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von Willebrand Factor and Factor VIII Clearance in Perioperative Hemophilia A Patients

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Abstract

Background von Willebrand factor (VWF) is crucial for optimal dosing of factor VIII (FVIII) concentrate in hemophilia A patients as it protects FVIII from premature clearance. To date, it is unknown how VWF behaves and what its impact is on FVIII clearance in the perioperative setting.

Aim To investigate VWF kinetics (VWF antigen [VWF:Ag]), VWF glycoprotein Ib binding (VWF:GPIbM), and VWF propeptide (VWFpp) in severe and moderate perioperative hemophilia A patients included in the randomized controlled perioperative OPTI-CLOT trial.

Methods Linear mixed effects modeling was applied to analyze VWF kinetics. One-way and two-way analyses of variance were used to investigate perioperative VWFpp/VWF:Ag ratios and associations with surgical bleeding.

Results Fifty-nine patients with median age of 48.8 years (interquartile range: 34.8–60.0) were included. VWF:Ag and VWF:GPIbM increased significantly postoperatively. Blood type non-O or medium risk surgery were associated with higher VWF:Ag and VWF:GPIbM levels compared with blood type O and low risk surgery. VWFpp/VWF:Ag was significantly higher immediately after surgery than 32 to 57 hours after surgery ($p < 0.001$). Lowest VWF:Ag quartile (0.43–0.92 IU/mL) was associated with an increase of FVIII concentrate clearance of 26 mL/h (95% confidence interval: 2–50 mL/h) compared with highest VWF antigen quartile (1.70–3.84 IU/mL). VWF levels were not associated with perioperative bleeding $F(4,227) = 0.54$, $p = 0.710$.

Keywords

- hemophilia A
- von Willebrand factor
- surgery
- factor VIII
- linear mixed effect modeling
- postsurgical bleeding

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Conclusion VWF:Ag and VWF:GPIbM levels increase postoperatively, most significantly in patients with blood type non-O or medium risk surgery. Lower VWF antigen levels did not lead to clinically relevant higher FVIII clearance. VWF:Ag or VWF:GPIbM levels were not associated with perioperative hemorrhage.

Introduction

A deficiency of coagulation factor VIII (FVIII) leads to diagnosis of hemophilia A, an X-linked bleeding disorder characterized by bleeding typically in joints and muscles, or bleeding after minor trauma and/or surgery. Mainstay of treatment is replacement therapy with FVIII concentrates which is administered both prophylactically in more severely affected patients, and on demand to treat bleeding events or to prevent bleeding during dental or surgical procedures in all patient categories.¹ Previously, we reported a study in 119 hemophilia A patients undergoing 198 surgeries and showed that perioperative FVIII concentrate dosing is challenging using current guidelines based on body weight.² In this retrospective study, 45% of all FVIII levels measured in the first 24 hours after surgery were below target levels as prescribed in Dutch guidelines,³ with a hypothetical higher risk of bleeding. In addition, 75% of FVIII levels measured 120 hours after surgery were above targeted FVIII levels with concomitantly unnecessary higher treatment costs. As von Willebrand factor (VWF) protects FVIII from proteolytic cleavage, premature activation, and clearance from the circulation, VWF is crucial to achieve adequate FVIII levels during FVIII concentrate dosing. Importantly, ratio between VWF propeptide (VWFpp) and VWF antigen (VWF:Ag) can be used as a marker for both VWF synthesis, secretion and clearance. More specifically, VWF:Ag and VWFpp are secreted equimolarly but are independently cleared with different half-lives of 8 to 12 and 2 hours, respectively.^{4,5} We hypothesized that specific knowledge on how VWF behaves and influences FVIII clearance in perioperative hemophilia A patients is relevant to optimize FVIII dosing.

The role of VWF in the perioperative period has previously been investigated in 30 healthy individuals, mainly women, undergoing orthopaedic surgery by Kahlon et al.⁶ This report showed that both VWF:Ag and VWF ristocetin cofactor activity decrease during a surgical procedure and increase directly afterwards with concomitant decrease and increase of FVIII levels. These results, however, cannot be translated to our population due to gender differences, as primarily men are diagnosed with hemophilia. In addition, levels of VWFpp were not measured in these patients. Moreover, FVIII in our population is derived from replacement therapy and therefore not released due to endogenous mechanisms.

Therefore, we aimed to investigate VWF kinetics in perioperative hemophilia A patients and the influence of VWF on FVIII clearance. This will provide novel insights into: (1) factors that modify VWF levels; (2) influence of VWF on FVIII concentrate pharmacokinetic parameters, especially

FVIII clearance; and (3) association of VWF levels with perioperative bleeding.

Methods

Patients

Patients were diagnosed with severe or moderate hemophilia A and included in the perioperative OPTI-CLOT trial.⁷ The OPTI-CLOT trial is a randomized controlled trial, which aims to compare pharmacokinetic (PK)-guided FVIII concentrate dosing with dosing based on body weight (standard treatment). Patients are stratified according to surgical risk (medium vs. low risk) and mode of FVIII concentrate administration (bolus administration vs. continuous infusion). All patients had baseline (lowest) FVIII activity levels ≤ 0.05 IU/mL, were ≥ 12 years of age, did not have FVIII inhibitory antibodies (Bethesda Units < 0.2 IU), and underwent elective surgery. Patients were enrolled from six academic hemophilia treatment centers in the Netherlands (Erasmus University Medical Center Rotterdam, University Medical Center Groningen, University Medical Center Utrecht, Radboud University Medical Center Nijmegen / Maxima Medical Center, Veldhoven, Leiden University Medical Center/ Haga Hospital, The Hague, Amsterdam University Medical Centers). This study was approved by the Institutional Review Board of the Erasmus University Medical Center and all patients gave written informed consent before enrollment according to the Declaration of Helsinki.

Patient and surgical characteristics were collected and included blood type, age, body weight, body mass index (BMI), ideal body weight, FVIII concentrate consumption, surgical risk score, and perioperative hemorrhage.^{8,9} Perioperative hemorrhage was based on the definition for a clinically relevant bleed as stated by the International Society of Thrombosis and Haemostasis. More specifically for our analyses, this included bleeding complications either leading to hemoglobin decrease of ≥ 1.24 mmol/L, necessitating additional FVIII concentrate treatment and/or red blood cell transfusion, and/or a second surgical intervention and/or prolongation of hospitalization.

Blood Sampling and Laboratory Measurements

Blood samples were drawn at baseline (≤ 3 days before surgery), immediately after first dose of FVIII concentrate ($t = 15$ –30 minutes), postoperatively in recovery room, beginning of first day after surgery ($t = 16$ –33 hours), and at the beginning of second postoperative day ($t = 33$ –57 hours). FVIII levels were measured locally at each treatment center, using a one-stage clotting assay. VWF:Ag, VWF glycoprotein Ib binding (VWF:GPIbM), and VWFpp were measured at two central laboratories (VWF:Ag and VWF:GPIbM in Erasmus

University Medical Center Rotterdam; VWFpp in Leiden University Medical Center). VWF:Ag was measured using polyclonal rabbit anti-human VWF antibody and horseradish peroxidase-conjugated anti-human VWF antibody (DakoCytomation, Glostrup, Denmark) in an enzyme-linked immunoassay. VWF activity was measured as VWF:GPIbM. VWF:GPIbM was measured with the Innovance VWF Ac reagent (Siemens Healthcare Diagnostics, The Hague, The Netherlands) on a Sysmex CS 5100 (Sysmex, EttenLeur, The Netherlands) using the manufacturer's protocol. In this test, polystyrene particles coated with anti-GPIb monoclonal antibodies were added and particle agglutination was measured as a change in turbidity. VWFpp was determined by enzyme-linked immunoassay using Sanquin antibodies (Amsterdam, The Netherlands).¹⁰

Population Pharmacokinetic Modeling

Individual FVIII PK parameters, for example, clearance and volume of distribution, were estimated using nonlinear mixed-effects modeling software NONMEM v7.4 (ICON Development Solutions, Ellicott City, Maryland, United States). To determine perioperative FVIII concentrate PK parameters, our published perioperative population PK model for FVIII concentrate dosing in severe and moderate hemophilia A patients was utilized.¹¹ The following PK parameters were estimated: clearance (CL), intercompartmental clearance (Q), volume of distribution of central (V1) and peripheral (V2) compartment, and elimination half-life (T1/2). R software v3.6.1 (R Core Team [2019]) and Xpose v4.5.3 were used for data exploration and model diagnostics.¹²

Statistical Analyses

Descriptive statistics were expressed as medians and interquartile range (IQR), or as numerical counts with percentages. To identify effect of different variables in the perioperative period on VWF:Ag or VWF:GPIbM, a linear mixed-effects model was applied on log-transformed VWF:Ag or VWF:GPIbM using the lme4 package in R. In this model with log(VWF:Ag) or log(VWF:GPIbM) as outcome, relationships with blood type, surgical risk, BMI, and age were investigated. This method was also used to identify VWF effect on FVIII PK parameters in the perioperative period. One-way analysis of variance (ANOVA) was used to identify statistical differences in ratios VWFpp/VWF:Ag or VWF:Ag/VWF:GPIbM in the perioperative period, both log transformed as a result of nonnormality. A two-way ANOVA was applied on log-transformed VWF:Ag and/or VWF:GPIbM and their association with postoperative hemorrhage. Post hoc tests were performed with a Bonferroni correction. A *p*-value of 0.05 was considered statistically significant. All statistical analyses were performed using R software v3.6.1 (R Core Team [2019]).

Results

Patients Characteristics

► **Table 1** presents general characteristics of the study population. In this analysis, a total of 59 patients were included from the perioperative OPTI-CLOT trial of which 38 patients

(64.4%) had severe hemophilia A. Median age was 48.8 years old (IQR: 34.8–60.0 years) with a median body weight of 87.0 kg (IQR: 50.4–133.5 kg). Nine of the 59 patients experienced a postoperative bleeding event.

Perioperative VWF and FVIII Levels

In the perioperative period, patients were treated with FVIII concentrate, aiming for target FVIII levels as stated in Dutch

Table 1 General characteristics of the study population

	No. (%) or median [IQR]
Patient characteristics	
Total no. of patients	59
Age (y)	48.8 [34.8–60.0]
Severe hemophilia (FVIII < 0.01 IU/mL)	38 (64.4)
Blood group O	34 (57.6)
Height (cm)	178 [172–185]
Bodyweight (kg)	87.0 [74.2–95.3]
Body mass index (kg/m ²)	26.4 [23.3–29.7]
Ideal body weight (kg)	71.0 [66.8–76.3]
History of inhibiting FVIII antibodies	11 (18.6)
Baseline VWF:Ag (IU/mL)	1.09 [0.88–1.42]
Baseline VWF:GPIbM (IU/mL)	0.89 [0.65–1.25]
Clotting factor VIII concentrates	
Octocog alfa ^a	17
Octocog alfa ^b	20
Morococog alfa ^c	4
Plasma derived FVIII concentrate ^d	3
Turocog alfa ^e	15
Surgical characteristics	
Surgical risk ^f	
Low	30
Medium	29
Mode of FVIII concentrate administration	
Bolus	30
Continuous	29
Postoperative hemorrhage	
No	50
Yes	9

Abbreviations: FVIII factor VIII; IQR, interquartile range; VWF:Ag, von Willebrand factor antigen; VWF:GPIbM, von Willebrand factor glycoprotein Ib binding.

^aKogenate.

^bAdvate.

^cRefacto AF.

^dAafact.

^eNovoEight.

^fSurgical risk was defined according to Koshy et al.⁸ Low surgical risk includes, e.g., port-a-cath removal/insertion and dental surgery. Medium risk includes, e.g., total hip or knee replacement and tonsillectomy.

guidelines. ►Fig. 1 shows that FVIII increases, as well as VWF:Ag and VWF:GPIbM levels. As is depicted in ►Fig. 1B, VWF:Ag increased postoperatively from preoperative median of 1.09 IU/mL (IQR: 0.88–1.42) to a postoperative median of 1.53 IU/mL (IQR: 1.14–1.82) 48 hours after surgery with significant interpatient variability. Interpatient variability and increase of VWF:GPIbM was even greater, as on average twofold differences were observed for each postoperative patient (►Fig. 1C) with preoperative median values of 0.89 IU/mL (IQR: 0.65–1.25) to a postoperative median of 1.74 IU/mL (IQR: 1.04–2.57) 48 hours after surgery. In contrast, VWFpp only increased immediately postoperatively. In the majority of patients, rapidly decreasing VWFpp levels were observed during the first day following surgery ($t = 16.0$ – 32.7 hours).

►Fig. 2A,C show fluctuations per individual of VWFpp/VWF:Ag ratio and local regression or locally estimated scatterplot smoothing (LOESS) line over time. A LOESS line is a nonparametric approach which aims to create a smooth line through all the data points available by fitting multiple regressions in local neighborhood. The VWFpp/VWF:Ag ratio differed between subsequent time points as determined by one-way ANOVA ($F(4,290) = 4.21$, $p = 0.003$). VWFpp/VWF:Ag was higher immediately after

surgery when compared with 48 hours after surgery ($p < 0.001$), supporting an increased acute production and/or release of large amounts of VWFpp due to surgical intervention. ►Fig. 2B,D show that VWF:Ag/VWF:GPIbM ratios decrease slightly over time with statistically significant differences when calculated over the total perioperative period as determined by one-way ANOVA ($F(4,232) = 4.25$, $p = 0.002$). Bonferroni post hoc testing showed that VWF:Ag/VWF:GPIbM is also statistically significant when (1) preoperative VWF:Ag/VWF:GPIbM is compared with VWF:Ag/VWF:GPIbM 48 hours after surgery ($p = 0.004$); and (2) VWF:Ag/VWF:GPIbM immediately after surgery is compared with VWF:Ag/VWF:GPIbM 48 hours postoperatively ($p = 0.024$).

VWF Dynamics in Perioperative Setting

To analyze how VWF levels, for example, VWF:Ag and VWF:GPIbM evolve over time due to alterations in synthesis, secretion and clearance, a linear mixed effect model was created. As VWF:Ag was not distributed normally, a log transformation was performed. In this model with $\log(\text{VWF:Ag})$ as outcome, relationships with blood type, surgical risk, BMI, and age were analyzed. Time was set at $t = 0$ at moment of first incision by

Fig. 1 Factor VIII (FVIII) and von Willebrand factor (VWF) in the perioperative period stratified by surgical risk score. Spaghetti plots of (A) FVIII; (B) VWF antigen (VWF:Ag); (C) VWF glycoprotein Ib binding (VWF:GPIbM); and (D) VWF propeptide (VWFpp). Each patient is represented by a black line. The red line indicates the local regression or locally estimated scatterplot smoothing (LOESS) line, which follows densest part of the data.

Fig. 2 Ratios of von Willebrand factor (VWF) propeptide (VWFpp)/VWF antigen (VWF:Ag) and VWF:Ag/VWF glycoprotein Ib binding (VWF: GPIbM) differ in the perioperative period. (A and B) Spaghetti plots of VWFpp/VWF:Ag and VWF:Ag/VWF:GPIbM in the perioperative period. Each patient is represented by a black line. The red line indicates local regression or locally estimated scatterplot smoothing (LOESS) line, which follows the densest part of the data. (A) VWFpp/VWF:Ag ratio, which can be used as a measure for VWF secretion in the acute phase. VWFpp/VWF:Ag is higher immediately after surgery compared with ratios before the surgery. (B) VWF:Ag/VWF:GPIbM ratio represents also the acute phase response of VWF. This ratio decreases over time. (C and D) Boxplots of VWFpp/VWF:Ag and VWF:Ag/VWF:GPIbM ratios for each perioperative time point. For each boxplot, whiskers depict 2.5th and 97.5th percentile of the data, whereas the box depicts the interquartile range. Median of data are depicted by black horizontal line inside the boxplot.

the operating surgeon and considered a nonlinear function in the model. First, the most extensive model with interaction terms between time and blood type, time and age, and age and BMI was investigated. A model with both random intercepts and random slopes was proven not superior to only random intercepts when testing with a restricted maximum likelihood test ($p = 0.42$). Therefore, analyses were continued with the extensive model with only random intercepts. Subsequently, all interaction terms were removed from the random intercept model to investigate if interaction terms improved the model. Models were fitted under maximum likelihood as the F -test could not be computed and denominator degrees of freedom could not be (reliably) defined. The likelihood ratio test (LRT) also showed that interaction terms were not able to improve the model ($p = 0.14$). Finally, the nonlinear characteristic of the time variable was investigated by creating a model with a linear function of time. Comparing these models with LRT resulted in a statistically significant difference ($p = 0.037$), meaning that nonlinear terms of time were important contributors to the model. Model assumptions were evaluated

with residual plots, and did not show violation of model assumptions, as is documented in ►**Supplementary Fig. S2** (available in the online version).

The final model describing $\log(\text{VWF:Ag})$ in the perioperative period is demonstrated in ►**Table 2**. The expected difference in $\log(\text{VWF:Ag})$ between patients with blood type O and non-O is -0.16 (95% confidence interval [CI] -0.30 to -0.01) if patients are comparable with regard to age, body weight, BMI, surgical risk, and when sampled at identical time points during perioperative follow-up. Transformation of data results in $\exp(-0.16) = 0.86$. Clinically, this means that perioperative hemophilia patients with blood type O may have 14% less VWF:Ag when compared with patients with blood type non-O, if all the other variables are kept constant. The expected difference in $\log(\text{VWF:Ag})$ between medium and low risk surgical procedures was 0.29 (95% CI 0.13 – 0.43) if patients were comparable with regard to age, body weight, BMI, surgical risk, and when sampled at identical time points during perioperative follow-up. Transformation of data resulted in $\exp(0.29) = 1.33$. When translated into clinical terms, this means that patients with a

Table 2 Associations between the determinants blood type, surgical risk, age, BMI, and the outcome log (VWF:Ag)

Fixed effects	Coefficient	95% Confidence interval	p-Value
Intercept	-0.132	-0.543 to 0.28	0.535
Time since start surgery (h)	0.002	0.001 to 0.004	0.000
Time since start surgery ² (h)	0.000	0.000 to 0.000	0.040
Blood type, type O	-0.157	-0.305 to -0.008	0.042
Surgical risk, medium risk	0.286	0.134 to 0.438	0.001
Age (y)	0.003	-0.002 to 0.008	0.188
BMI (kg/m ²)	0.003	-0.010 to 0.015	0.682

Abbreviations: BMI, body mass index; VWF:Ag, von Willebrand factor antigen. Note: Linear mixed-effects modeling was used to determine the associations with the outcome log(VWF:Ag). Time was set at $t = 0$ at moment of first incision by the operating surgeon and was defined as a nonlinear function. Especially, blood type non-O and medium surgical risk were associated with higher VWF:Ag levels perioperatively.

medium surgical risk may have 33% higher VWF:Ag levels compared with patients undergoing low surgical risk surgery if all other variables are kept constant. The influence of perioperative timing is reflected in the effect plot, which is included in ►Supplementary Fig. S1 (available in the online version). Similar results were obtained when creating a linear mixed effect model of log(VWF:GPIbM) of which results are included

in ►Supplementary Table S1 and ►Supplementary Fig. S3 (available in the online version). ►Fig. 3 also depicts impact of blood type (►Fig. 3A) and surgical risk (►Fig. 3B) on FVIII, VWF:Ag, and VWF:GPIbM. In this figure, it is clearly demonstrated that blood type non-O and medium surgical risk result in higher VWF:Ag and VWF:GPIbM levels when compared with blood type O and/ or low surgical risk.

Individual VWF and FVIII PK Parameters

The PK parameters clearance, volume of distribution (central and peripheral), and elimination half-life were calculated using a perioperative FVIII population PK model.¹¹ As this model is only valid starting from initiation of surgery, PK parameters were calculated from first postoperative time point onwards until 48 hours after surgery. ►Fig. 4 shows no differences between time points for clearance (one-way ANOVA [$F(2,152) = 0.02$, $p = 0.98$]), volume of distribution (one-way ANOVA [$F(2,152) = 0.12$, $p = 0.89$]), and elimination half-life (one-way ANOVA [$F(2,152) = 0.43$, $p = 0.65$]). To investigate how FVIII clearance evolves over time and to evaluate VWF:Ag influence on clearance, another linear mixed effect model was created. This final model with FVIII clearance as outcome, time as a linear function (in hours), and VWF:Ag (in IU/mL) was divided into four categories (quartiles), both were added as fixed effects in the model, with time as an additional random effect. The lowest VWF:Ag level quartile (0.43–0.92 IU/mL) was associated with a minimal increase of 26 mL/h (95% CI 2–50 mL/h) in FVIII clearance when compared with the highest VWF:Ag level quartile of (1.70–3.84 IU/mL) (►Table 3). In addition, subanalyses showed that FVIII clearance was not associated with mode of administration (bolus administration vs. continuous

Fig. 3 Both blood type and surgical risk affect perioperative von Willebrand factor (VWF) antigen (VWF:Ag) and VWF glycoprotein Ib binding (VWF:GPIbM) levels. For each boxplot, whiskers depict 2.5th and 97.5th percentile of the data, whereas the box depicts interquartile range. Median of data are depicted by the black horizontal line inside the boxplot. (A) Boxplots over time separated by blood type. Hemophilia A patients with blood type non-O have higher VWF:Ag and VWF:GPIbM levels compared with patients with blood type O. (B) Lower factor VIII (FVIII) levels for surgeries with a lower surgical risk, as the Dutch guidelines advise lower FVIII target levels. Both VWF:Ag and VWF:GPIbM are lower postoperatively in low risk surgeries.

Fig. 4 No differences between factor VIII (FVIII) pharmacokinetic (PK) parameters at various time points in perioperative follow-up period. For each boxplot, whiskers depict 2.5th and 97.5th percentile of the data, whereas the box depicts interquartile range. Median of the data are depicted by the black horizontal line inside the boxplot. (A) Similar FVIII clearance. (B) Similar volume of distribution in steady state (Vss) during the perioperative follow-up period. (C) The elimination half-life of FVIII does not differ postoperative.

infusion), when adding mode of administration in the model as an additional fixed effect.

Perioperative Bleeding

Both VWF:Ag and VWF:GPIbM were shown to increase postoperatively, suggesting an overall increase of procoagulant hemostatic factors. As some hemophilia A patients experience bleeding despite perioperative replacement therapy, associations between lower VWF:Ag and VWF:GPIbM and perioperative bleeding were investigated. Nine of the 59 study patients experienced bleeding, requiring additional FVIII concentrate treatment. In ►Fig. 5, results of two-way ANOVA are visualized which analyzes interactions between VWF:Ag and perioperative bleeding. No association between log(VWF:Ag), bleeding, and perioperative time point was found ($F(4,227)=0.54$, $p=0.710$) as patients with perioperative bleeding had similar log(VWF:Ag) levels when compared with patients without bleeding. Additionally, no association was found between log(VWF:

GPIbM) of perioperative patients with and without surgical bleeding ($F(4,227)=0.80$, $p=0.525$). Subanalysis showed that 4 of 24 (17%) patients had a VWFpp/VWF:Ag ratio > 1.5 immediately after surgery with a bleeding complication, while this was the case in 5 of 31 (16%) patients with a ratio < 1.5. A Fisher's exact test confirmed no statistically significant difference in risk between the subgroups (p -value = 1.000).

Discussion

The present study was designed to investigate VWF and its influence on FVIII clearance in perioperative hemophilia A patients. Both VWF:Ag and VWF:GPIbM increased postoperatively but with large interpatient variability. Blood type non-O and medium surgical risk, however, were associated with higher perioperative VWF:Ag or VWF:GPIbM levels compared with blood type O and low surgical risk. Importantly, differences in VWF were associated with only minimal changes in FVIII concentrate clearance. Furthermore, VWF:Ag and VWF:GPIbM levels were similar between patients with and without postoperative hemorrhage.

To the best of our knowledge, VWF levels in perioperative hemophilia A patients have not been studied in detail. Therefore, we are the first to describe VWF kinetics in hemophilia A patients undergoing elective surgery. Similar to results observed in mainly female patients without a bleeding disorder undergoing orthopaedic surgery, VWF:Ag and VWF:GPIbM increased over time.⁶ This can be explained by increased release of VWF from Weibel–Palade bodies in the vascular endothelium due to adrenergic stress reactions and other related mechanisms causing endothelial activation such as blood flow turbulence, blood pressure variation, medication, hemostatic challenge due to surgery, and possibly increased VWF release to compensate for perioperative increase of VWF clearance.^{13,14} In our study, besides a release of mature VWF (VWF:Ag), an increased release of VWFpp from Weibel–Palade bodies was observed as VWFpp/VWF:Ag ratios increased significantly directly after surgery with a subsequent decrease. Normally in steady state, VWFpp and VWF are present in plasma with a molar ratio of 1:10.¹⁵ When acute release of both VWFpp and mature VWF (VWF:Ag) occurs, the molar ratio between

Table 3 The association between VWF:Ag and FVIII concentrate clearance (mL/h) in the perioperative period

Fixed effects	Coefficient	95% Confidence interval	p-Value
Intercept	183	161 to 205	0.000
Time since start surgery (h)	0	0 to 0	0.647
VWF:Ag			
First quartile (0.43–0.92 IU/mL)	26	2 to 50	0.034
Second quartile (0.92–1.33 IU/mL)	23	0 to 46	0.056
Third quartile (1.33–1.70 IU/mL)	9	–10 to 28	0.367

Abbreviations: FVIII, factor VIII; VWF:Ag, von Willebrand factor antigen. Note: A linear mixed effects model was created with FVIII concentrate clearance as an outcome and time since start surgery and VWF:Ag as fixed effects. Time was set at $t=0$ at moment of first incision by the operating surgeon and was defined as a linear function. Time since start surgery was also set as a random effect. VWF:Ag was categorized according to quartiles. The reference category was the highest quartile with VWF:Ag levels between 1.70 and 3.84 IU/mL.

Fig. 5 von Willebrand factor (VWF) antigen (VWF:Ag) or VWF glycoprotein Ib binding (VWF:GPIbM) are not associated with postoperative bleeding. For each boxplot, whiskers depict 2.5th and 97.5th percentile of the data, whereas the box depicts the interquartile range. Median of the data are depicted by the black horizontal line inside the boxplot. Boxplots in light blue represent hemophilia A patients without postoperative bleeding, darker blue depicts patients with a postoperative bleeding. Of course, FVIII levels were low before surgery as all these patients received replacement therapy with FVIII concentrate. (B) No association in VWF:Ag levels between patients with and without a bleeding. (C) Finally, VWF:GPIbM levels were also similar between patients with and without a bleeding.

VWFpp and VWF:Ag may increase up to four- to fivefold.¹⁵ However, VWFpp/VWF:Ag ratio is expressed in units, thereby set to one, and not expressed in molar amounts. An acute release of both VWFpp and VWF:Ag results in equal increases in molar amounts, but when interpreted as units, the increase of VWFpp will be much higher. Initially, during an acute release phase, VWFpp/VWF:Ag ratio increases due to increases of both VWFpp and VWF:Ag. However, as VWFpp has a half-life of approximately 2 hours, and VWF:Ag a half-life of 8 to 12 hours, rapid VWFpp increase will also diminish within a short time period. Our findings support this hypothesis as VWFpp/VWF:Ag ratio was shown to normalize 48 hours after surgery. In addition, the VWFpp/VWF:Ag ratio will also normalize as a result of a probable increased consumption of VWF:Ag after surgery.

Acute phase VWF response may also be quantified by calculating VWF:Ag/VWF:GPIbM ratio. As VWF:Ag/VWF:GPIbM ratio decreased during subsequent postoperative days in our study, we hypothesized that this may be a consequence of the following pathophysiological mechanisms. First, higher a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) activity may be present due to surgery as a result of increased release of high molecular weight (HMW) VWF multimers. However, this is unlikely as Kahlon et al has shown that ADAMTS13 actually decreases after surgery. Second, constitutive secretion of VWF from the endothelium may increase, resulting in more low molecular weight (LMW) VWF during surgery.¹⁶ As strictly regulated VWF secretion from Weibel–Palade bodies results in more HMW VWF multimers, more constitutive secretion with more LMW VWF may lead to a smaller VWF:Ag/VWF:GPIbM ratio. Third, utilization of large HMW VWF multimers during surgical clot formation may lead to decreasing postoperative VWF:Ag/VWF:GPIbM ratios, as HMW VWF multimers have the highest platelet binding activity.

Most likely, observed interpatient variability of VWF is multifactorial. However, in our linear mixed-effect model, blood type and surgical risk were shown to be most relevant when predicting VWF:Ag fluctuations over time. As it is well known that VWF levels are on average 25% lower in patients

with blood type O, it was not surprising to find blood type as an important risk factor for lower VWF levels.¹⁷ Patients undergoing surgery with a medium surgical risk were associated with higher VWF:Ag levels compared with those with a low surgical risk, explained by greater physical adrenergic (shear) stress reactions as a consequence of more extensive surgery. Influence of VWF levels on age and BMI were unexpectedly small. Prior studies have identified age and BMI as important covariates when predicting VWF levels.^{18,19} Therefore, exclusion of these variables was overruled. Unfortunately, extensive testing of VWF:Ag modifying factors was limited, due to small patient numbers.

Although the highest quartile VWF:Ag levels (VWF 1.70–3.84 IU/mL) was associated with a decrease of 26 mL/h (95% CI 2–50 mL/h) in FVIII clearance when compared with lowest VWF:Ag level quartile (0.43–0.92 IU/mL), VWF effects on FVIII clearance were only minimal and not as important as expected. However, a recent pilot study by Loomans et al was also not able to show a decreased FVIII clearance with increased FVIII half-life after intravenous desmopressin infusion before FVIII concentrate administration. Study hypothesis was also that endogenous VWF increase after desmopressin would positively affect FVIII levels.²⁰ In our study, sufficiently high VWF levels, as is characteristic for the perioperative setting in non-von Willebrand disease patients, may lead to a threshold effect and therefore not significantly affect FVIII clearance. Therefore, if patients have sufficiently high VWF levels, dosing of FVIII concentrate need not be adapted based on these VWF levels. However, it is important to realize that PK parameters in our study were calculated with a perioperative population PK model without a time-dependent variable for clearance. This makes it more difficult to observe subtle changes of FVIII clearance over time.¹¹ Therefore, a limitation of our study is that the design may not be ideal to establish VWF effects on FVIII clearance during the perioperative time period. The novel perioperative population FVIII PK model under construction and enriched with prospectively collected VWF and FVIII levels from our randomized controlled OPTI-CLOT trial will lead to further elucidate FVIII clearance mechanisms.

Only a small number of study patients, for example, 9 out of 59 (15%), experienced perioperative bleeding. Bleeding was not associated with VWF levels or VWFpp/VWF:Ag ratio. In addition, no statistically significant differences were observed in VWF:Ag or VWF:GPIIb/IIIa when comparing patients with surgical bleeding and patients without bleeding. We could not prove the hypothesis that VWFpp/VWF:Ag ratios higher than 1.5 were associated with perioperative bleeding as higher VWF clearance could potentially lead to an inadequate primary hemostasis. Capacity for statistical analyses was however limited due to small patient and complication numbers.

In conclusion, we are the first to report on VWF kinetics and FVIII clearance in perioperative hemophilia patients. VWF increased perioperatively in hemophilia A patients with blood type, and surgical risk as most important predictors of VWF increase. VWF levels only showed a small effect on FVIII clearance and were not associated with perioperative hemorrhage. We recommend further investigation into VWF and its role in the perioperative period of hemophilia A patients by refinement of current population PK models with VWF data and ultimate population PK-pharmacodynamic modeling to further unravel pathophysiological mechanisms of the hemostatic system.

What is known about this topic?

- von Willebrand factor (VWF) protects FVIII from premature clearance.
- VWF may be crucial for optimal dosing of factor VIII concentrate (FVIII) in hemophilia A patients.
- It is unknown how VWF behaves and what its impact is on FVIII clearance in the perioperative setting.

What does this paper add?

- VWF levels increase postoperatively, most significantly in patients with blood type non-O or medium risk surgery.
- Lower VWF antigen levels are associated with only minimally higher FVIII clearance.
- VWF levels are not associated with perioperative hemorrhage.

Authors' Contributions

M.C., I.M., and H.C.A.M.H. were responsible for protocol design and study implementation. I.M. enrolled patients, performed blood sampling for PK analysis, collected data, performed statistical analyses, and is main author of the manuscript together with M.C. L.B. performed population pharmacokinetic calculations. R.S., B.L., L.H., F.M., K.F., F.L., and K.M. monitored patient inclusion. M.C., R.M., J.E., F.L., K.F., K.M., and M.d.M. gave critical guidance during the project. M.C. and R.M. supervised the study. All authors substantially contributed to the writing and critically revised the manuscript, with approval of the final draft.

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Conflict of Interest

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