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Population pharmacokinetics of recombinant factor VIII:C (ReFacto®) in adult HIV-negative and HIV-positive haemophilia patients

Anastasia Karafoulidou · Elena Suarez · Ioanna Anastasopoulou · Olga Katsarou · Anna Kouramba · Paraskevi Kotsi · Anastasios Zografidis · John C. Lukas

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Abstract

Purpose The aim of this study was to explore possible differences in the pharmacokinetics (PK) of recombinant factor VIII:C (ReFacto® - ReFacto) in HIV+ vs. HIV- patients and also differences in the chromogenic substrate bioassay (CHS) and one-stage clotting (OSC) methods.

Methods Twenty-eight haemophilia A adults (20 HIV- and eight HIV+) were assayed with both the CHS and OSC methods. An average of two and six samples were collected per patient for HIV-/+, respectively, after one, and occasionally two more, prophylactic doses (mean 2,003 IU; range 1,000–4,300 IU). The observations were analysed with the mixed-effects (population) compartmental PK modelling package NONMEM (nonlinear mixed-effects modelling) and the FOCE (first-order conditional estimation) method. Base modelling was performed independently for the CHS and OSC bioassays for comparison, and

covariate models and simulation tests were done only for the commonly used OSC bioassay. The final covariate model was validated using the bootstrap method. Monte Carlo simulations were used to estimate the expected probability of exceeding 20%, 40% or 60% of normal factor VIII:C in plasma after a single dose, corresponding to required levels for preventing mild, moderate and life-threatening haemorrhages.

Results One-compartment base-model population PK parameters were [mean parameter (interpatient variability %)] for CHS: clearance (CL)=2.56 dl h⁻¹ (33.2%); volume of distribution (V)=34.8 dl (12.8%); and for OSC: CL=3.83 dl h⁻¹ (47.8%), V=53.7 dl (22.4%). The volumes differed significantly between the CHS and OSC methods ($p<0.0001$), and variabilities were higher for OSC. Nevertheless, the empirical half-lives ($t_{1/2} = 1.44 \times V/CL$) were similar for CHS and OSC, [(mean ± standard deviation (SD)), 9.5 ± 3 h and 10.2 ± 4 h, respectively. In covariate modelling with the OSC-derived model, HIV status (VIR) was a significant categorical predictor ($p<0.005$) for V. The final covariate models with OSC were for CL=3.93 + 0.09 × (WT-75) and for V=48.6 × (1 + 0.36 × VIR) + 0.55 × (WT-75); therefore, V for the typical HIV+ patient was 36% higher than for the HIV- patient.

Conclusions Both HIV- and HIV+ patients showed 100% success with the 20% threshold at doses >20 IU/kg. HIV- patients receiving >50 IU/kg had a 100% expected chance of success for all thresholds. HIV+ patients for moderate or life-threatening haemorrhage treatment need 10 IU/kg more than the HIV- patient equivalent to have the same probability of success.

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Keywords Haemophilia · Factor VIII · Population Pharmacokinetics · HIV positive · Monte Carlo simulation proportions

Introduction

Factor VIII is a large protein with two components, coagulant factor VIII (factor VIII:C) and von Willebrand Factor (vWF), which binds to and protects factor VIII:C. Haemophilia A is a sex-linked disorder occurring in one out of 10,000 males where factor VIII:C is reduced or defective whereas vWF has normal levels. Haemophilia patients typically suffer from deep-tissue bleeding, haemarthrosis and haematuria and frequent surgical interventions to treat orthopaedic conditions. Haemophilia A is classified as severe (levels are <1% of normal), moderate (1–5%), mild (6–24%), subnormal (25–495) and normal (50–200%).

The introduction of replacement therapy with factor VIII:C concentrates 40 years ago revolutionised the care of patients with haemophilia A (congenital factor VIII deficiency or classic haemophilia). The factor is dosed intravenously (i.v.) as on-demand treatment for spontaneous bleeding episodes, before surgical procedures or as long-term prophylactic treatment to prevent haemophilic arthropathy. The relatively recent (2002) B-domain-deleted recombinant (BDDr) albumin-free antihaemophilia factor ReFacto[®] has been shown to have similar haemostatic properties to full-length serum-derived factor VIII:C [1, 2]. The dose amount of factor VIII:C is expressed in international units (IU), and activity in plasma can be expressed as a percent of normal, then 100% of normal is equivalent to 1 IU/dl. For each type of treatment, haemorrhage or surgery, target levels of activity have been established [3] ranging from 20 IU/dl for early haemarthrosis to 100 IU/dl for major surgery. The corresponding recommended dosage treatment is 20–50 IU/kg, depending on the type of treatment (occasional or long-term). Conventionally, 1 IU/kg of factor VIII:C is expected to increase plasma levels by 2 IU/dl [3, 4].

Nevertheless, monitoring may be required for factor VIII:C, as the dose to achieve specific levels varies among patients due to tolerance development, von Willebrand's disease or concomitant disease and treatment. HIV infection is in fact frequent among haemophilia patients. About 50% of patients treated between 1978 and 1985 with non-heat-treated factor VIII:C concentrates worldwide became infected with HIV. Most of these patients receive highly active antiretroviral therapy (HAART). It is still unclear how this comedication may influence the pharmacokinetics (PK) of factor VIII:C. In clinical practice, the dose to achieve similar efficacy is higher in HIV+ compared with HIV- patients [5, 6]. The overall unpredictability in achieving the desired levels can be attributed to variation in the drug's disposition kinetics between patients [7].

The recommended monitoring is via the area under the plasma concentration versus time curve up to 48 h postdose

(AUC[0–48]) using at least five time points [7, 8]. Once the (usually numerous) samples are collected and assayed, noncompartmental analysis (NCA) is easy to perform and amenable to automation. However, the NCA has less utility as a predictive tool, and its use compared with the advantages offered by compartmental PK is criticized [9–11]. Several studies have addressed the compartmental PK properties of factor VIII [12]. Such PK models address the underlying process of absorption, distribution, and elimination as model parameters, allowing for a better prediction. Population PK analyses resolve the observed variability into its interpatient and random assay within patient error components, but whereas these methods [13] have a long history of usage for the more common blood-clotting drugs, that is not the case with haemophilia treatments. Only recently a population analysis has been reported for factor VIII dose individualisation in healthy adults with haemophilia A before surgery [12, 14]. However, PK – and particularly population models – can address several of the above problems with factor VIII:C treatment. These include the reduction in the number of samples required for monitoring, as well as relating in a dose-independent manner the kinetics and the interpatient variability with covariates and patient subpopulations. Particularly regarding treatment for paediatric populations, both of the above capabilities are critical.

Yet another possibly confounding issue with factor VIII:C is the existence of two bioassay methods: One-stage clotting (OSC) depends on the measurement of clotting time in the presence of phospholipids and an activator and a system where all coagulation factors are present except factor VIII:C, which is derived from the tested sample. In contrast, the chromogenic substrate bioassay (CHS), recommended for the measurement of BDDr factor VIII:C [15] does not require the presence of all the other coagulation factors necessary for the OSC. The specific chromogenic substrate is hydrolysed by activated coagulation factor X (FXa), and its spectrophotometric intensity is proportional to the factor VIII:C present in the sample. However, the OSC is widely applied in haemostasis laboratories worldwide, as it appears to better reflect *in vivo* coagulation.

In this study, a population PK analysis was performed with ReFacto[®] observations assayed both with CHS and OSC methods in a mixed HIV- and HIV+ adult population in prophylaxis or episodic treatment. The primary purpose was to describe the population PK of a typical patient group in our hospital unit, including both HIV+ and HIV- patients, and where both OSC and CHS assays are customarily applied. The CHS and OSC population parameters were contrasted, but the focus of the covariate analysis was for the more commonly used OSC assay. Covariate predictor models were developed to explain the

interpatient kinetic variability, including the possible differences due to viral status. Then, Monte Carlo simulation was used to explore the probability of attainment of several levels of increase in coagulation rate for the 20–60 IU/kg dose recommendations, relevant to ambulatory patients such as ours.

Methods

Patients

The study was performed in the Haemophilia Center of Laiko University Hospital (Athens, Greece) with adult haemophilia A patients under on-demand treatment. Mostly severe haemophilia patients over 18 years old were included. All subjects signed an informed consent form related to the blood samples for PK determination, and approval of the Ethics Committee of the hospital was obtained. At the end of the study period, 28 adult Caucasian male haemophilia subjects had been included. Eight patients (28% of total) were HIV+ under HAART. Of 28 patients, 14 (50%) had severe, five (18%) had moderate, and seven (25%) had mild haemophilia. All HIV+ patients had severe haemophilia, except one patient with mild. No patient had antifactor VIII antibodies detected, and all had nominal levels of plasma von Willebrand factor and platelet counts $>100 \times 10^9/L$. All HIV+ patients were hepatitis C virus antibody (HCV-AB) positive, but none of them had decompensated liver cirrhosis.

A predose sample at time 0 was obtained from all subjects and ReFacto® (Wyeth, Andover, MA, USA) factor VIII:C was infused by the vein route in a bolus infusion. Postdose samples were collected from a different peripheral vein. The population PK methodology permitted reduction in the per-patient sample number to approximately three per patient. The HIV- patient sample ($N=20$) was divided into three groups, with different Clinical Report Forms for each, containing three recommended time points staggered across the total intended samples. In the end, the overall data set was to contain samples at 30 min, 1, 3, 6, 9, 12, 24 and 48 h postdose. The HIV+ patients were intensively sampled at all the above time points. Eventually, 140 activity level observations (ReFacto) were available for PK modelling.

Laboratory methods

Blood samples were collected into siliconised glass tubes containing trisodium citrate (volume 9:1). Samples were centrifuged for 15 min at 2,500 g at room temperature. Plasma was separated immediately after centrifugation in small aliquots, which were stored at -80°C . Factor VIII levels were determined using either CHS (Coamatic Factor VIII:C, Chromogenix Instrumentation Laboratory SpA

Milan, Italy), or the OSC (STA-factor VIII deficient plasma, Diagnostica Stago, Asnieres sur Seine, France) on an STA analyzer (by Diagnostica Stago). A standard curve was performed for both the assays before each run. Concerning the chromogenic substrate method, the ReFacto laboratory standard (99/694-9, four-factor FVIII IU) provided by Wyeth was used. The standard was diluted with factor-VIII-deficient plasma (Diagnostica Stago) to yield a final solution of approximately 1 IU/ml that was used for the calibration curve. The standard curve for the OSC assay was performed using the STA unicalibration by Diagnostica Stago. Levels in plasma were assessed as international units per millilitre expressed in this study as percent from normal.

Pharmacostatistical modelling

Data were analysed using a mixed-effects population PK modelling package [nonlinear mixed-effects modelling (NONMEM) version 6, NONMEM Project Group, University of Southern California, CA, USA] [16]. It employs an extended maximum likelihood (EML) method to estimate the PK parameter distributions in the populations, i.e. means and interpatient variability standard deviations (SD), expressed here as coefficient of variation percent (CV%). Importantly, the standard errors (SE) of these estimates are also obtained, providing a measure of goodness of fit for the parameters. The package also includes a Bayes estimator for obtaining the individual patient PK parameters. The Bayes estimator only requires a few or even a single blood sample and the knowledge of the population PK parameters previously extracted. Finally, the parametric population PK method permits modelling the influence of continuous covariates (predictors), (e.g. body weight or age), as well as categorical ones (e.g. HIV subpopulations) directly on the PK parameters.

Both one- and two-compartment i.v. bolus PK models were tested. The NONMEM and first-order conditional estimation (FOCE) method was used for all model development. Model diagnosis for selection among alternative PK structures, as well as the covariate elimination, was based on the following criteria: (a) The objective function change criterion. According to standard statistical methods, the change in the EML objective function is approximately chi-square (χ^2) distributed and can be used for model comparison with degrees of freedom equal to the difference in number of parameters. (b) The precision (SE) for the estimates of the estimated parameters. These estimates are assumed significant if their 95% confidence interval ($CI_{95\%}$) does not include zero.

Three covariates, weight, age and viral status (WT, AGE, VIR), were available for inclusion in the final covariate model. Continuous covariates (WT, AGE) were centered at the median, and the categorical covariate was factored as 0, 1.

Covariate model development followed three steps: (1) Forward inclusion sequence of each covariate separately at $p < 0.05$ for change in EML objective function ($\chi^2 = 3.8$) (one degree of freedom). (2) Full model run with all significant covariates from step 1. (3) Backward modeling steps with $p < 0.005$ ($\chi^2 = 7.8$) after removal of a single covariate term. Terms that were significant from step 3 were kept and constituted the final covariate model. The more stringent criteria for retention in the backward steps assures a more robust model. All analyses were performed in accordance with current US Food and Drug Administration (FDA) guidelines [17].

Due to the limited number of patients in the HIV+ subpopulation ($n = 8$), the bootstrap was used to verify the covariate model for VIR. For each of $n = 500$ occasions, the HIV- and HIV+ were resampled separately, then complete fits for the final model were performed and the distribution of the $n = 500$ estimates of the VIR coefficient and the corresponding distribution of EML objective functions were explored. The same procedure was followed for the null model (the coefficient set to zero), and the distribution of the objective functions contrasted with that for the final model.

Monte Carlo simulations

Simulations were performed in order to compare the probability of exceeding factor VIII:C plasma levels of 20%, 40% and 60% of normal for both HIV- and HIV+ subpopulations after a range of single doses. The above targets of normal factor VIII:C plasma levels correspond to the levels for early haemarthrosis—minor bleeding, extensive haemarthrosis and life-threatening haemorrhage, respectively. The final PK model in NONMEM was used to simulate virtual patients dosed at nine levels (20–60 IU/kg, at 5 IU/kg increments) ($N = 1000$ patients per level) and the proportion of patients above the three thresholds were calculated. For all simulations, the WT distributions for each subpopulation were sampled in parallel in order to calculate each patients' WT-dependent covariates [e.g. dose, clearance (CL), volume of distribution (V)]. The handling of simulated data and graphs was performed using S-plus (v.6.1, Insightful, Seattle, WA, USA).

Results

Factor VIII:C – ReFacto® was administered to 20 HIV- and 8 HIV+ patients, for a simple pharmacokinetic study in 20 patients and for haemarthrosis treatment in five patients, whereas patients received two doses in 12-h intervals as prophylactic therapy for minor surgical procedures. The doses differed between HIV- and HIV+, being higher for the latter (Table 1). The baseline (predose sample) levels for all

patients were lower than 23% for CHS and <18% for OSC, with the average baseline <3%, as most patients had severe haemophilia A. The dose-standardised AUC versus time curves over 24 h (AUC [0–24]/dose), obtained with the trapezoidal rule, varied less for CHS and OSC, respectively (Table 1).

Some of the overall ReFacto assay variability is visible in Fig. 1, which shows the observed coagulation activity levels in international units per decilitre (or percent improvement from normal) for the OSC. The CHS method assay shows higher coagulation activity compared with the OSC, as expected, with simultaneous CHS and OSC assays from the same blood sample (FDA, Summary Basis of Approval report for ReFacto, Ref. No. 98–0137). Figure 2 depicts a comparison of CHS vs. OSC reported ReFacto coagulation rates with percent $[100 \times (\text{OSC} - \text{CHS}) / \text{OSC}]$ differences ranging between 0% and 70% on the average for low- and high-activity levels, respectively, and 30% variability around these values.

Population PK analysis

An i.v. bolus, one-compartment disposition model represented the ReFacto activity level observations obtained from either the CHS or OSC assay better than two compartment alternatives. The factor VIII:C baseline were modelled with a mixed effects parameter (population mean) (C0) multiplying the observed baseline. The baseline was assumed to be patient specific and not change with time.

The base compartmental population PK parameters are listed in Table 2 for both bioassays. None of the $CI_{95\%}$ contained zero; precision was good, and overall variabilities were <50%. Notably, estimates for V differed significantly between the CHS and OSC assays ($p < 0.05$). Interpatient variability was elevated with the OSC method for both CL and V compared with the CHS, but parameter estimate precision was higher for OSC (lower CV%). In contrast, the residual error (σ) was higher for CHS. The population half-life was also lower (but not significantly) for CHS compared with that obtained from OSC. Importantly, the range of half-lives was also smaller with the CHS vs. the OSC method (Table 3).

Individual patient PK parameters were obtained with empirical Bayes estimation in NONMEM using the base model for OSC. In graphical exploration of the individual PK parameters for OSC vs. covariate relationships, V had an apparent trend with viral status. In forward inclusion, WT was a significant predictor covariate for CL, and WT and VIR were significant for V. The full model included three covariate terms on CL and V that were retained in backward removal. The final covariate model and parameter estimates for the OSC assay is listed Table 4 (28.7 objective function reduction vs. the base model). The

Table 1 Demographic and empirical pharmacokinetic (PK) parameter patient characteristics

Variable	Number	Mean ± standard deviation	Median	Range	Symbol
Age (years)	28	40.1±15.8	34	18 – 70	AGE
Weight (kg) ^a	28	74.8±11.1	75	54 – 104	WT
N patients HIV (+)	8 (29%)	-	-	-	VIR=1
N patients HIV (-)	20 (71%)	-	-	-	VIR=0
Dose of ReFacto® (IU) ^b					
HIV-	20	1449±524	1,500 (17 IU/kg)	1,000 – 2,500	AMT
HIV+	8	3583±715	3,500 (48 IU/kg)	1,620 – 4,300	AMT
Baseline OSC (IU/dl)					
HIV-	20	4.2±4.5	1.9	0 – 17	C0
HIV+*	8	1.5±2.9	0.3	0.1 – 9	C0
AUC[0–24] _{CHS} /DOSE (h/dl)**	28	0.245±0.110	0.219	0.133 – 0.632	AUC[0–24]
AUC[0–24] _{OSC} /DOSE (h/dl)*	28	0.181±0.110	0.146	0.072 – 0.486	AUC[0–24]

AGE age, WT weight, VIR HIV status, AMT, C0, AUC[0–24] area under the plasma concentration versus time curve over 24 h

^a No difference in WT for HIV- and HIV+ (mean of 76 and 74 kg, respectively)

^b Significant difference in doses for HIV- and HIV+ (*t* test *p*<0.05)

* All HIV+ patients had severe haemophilia (predose <1%) except one (predose=9%)

** Calculated with trapezoidal rule on observations after the first dose (AUCs differ; *t* test *p*=0.04)

unexplained variance in CL was reduced by 34% and that for V by 66% in the final model. The precision (CV%) for the CL and V estimates was also slightly improved in the final model, but those of the variances slightly increased.

The bootstrap distribution of the VIR coefficient parameter was significant (its CV% was <50%) in >75% of the runs. The EML objective function distribution mode for the null model (coefficient set to zero) was 548.9 vs. 533.4 for the full final model (a difference of 7.8 for 1

degree of freedom was significant at *p*<0.005). The viral status coefficient and hence the VIR covariate model were considered appropriate. Goodness-of-fit panel plots for the final covariate model with OSC are shown in Fig. 3. The model shows little structural bias (upper and lower left panels) seen in the spline fit, staying close to the identity and with the data clustering evenly and closely around the identity line. The individual patients (post hoc method) estimated response is very close to the observed for OSC

Fig. 1 Large plot: Time evolution profiles of ReFacto measured with one-stage clotting (OSC) Stago in HIV- (open circles with lines) (*n*=20) and HIV+ (thatched squares with dotted lines) (*n*=8) patients, with some separation due to different dose levels. Inset: The dose-corrected (first day dose only) circulating factor VIII after ReFacto i.v. (international units per decilitre is considered equivalent to percent of normal)

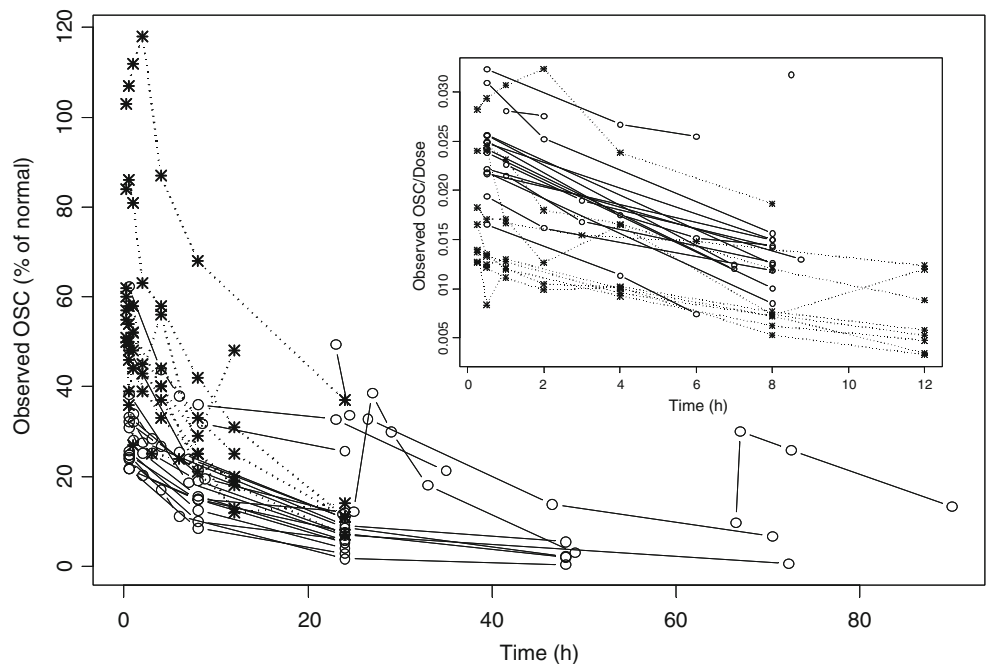
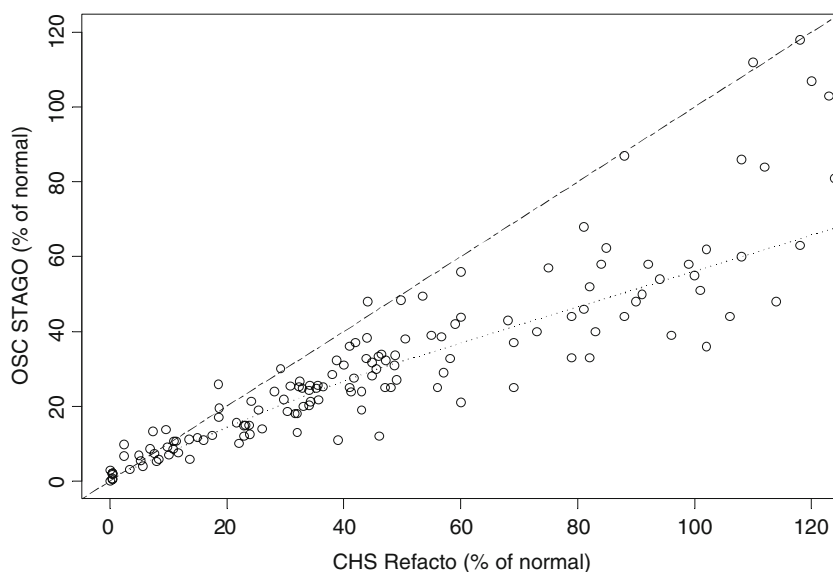


Fig. 2 Comparison between one-stage clotting (OSC) and chromogenic substrate bioassay (CHS) methods of ReFacto assay. *Dot-dash* is the identity line, and the *dotted spline* marks the mean trend



(upper right panel, observed vs. predicted factor VIII circulating levels). The model also represents well the observations in the few repeated dosing occasions (lower right panel, residuals across all treatment times).

Simulations

Figure 4 shows the expected proportion of patients (or probability for) reaching levels above threshold targets for HIV- (solid lines) and HIV+ (dotted lines). The tested targets were 20% (squares), 40% (triangles) and 60% (crosses) of normal factor VIII:C levels in plasma. It should be noted that the simulations are conditional on the patient's weight, so at each international unit per

kilogram level, the proportions are adjusted for (integrated across) all weights, similar to the ones in our population (Table 1).

Above 30 IU/kg, 100% of HIV- patients are expected to exceed both the 20% and 40% of normal thresholds and 50% of patients exceed the 60% level. Above 50 IU/kg, all thresholds are exceeded by all HIV- patients. However, for the HIV+ patients, a 10-IU/kg shift on the scale (more dose) is required to have the same expected success. With the more severe conditions, the HIV+ patients require >60 IU/kg to achieve 100% probability of success. Nevertheless, for the minor haemorrhage target of 20% of normal, both patient populations achieve near 100% recovery at low doses >20 IU/kg.

Table 2 Base one-compartment population (NONMEM – FOCE method) pharmacokinetic (PK) estimates for ReFacto in adults ($n=28$), measured either with the chromogenic substrate (CHS) or one-

stage clotting – Stago (OSC) bioassays. The within-subject variability includes model misspecification and sampling errors. (NONMEM objective functions: CHS=723.7; OSC=576.6)

	CHS	OSC
Parameter	Estimate (CV%) *	Estimate (CV%) *
CL (dl h ⁻¹)	2.56 (8.6)	3.83 (9.3)
V (dl)	34.8 (4.6)	53.7 (5.3) **
C0 multiplier	0.81 (21.)	0.86 (16.)
Between-subject variability		
ω_{CL} (%)	33.2 (31)	47.8 (27)
ω_V (%)	12.8 (58)	22.4 (23)
Within-subject variability		
σ (%)	23.3 (34)	15.3. (20)

CL clearance, V volume, C0, CV% coefficient of variation percent

* For the parameters, CV% of the estimate = $100 \times \text{standard error}/\text{estimate}$; for the variabilities, CV% is of the variance estimate

** Significantly different volumes of distribution between CHS and OSC ($p<0.0001$; by t test)

Table 3 Population-model-derived half-lives for chromogenic substrate (CHS) and one-stage clotting (OSC) bioassays

Variable	Number	Mean ± standard deviation	Range
$t_{1/2}$ CHS (h)	28	9.5±2.8	5.6 – 16.8
$t_{1/2}$ OSC (h)	28	10.2±4.2	3.9 – 23.8

Discussion

It is difficult to establish a fixed dose schedule for haemophilia A patients treated with factor VIII:C concentrates [4, 7, 18]. Very large inter and intra-patient variability in reaching specific factor VIII:C levels is observed after typical doses of 25-50 IU/kg [4]. The causes for the variability are complex and not easily resolved by linear correlations of a surrogate exposure measure with the dose. They are related to differences in haemophilia severity, to usually unknown von Willebrand factor levels or to concomitant diseases. Some haemophilia patients, many of them at a young age, became silently infected with HIV, concurrently with hepatitis C [19]. These patients have higher clotting factor utilisation [5]. Differences between the CHS and OSC assay methods, which can be clinically significant at low quantification levels, may also contribute to the overall

complexity [7]. In any case, the B-domain-deleted formulations do not seem to differ from other formulations [1, 2, 7].

From a practical point of view, the interpatient variability is attributed to the PK, and consequently, characterisation of individual patient PK is necessary for appropriate dosing of this costly treatment. Indeed, a recent joint report from the World Health Organization (WHO) World Federation of Haemophilia and International Society of Thrombosis and Hemostasis states that the use of coagulant products should be optimised and that considering the PK and its variability is fundamental to dosing.

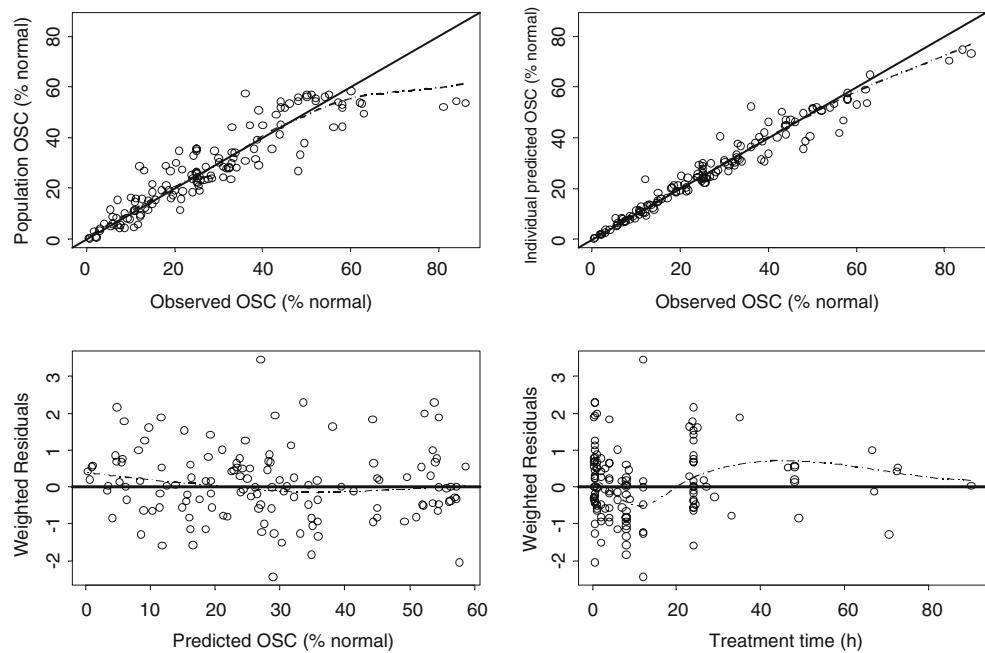
In this study, a population PK analysis was performed of ReFacto after a single dose of ReFacto® in a group of 28 haemophilia A adults (20 HIV- and 8 HIV+), assayed both with the CHS and OSC methods. One-compartment PK was found appropriate for both assays. Significant differences were found here in the volume of distribution (V) but

Table 4 Final covariate model for one-stage clotting (OSC) bioassay (objective function = 548)

Parameter	Value	Reduction vs. base model for OSC
CL _{median patient} (dl h ⁻¹)	3.93 (8.7)*	-
CL _{weight} (dl h ⁻¹ kg ⁻¹)	0.09 (24.6)*	-
V _{median patient} (dl)	48.6 (3.5) *	-
V _{weight} (dl kg ⁻¹)	0.55 (28.8)*	-
θ_6 (%)	36% (29%)*	-
θ_3	0.80 (16.1)*	-
Between-subject variability		
ω_{CL} (%)	38.9 (33.1) **	34% ***
ω_V (%)	13.0 (52.9) **	66% ***
Within-subject variability		
σ (%)	15.2 (20.9) **	Similar to base OSC model
Covariate effects (%)		
CL		
WT		-
Min: 54 kg	- 48% ****	
Max: 104 kg	+ 66% ****	
V : VIR=0		
WT		
Min: 54 kg	- 24% ****	
Max: 104 kg	+ 33% ****	
V : VIR=1		
WT		-
Min: 54 kg	- 17% ****	
Max: 104 kg	+ 24% ****	

CL clearance, V volume, VIR HIV status, WT weight, Min minimum, Max maximum
 $CL = CL_{median} + CL_{weight} \times (WT-75)$; $V = V_{median} \times (1 + \theta_6 \times VIR) + V_{weight} \times (WT-75)$;
 Baseline= $\theta_3 \times C_0$
 * CV% of the estimate = $100 \times \text{standard error}/\text{estimate}$
 ** CV% of the variance estimate
 *** $p < 0.001$, % reduction of the variances
 **** % with respect to the median. For example: $CL_{54kg} = 3.93 + 0.09 \times (54 - 75)$

Fig. 3 Goodness-of-fit plot for population and individual (post hoc) predictions vs. observed one-stage clotting (OSC) ReFacto assay (upper panels) and weighted residuals vs. predictions and time (lower panels) from a one-compartment pharmacokinetic (PK) model. *Solid line* is the identity line (upper two panels) or the zero line (lower panels). The *dotted line* is a spline fit



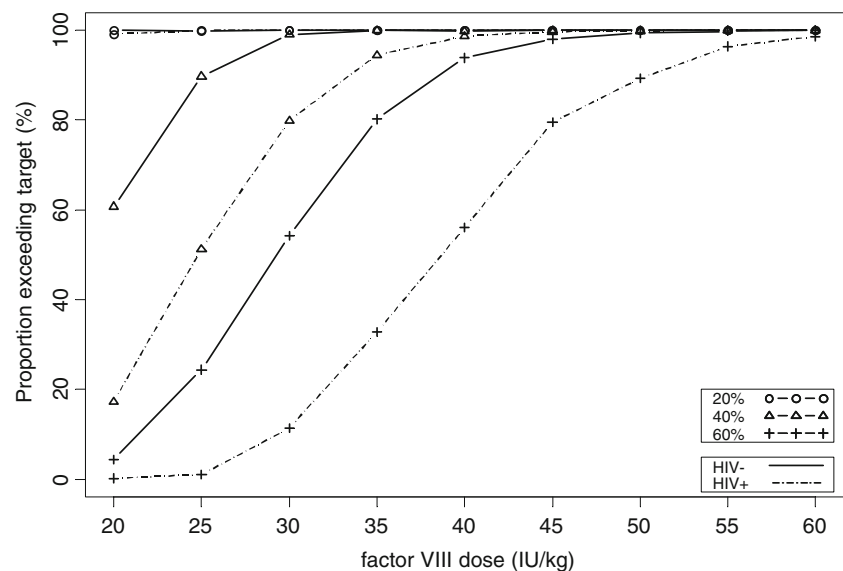
not for the half-lives between assay methods. When targeting a specific percent improvement in coagulation, initial dosing estimates will differ depending on the assay because of the difference in V between the CHS and OSC.

Importantly, the interpatient variabilities (for CL and V) were $>30\%$ higher with the OSC compared with the CHS method, suggesting that although the OSC is widely used, the recombinant-specific method would provide increased reliability when deciding on the next dose based on individual PK parameters. Nevertheless, the OSC method had 25% lower sampling error (16.2% vs. 21.8% for OSC and CHS, respectively), so in terms of the use of one or the other method parameters in estimating the uncertainty for

the next dosed patient, the two methods could be equivalent. The difference between methods in the interpatient variabilities (less for CHS) could mean that the CHS is actually a better assay. The difference in the sampling error (less for OSC) could be related to the CHS method being more prone to methodological faults in clinical laboratories. In this study, because of its wider use, the OSC method formed the focus of the rest of the analysis, covariate model and simulations.

Age, weight, and viral status were tested for significance as predictors (covariates) of the PK parameters by measuring the reduction in the unexplained interpatient variability after their inclusion/exclusion. In the final covariate model,

Fig. 4 Proportion of patients (probability percent) achieving specific target factor VIII:C levels after doses of 20–60 IU/kg for HIV- (solid lines) and HIV+ (dotted lines) patients. Target (maximum) levels were 20% (dots), 40% (upper triangles) and 60% (crosses) of normal



CL and V of ReFacto were predicted by WT. This relationship between CL, V and WT has been observed earlier for factor VIII [7]. However, no previous studies quantified the influence of HIV status on factor VIII PK. Here, we found that viral status did not relate to CL but was a significant factor for V, with that parameter increased by 36% in HIV+ patients. The WT and VIR covariates accounted for 34% and 66% of the unexplained variation across patients for CL and V, respectively. However, a portion of unexplained variability still remains, so monitoring may still be necessary, depending on the treatment and the patient's state [8, 11].

Regarding the dependence of V alone on viral status, it is not surprising that CL is not affected in these patients. The hepatic function is altered in HIV+ due to concomitant HCV, and possibly also HAART [19], but as only synthesis of factor VIII occurs in the liver and elimination occurs in blood, the pathology would be reflected in the baseline coagulation rather than in altered elimination. The increase by 36% in V for HIV+ patients, from 48.6 dl to 66 dl for the median-weight patient, explains the need for higher doses. The difference in V could be related to changes in the body mass/ body fat ratio or possible distribution outside the blood volume due to the disease and HAART provoking endothelial inflammation [20]. This effect would corroborate the increased incidence of haemorrhages in these patients [5, 6]. Our HIV+ patients had, indeed, lower baselines and required higher doses to achieve similar therapeutic levels as the HIV- patients.

Simulating based on this model showed that all HIV- and HIV+ patients achieved mean plasma levels >20% of normal for any of the recommended doses. However, the required levels for moderate or life-threatening haemorrhages were reached differently for the two patient subpopulations, with HIV+ patients requiring approximately 10 IU/kg more dose to achieve the same target with similar probability as the equivalent HIV- patient. Seen another way, HIV+ patients had, after for example 50 IU/kg of ReFacto, a probability reduced by 10–20% of reaching the same 60% of normal level as HIV- patients. Converting the thresholds into dose recommendation, the 20% of normal threshold requires doses around 20 IU/kg for both HIV- and HIV+ patients. The 40% threshold requires doses >30 IU/kg and >40 IU/kg for HIV- and HIV+, respectively, and the 60% threshold necessitates doses >50 IU/kg and >60 IU/kg for HIV- and HIV+ patients, respectively.

The assay dependence of PK parameters warrants consideration in future studies. The viral-status-dependent difference in the ReFacto PK (volume of distribution) observed here, unrelated to weight, could be associated either to the disease or to HIV treatment, and this was not clarified here. Further exploration of this effect in larger HIV+ patient populations is of interest. For other than minor haemorrhages, to achieve the

same factor VIII:C level, approximately 10 IU/kg more were needed in HIV+ patients.

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