DR JILL M JOHNSEN (Orcid ID : 0000-0002-2279-2550)
DR STEVEN W PIPE (Orcid ID : 0000-0003-2558-2089)
DR JAMES O'DONNELL (Orcid ID : 0000-0003-0309-3313)

Article type : Review Article

BIOLOGICAL MECHANISMS UNDERLYING INTER-INDIVIDUAL VARIATION IN FACTOR VIII CLEARANCE IN HEMOPHILIA

Peter L. Turecek [1], Jill M. Johnsen[2,3], Steven W. Pipe[4], and James S. O'Donnell[5,6,7] on behalf of the iPATH study group.

[1] Baxalta Innovations GmbH, A Member of the Takeda Group of Companies, Vienna, Austria.
[2] Bloodworks Northwest Research Institute, Seattle.
[3] Department of Medicine, University of Washington, Seattle, Washington, USA.
[4] Departments of Pediatrics and Pathology, University of Michigan, Ann Arbor, Michigan, USA.
[5] Haemostasis Research Group, Irish Centre for Vascular Biology, Royal College of Surgeons in Ireland.
[6] National Children's Research Centre, Our Lady's Children's Hospital, Dublin, Ireland.
[7] National Coagulation Centre, St James's Hospital, Dublin, Ireland.

Running Title:	**FVIII clearance variation in PWH**
Text word count:	**3804**
Abstract word count:	**215**
Figure / Table count:	**2/1**
Reference count:	**60**

Editorial correspondence should be addressed to:
Prof. James O'Donnell,

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/HAE.14078

Irish Centre for Vascular Biology,
Royal College of Surgeons in Ireland,
Ardilaun House, 111 St. Stephen's Green, Dublin 2, Ireland.
Tel +353 (1) 416 2141; Fax +353 (1) 410 3570;
e-mail jamesodonnell@rcsi.ie

ABSTRACT

Previous studies have highlighted marked inter-individual variations in factor VIII (FVIII) clearance between patients with haemophilia (PWH). The half-life of infused FVIII has been reported to vary from as little as 5.3 hours in some adult PWH, up to as long as 28.8 hours in other individuals. These differences in clearance kinetics have been consistently observed using a number of different plasma-derived and recombinant FVIII products. Furthermore, recent studies have demonstrated that half-life for extended half-life (EHL-) FVIII products also demonstrates significant inter-patient variation. Since time spent with FVIII trough levels less than 1% has been shown to be associated with increased bleeding risk in PWH on prophylaxis therapy, this variability in FVIII clearance clearly has major clinical significance.

Recent studies have provided significant novel insights into the cellular basis underlying FVIII clearance pathways. In addition, accumulating data have shown that endogenous plasma VWF levels, ABO blood group and age, all play important roles in regulating FVIII half-life in PWH. Indeed, multiple regression analysis suggests that together these factors account for approximately 34% of the total inter-individual variation in FVIII clearance observed between subjects with severe hemophilia A. In this review, we consider these and other putative modulators of FVIII half-life, and discuss the biological mechanisms through which these factors impact upon FVIII clearance *in vivo*.

KEYWORDS

– Haemophilia A, Factor VIII, von Willebrand factor, Clearance, Pharmacokinetics

INTRODUCTION

Haemophilia A is an X-linked inherited bleeding disorder caused by deficiency or dysfunction of procoagulant factor VIII (FVIII). Approximately 25 in 100,000 male births are affected by hemophilia A.[1] Consequently, there are estimated to be more than 1 million persons with hemophilia worldwide.[1] Patients with severe hemophilia A (PWH) have plasma FVIII < 1 IU/dL (<1% normal). These individuals typically develop spontaneous bleeding in joints and muscles from early childhood which result in progressive musculoskeletal deterioration.[2] In order to reduce bleeding, patients with severe hemophilia A require regular

FVIII replacement treatment. Several studies have confirmed that FVIII prophylaxis reduces the number of spontaneous joint bleeds, thereby significantly attenuating hemophilic arthropathy.[2]

Initial FVIII replacement studies first with single donations, and later with FVIII concentrates derived from human plasma, commenced in the 1950s based on the observation that spontaneous hemarthroses were much less common in patients with moderate hemophilia (FVIII 1 – 5 IU/dL; 1 – 5%). Thus, patients with severe hemophilia were treated with the aim of maintaining trough FVIII levels > 1%. From the 1990s, availability of recombinant FVIII products with standard half-life and later also modified FVIII with extended half-life enabled targeting higher trough levels of 3%, 5%, or even 10%, which could further reduce the likelihood of bleeding and might improve the joint-health status of PWH.[3,4]. Based upon an estimated FVIII plasma half-life of approximately 12 hours, prophylaxis was typically administered as a dose of 25-40 IU/kg three times weekly. Critically however, accumulating data have demonstrated that this '*one size fits all'* weight-based approach to hemophilia prophylaxis has inherent limitations.[5] In particular, it is clear that in vivo clearance of FVIII varies markedly between different PWH.[6,7] Despite this significant inter-individual variation in FVIII half-life, the mechanisms underlying FVIII clearance have remained poorly defined for many years. Recent studies have provided important insights into the biological mechanisms underpinning physiological and pathological FVIII clearance.[8,9] In this manuscript, we review the molecular and cellular mechanisms responsible for modulating inter-individual variations in FVIII clearance in PWH, and consider its clinical significance.

Inter-individual variation in FVIII clearance between PWH

Numerous pharmacokinetic (PK) studies have consistently demonstrated marked inter-individual variations in FVIII clearance between PWH (**Table 1**).[6,10-17] Collectively, these studies suggest that FVIII half-life may vary from as little as 5.3 hours in some PWH, up to as long as 28.8 hours in other individuals. These differences in clearance kinetics have been observed using a number of different plasma-derived and recombinant FVIII products (including full length and B-domain deleted rFVIII) and appear to be independent of the dose of FVIII administered.[10-12] Moreover, similar inter-individual variations have been observed in studies of PWH from different ethnic and racial origins.[14] FVIII clearance kinetics are significantly more rapid in young children compared to adult PWH.[16,18] For example, Bjorkman *et al* found that the terminal half-life of FVIII in children aged 1-6 years was 9.4 hours compared to 11.1 hours in a combined group of adolescents and adults (aged 10-65 years).[16] Nevertheless, significant inter-individual differences in FVIII clearance have also been reported in studies of pediatric cohorts.[14,15] In contrast, for a given individual patient, FVIII half-life remains relatively consistent during adulthood at least until the age of 40

years.[6,10,16,19] However, it has been shown that the half-life of infused FVIII is significantly reduced in PWH during acute bleeding episodes and in the perioperative period.[20]

FACTORS INFLUENCING INTER-INDIVIDUAL VARIATION IN FVIII CLEARANCE

1. VWF levels and binding affinity

Under normal conditions, the majority of plasma FVIII circulates in high affinity complex (K_d ~ 0.2-0.5 nmol/L) with von Willebrand factor (VWF) (**Figure 1)**.[8,21] The plasma concentrations of FVIII and VWF are 200ng/ml (0.8nM) and 10µg/ml (35nM) respectively. Consequently, under steady-state conditions there is a 50 Molar excess of VWF. In normal individuals it has been estimated that approximately 95-97% of FVIII is bound to VWF, whilst the remaining 3-5% circulates as free FVIII.[8] Importantly however, this interaction between VWF and FVIII is reversible and exists as a dynamic equilibrium.[22] Interaction with VWF plays an important role in protecting FVIII against premature proteolytic degradation and clearance. In the absence of VWF-binding, the half-life of free FVIII is reduced approximately 6-fold. This enhanced clearance underlies the reduced plasma FVIII levels typically seen in patients with Type 2N (characterized by reduced VWF binding to FVIII) or Type 3 VWD (almost complete VWF deficiency). Conversely, VWF half-life is not affected by the presence or absence of FVIII.

Given this key role for VWF in regulating FVIII clearance in normal individuals, it is perhaps unsurprising that plasma VWF:Ag levels at time of FVIII treatment correlate with the half-life of infused FVIII. Several studies have demonstrated that FVIII half-life is significantly longer in PWH who have higher endogenous plasma VWF:Ag levels.[10,12-15,17,21] This effect of VWF levels in regulating FVIII clearance is consistent for both pd- and rFVIII products.[6,16] For example, in a study of 12 patients Fijnvandraat *et al* observed a strong correlation between pre-infusion VWF:Ag levels and half-life for a BDD-rFVIII product (r = 0.87; P = 0.0003).[10] Subsequently in a larger cohort of 32 PWH, Vlot *et al* confirmed a positive correlation between VWF:Ag levels and pd-FVIII half-life (r = 0.52; P = 0.001).[11] Using linear regression analysis, the authors estimated that VWF levels accounted for approximately 25% of the total inter-patient variability observed in FVIII half-life.[11] Calculations suggest that each increase of 0.1 IU/dL VWF:Ag is associated with an increase of 16.6 (95% CI 9-24) minutes in the half-life of infused FVIII ($P < 0.01$).[12] Recent pediatric studies have showed that FVIII pharmacokinetics in children with haemophilia A are also significantly influenced by endogenous plasma VWF:Ag levels.[14,15]

In addition to the importance of pre-infusion plasma VWF:Ag levels in regulating the half-life of FVIII therapy in PWH, recent studies have demonstrated that alterations in the FVIII

binding capacity of VWF may also contribute to inter-individual variations in FVIII clearance. Swystun *et al* investigated FVIII binding activity in 43 pediatric PWH.[15] Sequencing of the FVIII-binding region of VWF identified five patients heterozygous for two low-frequency variants (p.Arg826Lys and p.Arg852Glu) both of which were shown to attenuate FVIII-binding. Another study suggested that structural elements of the huge VWF protein chains and the degree of multimerization may influence FVIII binding.[23] Collectively, these findings suggest that subtle reductions in the FVIII-binding capacity of endogenous VWF have the potential to lead to enhanced clearance of infused FVIII in PWH.

2. ABO blood group

Although traditionally regarded as red blood cell antigens, the carbohydrate structures that constitute the ABO blood group system (*A, B* and *H* determinants) are actually expressed on a number of other tissues and cell types including endothelial cells (EC) and platelets. Recent evidence suggests that the majority of plasma VWF and FVIII are derived from constitutive EC secretion. Prior to this secretion, VWF and FVIII undergo complex post-translational modification that includes significant glycosylation.[24] Unlike the vast majority of other plasma proteins, mass spectrometry studies have demonstrated that plasma VWF and FVIII both express covalently linked ABO(H) structures on their glycan structures. This is important because ABO blood group has a major effect on plasma levels of the VWF-FVIII complex. In particular, blood group O individuals have VWF:Ag levels that are approximately 20-30% lower compared to non-O individuals.[25] ABO group has similar effects on plasma FVIII:C levels in normal individuals, although these effects appear to be predominantly mediated via the changes in VWF levels. The mechanism(s) through which ABO blood group influences plasma VWF:Ag levels has not been fully elucidated, but current evidence suggests that VWF clearance is significantly enhanced in group O compared to non-O individuals.[26] Interestingly, platelet VWF does not carry AB blood group determinants and levels are not influenced by ABO blood group.[27]

Given the major effect of plasma VWF:Ag levels in determining the half-life of infused FVIII in PWH, it is perhaps unsurprising that several studies have reported that ABO blood group also influences FVIII pharmacokinetics.[11,13,17] Vlot *et al* showed that FVIII half-life was significantly reduced in group O PWH compared to group A PWH (15.3 versus 19.7 hours respectively; $P = 0.003$).[11] Similarly, Fischer *et al* observed significantly enhanced FVIII clearance in group O compared to non-O adult PWH (11.5 versus 14.3 hours; $P = 0.004$).[13] This later study further observed that plasma VWF:Ag levels correlated more strongly with FVIII half-life in non-O compared to group O PWH. Despite the fact that ABO blood group prevalence varies between racial groups, the ABO effect in modulating FVIII clearance in PWH has been consistently observed in studies that have enrolled a variety of different

ethnicities. Finally, although FVIII clearance is significantly faster in children compared to adult PWH,[18] nonetheless an ABO effect on FVIII half-life is still apparent in pediatric PK studies.[14,15]

3. Age

It is well recognized that plasma VWF:Ag levels increase progressively with age. This observation has been reported in normal individuals, as well as in patients with VWD.[28,29] Indeed, plasma VWF:Ag levels have been shown to correct into the normal range with advancing age in many patients with mild quantitative VWD.[30] Furthermore, significant age-related increases in plasma VWF levels have also been observed in PWH. van Dijk *et al* demonstrated that every ten years increase in age in PWH was associated with a 0.16 IU/dL increase of in VWF:Ag levels.[12] Interestingly, the age-related increase in plasma VWF:Ag has also been reported to be more marked in non-O compared to group O individuals.[28]

Since endogenous VWF levels have an effect in regulating infused FVIII half-life in PWH, several groups have investigated whether age may also have an effect. Some inconsistency in results has been observed between studies, which may reflect limited sample numbers, as well as other differences in study design. Nevertheless, a number of studies have reported that FVIII clearance is reduced in older patients.[11,13,17,19] For example, Vlot *et al* observed a trend towards increased FVIII half-life with age, although this failed to achieve statistical significance (P = 0.08).[11] In addition, Fischer *et al* demonstrated a weak positive correlation between age and FVIII half-life on univariate analysis (Pearson-rank = 0.5; P = 0.028).[13] More recently, Kepa *et al* also showed that age had a significant association with FVIII clearance. When patients were grouped into age decades, the authors noted that this age effect was considerably more evident in PWH aged greater than 40 years.[17] Importantly, regression analysis confirmed that the association between age and FVIII clearance was not just attributable to increased VWF levels, as age remained an independent predictor even following correction for VWF. Although the biology underlying this age-related increase in VWF has not been fully defined, recent data suggest that age may impact upon both the secretion and clearance of VWF.[28]

4. Additional factors

It is well established that neutralizing high-titer FVIII-specific antibodies in PWH result in the rapid clearance of infused FVIII. These polyclonal antibodies tend to be IgG1 or IgG4 and are high-affinity in nature.[31] Non-neutralizing anti-FVIII antibodies can also be identified in a significant number of PWH who do not have clinically evident inhibitors.[32] These antibodies are commonly IgG1 or IgG3 and bind FVII with low to moderate affinity. In a recent study of

42 adult PWH, Hofbauer *et al* investigated whether these non-neutralizing FVIII-specific antibodies had any effect on the clearance of infused FVIII.[33] Overall, 15 (37%) of these patients studied were found to have FVIII-binding antibodies with titers ≥ 1:20. Moreover, in 9 of these subjects, the titer of FVIII-specific antibodies was ≥ 1:40. Interestingly, FVIII half-life was significantly reduced in this cohort with high titer non-neutralizing FVIII-specific antibodies (median 7.8 versus 10.4 hours respectively; P = 0.004). The effect of the non-neutralizing antibodies was independent of VWF:Ag levels and was estimated to account for 17% of the total inter-individual variability in FVIII half-life.[33]

The fact that ABO blood group influences FVIII clearance has led to the suggestion that other blood groups may also be important. Vlot *et al* found that Rhesus phenotype (RhD), a blood group system determined by a protein in the red blood cell membrane, had no effect on FVIII pharmacokinetics in PWH.[11] In contrast, the Secretor carbohydrate blood group shares some similarities with the ABO system in that it is defined by the presence or absence of specific terminal sugar residues on glycan chains. A number of groups have reported a weak association between Secretor blood group and plasma VWF:Ag levels.[34,35] Further studies will be required to determine whether this effect on VWF levels translates into a secondary effect on FVIII half-life in PWH. Finally, studies have investigated whether FVIII PK may be influenced by FVIII genotype in PWH.[17] However, no significant difference in FVIII recovery or clearance were observed between patients with *F8* gene inversions, deletions or point mutations respectively.

Clearance of free-FVIII compared to VWF-bound FVIII

As previously discussed, under normal steady state conditions, approximately 95-97% of plasma FVIII circulates bound to VWF.[8,36] The remaining 3-5% circulates in plasma as free-FVIII. Consequently, it seems likely that the majority of infused FVIII in PWH will be cleared in complex with endogenous VWF. Importantly, although the amount of free-FVIII in plasma at any particular time point is limited, this fraction of FVIII has a very short in vivo half-life (~2 hours compared to ~12 hours for VWF-bound FVIII). Consequently, as much as 25% of total infused FVIII in PWH may actually be cleared in the form of free-FVIII.[37] The relative proportion of infused FVIII cleared as free-FVIII will clearly be highly dependent upon the affinity of the FVIII-VWF binding interaction **(Figure 2)**.

Recent studies have provided significant insights into the biological pathways involved in regulating the clearance of both free-FVIII and VWF-bound FVIII.[8,9] These data have demonstrated that hepatic macrophages and liver sinusoidal EC (LSECs) play key roles in regulating *in vivo* clearance of VWF and FVIII.[38-40] In addition, a number of cell surface receptors have also been implicated in modulating VWF-FVIII binding interactions.[9] On macrophages, these include the low-density lipoprotein receptor-related protein-1 (LRP1),[41]

the scavenger receptor class A member I (SR-A1)[42] and the macrophage galactose-type lectin (MGL).[43] Additional receptors expressed on LSECs that may be important include stabilin-2 (STAB2)[40] and C-type lectin domain family 4 member M (CLEC4M).[44] A number of other miscellaneous receptors have also been reported to bind FVIII in vitro. These include the asialoglycoprotein receptor (ASGPR) which is predominantly expressed on hepatocytes, as well as LDL-R, CD206, Siglec-5 and heparin sulfate proteoglycans (HSPGs).[8] Although the relative importance of these different clearance receptors in terms of regulating the clearance of free- and VWF-bound FVIII remains unclear, many of them have been shown to be able to bind and internalize VWF and FVIII. Interestingly, for some of these clearance receptors (e.g. LRP1 and ASGPR), the ability of FVIII to interact with the receptor is significantly attenuated when FVIII is in complex with VWF.[8] Nevertheless, significant correlations between some receptor polymorphisms and human FVIII levels have been reported.[45,46] In addition, Lunghi *et al* recently reported that a polymorphism in the LDLR (c.1773C/T) was associated with differences in the initial phase of FVIII distribution in PWH.[47] All together, based on current evidence, it seems that variation in clearance pathways for free- and VWF-bound FVIII are highly likely to contribute to inter-individual variations in FVIII clearance in PWH **(Figure 2).**[9,48]

Inter-individual variations in VWF clearance pathways

As detailed above, current findings suggest that VWF represents the most important determinant of inter-individual variation in FVIII clearance in PWH. In keeping with this hypothesis, the effects of ABO blood group and age on FVIII half-life seem to result predominantly via their respective effects upon VWF levels. The observation that pre-infusion plasma VWF:Ag levels positively correlates with FVIII half-life in PWH is interesting. One simple explanation for this correlation would be that in PWH with higher endogenous VWF levels, more infused FVIII therapy is able to bind to VWF.[13] Consequently, since there is less free-FVIII in plasma, overall FVIII clearance rate is attenuated. A number of lines of evidence suggest that this may not be the case. First, VWF is present in huge molar excess in plasma compared to the infused FVIII.[36] Second, several studies have demonstrated that induced increases in plasma VWF levels prior to FVIII infusion do not affect FVIII half-life in hemophiliac plasma. In a murine model of hemophilia, Fischer *et al* showed that treatment with rIL-11 resulted in a 1.7-fold increase in plasma VWF levels compared to untreated controls.[13] Subsequently, the half-life of infused FVIII was assessed in the rIL-11 treated and control cohorts. Despite the higher basal VWF levels in the rIL-11 treated group, no difference in FVIII half-life was observed.[13] Similarly, human studies have shown that DDAVP administration can significantly increase endogenous plasma VWF:Ag levels in PWH.

However, DDAVP pre-treatment two hours prior to infusion of FVIII had no significant effect on FVIII clearance.[49]

Some plasma derived FVIII concentrates also contain VWF in substantial and pharmacologically relevant amounts. Nevertheless, pharmacokinetics of such FVIII-VWF concentrates seem not to be essentially different from pharmacokinetics of FVIII products devoid of VWF. Both groups of products, plasma-derived with and without VWF and recombinant FVIII concentrates, all without VWF are considered to have virtually indistinguishable pharmacokinetics of FVIII.[14,50]

An alternative mechanism that could explain the positive correlation between plasma VWF levels and FVIII half-life in PWH is that the VWF level is really a surrogate measure of endogenous VWF clearance rates. Hence, PWH with slower VWF clearance rates will tend to have higher basal plasma VWF:Ag levels. According to this hypothesis, these PWH would also have longer FVIII half-lives since the majority of infused FVIII is cleared through VWF-dependent clearance pathways. This mechanism is consistent with the data demonstrating that targeted increased in plasma VWF levels before FVIII administration do not prolong FVIII half-life.[13,49] Several studies have attempted to assess endogenous VWF clearance rates in PWH using VWF propeptide to antigen (VWF:pp/VWF:Ag) ratios. Swystun *et al* recently reported a strong association between FVIII pharmacokinetics in PWH and the VWF:pp/VWF:Ag ratio ($P < 0.0001$).[15] In particular, the VWF:pp/VWF:Ag ratio negatively correlated with FVIII half-life in non-O pediatric PWH.[15] Similarly, Fischer *et al* observed a strong correlation between VWF:pp/VWF:Ag ratio and FVIII half-life in blood group O PWH (Pearson-rank = 0.7; $P = 0.001$).[13] Collectively, these findings emphasize the fact that FVIII half-life in PWH is critically dependent upon inter-individual variations in clearance rates for endogenous VWF. The factors responsible for regulating variability in VWF clearance between individual patients with haemophilia remain to be defined but likely relate in part to (i) variations in VWF glycosylation[51,52] and/or (ii) variations in VWF clearance pathways.[9,48]

Variation in FVIII clearance – clinical significance

Understanding the molecular mechanisms responsible for the marked inter-individual variability in FVIII clearance kinetics observed between individual PWH has major clinical significance. In particular, Collins *et al* demonstrated that for patients with severe hemophilia A on prophylaxis, increasing time per week with plasma FVIII less than 1% was associated with significantly increased risk for bleeding episodes.[18] This finding was true for both hemarthroses and total bleeds. Moreover, the observation was consistent for both children and adult PWH. For example, in 99 patients aged between 10 and 65 years, each hour spent with FVIII levels below 1% were associated with a 1.4% increase in annual bleed rate (CI 0.21 – 2.62%).[18] Subsequent modelling studies have shown that FVIII half-life and frequency

of infusions are critical determinants of the amount of time each PWH spends per week with trough FVIII levels < 1%.[7] In contrast, in vivo FVIII recovery and infused FVIII dose per kg were less important. Cumulatively, these data have led to the concept that personalized treatment regimens for PWH should be considered. Defining individual-specific FVIII PK parameters constitutes a critical first step in terms of developing any precision-medicine approach to the treatment of hemophilia. This approach has already been investigated in a number of clinical studies and has been become significantly more practical with the recent introduction of population-based PK studies. This subject has been addressed in a number of recent comprehensive review articles.[53,54]

With respect to optimization of treatment for PWH, it is also important to highlight that a number of different extended half-life (EHL-) rFVIII treatments have been developed in recent years that use a variety of different genetic engineering strategies to extend the half-life of FVIII.[55] Interestingly however, significant inter-individual variation in clearance of these long-acting FVIII preparations has already been reported.[56-58] In the clinical context, knowledge of individual pharmacokinetics for EHL-FVIII is likely to be of even greater translational importance.[59] Although the biological factors responsible for mediating inter-individual variation in clearance of different EHL-rFVIII products remains poorly understood, it is interesting that associations with endogenous plasma VWF levels and ABO blood group have already been reported.[57,60] These data are consistent with the concept that EHL-FVIII molecules are still being cleared in complex with endogenous VWF.

Conclusions

In conclusion, numerous studies have highlighted the fact that marked inter-individual variation in FVIII clearance rates exists between PWH. This variation has direct clinical relevance in that it impacts the efficacy of weight-based FVIII prophylaxis regimens.[12] Current state-of-the-art data suggest that endogenous VWF levels, ABO blood group and age all play important roles in regulating FVIII half-life in PWH. Critically however, multiple regression analysis suggests that cumulatively VWF, ABO group and age can only explain 34% of the total inter-individual variation in FVIII clearance observed between subjects with severe hemophilia A.[17] Further adequately powered studies that include deep clinical phenotype, detailed PK analysis and genomic data will be necessary to elucidate the biological mechanisms responsible for the remaining 66% in FVIII clearance variability.

ACKNOWLEDGEMENTS

This publication has emanated from research supported in part by a research grant from Science Foundation (SFI) under the SFI Strategic Partnership Programme Grant number 16/SPP3303" and research support from Shire US Inc., a member of the Takeda group of companies, Lexington, MA, USA.

AUTHORSHIP

Contribution: All authors were involved in writing and reviewing the paper.

Conflict-of-interest disclosure:

P.L.T. is full-time employee of Baxalta Innovations GmbH, a member of the Takeda group of companies, and shareholder of Takeda Pharmaceutical Company Limited.

J.M.J. has served as a consultant for CSL Behring and Octapharma and has received research grant funding from Octapharma.

SWP has served as a consultant to Apcintex, Bayer, Biomarin, Catalyst Biosciences, CSL Behring, HEMA Biologics, Freeline, Novo Nordisk, Pfizer, Roche/Genentech, Sangamo Therapeutics, Sanofi, Takeda, Spark Therapeutics, and uniQure.

J.S.O'D has served on the speaker's bureau for Baxter, Bayer, Novo Nordisk, Boehringer Ingelheim, Leo Pharma, Takeda and Octapharma. He has also served on the advisory boards of Baxter, Bayer, Octapharma CSL Behring, Daiichi Sankyo, Boehringer Ingelheim, Takeda and Pfizer. J.S.O.D has received research grant funding awards from Baxter, Bayer, Pfizer, Shire (now part of Takeda), Takeda and Novo Nordisk.

LEGENDS

Table 1: Examples of studies investigating FVIII half-life determinants in PWH

n = number.

Figure 1: FVIII interaction with VWF in normal plasma.

VWF and FVIII are both expressed in EC and secreted into plasma. Normal plasma concentrations for VWF and FVIII are 35nM and 0.8nM respectively. The majority of plasma FVIII (approximately 95-97%) circulates as part of a high affinity complex with VWF. Thus. free-FVIII and VWF-bound FVIII exists in dynamic equilibrium.

Figure 2: Inter-individual variation in FVIII clearance in PWH

Previous studies have highlighted marked inter-individual variations between PWH in terms of clearance rates for infused FVIII therapy. Although recent data have provided important

insights into the cellular and receptors involved in the clearance of both free-FVIII and VWF-bound FVIII, the biological mechanisms responsible for the variation in FVIII half-life remains poorly understood. This may be due to (i) variability in the VWF-FVIII binding affinity*; (ii) variation in the clearance of Free-FVIII** and/or (3) variation in the clearance of VWF-bound FVIII***

LSECs = liver sinusoidal endothelial cells; STAB2 = stabilin-2; CLEC4M = C-type lectin domain family 4 member M; MGL = macrophage galactose-type lectin; LRP1 = low-density lipoprotein receptor-related protein-1; SR-A1 = scavenger receptor class A member I; ASGPR = asialoglycoprotein receptor.

REFERENCES

1. Iorio A, Stonebraker JS, Chambost H, et al. Establishing the Prevalence and Prevalence at Birth of Hemophilia in Males: A Meta-analytic Approach Using National Registries. *Ann Intern Med*. 2019.

2. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med*. 2007;357(6):535-544.

3. Den Uijl IE, Mauser Bunschoten EP, Roosendaal G, et al. Clinical severity of haemophilia A: does the classification of the 1950s still stand? *Haemophilia*. 2011;17(6):849-853.

4. Warren BB, Jacobson L, Kempton C, et al. Factor VIII prophylaxis effects outweigh other hemostasis contributors in predicting severe haemophilia A joint outcomes. *Haemophilia*. 2019;25(5):867-875.

5. Valentino LA. Controversies regarding the prophylactic management of adults with severe haemophilia A. *Haemophilia*. 2009;15 Suppl 2:5-18, quiz 19-22.

6. Bjorkman S, Folkesson A, Jonsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3-74 years: a population analysis based on 50 patients with long-term prophylactic treatment for haemophilia A. *Eur J Clin Pharmacol*. 2009;65(10):989-998.

7. Collins PW, Bjorkman S, Fischer K, et al. Factor VIII requirement to maintain a target plasma level in the prophylactic treatment of severe hemophilia A: influences of variance in pharmacokinetics and treatment regimens. *J Thromb Haemost*. 2010;8(2):269-275.

8. Pipe SW, Montgomery RR, Pratt KP, Lenting PJ, Lillicrap D. Life in the shadow of a dominant partner: the FVIII-VWF association and its clinical implications for hemophilia A. *Blood*. 2016;128(16):2007-2016.

9. O'Sullivan JM, Ward S, Lavin M, O'Donnell JS. von Willebrand factor clearance - biological mechanisms and clinical significance. *Br J Haematol*. 2018;183(2):185-195.

10. Fijnvandraat K, Peters M, ten Cate JW. Inter-individual variation in half-life of infused recombinant factor VIII is related to pre-infusion von Willebrand factor antigen levels. *Br J Haematol*. 1995;91(2):474-476.

11. Vlot AJ, Mauser-Bunschoten EP, Zarkova AG, et al. The half-life of infused factor VIII is shorter in hemophiliac patients with blood group O than in those with blood group A. *Thromb Haemost*. 2000;83(1):65-69.

12. van Dijk K, van der Bom JG, Lenting PJ, et al. Factor VIII half-life and clinical phenotype of severe hemophilia A. *Haematologica*. 2005;90(4):494-498.

13. Fischer K, Pendu R, van Schooten CJ, et al. Models for prediction of factor VIII half-life in severe haemophiliacs: distinct approaches for blood group O and non-O patients. *PLoS One*. 2009;4(8):e6745.

14. Chen ZP, Li PJ, Li G, et al. Pharmacokinetic Studies of Factor VIII in Chinese Boys with Severe Hemophilia A: A Single-Center Study. *Chin Med J (Engl)*. 2018;131(15):1780-1785.

15. Swystun LL, Ogiwara K, Rawley O, et al. Genetic determinants of VWF clearance and FVIII binding modify FVIII pharmacokinetics in pediatric hemophilia A patients. *Blood*. 2019;134(11):880-891.

16. Bjorkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. *Blood*. 2012;119(2):612-618.

17. Kepa S, Horvath B, Reitter-Pfoertner S, et al. Parameters influencing FVIII pharmacokinetics in patients with severe and moderate haemophilia A. *Haemophilia*. 2015;21(3):343-350.

18. Collins PW, Blanchette VS, Fischer K, et al. Break-through bleeding in relation to predicted factor VIII levels in patients receiving prophylactic treatment for severe hemophilia A. *J Thromb Haemost*. 2009;7(3):413-420.

19. Bjorkman S, Blanchette VS, Fischer K, et al. Comparative pharmacokinetics of plasma- and albumin-free recombinant factor VIII in children and adults: the influence of

blood sampling schedule on observed age-related differences and implications for dose tailoring. *J Thromb Haemost*. 2010;8(4):730-736.

20. Ruffo S, Messori A, Longo G, Matucci M, Morfini M, Rossi-Ferrini P. A microcomputer program for individualizing factor VIII dosage in hemophilia patients undergoing major surgery. *Comput Methods Programs Biomed*. 1986;23(1):37-46.

21. Vlot AJ, Koppelman SJ, van den Berg MH, Bouma BN, Sixma JJ. The affinity and stoichiometry of binding of human factor VIII to von Willebrand factor. *Blood*. 1995;85(11):3150-3157.

22. Dimitrov JD, Christophe OD, Kang J, et al. Thermodynamic analysis of the interaction of factor VIII with von Willebrand factor. *Biochemistry*. 2012;51(20):4108-4116.

23. Turecek PL, Spannagl M, Kragh T, et al. The role of ultralarge multimers in recombinant human von Willebrand factor - a review of physico-and biochemical studies and findings in in vivo models and in humans with von Willebrand disease. *Hamostaseologie*. 2017;37(S 01):S15-S25.

24. Preston RJ, Rawley O, Gleeson EM, O'Donnell JS. Elucidating the role of carbohydrate determinants in regulating hemostasis: insights and opportunities. *Blood*. 2013;121(19):3801-3810.

25. Jenkins PV, O'Donnell JS. ABO blood group determines plasma von Willebrand factor levels: a biologic function after all? *Transfusion*. 2006;46(10):1836-1844.

26. Gallinaro L, Cattini MG, Sztukowska M, et al. A shorter von Willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand factor. *Blood*. 2008;111(7):3540-3545.

27. McGrath RT, van den Biggelaar M, Byrne B, et al. Altered glycosylation of platelet-derived von Willebrand factor confers resistance to ADAMTS13 proteolysis. *Blood*. 2013;122(25):4107-4110.

28. Albanez S, Ogiwara K, Michels A, et al. Aging and ABO blood type influence von Willebrand factor and factor VIII levels through interrelated mechanisms. *J Thromb Haemost*. 2016;14(5):953-963.

29. Sanders YV, Giezenaar MA, Laros-van Gorkom BA, et al. von Willebrand disease and aging: an evolving phenotype. *J Thromb Haemost*. 2014;12(7):1066-1075.

30. Lavin M, Aguila S, Schneppenheim S, et al. Novel insights into the clinical phenotype and pathophysiology underlying low VWF levels. *Blood*. 2017;130(21):2344-2353.

31. Whelan SF, Hofbauer CJ, Horling FM, et al. Distinct characteristics of antibody responses against factor VIII in healthy individuals and in different cohorts of hemophilia A patients. *Blood*. 2013;121(6):1039-1048.

32. Hofbauer CJ, Whelan SF, Hirschler M, et al. Affinity of FVIII-specific antibodies reveals major differences between neutralizing and nonneutralizing antibodies in humans. *Blood*. 2015;125(7):1180-1188.

33. Hofbauer CJ, Kepa S, Schemper M, et al. FVIII-binding IgG modulates FVIII half-life in patients with severe and moderate hemophilia A without inhibitors. *Blood*. 2016;128(2):293-296.

34. O'Donnell J, Boulton FE, Manning RA, Laffan MA. Genotype at the secretor blood group locus is a determinant of plasma von Willebrand factor level. *Br J Haematol*. 2002;116(2):350-356.

35. Schleef M, Strobel E, Dick A, Frank J, Schramm W, Spannagl M. Relationship between ABO and Secretor genotype with plasma levels of factor VIII and von Willebrand factor in thrombosis patients and control individuals. *Br J Haematol*. 2005;128(1):100-107.

36. Terraube V, O'Donnell JS, Jenkins PV. Factor VIII and von Willebrand factor interaction: biological, clinical and therapeutic importance. *Haemophilia*. 2010;16(1):3-13.

37. Swystun LL, Notley C, Georgescu I, et al. The endothelial lectin clearance receptor CLEC4M binds and internalizes factor VIII in a VWF-dependent and independent manner. *J Thromb Haemost*. 2019;17(4):681-694.

38. Lenting PJ, Westein E, Terraube V, et al. An experimental model to study the in vivo survival of von Willebrand factor. Basic aspects and application to the R1205H mutation. *J Biol Chem*. 2004;279(13):12102-12109.

39. Chion A, O'Sullivan JM, Drakeford C, et al. N-linked glycans within the A2 domain of von Willebrand factor modulate macrophage-mediated clearance. *Blood*. 2016;128(15):1959-1968.

40. Swystun LL, Lai JD, Notley C, et al. The endothelial cell receptor stabilin-2 regulates VWF-FVIII complex half-life and immunogenicity. *J Clin Invest*. 2018;128(9):4057-4073.

41. Rastegarlari G, Pegon JN, Casari C, et al. Macrophage LRP1 contributes to the clearance of von Willebrand factor. *Blood*. 2012;119(9):2126-2134.

42. Wohner N, Muczynski V, Mohamadi A, et al. Macrophage scavenger receptor SR-AI contributes to the clearance of von Willebrand factor. *Haematologica*. 2018;103(4):728-737.

43. Ward SE, O'Sullivan JM, Drakeford C, et al. A novel role for the macrophage galactose-type lectin receptor in mediating von Willebrand factor clearance. *Blood*. 2018;131(8):911-916.

44. Rydz N, Swystun LL, Notley C, et al. The C-type lectin receptor CLEC4M binds, internalizes, and clears von Willebrand factor and contributes to the variation in plasma von Willebrand factor levels. *Blood*. 2013;121(26):5228-5237.

45. Cunningham N, Laffan MA, Manning RA, O'Donnell JS. Low-density lipoprotein receptor-related protein polymorphisms in patients with elevated factor VIII coagulant activity and venous thrombosis. *Blood Coagul Fibrinolysis*. 2005;16(7):465-468.

46. Morange PE, Tregouet DA, Frere C, et al. Biological and genetic factors influencing plasma factor VIII levels in a healthy family population: results from the Stanislas cohort. *Br J Haematol*. 2005;128(1):91-99.

47. Lunghi B, Bernardi F, Martinelli N, et al. Functional polymorphisms in the LDLR and pharmacokinetics of Factor VIII concentrates. *J Thromb Haemost*. 2019;17(8):1288-1296.

48. Ward S, O'Sullivan JM, O'Donnell JS. von Willebrand factor sialylation-A critical regulator of biological function. *J Thromb Haemost*. 2019;17(7):1018-1029.

49. Deitcher SR, Tuller J, Johnson JA. Intranasal DDAVP induced increases in plasma von Willebrand factor alter the pharmacokinetics of high-purity factor VIII concentrates in severe haemophilia A patients. *Haemophilia*. 1999;5(2):88-95.

50. Carcao M. Changing paradigm of prophylaxis with longer acting factor concentrates. *Haemophilia*. 2014;20 Suppl 4:99-105.

51. Aguila S, Lavin M, Dalton N, et al. Increased galactose expression and enhanced clearance in patients with low von Willebrand factor. *Blood*. 2019;133(14):1585-1596.

52. O'Sullivan JM, Aguila S, McRae E, et al. N-linked glycan truncation causes enhanced clearance of plasma-derived von Willebrand factor. *J Thromb Haemost*. 2016;14(12):2446-2457.

53. Hazendonk H, van Moort I, Mathot RAA, et al. Setting the stage for individualized therapy in hemophilia: What role can pharmacokinetics play? *Blood Rev*. 2018;32(4):265-271.

54. Iorio A. Using pharmacokinetics to individualize hemophilia therapy. *Hematology Am Soc Hematol Educ Program*. 2017;2017(1):595-604.

55. Tiede A. Half-life extended factor VIII for the treatment of hemophilia A. *J Thromb Haemost*. 2015;13 Suppl 1:S176-179.

56. Lalezari S, Martinowitz U, Windyga J, et al. Correlation between endogenous VWF:Ag and PK parameters and bleeding frequency in severe haemophilia A subjects during three-times-weekly prophylaxis with rFVIII-FS. *Haemophilia*. 2014;20(1):e15-22.

57. Carcao MD, Chelle P, Clarke E, et al. Comparative pharmacokinetics of two extended half-life FVIII concentrates (Eloctate and Adynovate) in adolescents with hemophilia A: Is there a difference? *J Thromb Haemost*. 2019;17(7):1085-1096.

58. Powell JS, Josephson NC, Quon D, et al. Safety and prolonged activity of recombinant factor VIII Fc fusion protein in hemophilia A patients. *Blood*. 2012;119(13):3031-3037.

59. Ragni MV, Croteau SE, Morfini M, et al. Pharmacokinetics and the transition to extended half-life factor concentrates: communication from the SSC of the ISTH. *J Thromb Haemost*. 2018;16(7):1437-1441.

60. Tang L, Leong L, Sim D, et al. von Willebrand factor contributes to longer half-life of PEGylated factor VIII in vivo. *Haemophilia*. 2013;19(4):539-545.

Study	n	Age range	Hemophilia	FVIII product	PK study	Half-life (Average)	Half-life (Range)	Significant determinants
Fijnvandraat *et al*, 1995	12	25 - 44 years (mean = 32)	Severe	rFVIII	11 time points	12.5 hours	6 - 28.8 hours	Pre-infusion VWF
Vlot *et al*, 2000	32	15 - 43 years	Severe (n=30) Mild (n=2)	pd-FVIII & rFVIII	11 time points	18.2 hours & 17.6 hours	13.2 - 23.2; 13.5 - 21.7 hours	Pre-infusion VWF ABO group Age (p=0.08)
Van Dijk *et al*, 2005	42	15 - 43 years (mean = 28.8)	Severe	pd-FVIII & rFVIII	10 time points	11.8 hours	7.4 - 20.4 hours	Pre-infusion VWF ABO group Age
Barnes *et al*, 2006	20	4 – 18 years (mean = 12.8)	Severe (n=16) Moderate (n=4)	rFVIII	11 time points 5 time points	10.7 hours	7.8 – 15.3 hours	Pre-infusion VWF Low titre inhibitors
Fischer *et al*, 2009	38	10 - 47 years (mean = 26.3)	Severe	pd-FVIII & rFVIII	10 time points	12.9 hours	7.4 - 20.4 hours	Pre-infusion VWF ABO group VWF:pp/Ag ratio

Kepa *et al*, 2015	42	24 - 44 years (median = 25.7)	Severe (n=37) Moderate (n=5)	pd-FVIII & rFVIII	11 time points	10 hours	6.2 - 20.7 hours	Pre-infusion VWF ABO group Age
Chen *et al*, 2018	36	4 – 16 years (median = 7.8)	Severe	pd-FVIII & rFVIII	5 time points	11.0 hours	5.5 – 20.0 hours	Pre-infusion VWF ABO group

Figure 1: FVIII interaction with VWF in normal plasma

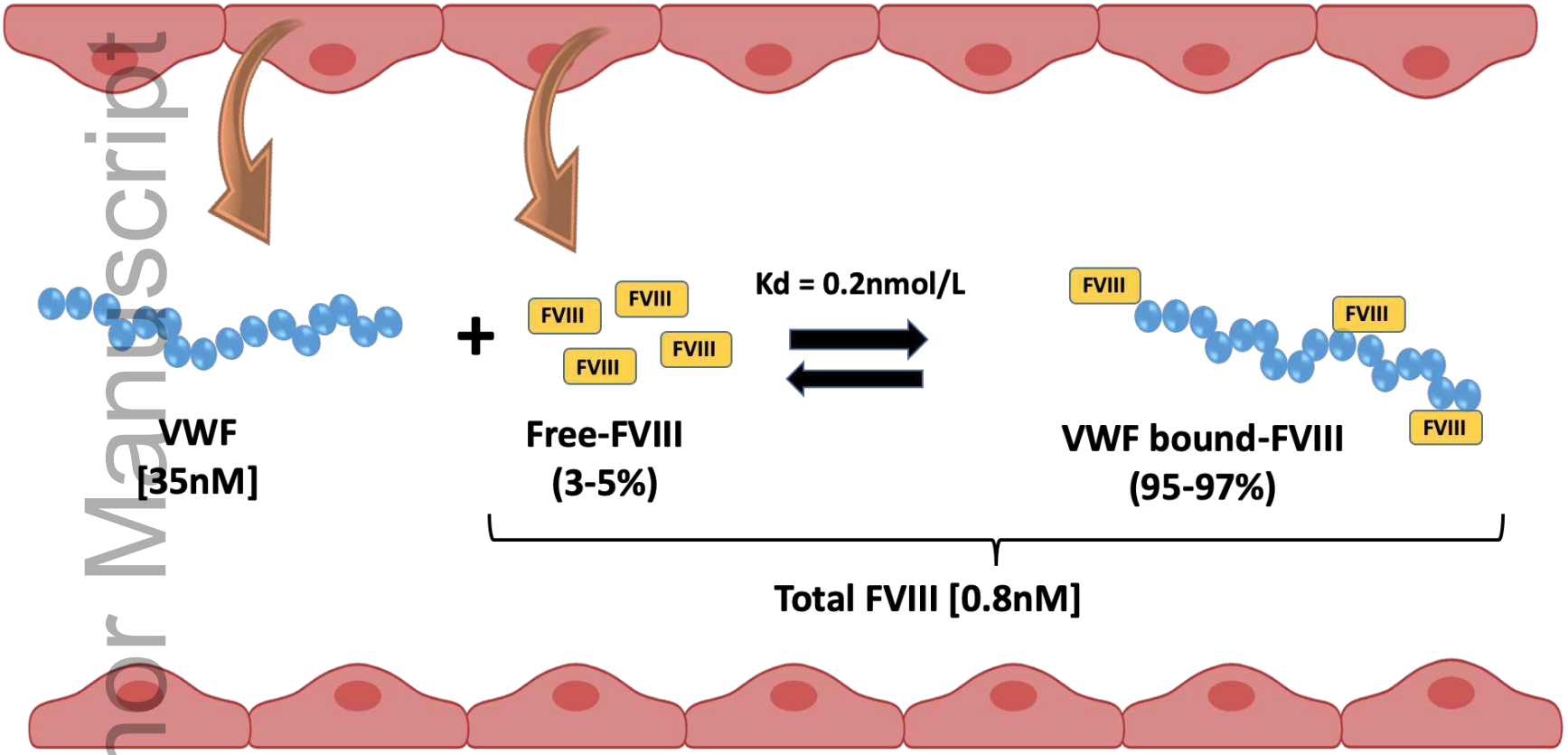

Figure 2: Inter-individual variation in FVIII clearance in PWH