ORIGINAL ARTICLE *Clinical haemophilia*

Association of peak factor VIII levels and area under the curve with bleeding in patients with haemophilia A on every third day pharmacokinetic-guided prophylaxis

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Introduction: We previously showed that pharmacokinetic-guided prophylaxis (PKP) allows the dosing interval to be extended while maintaining a specific trough level. However, the associations of peak factor VIII (FVIII) levels and area under the curve (AUC) with breakthrough bleeding have not been investigated. **Aim:** The aim of this study was to analyse data from the PKP arm to determine whether peak FVIII levels, AUC and time with FVIII levels in a haemostatically effective range are independent predictors of bleeding during prophylaxis. **Methods:** *Post hoc* analysis of the association of FVIII levels and AUC with annualized bleeding rate in 34 patients on PKP. **Results:** During 1 year of PKP, 131 bleeding episodes occurred in 24/34 patients. Average peak FVIII levels ranged from 24 to 168 IU dL^{-1}, with higher values associated with a decreased risk for all bleeding (joint and non-joint; $P < 0.01$) and joint bleeding ($P < 0.01$). Following rFVIII infusion, median percent of time spent with FVIII levels >20 IU dL^{-1} was 22%; median AUC was 1363. Both values were significantly associated with a lower ABR when targeting a 1% trough at 72 h. **Conclusion:** When PKP was administered every third day, higher peak FVIII levels, higher AUC and more time spent per week with FVIII levels >20 IU dL^{-1} provided increased protection from joint and non-joint bleeding. These data highlight the potential impact of variability in individual pharmacokinetic and bleeding risk and support the need for high peak levels and AUC in some patients treated every third day. The findings do not necessarily apply to alternate-day or other prophylactic dosing regimens.

Keywords: bleeding, haemarthrosis, haemophilia, joint bleeding, pharmacokinetic-guided treatment, prophylaxis, rAHF-PFM

Introduction

Prophylaxis is considered optimal care for children and adults with severe haemophilia A [1,2] because of its proven ability to reduce joint and other bleeding episodes [3–7]. On the basis of experience from Sweden, where prophylaxis was pioneered in the late 1950s [8], target factor VIII (FVIII) trough levels for prophylaxis have traditionally been set at ≥1 IU dL^{-1} above baseline, with the intent of converting the severe bleeding phenotype into the less frequent bleeding pattern of moderate haemophilia [9]. Yet 1 IU dL^{-1} is an arbitrary value above which neither all haemorrhages are prevented nor below which bleeding inevitably occurs [10,11]. Some patients, including those with arthropathy or who have active lifestyles, may require higher FVIII trough levels [12], whereas others may have trough levels lower than 1 IU dL^{-1} but do not bleed [3]. Furthermore, peak FVIII levels, area under the curve (AUC) and time spent with FVIII levels >15 IU dL^{-1} [13]) may also be important in protecting against haemarthroses and other bleeding episodes, particularly during periods of high activity, when patients are at increased risk for traumatic bleeding.

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A 'one size fits all' approach to prophylaxis is not necessarily ideal, possibly leading to inefficient or unnecessarily costly dosing. Using an individual's pharmacokinetic (PK) response to factor VIII (FVIII) infusions to calculate dose and dosing frequency is one strategy for personalizing the prophylactic regimen [12,14,15]. In the study of prophylaxis with ADVATE Antihemophilic Factor (Recombinant), Plasma/Albumin Free Method (rAHF-PFM) (NCT00666406), PK-guided prophylaxis (PKP) given every third day was comparable to standard weight-based every-other-day prophylaxis (SP) [6]. In both arms, the annualized bleeding rate (ABR) [6] and the median annualized joint bleeding rate (AJBR) [16] were significantly reduced as compared with on-demand therapy (OD), and the PKP arm had the advantage of requiring 1 less infusion weekly [6].

Here, we report the results of a *post hoc* analysis of data for the intent-to-treat PKP group to determine whether peak FVIII levels, AUC and time with FVIII levels >20 IU dL^{-1} are independent predictors of prophylactic efficacy for patients using PKP every third day.

Methods

Clinical study summary

The details of the rAHF-PFM prophylaxis study (NCT00243386) have been described [6]. To summarize, 66 previously on-demand-treated males with moderately severe to severe haemophilia A (FVIII ≤2%) aged 7 to 59 years (median: 26 years) who experienced ≥8 joint haemorrhages in the previous year were treated OD for 6 months. These patients (the intent-to-treat population) were then randomly assigned to 12 months of SP (20–40 IU kg^{-1} every other day) or PKP (20–80 IU kg^{-1} every third day), with the PKP regimen intended to maintain FVIII trough levels ≥1 IU dL^{-1}. All prophylactic doses were to be infused in the morning. The characteristics of the study patients were similar for the two prophylaxis arms, with all but one patient in the SP arm and two patients in the PKP arm having 1–10 target joints (defined as ≥4 bleeds into the same joint within a 6-month period or >20 lifetime bleeds into a joint) at the start of prophylaxis. (Five of 32 [16%] patients in the SP arm and 13/34 [38%] in the PKP arm had experienced ≥3 joint bleeds in the year preceding study randomization.) No information was collected with regard to patients' lifestyle or physical activity.

Median (interquartile range [P25, P75]) ABR during prophylaxis was 1.0 (0.0, 3.5) and 2.0 (0.0, 6.9) for the SP and PKP arms, respectively, whereas the median ABR (IQR) during OD was 43.9 (34.6, 56.5), a statistically significant difference (P < 0.0001) [6]. Twenty-two patients (33.3%) were haemorrhage-free while receiving prophylaxis (either regimen), whereas all patients experienced bleeding during the OD period. Similarly, the AJBR was significantly lower for per protocol patients (n = 53) treated with SP (55 total haemarthroses; AJBR median: 0.48 [1.96]) or PKP (72 total haemarthroses; AJBR median: 1.00 [4.07]), as compared with OD (1164 total haemarthroses; AJBR median: 38.65 [24.81]; P < 0.0001) [16]. Median (range) FVIII trough levels were 3.0 IU dL^{-1} (0.5–45.0) during SP (56 observations in 24 patients) and 1.0 IU dL^{-1} (0.5–10.0) during PKP (52 observations in 23 patients). FVIII consumption and adverse event rates for both prophylactic regimens were equivalent, and no patient developed a hypersensitivity reaction related to rAHF-PFM or a FVIII inhibitor while on study.

Data for all 34 patients in the every third day intent-to-treat PKP arm were evaluated. A total of 4089 prophylactic infusions were administered during a median of 364 treatment days (range: 97–394). The median dose per infusion was 43.0 (range: 13.0–107.1) IU kg^{-1}, and the median number of units administered per patient was 2979 (range: 1073–5600). Bleeding episodes were recorded in patient diaries and verified by the investigator. Data collected included time and date of bleeding, anatomical site(s), identification of bleeding as joint or non-joint, aetiology (spontaneous, traumatic or undetermined) and severity.

Statistical analyses

The predicted concentration level (IU dL^{-1}) at the time of bleeding was projected on the basis of individual PK parameters, assuming that each patient was at his natural baseline. Average peak FVIII levels were estimated using individual in vivo recovery values and average dose/prophylactic infusion.

A negative binomial multivariate regression model was used to evaluate the association between ABR and (i) average peak FVIII level (IU dL^{-1}), (ii) time spent >20 IU dL^{-1} FVIII activity (%) and 3) weekly AUC (IU × h dL^{-1}); age and body mass index were covariates [17]. ABR was calculated as the number of bleeding events divided by the length (in years) of the treatment regimen. Average peak FVIII level was calculated as the average dose (IU kg^{-1}) during the study multiplied by incremental recovery (IU dL^{-1} IU^{-1} kg^{-1}). Time spent and weekly AUC above 5, 10, 15, 20, 30 and 40 IU dL^{-1} FVIII activity were estimated from each patient's infusion log and time per week together with his PK parameters. Correlation between two variables was described using non-parametric spearman method.

Results

A total of 845 bleeding episodes (188 traumatic, 657 spontaneous; 750 joint, 95 non-joint) occurred in the 34 patients during 6 months of OD that preceded PKP. The mean ±SD (range; median) ABR and AJBR during on-demand treatment was 49.2 ± 20.7 (13–90.8; 44.8) and 43.5 ± 21.3 (6.5–90.8; 39.0) respectively.

Number of haemorrhages and time of bleeding

During PKP, 131 bleeding episodes occurred in 24/34 patients (70.6%). Among the 121 episodes of joint bleeding, 4 (all spontaneous) occurred within the first 12 h after infusion, 20 (6 spontaneous) occurred 12–24 h post infusion, 49 (21 spontaneous) occurred 24–48 h post infusion and 40 (20 spontaneous) occurred 48–72 h post infusion (Table 1). No significant increase was observed in the number of bleeding episodes occurring more than 48 h after infusion as compared with those occurring within the first 48 h.

Pattern of spontaneous and traumatic bleeding

The median predicted concentration (range) at the time of bleeding among patients in the PKP arm was 2.71 IU dL^{-1} (0–26.55) for 57 spontaneous haemorrhages, 3.57 IU dL^{-1} (0–41.6) for 74 traumatic haemorrhages, 3.43 IU dL^{-1} (0–41.67) for 121 haemarthroses

and 2.87 IU dL^{-1} (0–11.26) for 10 non-joint bleeding events (Figs 1 and 2). No statistically significant difference in predicted FVIII levels was observed either between spontaneous or traumatic or joint and non-joint haemorrhages. Traumatic joint bleeding occurred with predicted FVIII levels as high as 42%, but most haemarthroses occurred at levels <10 IU dL^{-1} (median predicted value 3.43 [IQR = 7.18 [1.33–8.51]).

Peak levels and bleeding risk

Average peak FVIII levels (IVR × average dose) in the PKP arm ranged from 24.3 to 167.5% (median: 70.9%), with lower values associated with an increased risk for all bleeding (P = 0.0004) and joint bleeding (P = 0.0013) (Fig. 3).

AUC and peak FVIII levels in non-bleeding and bleeding patients

The average predicted AUC was 136 between 24 and 48 h and 33 between 48 and 72 h post infusion. To further examine the possible relationship between high AUC, peak FVIII levels and bleeding risk in the immediate hours following infusion, we compared the weekly AUC 0–12 h post infusion for patients who did and did not report bleeding (Table 2). No significant difference was observed between the two groups, likely attributable to the small sample size. Nonetheless, patients who had no bleeding during the 12-month study period appeared to have higher post infusion AUC 0–12 h as well as higher peak FVIII levels.

Time spent at FVIII levels >20 IU dL^{-1} and bleeding risk

Among patients in the PKP arm, the median percent of time spent with FVIII levels >20 IU dL^{-1} was 22.3%

Table 1. Number of haemorrhages (patients) and time at which bleeding occurred post infusion in the pharmacokinetic-guided prophylaxis arm.

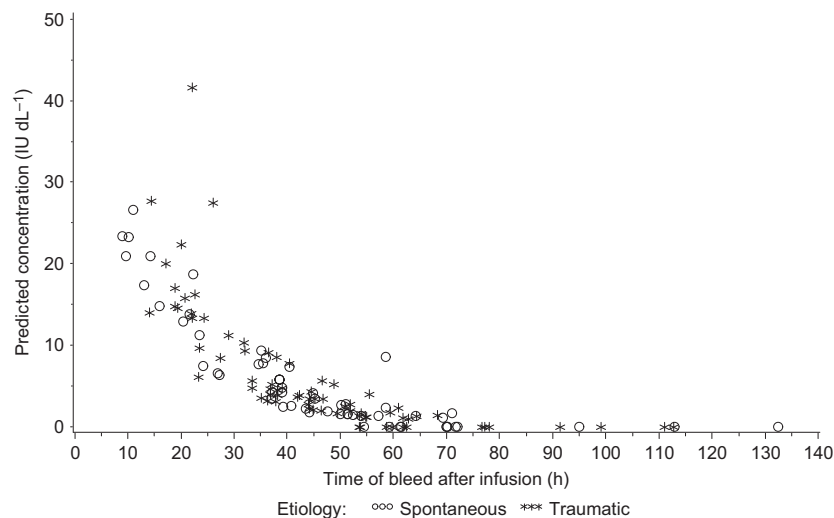| Bleeding type	12 h	12-24 h	24-48 h	48-72 h	>72 h*	All
Spontaneous	4 (4)	7 (4)	21 (13)	22 (12)	3 (3)	57
Traumatic	0 (0)	14 (9)	31 (10)	22 (11)	7 (4)	74
Joint	4 (4)	20 (11)	49 (17)	40 (15)	2 (1)	121
Non-joint	0 (0)	1 (1)	3 (2)	4 (3)	2	10

*Indicates non-adherence to the treatment regimen.

Fig. 1. Spontaneous and traumatic bleeding episodes over time in patients on pharmacokinetic-guided prophylaxis (PKP).
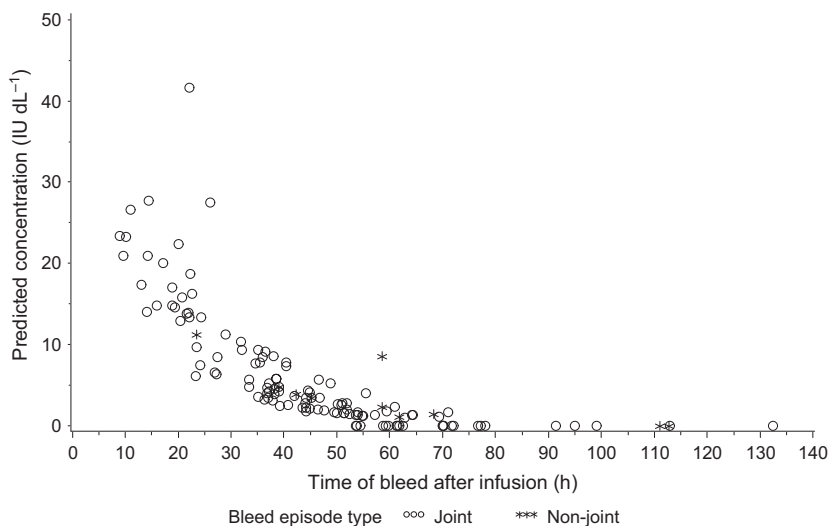
Fig. 2. Joint/non-joint bleeding episodes over time in patients on pharmacokinetic-guided prophylaxis (PKP).
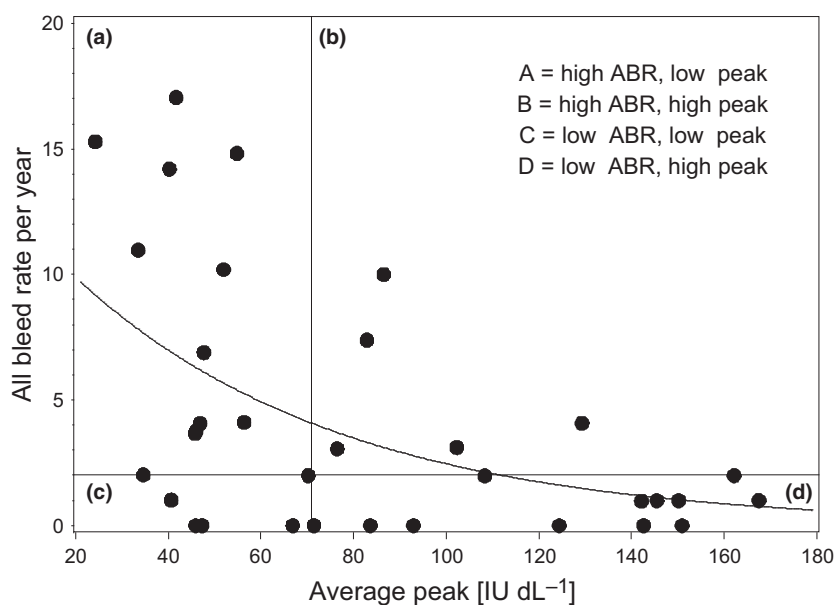

Fig. 3. Average peak factor VIII (FVIII) levels and risk for bleeding in patients on pharmacokinetic-guided prophylaxis (PKP). The trend curve presents the predicted ABR derived from the negative binomial multivariate regression model across the average peak FVIII level for an average patient (with regard to age and body mass index). The average peak FVIII levels are related to the risk of all bleeding ($P = 0.0004$; coeff: -0.02) and joint bleeding ($P = 0.0013$; coefficient: -0.02). Four quadrants are identified to signify the relationship between bleeding rates and average peak FVIII levels. The horizontal line represents median ABR (2.0) for the overall population, and the vertical line represents median theoretical peak FVIII level achieved following prophylactic infusion.

(range: 2.9–46.1), resulting in a median AUC of 1363 (range: 130–4265). Both values were significantly associated with a lower ABR, and during the period that the FVIII concentration was ≥ 27 IU dL^{-1}, PKP-treated patients experienced no spontaneous bleeding. This relationship was even more pronounced when a higher percentage of time was spent with FVIII levels >30 IU dL^{-1} (Fig. 4) or higher peak levels (Figs 1 and 2) were achieved. The modelled data showed a consistent and strong positive correlation among peak level, time above percent and weekly AUC (Table 3).
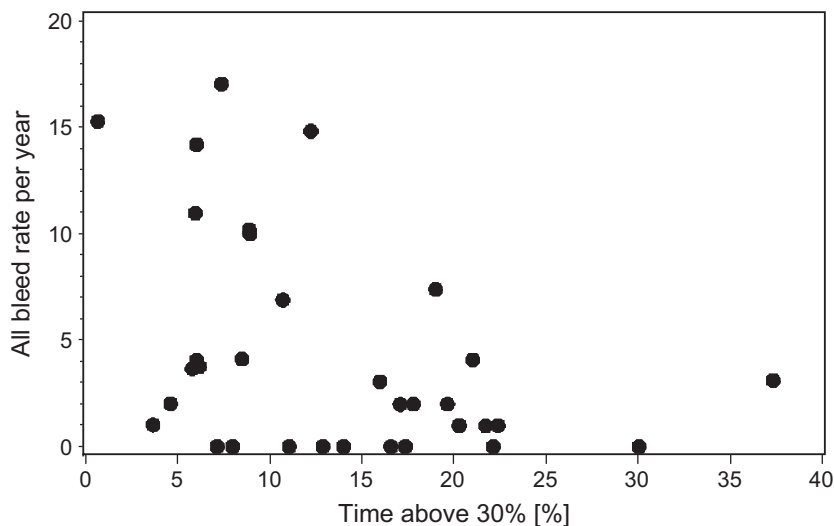
Discussion

In this *post hoc* analysis of the rAHF-PFM prophylaxis study data, patients receiving PKP every third day were dosed to maintain a target trough FVIII level

of ≥ 1 IU dL^{-1} [6], thereby eliminating time spent <1 IU dL^{-1} as a variable contributing to bleeding risk. Evaluating PK parameters in relation to the reported bleeding events led to three observations. First, higher peak FVIII levels and AUC provided increased protection against joint and non-joint bleeding. Specifically, spontaneous bleeding was prevented when patients in the PKP arm had a FVIII concentration ≥ 27 IU dL^{-1}.

Broderick and colleagues similarly recognized the link between high peak levels and a reduction in bleeding [18]. In a case-crossover study nested within a prospective cohort study that included 104 children and adolescents with haemophilia, they found the transient increase in bleeding risk associated with vigorous physical activity was mitigated with estimated peak factor concentrations of 70–100 IU dL^{-1}. Conversely, low peak levels may have contributed to

Table 2. Peak factor VIII (FVIII) and area under the curve (AUC) levels within 12-h post infusion in non-bleeding and bleeding patients.

	Non-bleeding patients (N = 9)					Bleeding patients (N = 25)					
	Min	Q25	Median	Q75	Max	Min	Q25	Median	Q75	Max	P-value*
Average peak FVIII (IU dL^{-1})	45.9	66.9	83.6	124.4	150.9	24.3	45.9	56.4	108.3	167.5	0.4793
Weekly AUC for 12 h after infusion	852.2	1134.5	1263.0	1846.0	2882.0	490.3	774.5	1097.5	1712.1	2353.4	0.3765

*P-value is from *t*-test.

Fig. 4. Time spent post infusion with factor VIII (FVIII) levels >30% and bleeding risk in the pharmacokinetic-guided prophylaxis (PKP) arm.

Table 3. Average peak level, time above % and weekly area under the curve (AUC) covariance in the pharmacokinetic-guided prophylaxis (PKP) arm: correlation coefficients among the variables.

Variable	PKP (N = 34)	
	Time >5%	AUC >5%
	Time >10%	AUC >10%
	Time >20%	AUC >20%
	Time >30%	AUC >30%
	Time >40%	AUC >40%
Average peak (IU dL^{-1})	*ns*	0.82 (s)
	0.43 (s)	0.82 (s)
	0.65 (s)	0.87 (s)
	0.90 (s)	0.94 (s)
	0.94 (s)	0.95 (s)
Time >5%	Not applicable	0.60 (s)
Time >10%		0.81 (s)
Time >20%		0.90 (s)
Time >30%		
Time >40%		0.99 (s)

Non-parametric correlation coefficient: (s) indicates *P* < 0.05.

the increase in bleeding events observed by Lindvall *et al.* [19] in some patients who crossed over to daily low-dose prophylaxis designed to maintain at least the same FVIII trough level as obtained with their standard prophylactic regimens.

Second, ABR declined as the percent of time spent weekly with FVIII levels >20–30 IU dL^{-1} rose. Specifically, among eight patients who spent ≥20% of time about 30 IU dL^{-1}, only two had an ABR >2. Nearly 40 years ago, Aronstam described a 60% reduction in bleeding frequency in boys with severe

haemophilia A treated with FVIII-containing 'materials' to increase their factor levels to 15–30% of normal [20]. In 2011, den Uijl *et al.* observed that patients with haemophilia A who had a baseline FVIII activity exceeding 15 IU dL^{-1} experienced no joint bleeding [13]. This result is not directly equivalent to the situation observed in our study, however, as a steady state of 15 IU dL^{-1} differs from the peaks and troughs we described. In addition, our FVIII level data are modelled on the basis of estimated PK parameters and patient-reported infusion and bleed event (including aetiology) records, which may introduce some degree of error. Nevertheless, Broderick also noted the connection between higher FVIII levels and bleed reduction, reporting that the risk of bleeding decreased by 2% for each 1% increase in factor activity [18]. When factor levels were maintained at approximately 50 IU dL^{-1} during physical activity, bleeding risk was lower than that for inactive patients treated on-demand.

Finally, while traumatic bleeding occurred throughout the range of predicted FVIII plasma levels (as high as 58 IU dL^{-1}), most occurred at FVIII levels <10 IU dL^{-1}. These observations are consistent with those reported by den Uijl *et al.* [13].

In patients with haemophilia A, joint bleeding results in increased morbidity, decreased health-related quality of life and higher treatment costs associated with the management of chronic arthropathy [21–24].

Prophylaxis is highly effective in preventing joint and other serious bleeding episodes [3–6,25], but a personalized approach to treatment [15] that takes into consideration an individual's PK – FVIII peaks and AUC as well as troughs – may have the potential to optimize the treatment regimen.

It is important to recognize that the findings from our analyses are specific to patients with severe haemophilia A (baseline FVIII ≤2%) receiving PKP administered every third day. The results cannot necessarily be extrapolated to other regimens and may not be applicable to alternate-day dosing. Indeed, we have previously reported that AUC was not associated with breakthrough bleeds in patients receiving prophylaxis three to four times per week [26]. Furthermore, almost all patients had pre-existing joint disease, with nearly 40% having ≥3 target joints at study initiation. The impact of joint status on FVIII levels at the time of bleeding is unclear. Finally, consistent with the results of den Uijl *et al.* [13], the influence of peak FVIII levels is likely to diminish as baseline FVIII levels approach the normal range.

The observation that lower peak levels were associated with increased bleeding in the PKP arm has interesting implications. Patients with the longest-half-lives required the lowest FVIII doses to sustain a level >1 IU dL^{-1} by 72 h and, therefore, also had the lowest peaks. Thus, paradoxically, those with the longest half-lives appeared to do less well on every third day prophylaxis targeted at a trough of 1 IU dL^{-1} than did those with shorter half-lives. In addition, owing to the shape of the PK curves in people with longer half-lives, those with lower peaks also spent more time with low FVIII levels between infusions. Whether these findings have implications for more frequent dosing intervals and/or for treatment with extended half-life molecules require further investigation.

Limitations to the analysis

Our analysis has several limitations. First, the data set is comprised of only 34 patients receiving PKP, and the analysis was performed *post hoc*. Second, information about FVIII activity at the time of each bleeding episode was extrapolated from PK data obtained at study entry and was calculated using patient-reported time of infusion and time of haemorrhage without adjustment for potential residual FVIII from prior dose. Third, the impact of a patient's lifestyle and participation in sports activities on bleeding could not be ascertained, as these data were not collected, but it is possible that they may have influenced bleeding in some patients. Finally, the possibility that some joint pain-related events may have been misclassified as haemarthroses, and that some bleeding episodes may

not have been recognized or were misclassified as traumatic (vs. spontaneous) cannot be ruled-out.

In conclusion, the findings from this *post hoc* analysis of data for the PKP arm of the rAHF-PFM prophylaxis study add to previous observations that time spent with FVIII levels <1% positively correlates with bleeding risk by demonstrating that in patients receiving every third day prophylaxis targeting a trough of 1 IU dL^{-1}, peak FVIII levels, AUC and time spent at high FVIII plasma levels are also associated with the risk for joint and non-joint bleeding. The data highlight the potential impact of variability in individual PK and the likelihood for haemorrhage and may support the need for higher peak levels and AUC in some patients on every third day dosing, such as those with underlying joint disease or during periods of increased activity. These conclusions do not necessarily apply to patients receiving alternate-day dosing, and further studies in this group are needed.

Acknowledgements

Author contributions

Myungshin Oh performed the statistical analyses; L. A. Valentino, S.W. Pipe, P.W. Collins, V.S. Blanchette, E. Berntorp, K. Fischer, B. Ewenstein and G. Spotts analysed and interpreted data; L. A. Valentino and S.W. Pipe prepared the manuscript, which was critically reviewed by the other co-authors.

Disclosures

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