SUPPORTING INFORMATION

Minimum factor VIII levels to prevent joint bleeding in mild hemophilia A

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Table S1. Association between FVIII level and frequency of other than joint bleeds.

	Negative Binomial Regression Models for ABR[1]					
	Univariate (n = 251)			Multivariate[2] (n = 251)		
	ABRR	95% CI	p	ABRR	95% CI	p
Mean FVIII:C (%)	0.9813	(0.9698; 0.9928)	0.0014	0.9813	(0.9699; 0.9927)	0.0014
	Negative Binomial Regression Models for Spontaneous ABR[1]					
	Univariate (n = 234)			Multivariate[2] (n = 234)		
	ABRR	95% CI	p	ABRR	95% CI	p
Mean FVIII:C (%)	1.0058	(0.9725; 1.0385)	0.73	1.008	(0.9753; 1.0410)	0.61

[1] calculated considering as time of observation the period between January 2005 and last follow-up and, as bleeds, only other bleeds; [2]adjusted for age and ABO blood group.
Other bleeds included all the major and clinically relevant non major bleeds other than joint bleeds. For spontaneous ABR* only spontaneous bleeds were considered.
Abbreviations: ABR: annualized bleeding rate; CI: confidence interval; FVIII:C: factor VIII coagulant activity; OR: unitary odds ratio.

Table S2. Association between FVIII level and overall bleeding frequency.

	Negative Binomial Regression Models for ABR					
	Univariate (n = 243)			Multivariate[1] (n = 243)		
	ABRR	95% CI	p	ABRR	95% CI	p
Mean FVIII:C (%)	0.9758	(0.9643; 0.9873)	<0.0001	0.9757	(0.9643; 0.9872)	<0.0001
	Negative Binomial Regression Models for Spontaneous ABR					
	Univariate (n = 223)			Multivariate[1] (n = 223)		
	ABRR	95% CI	p	ABRR	95% CI	p
Mean FVIII:C (%)	0.9910	(0.9562; 1.0252)	0.63	0.9956	(0.9606; 1.0301)	0.81

[1] adjusted for age and ABO blood group.
ABR was calculated considering as time of observation the period between January 2005 and last follow-up and, as bleeds, all bleeds (joint and other clinically relevant non major and major bleeds). For spontaneous ABR only spontaneous bleeds were considered.
Abbreviations: ABR: annualized bleeding rate; CI: confidence interval; FVIII:C: factor VIII coagulant activity; OR: unitary odds ratio.

METHODS

Statistical analysis

In order to evaluate the association between mean FVIII level and joint bleeding frequency (as a count outcome), we considered the following models as candidates:

- Model 1: Poisson regression model (POIS);
- Model 2: Negative Binomial regression model (NB);
- Model 3: Zero-inflated Poisson regression model (ZIP);
- Model 4: Zero-inflated Negative Binomial regression model (ZINB).

Adjustments for the different exposure times of observational units were performed for all the four models incorporating an offset term (i.e. time during which the subjects are at risk of incurring joint bleeding, which is, in this instance, age at the end of follow-up). Since the regression coefficient for an offset variable is set to be 1, rates rather than counts can be modelled as outcome of interest (1). In this instance, the rate of interest is AJBR (annualized joint bleeding rate), which represents the occurrence of bleedings per unit-exposure.

Therefore, models 1 and 2 above can be expressed as follows:

$$\log(\mu_i) = \alpha + \beta * \text{FVIII}_i + \log(t_i) \quad \text{->} \quad \log(\frac{\mu_i}{t_i}) = \alpha + \beta * \text{FVIII}_i \ (1)$$

where:
i = 1, …, n
t = time period observed (exposure time)
$\log(t_i)$ = offset variable
$\frac{\mu_i}{t_i}$ = rate (i.e. $\frac{count\ of\ bleedings}{exposure\ time} = AJBR$)

provided that

$$Count\ of\ bleedings \sim POIS\ (\mu)$$

with $Mean(Count\ of\ bleedings) = Variance(Count\ of\ bleedings) = \mu$

or

$$Count\ of\ bleedings \sim NB\ (\mu, \theta)$$

with $\theta = dispersion\ parameter$

$Mean(Count\ of\ bleedings) = \mu$ and $Variance(Count\ of\ bleedings) = \mu + \frac{\mu^2}{\theta}$

Models 3 and 4 are made of two sub-models: one modelling the positive counts >0 as in (1) and the other modelling the zero process as follows:

$$\log\left(\frac{\pi_i}{1-\pi_i}\right) = \alpha + \beta * \text{FVIII}_i + \log(t_i) \quad (2)$$

where:
$i = 1, \ldots, n$
t = time period observed (exposure time)
$\log(t_i)$ = offset variable
$\frac{\pi_i}{1-\pi_i}$ = odds (i.e. $\frac{probability\ of\ bleeding}{probability\ of\ non-bleeding}$)

provided that

$$Presence\ of\ bleeding \sim BERNOULLI\ (\pi)$$

with $\pi = probability\ of\ bleeding\ (i.e. proportion\ of\ bleeds)$

$Mean(Presence\ of\ bleeding) = \pi$ and $Variance(Presence\ of\ bleeding) = \pi * (1 - \pi)$

It should be noted that the set of regressors of the count data sub-model (Poisson or NB) (1) and the Logistic Regression sub-model (2) are the same in this application. Negative Binomial model is here considered as a potential model because it is a robust alternative to the more known Poisson model, allowing the variance parameter to exceed the mean. Zero-inflated models are taken into account for dealing with the potential excess zeros issue. Model selection was carried out by assessing model goodness-of-fit (GOF) by Akaike's Information Criterion (AIC) value. The Negative Binomial model resulted to be the best model to fit the data, whereas the worse one was Poisson regression (Table S3). The selected model was chosen to explain the variability in bleeding frequency as a function of the mean factor VIII level. The linear regression model was not considered, not being a suitable model for the analysis of low-frequency bleeding data [1].

Table S3. Count model goodness-of-fit assessed by AIC value.

Proposed models	AIC
POIS	580.12
NB	531.16
ZIP	556.78
ZINB	535.16

Abbreviations: AIC: Akaike's Information Criterion; NB: Negative Binomial regression model; POIS: Poisson regression model; ZINB: Zero-inflated Negative Binomial regression model; ZIP: Zero-inflated Poisson regression model.

References

[1] den Uijl IE, Fischer K, Van Der Bom JG, Grobbee DE, Rosendaal FR, Plug I. Analysis of low frequency bleeding data: the association of joint bleeds according to baseline FVIII activity levels. Haemophilia. 2011 Jan;17(1):41-4. doi: 10.1111/j.1365-2516.2010.02383.x. Epub 2010 Sep 2. PMID: 20825504.

[2] Kaatz S, Ahmad D, Spyropoulos AC, Schulman S; Subcommittee on Control of Anticoagulation. Definition of clinically relevant non-major bleeding in studies of anticoagulants in atrial fibrillation and venous thromboembolic disease in non-surgical patients: communication from the SSC of the ISTH. J Thromb Haemost. 2015 Nov;13(11):2119-26.