Pharmacokinetics in routine haemophilia clinical practice: rationale and modalities—a practical review

Cedric Hermans and Gerry Dolan

Abstract: Prophylactic therapy with exogenous clotting factor concentrates in haemophilia A and B aims to achieve levels of circulating FVIII or FIX that are adequate for the prevention or reduction of spontaneous joint bleeding. Historically, a minimum trough level of at least 1% of the normal levels of circulating clotting factor has been targeted using standardised protocols. However, clearance of clotting factor varies between products and patients, and other pharmacokinetic (PK) parameters such as the frequency and magnitude of peaks may be important for ensuring optimal coverage. Thus, it is increasingly recognised that an individualised, PK-based approach to prophylaxis is necessary to achieve optimal protection.

This review focuses on the clinical implications of using PK-guided, individualised prophylaxis in haemophilia to improve patient outcomes and considers practical methods of establishing patients’ PK parameters. The most useful PK parameters will depend on the aim of the specific treatment (e.g. preventing activity-related and traumatic bleeds or addressing subclinical bleeding). In clinical practice, lengthy and frequent post-infusion sampling for PK analysis is costly and a significant burden for patients. However, a Bayesian analysis allows for the estimation of different PK parameters (e.g. half-life, factor concentrations over time, etc.) with only a minimum number of samples (e.g. 4, 24 and 48 h for haemophilia A), by using the patient’s data to adjust a relevant population PK value towards the actual value. Numerous tools are available to aid in the practical use of Bayesian PK-guided dosing in the clinic, including the Web-based Application for the Population Pharmacokinetic Service hosted by McMaster University, Canada. The PK data can be used to determine the appropriate prophylaxis regimen for the individual patient, which can be monitored by assessment of the trough level at each clinic visit.

Collection of PK data and subsequent PK-guided dosing should become standard practice when determining treatment strategies for people with haemophilia.

Keywords: extravascular FIX, haemophilia, individualised, pharmacokinetics, prophylaxis

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Introduction

Haemophilia A and B: classification and treatment

Haemophilia A and B are inherited bleeding disorders resulting from a partial or complete deficiency of factor VIII (FVIII) or factor IX (FIX), respectively. The severity of haemophilia has traditionally been classified according to a patient’s residual endogenous level of FVIII or FIX (levels below normal [50–150 International Units (IU)/dl]), with <1 IU/dl (or <1%) of the normal plasma levels being classified as ‘severe’. Most individuals with severe haemophilia will experience spontaneous bleeding in the joints or muscles, although the risk of bleeding is not confined to these sites and bleeding may occur anywhere in the body, including life-threatening intracranial
haemorrhage. It is well recognised, however, that for a notable proportion of individuals, no clear correlation between factor levels and bleeding phenotype (number of spontaneous or provoked bleeding episodes) is observed. Even within the group of patients with severe disease, there is substantial variation in bleeding phenotype. Similarly, patients with endogenous FVIII or FIX in the range 1–5% (classified as ‘moderate’) can present phenotypically as ‘severe’.

The primary goal of therapy in haemophilia A and B is to prevent bleeding episodes through replacement of the deficient clotting factor, usually by intravenous administration of exogenous clotting factor concentrate (CFC) derived from plasma or produced using biotechnology. Irrespective of the type of CFC used, the pharmacokinetics (PK) of replacement FVIII and FIX shows a rapid increase in measured plasma concentration, resulting in a ‘peak’ of activity, followed by progressive elimination (clearance) from the blood. The rate of clearance following infusion varies between individuals and at different life stages. The specific characteristics of the therapeutic CFC also influence clearance; for example, with standard half-life (SHL) FVIII products, exogenous FVIII is cleared from the blood relatively rapidly, with a half-life of 8–12 h with individual variation seen with different brands, whereas the half-life may be longer for FVIII products that have been engineered to extend the duration of plasma activity.

Prophylaxis protocols
Prophylaxis is considered to be the gold standard for haemophilia treatment: exogenous FVIII or FIX CFC is regularly administered, with the aim of achieving levels of circulating protein that are adequate for prevention or reduction of spontaneous joint bleeding, thus preserving normal musculoskeletal function. Historically, this meant targeting a minimum trough level of circulating FVIII or FIX, often defined as at least 1% of the normal levels. Notably, no fixed trough level applies to all patients, and data from a Swedish study found that some patients experienced joint bleeds with a FVIII trough level of >3 IU/dL. These findings indicate that the optimal trough level to prevent bleeding must be determined on a person-by-person basis, and simply aspiring to achieve a level of 1% for all patients is not appropriate.

Prophylaxis has historically been based on one of three well-studied protocols (Table 1). These protocols rely on the potential/ability of infused CFC to increase the circulating levels of FVIII and FIX soon after infusion, expressed as the recovery (~2 for FVIII and 1 for FIX) and a 50% elimination of the infused dose within 8–12 h for FVIII and 16 h for FIX. More recently, alternative/modified prophylaxis protocols have been trialled and efforts have been made to study low-dose prophylaxis as a real-world alternative to on-demand therapy for people in severely cost-constrained environments.

| Prophylaxis protocol | Dose frequency | |
|---------------------|---------------|---|
| Sweden/Malmö (high dose) | Every 2 days (25–40 IU/kg) | Twice-weekly (40–60 IU/kg) |
| Dutch/Utrecht (intermediate dose) | 3 days per week (15–25 IU/kg) | Twice-weekly (20–40 IU/kg) |
| Canadian dose escalation | Once-weekly; stepwise escalation if breakthrough bleeding occurs (50 IU/kg) | |

*Dosing may vary in different publications. IU, international units.
prevent bleeding,9,10 other parameters such as the frequency and magnitude of peaks may be important for ensuring optimal coverage during periods of physical activity.11 An individualised, PK-based approach to prophylaxis is therefore crucial for achieving optimal bleed protection.

Role of PK in individualising prophylaxis. Calculating the number of FVIII units required per dose of CFC for a patient with haemophilia A already includes the recovery value (peak plasma level of clotting factor/dose), which varies significantly with body mass index (BMI). The standard formula is as follows (assuming a FVIII recovery value of 2 for all patients regardless of their individual PK profile)12:

\[
\text{body weight (kg) \times desired FVIII increase (\%) / 2}
\]

A study examining the impact of being underweight or overweight on FVIII dosing in people with haemophilia A showed that patients who were underweight (BMI < 20.3 kg/m²) or overweight (BMI > 29.6 kg/m²) exhibited median FVIII recovery values of 1.60 and 2.70, respectively.12 In addition, Berntorp and colleagues noted that CFC half-life and clearance have also been shown to vary considerably between patients, independent of BMI, as illustrated in Figure 1.10,13 Exogenous CFCs behave kinetically differently in different individuals, suggesting that rigid treatment regimens with fixed dosing and timing of injection protocols are unlikely to deliver the objective of effective prophylaxis in all patients. The observation that increased time spent below the minimum trough level for an individual patient, and certainly below 1 IU/dl, is associated with increased total bleeds and haemarthroses,1,14 confirms the clinical consequences of not achieving continuous protection from bleeds. Prophylaxis regimens should therefore be adjusted to optimise bleed protection for each individual patient.15

This review will focus on the clinical implications of using PK-guided, individualised prophylaxis in haemophilia to improve patient outcomes, and will look at the practical methods of establishing patients’ PK parameters.

**Understanding the PK of CFCs**

**PK parameters: the basics**

Current consensus is that prophylaxis should be individualised according to the patient’s age, joint status, bleeding phenotype and level of activity.1 Access to sufficient CFCs is likewise important.1 How should the clinician identify the appropriate prophylaxis protocol for each patient?

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**Figure 1.** Effect of half-life on FVIII level following a bolus infusion. Following a standard weight-based bolus infusion of 30 IU/kg in patients aged 10–65 years, the time taken for FVIII to reach 1% can vary by as much as 59 h within the normal range of half-life. A short half-life is the 5th percentile and a long half-life is the 95th percentile of the normal range. Figure adapted from Collins et al.10

FVIII, factor VIII; IU, international unit.
PK is the study of the fate of substances, such as pharmaceutical drugs, that are administered to living organisms, and is concerned primarily with drug absorption, distribution, metabolism and elimination.\textsuperscript{5,16} As such, a range of parameters can be calculated to describe the PK of a drug, including absorption, bioavailability, volume of distribution, elimination half-life and clearance.

In practical terms, if a patient’s pharmacological response correlates well with the measured drug concentration in a clinical sample, then PK parameters can be used by the clinician to optimise approaches to dosing for that patient.\textsuperscript{17} For example, a previous study of methotrexate for the treatment of psoriasis revealed a strong correlation between the PK parameter ‘area under the curve’ (AUC) at the steady state and the anti-psoriatic effect of treatment.\textsuperscript{18} This study also demonstrated that, although PK varied widely between patients, each individual patient had a consistent PK profile, thus concluding that dose individualisation at the beginning of therapy would be beneficial.\textsuperscript{18} Similarly, gentamicin, an aminoglycoside antibiotic used for treatment of infections and for surgical prophylaxis, has been associated with serious dose-related adverse effects.\textsuperscript{19} It is therefore recognised that patients must receive the correct individualised dose and be monitored regularly during treatment.\textsuperscript{19} The dose required is adjusted according to numerous factors, including weight and kidney function, and monitoring whilst on treatment ensures that therapeutic, but not toxic, levels of gentamicin are being reached in each patient.\textsuperscript{19} Several possible dosing regimens are used, one of which is tailoring treatment according to the patient’s PK profile, which ensures accurate dosing, improved efficacy and reduced potential for toxicity—an approach that is considered particularly useful for patients with impaired renal function.\textsuperscript{19}

PK of CFCs

As most of the currently available CFCs are delivered via intravenous infusion, absorption is largely irrelevant and the important determinants of circulating plasma level are the dose and time of infusion, and the patient’s individual PK response to that dose.\textsuperscript{10,20} As a result, a patient’s PK response can be used to calculate the optimal dosing regimen required to maintain their CFC levels above a defined threshold, and to monitor their circulating CFC plasma levels during prophylaxis.\textsuperscript{20} This also ensures that they have coverage during periods of increased activity.

The standard visual expression of PK values is a decay curve, a plot of plasma–drug concentration over time. Decay curve graphs are produced by administering a known dose of the CFC to the patient and subsequently measuring plasma levels at various intervals (e.g. pre-dose, then 30 min, 1, 3, 6, 12, 24 and 48 h post-infusion for FVIII).\textsuperscript{20} A number of PK parameters have been identified as being useful for optimising CFC dosing in the treatment of haemophilia (Table 2).

Decay curves can be analysed using either a model-dependent or model-independent approach (Figure 2). A model-dependent approach, most widely used in routine clinical practice, relies on a model relating the factor level to time following infusion to estimate the time above a 1% threshold, half-life and IVR. For FVIII, a two-compartment model can be applied, comprising an initial distribution phase followed by an elimination phase.\textsuperscript{22} For FIX, a three-compartment model may be more appropriate (see Important differences for FIX).\textsuperscript{22} A model-independent approach differs in that it is based on the calculation of the AUC. Kinetic parameters estimated using this approach are CL, Vss and MRT. These two modelling approaches are complementary and should be considered jointly when estimating an individual’s PK parameters; however, specialised software is required to calculate PK using these approaches.

Several factors influence the PK of exogenous FVIII, including size of the molecule, distribution, clearance (CFCs are not cleared by the kidneys, but by the liver),\textsuperscript{23} binding to other proteins [e.g. FVIII binding to von Willebrand factor (vWF—a blood glycoprotein involved in haemostasis)],\textsuperscript{24} and modifications to exogenous CFCs (e.g. PEGylation or albumin/Fc fusion) that can affect distribution and rate of elimination.\textsuperscript{15} One other factor to consider is that the levels of circulating CFC must be accurately measured. However, laboratory results can vary depending on the assay used (e.g. one-stage or chromogenic substrate assay), the product-specific laboratory standard or the activated partial thromboplastin time (aPTT) reagent used, meaning that the precision and accuracy of laboratory assaying is a key consideration when using plasma activity level to estimate PK parameters for individualised dosing.\textsuperscript{15}
Selecting the correct parameters

Discussion in the community about which PK parameters are most useful for treatment decision-making in haemophilia is ongoing. CL, MRT and Vss provide a good overview of a patient’s PK response to infused CFC; however, trough level and length of time spent with a low level of coagulation factor may be better for determining the effectiveness of prophylaxis in preventing spontaneous bleeds. As trough level is

| Table 2. Standard PK parameters used to characterise clotting factors. |
|---|
| PK parameter | Description |
| Peak level \( (C_{\text{max}}) \) | Maximum clotting factor concentration following infusion |
| Trough level | Minimum clotting factor concentration reached following infusion and before the next dose is administered [can be used as a basic clinical measure of PK for dosing] |
| Half-life \(^{21}\) | Time taken for clotting factor concentration to reduce by 50% after equilibrium has been reached [e.g. from 100% to 50%, or from 25% to 12.5%] |
| CL \(^{21}\) | Volume of plasma cleared of clotting factor per unit time \[\text{dose administered/AUC (ml/h/kg)}\] |
| MRT \(^{21}\) | Average time [h] that a single molecule of clotting factor remains in the body |
| \( V_{ss} \) \(^{21}\) | Apparent volume [ml] in which an amount of clotting factor is distributed \[\text{CL} \times \text{MRT}\] following infusion when equilibration between plasma and surrounding tissues |
| IVR \(^{21}\) | Peak factor activity following infusion divided by expected peak of clotting factor activity \[\text{dose administered/estimated plasma volume of patient, expressed as U dl}^{-1} \text{per U kg}^{-1}\] |
| Incremental recovery | The peak factor level recorded in the first hour of infusion |
| AUC \(^{21}\) | The integral of the concentration–time curve. Relates to the total exposure of the body to the clotting factor over time. |

AUC, area under the curve; CL, clearance; IVR, in vivo recovery; MRT, mean residence time; PK, pharmacokinetic; U, units; \( V_{ss} \), Volume of distribution at steady state.

Figure 2. Methods of analysis of coagulation factor versus time curves. Representative FVIII decay curves for both model-dependent (left) and model-independent (right) analyses. For FVIII, a two-compartment model can be applied whereby there is an initial distribution phase and then an elimination phase. The model-independent approach is based on the calculation of the AUC.

AUC, area under the curve; FVIII, factor VIII.

Selecting the correct parameters

Discussion in the community about which PK parameters are most useful for treatment decision-making in haemophilia is ongoing. CL, MRT and \( V_{ss} \) provide a good overview of a patient’s PK response to infused CFC; however, trough level and length of time spent with a low level of coagulation factor may be better for determining the effectiveness of prophylaxis in preventing spontaneous bleeds. As trough level is
highly dependent on half-life, knowing a patient’s individual half-life is important for identifying the optimal dose. The most useful PK parameters will therefore depend on the exact aim of the specific treatment. For example, if the aim is to prevent activity-related and traumatic bleeds, peak plasma levels may be a key consideration, whereas if the goal is to address subclinical bleeding, AUC values may play an important role.

Björkman and colleagues analysed PK data from three studies and revealed differences in IVR, weight-adjusted CL and half-life when comparing the 1–6 and 10–65 years of age groups. PK cannot be accurately predicted from observed patient characteristics (e.g. age and weight) and must be determined for each individual. Reproducibility of PK parameters within a patient is essential for successful dose optimisation. Of note, proportions of intrapatient variance are much smaller than proportions of interpatient variance. It has been demonstrated that weight-adjusted CL is highly reproducible, meaning that CL, and thus AUC/dose, are the optimal parameters to use for product comparison studies. Additionally, reducing the blood sampling schedule is possible when measuring PK for individual patients, as long as the blood samples are taken at appropriate time points.

Numerous characteristics can impact on the calculated half-life of CFCs. For example, the half-life of FVIII has been shown to be reduced by approximately six-fold in the absence of vWF, whereas the half-life of vWF does not seem to be affected by the presence of FVIII. The patient’s age can also have an effect: shorter terminal half-life of recombinant FVIII was observed in children aged 1–6 years compared with people aged 10–65 years (9.4 versus 10.5 h, respectively). An increasing half-life has been correlated with increasing age (>10 years of age).

Considerations for FIX
Historically, prophylaxis with FIX has been largely based on observations in patients with moderate haemophilia A, who have fewer bleeds and less joint disease compared with those with severe disease. However, the PK of FIX appear to be more complex, and is less well-characterised and studied, compared with FVIII PK. Data from existing studies of the infusion of SHL FIX CFCs have demonstrated a lower IVR, a three-phase decay curve and a fast distribution half-life – all distinct from the PK of FVIII. Our understanding of the distribution of FIX is rapidly evolving and the concepts are not yet fully validated, requiring further study.

The notable differences in the PK of FIX compared with FVIII are most likely due to differences in their size and protein-binding characteristics. Although both have key roles together in haemostasis, the binding of these factors to other proteins and their localisation differ. FVIII, because of its relatively large size, predominantly circulates in the blood and forms a stable complex with vWF. When FVIII is not bound to vWF, it is rapidly degraded. The representative decay curves (Figure 2) are based on PK typically observed for FVIII. By contrast, FIX is much smaller in size and does not form a stable complex while circulating in the blood. In addition, unlike FVIII, which is restricted to the intravascular space, there is compelling evidence of an extravascular store of FIX (Figure 3). There are data to suggest that FIX binding to collagen IV in the extravascular endothelium may play a role in haemostatic function. However, the exact clinical significance of this is not yet fully understood and is still under investigation.

Early in vivo evidence for the existence of the extravascular space comes from experiments on baboons, carried out in the late 1980s, where, following administration of bovine FIX, the level of the infused bovine FIX decreased and a comparable and proportional rise in baboon FIX was observed over the same period. Another experiment revealed that, after 30 mins, bovine FIX levels in the blood were reduced to ~30% of the initial quantities. The bovine FIX was found to be widely distributed in the tissues, predominantly in highly vascular organs, such as the lung, kidney, liver, spleen and heart, and appeared to be bound to the blood vessel surface. Stern and colleagues concluded that there is a rapid and reversible equilibrium between blood and extravascular FIX. Feng and colleagues further analysed the data and proposed that the
The extravascular compartment may contain three times more FIX compared with that in circulation. In vitro competition experiments using bovine FIX and factor X demonstrated that FIX binding to endothelial cells in the vascular walls is tight, specific and reversible. The binding site of FIX was later revealed to involve the omega loop of FIX's Gla domain, and that the endothelial cell binding site is type IV collagen. The rapid initial loss of FIX from circulation appears to be due to its binding to collagen IV. When FIX is injected, 50–80% disappears within 5 min, and when continuously infused, the amount of FIX required for a level of 100% decreases with time, likely due to these binding sites becoming saturated.

From these data, it appears that FIX rapidly diffuses into the extravascular space, where it spends ~44% of its MRT. This results in the elimination half-life being longer compared with that of FVIII (18–20 h versus 12–14 h, respectively), likely due to FIX returning from the extravascular space to the plasma pool. A study in mice with haemophilia B revealed that infused FIX could still protect from bleeding 7 days following injection, although the plasma levels were <1% by Day 3, suggesting that the extravascular store of FIX is important for coagulation. Cooley and colleagues have provided further mouse data supporting the existence of the extravascular store of FIX and the potential importance of this store in the coagulation cascade.

As noted previously, in comparison with FVIII PK, which follow a two-phase model, the PK of FIX have been demonstrated to be better described by a non-linear, three-compartment model, as illustrated in Figure 4. Back-flow of FIX from the extravascular compartment to the central compartment would affect observed half-life and trough levels of FIX, which then would not directly correlate with CL. This model may explain why the terminal half-life of FIX is longer than that of FVIII, even though FIX CL appears to be higher.

A study by Hua and colleagues confirmed that sampling time is important for an accurate assessment of the PK of infused FIX: prolonging sampling collection times to 96 h gave longer half-life estimates compared with FIX activity observations made to more traditional 50- or 72-h schedules (half-life in patients aged ≥18 years of age averaged 40, 27 and 30 h, respectively). Unfortunately, data showing how the PK of FIX are associated with age and body weight are scarce, although the study by Hua and colleagues did confirm that half-life is shorter in younger versus older patients (half-life in patients aged 6–12 years of age averaged 28, 18 and 24 h, respectively). As the IVR of different SHL FIX products in different studies is highly variable (25–75%), and the PK of plasma-derived and recombinant FIX differ, treatment individualisation is likely to provide a more optimised approach for patients. The need for a more personalised approach in an era of

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**Figure 3.** FVIII and FIX distribution. Schematic demonstrating the theoretical difference in the distribution of FVIII and FIX. FVIII is restricted to the intravascular space, likely due to its large molecular weight. By contrast, FIX is a smaller protein and can distribute between both the intravascular and extravascular space. Therefore, the volume distribution for FIX will differ between the two.

FVIII, factor IX; FIX, factor VIII; K, elimination rate constant [K=ln(peak/trough)/time].

expanding treatment options in haemophilia B is further supported by emerging real-world data for patients switching to extended half-life (EHL) FIX products. Reports from a small cohort of haemophilia B patients in the United States (US) showed unexpected spontaneous or minimally traumatic bleeding, or poorly controlled bleeding following a switch to EHL FIX products, warranting switchbacks to SHL FIX products for some patients. The observed, individual response of patients further supports the need for continued, real-world monitoring following switching, to fully understand the role of individualised PK-based dosing in haemophilia B.

Application of PK to haemophilia treatment

How can PK parameters be calculated in the clinic?

Although data clearly show that PK-guided prophylaxis can help to manage under- or over-treatment of patients, and potentially optimise outcomes, this approach is still not used routinely in many centres, perhaps due to the burden of classical PK sampling requirements on clinicians and patients. The International Society on Thrombosis and Haemostasis guideline for PK studies of novel FVIII concentrates recommends that, for a full PK profile, there is a washout period of 72 h, followed by obtaining 7–10 samples over 32–48 h [e.g. at pre-dose (baseline), then 10–15 and 30 min, and 1, 3, 6, 9, 24, 28, 32 and 48 h post-infusion]. In addition, the patient may require overnight hospital admission to support these time points. The introduction of new EHL products has led to extended sampling times out to 72 h post-infusion. As a result, utilising these extended sampling times in studies of EHL products – using SHL rFIX as a comparator – has revealed a longer terminal half-life compared with our previous understanding.

**Figure 4.** Three-compartment model. A three-compartment model has been proposed to describe the PK of FIX. Concentrate is infused into the plasma, then rapidly redistributes to compartment 2 [e.g. a protein binding site] and 3 [e.g. the extracellular space]. This steep drop in plasma concentration correlates with section A of the graph. At saturation of compartment 2 binding sites or at equilibrium in compartment 3, the concentrate begins to redistribute back towards the central compartment, resulting in a relative increase in plasma concentration – this appears on the concentration – time curve as a slower [section B] and then slowest [section C] decrease in concentration. Figure adapted from Iorio et al. ©Georg Thieme Verlag KG. FIX, factor IX; PK, pharmacokinetic.
colleagues, a reduced blood sampling schedule is possible. A Bayesian analysis can be performed, requiring no washout period and involving fewer patient blood samples. This approach allows for the estimation of complex PK parameters with only a minimum number of samples. One analysis of data demonstrated that samples taken at 4, 24 and 48 h provided similar results to a conventional study using 7–10 samples. To prevent inaccuracies in the data, sample intervals must be sufficiently spaced, and no earlier than 4 h post-dosing to avoid the irregularities in the initial part of the FVIII activity versus time curve, or later than 48 h, as these risk reaching the limits of the assay. Bayesian analysis using a population PK model can be performed on data from almost any dosing schedule, requiring the doses and infusion times for the most recent five half-lives prior to the study dose. This analysis is performed by first assuming that the individual’s PK values are identical to those of a relevant population of patients, followed by using the patient’s data to adjust the estimate towards their actual value. Using this modified approach, it is possible to calculate all of the parameters used for dose adjustment, such as the AUC and $C_{\text{max}}$.

**Community support tools to aid PK-based dosing**

Population PK may be a useful approach to support individualised dosing – particularly when multiple PK samples for an individual patient cannot be measured/ endured. With a population-based approach, a PK model can be generated using a small number of samples from the individual. In a 2019 study, Megías-Vericat and colleagues demonstrated that PK-guided prophylaxis using Bayesian analysis with limited sampling supported bleeding control in people with haemophilia A without increasing FVIII consumption. This methodology can facilitate calculation of PK parameters in routine clinical practice, reducing inconvenience to the patient.

Numerous tools are available to aid in the practical use of Bayesian PK-guided dosing in the clinic. Some are product-specific and are available from the manufacturers. However, in recent years, there has been a move towards a more globally accessible online tool, resulting in the Web-based Application for the Population Pharmacokinetic Service (WAPPS-Hemo), hosted by McMaster University (Hamilton, Ontario, Canada).

Pharmaceutical companies and independent investigators are invited to contribute existing PK data for individuals with haemophilia A and B, to gather FVIII and FIX data across a range of products to develop a larger database in support of more robust population PK models. The resulting database allows those participating to securely input FVIII and FIX plasma levels from sparse samples to calculate population PK estimates for individual patients treated with all existing CFCs using the designed models. Of note, a key limitation of the WAPPS-Hemo tool is the requirement for users to have a basic understanding of PK.

The overall aim of WAPPS-Hemo is to provide a centralised, web-accessible service to improve haemophilia treatment, by facilitating assessment of individual PK parameters, allowing good-quality estimates of individual PK parameters from fewer samples and to improve knowledge of the PK of FVIII and FIX. Population-based PK using tools such as WAPPS-Hemo should be promoted as routine replacement therapy evaluation and follow up of each patient on prophylaxis, which may include annual sampling.

**How to use population PK**

In support of more routine use of PK in clinical practice, we propose the following practical guide for the adoption of population PK in the clinic (Table 3).

**PK in clinical practice**

*Strategies for implementing PK-guided dosing into routine clinical practice.* Prophylactic protocols should take into account the local availability of CFCs, venous access, patient’s lifestyle and need for coverage, and the motivation of the patient to adopt the proposed regimen. The patient should understand why the regimen has been proposed, and should be capable of adhering to it. When the patient has started the new regimen, they should be monitored for their ability to comply with the prescribed regimen over time, and there should be objective assessment of the outcome of prophylaxis. Even calculation of the trough level at each clinic visit may be a useful, simple and highly practical approach to validating aspects of the prophylactic regimen (and to allow changes to be
made to the regimen, if required). The time delay between infusion and sampling should be carefully recorded.

**Steps to implementing a PK-guided approach to prophylaxis in the clinical setting**

1. Determine the PK behaviour of the particular concentrate used by the individual patient;
2. Evaluate the patient’s joint status (e.g. through the use of clinical score or imaging techniques such as ultrasound or magnetic resonance imaging), physical activity/lifestyle and bleeding phenotype;47
3. Use PK data to determine the appropriate treatment regimen (dose per infusion/frequency of infusion) that would generate the appropriate peaks (timing and magnitude) and maintain a trough level that would be sufficient to prevent spontaneous bleeding according to the individual patient profile;
4. Discuss with the patient their goals and expectations from treatment regimen, and factor this into decision-making. Additionally, promote adherence to the treatment regimen by educating patients to understand the importance of PK

**Trough levels in practice.** Figure 5 illustrates trough levels in practice.

**EHL FVIII and FIX**

A major advancement over the last decade has been the development and validation of EHL FVIII and FIX molecules using various technologies. These EHL products have been developed utilising various technologies to prolong the circulating half-life of CFCs, such as Fc fusion, PEGylation and albumin fusion,15 resulting in a

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**Table 3. How to use population PK.**

| Question                              | Answer                                                                                                                                                                                                 |
|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| How should I organise the clinic visit? | Time of infusion and/or visit should be organised to ensure that adequate sampling can be taken at the required time points, while taking into consideration patient convenience |
| What are the ideal measurement time points? | FVIII should be measured ideally between 4 and 48 h post infusion20                                                                                                                                     |
| What population PK software/tools should I use? | You will need availability/access to tools such as WAPPS-Hemo, or product-specific tools developed by the product manufacturer                                                                 |
| What information is required?          | Product factors including type and dose administered                                                                                                                                                   |
|                                       | Timing of infusion and subsequent blood sampling                                                                                                                                                        |
|                                       | Patient factors for example body weight, height, age, blood group, vWF levels (ideally) and baseline factor activity level                                                                               |
| What assay should I use to measure factor levels? | The most appropriate, well-validated assay should be used, taking into consideration the variability observed depending on the assay type, standard used and aPTT reagent used. All samples should be assessed using the same assay protocol. Ideally a product-specific assay should be used. |
| Once the results are received, what should I do with these data? | Integrate the results into patient files, notes, etc.                                                                                                                                                   |
|                                       | Communicate and explain the results to the patient                                                                                                                                                      |
|                                       | Adapt the treatment regimen, if required, based on the results                                                                                                                                          |

*APTT, activated partial thromboplastin time; FVIII, factor VIII; PK, pharmacokinetic; vWF, von Willebrand factor.*
large molecular size due to the addition of the conjugated protein. In haemophilia B, whether this increased size influences the distribution into the extravascular compartment for FIX is unknown; however, initial measured recovery values following infusion are 20–94% greater for EHL FIX products compared with that of wild-type FIX, suggesting that there may be a difference in distribution. Currently, limited data exist to determine whether modified FIX proteins have a different binding affinity to collagen IV and whether or not this is important with respect to haemostatic function. Cooley and colleagues have suggested that future research on EHL FIX products should include direct comparisons with FIX_{WT} and focus more on clinical outcomes rather than plasma serum levels.

A clear understanding of the PK properties and the implementation of PK analysis in the clinic is key to accurate assessment and support for patients. As new products become available, appropriate PK measurements should be performed to ensure optimal, individualised treatment for patients, including before and after initiating any new treatment regimen. This will be increasingly important to enable patients to realise the full potential benefit of new transformative therapies, such as gene therapy.

**A look to the future**

A key question regarding CFCs is, what should be the treatment goal? For certain patients, higher clotting factor trough levels would provide...
improved protection during physical activity or may prevent spontaneous breakthrough bleeding or subclinical bleeding and subsequent joint disease. However, less active and other patients may prefer prophylaxis with fewer injections, which may improve treatment adherence.48 However, it is worth noting that poor adherence is a complex, multifactorial challenge that warrants further understanding at an individual patient level. It is therefore important that treatment is individualised to ensure that the optimal outcome is achieved for each patient. Collection of PK data and subsequent PK-guided dosing are instrumental for ensuring optimal treatment and should become standard practice when determining treatment strategies for people with haemophilia.

Using population-based PK models will help to overcome the cost barriers and logistical burden of performing PK evaluation in every patient and the challenges with obtaining multiple samples from young children.9 Further research is required to improve our understanding of how best to optimise care through PK-guided dosing. Aspects to be taken into consideration when tailoring therapy for both haemophilia A and B include not only patients’ PK responses to infused coagulation factor (including conjugated products), but also their bleeding phenotype, lifestyle (activity, job, etc.), activity levels, presence of target joints and history of haemophilic arthropathy.20 Considering the inter-individual variability of clotting factor elimination, and as recommended by the recent WFH guidelines,1 individual PK-guided dosing should be used to maintain clotting factor levels above those required to prevent bleeding complications for each patient, as opposed to a general target level for all patients, taking into account the variables mentioned above.

There may be particular complexities in applying measured FIX plasma levels to PK-driven prophylaxis for haemophilia B, as for some, if not all patients, the plasma FIX level may not fully reflect the full FIX reservoir that is available for haemostasis at the time of sampling.49

A comprehensive, in-depth review of the history and current landscape of the role of PK in haemophilia emphasises that ‘one size does not fit all’.16 Whatever the ultimate goal of therapy, delivering personalised, PK-guided prophylactic dosing should help to optimise patient outcomes and use of treatment, now and in the future.

**Recommendations for clinical practice**
- Promote a good understanding of the PK behaviour of CFCs in people with haemophilia among clinicians, other healthcare professionals (e.g. nurses, physiotherapists) and patients;
- Support a shared understanding in the clinic of PK parameters (significance of peaks and troughs in individual patients);
- Obtain PK parameters (recovery, trough and, ideally, half-life and AUC) using population-based approaches in each patient on prophylaxis and also when considering switching (at time of product switch);
- Use the PK profile to determine the individualised prophylactic treatment regimen, considering bleeding phenotype, joint status, physical activity (including periods of physical activities and sport), patient motivation and expected adherence to a given regimen;
- Regularly monitor and reassess the individualised prophylactic treatment regimen over the patient’s life, and adjust if required.

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