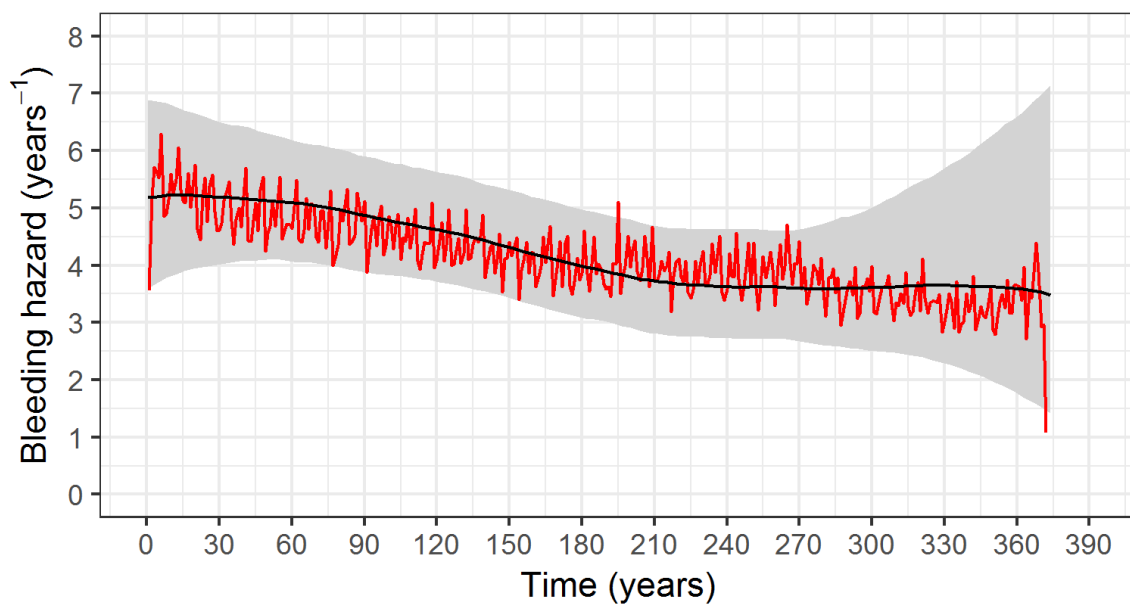


Figure S1 - Kernel-based visual hazard comparison plot. The red line represents the mean bleeding hazard rate of the model over time, calculated from the individual posthoc bleeding hazard estimates of all non-censored patients, and the black line with shaded area represents the non-parametric kernel estimate of the bleeding hazard rate and its 95% confidence interval, respectively.

Observed Kaplan-Meier curves for the first 3 bleeding episodes and the 95% confidence interval (CI) of the model predictions using the model including only spontaneous joint bleeds

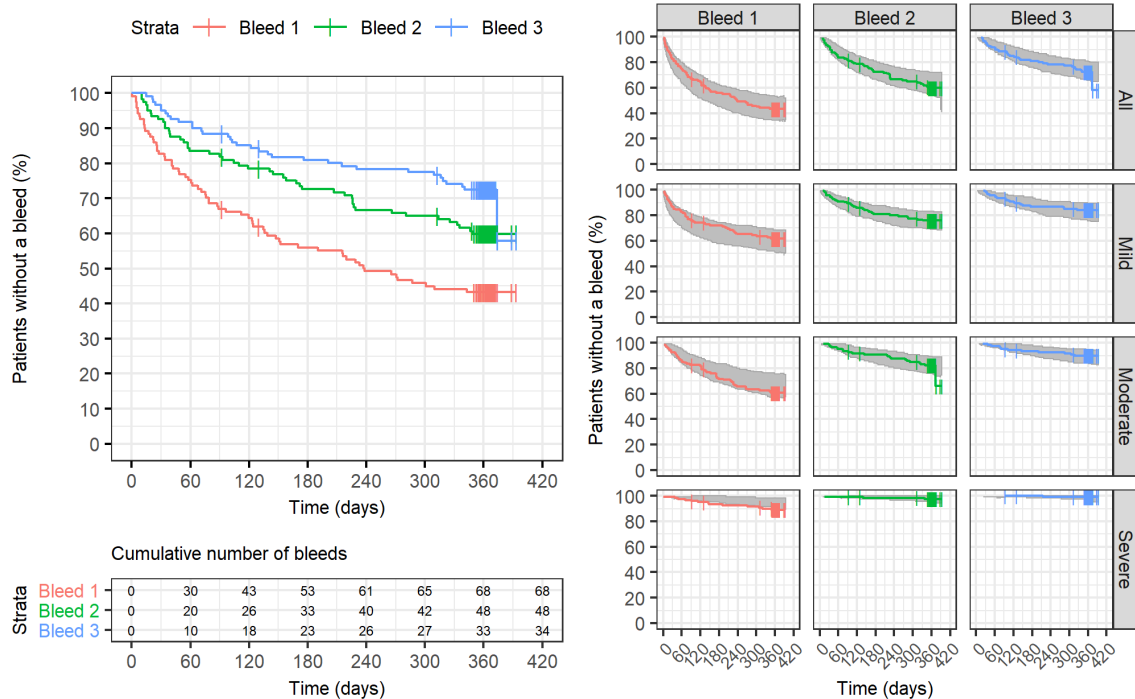


Figure S2 – Observed and model-predicted Kaplan-Meier curves depicting the percentage of bleed-free patients vs. time after start of the LEOPOLD studies (LEOPOLD I and LEOPOLD II) using the model including only joint spontaneous bleeds, for the first, second and third individual bleed. Left panel: observed Kaplan-Meier curves (plot) and cumulative number of bleeds throughout time (table). Right panel: observed Kaplan-Meier curves by number of bleed (first, second or third in the study) and bleeding severity (mild, moderate or severe) overlaid with the 95% confidence interval of the model-predicted Kaplan-Meier curves (shaded area), based on 200 simulations. Vertical lines indicate that a patient was censored.

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