



# Comparison between coagulation factor VIII quantified with one-stage activity assay and with mass spectrometry in haemophilia A patients: Proof of principle

Anouk A. M. T. Donners<sup>1</sup> | Erik M. van Maarseveen<sup>1\*</sup> | Yrea R. J. Weetink<sup>1</sup> | Mohsin El Amrani<sup>1,2</sup> | Kathelijnn Fischer<sup>3</sup> | Carin M. A. Rademaker<sup>1</sup> | Toine C. G. Egberts<sup>1,4</sup> | Albert Huisman<sup>2</sup> | Ruben E. A. Musson<sup>2</sup>

<sup>1</sup>Department of Clinical Pharmacy, University Medical Centre Utrecht, Utrecht, The Netherlands

<sup>2</sup>Clinical Chemistry and Haematology, University Medical Centre Utrecht, Utrecht, The Netherlands

<sup>3</sup>Van Creveldkliniek, University Medical Centre Utrecht, Utrecht, The Netherlands

<sup>4</sup>Division of Pharmacoepidemiology and Clinical Pharmacology, Department of Pharmaceutical Sciences, Faculty of Science, Utrecht University, The Netherlands

## Correspondence

Anouk A. M. T. Donners, Department of Clinical Pharmacy, University Medical Centre Utrecht, Postbus 85500, 3508 GA Utrecht, The Netherlands.  
Email: a.a.m.donners@umcutrecht.nl

## Abstract

**Introduction:** Haemophilia A is a hereditary bleeding disorder caused by a factor VIII (FVIII) deficiency. As biomarker, FVIII activity is used to classify disease severity and to monitor treatment. The one-stage clotting assay (OSA) is performed to measure FVIII activity, but OSA's limitations may result in misclassification of disease severity or suboptimal monitoring of treatment. Measurement of FVIII plasma concentration with liquid chromatography-tandem mass spectrometry (LC-MS/MS) might overcome these challenges. The objective is to investigate the correlation between FVIII activity and concentration, and determinants for differences between the two methods.

**Methods:** In this cross-sectional study, all haemophilia A patients receiving standard-of-care were eligible for inclusion. Within the activity categories of <1 IU/dL, 1-5 IU/dL, >5-40 IU/dL, >40-150 IU/dL and >150-600 IU/dL, we randomly selected 15-20 plasma samples and compared FVIII concentration (LC-MS/MS) to FVIII activity (OSA) with linear regression and Bland-Altman analysis. Potential determinants for differences were analysed with linear regression.

**Results:** Inclusion was 87 samples. Bland-Altman analysis demonstrated an overall mean difference of -1% with an SD of 64% between the two methods. Large differences were correlated with the presence of anti-FVIII antibodies (133% [95% CI: 81, 185] n = 5) and use of exogenous FVIII products (-37% [95% CI: -65, -9] n = 58), for example plasma-derived and B-domain-modified FVIII products.

**Conclusions:** Despite good overall correlation between the two methods, relative differences were large, especially for samples with anti-FVIII antibodies or exogenous FVIII products. These differences may have clinical impact. More research is

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needed to determine the value of FVIII plasma concentration in comparison with FVIII activity.

#### KEYWORDS

blood coagulation tests, factor VIII, haemophilia A, mass spectrometry, proof of concept

## 1 | INTRODUCTION

Haemophilia A is a hereditary bleeding disorder resulting from a deficiency or dysfunction of endogenous coagulation factor VIII (FVIII) with a prevalence of 1:5000 male live births.<sup>1-3</sup> The International Society of Thrombosis and Haemostasis classifies the severity of haemophilia A based on the endogenous FVIII activity as severe (<1 IU/dL), moderate (1-5 IU/dL) or mild (>5-40 IU/dL), all three with a specific phenotype.<sup>4</sup> Patients with severe haemophilia (approximately 40% of haemophilia patients) have spontaneous or provoked bleedings in soft tissue and joints, causing arthropathy, impaired quality of life and higher risks of intracranial haemorrhage or early death. Patients with moderate haemophilia, in contrast, are less affected but suffer from, for example, prolonged bleeding or easy bruising. Patients with mild haemophilia only experience bleeding problems during and after major trauma or surgery.<sup>5-7</sup> The standard of care in the developed regions with access to costly FVIII products preferably entails an intravenous substitution of exogenous FVIII products based on disease severity and bleeding phenotype. Typically, severe patients receive regular prophylactic infusions with FVIII, and mild or moderate patients are treated in case of bleedings only (on-demand). The dose is often based on an individualised pharmacokinetic profile of a patient's FVIII activity. To minimise bleeding risk and to prevent bleedings, many protocols aim at maintaining minimum trough levels of FVIII activity (>1 IU/dL) in patients with severe haemophilia.<sup>8</sup>

The FVIII activity is currently used as a biomarker to assess disease severity and for monitoring treatment with FVIII products which is dependent on an accurate and precise quantification. The FVIII activity can be measured in clinical laboratories with the one-stage clotting assay (OSA) and/or the chromogenic assay (CSA). The OSA is based on the activated partial thromboplastin time (aPTT), making it easily automated, simple, fast and inexpensive compared to CSA. The CSA is perceived to be more complex and technically challenging as a consequence of the two-stage principle with factor X activation and an additional chromogenic substrate step.<sup>9</sup> For diagnosing, it is recommended to perform multiple OSA measurements, to combine both OSA and CSA to ascertain the absence of discrepancies or to evaluate the mutation profile of a patient.<sup>10</sup>

Unfortunately, FVIII activity measuring has several limitations. Not only can these limitations result in misclassification of disease severity leading to under- or overestimation of the bleeding phenotype in specific subgroups, but also can result in suboptimal treatment monitoring of patients receiving FVIII replacement products.<sup>11,12,13</sup> Both OSA and CSA are hampered by interference of different drugs (eg heparin, direct oral anticoagulants) and endogenous inhibitors such as lupus

anticoagulant. Results from the assays are also affected by interlaboratory variability, caused by the use of a wide variety of instruments, reagents, standards and dilution algorithms.<sup>14, 15</sup>

We have recently developed and published a novel method to determine the human FVIII plasma concentration with liquid chromatography-tandem mass spectrometry (LC-MS/MS).<sup>16</sup> The LC-MS/MS technique enables quantification of the FVIII molecule with a high sensitivity and specificity. Although this method is further upstream than activity measurements and has its shortcomings as well, the new method might also have some advantages. For example, sampling could be done by patients themselves at home using the dried blood spot technique. The primary objective of this proof of principle study is therefore to investigate the correlation between FVIII activity measured with OSA compared to FVIII plasma concentration measured with LC-MS/MS in patients with haemophilia A, and to identify determinants for differences between the two methods.

## 2 | MATERIALS AND METHODS

### 2.1 | Setting and participants

This cross-sectional study was conducted at the University Medical Centre Utrecht, and specifically at the laboratories of the Department of Clinical Chemistry and Haematology and the Department of Clinical Pharmacy. All haemophilia A patients or female carriers receiving standard-of-care treatment were eligible for inclusion. Their remnant material was stored in accordance with the local opt-out procedure. Within each of the clinically used FVIII activity categories (<1 IU/dL, 1-5 IU/dL, >5-40 IU/dL, >40-150 IU/dL, >150-600 IU/dL), 15-20 samples were randomly selected in the period of August 2017 to March 2018. The FVIII plasma concentration was measured with LC-MS/MS and compared to the FVIII activity measured with OSA. Per patient, a maximum of one sample was included per category. The local institution's ethics committee approved the procedure and provided a waiver for patients' consent. This study was conducted in accordance with the current revision of the Declaration of Helsinki as revised in 2013.

### 2.2 | FVIII activity with OSA

After blood had been drawn for usual care, the FVIII activity was measured at the ISO15189-certified Laboratory of Clinical

Chemistry and Haematology and reported as a percentage of FVIII activity compared to reference plasma. The FVIII activity was measured on a STA-Rack evolution coagulation analyser using STA CK Prest aPTT reagent (Diagnostica Stago, Asnières-sur-Seine, France). The FVIII-deficient plasma and reference plasma were obtained from Precision Biologic (Dartmouth, NS, Canada). The assay had a within-run %CV of 3.8% at a FVIII activity of 1.3 IU/dL, a between-run %CV of 4.6% at a FVIII activity of 34 IU/dL, a between run %CV of 5.6% at a FVIII activity of 88 IU/dL, a linearity range <1-600 IU/dL, a recovery of 100.7% and a 1.0 IU/dL lower limit of quantification. Robust internal and external quality assessment schemes were performed on the assay, and local performance characteristics were within the pre-defined limits stated by the manufacturers.

### 2.3 | FVIII plasma concentration with LC-MS/MS

The FVIII plasma concentration was measured using the LC-MS/MS method at the ISO15189-certified Laboratory of Clinical Pharmacy. The LC-MS/MS method development is extensively described in the previously published manuscript of El Amrani et al<sup>16</sup> and was validated in accordance with European Medicines Agency guidelines on bioanalytical methods. In brief, the method starts with the dissociation of the Von Willebrand factor from FVIII by triggering the coagulation cascade, which generates an unbound FVIII molecule. Subsequently, FVIII is selectively extracted by immunoaffinity interaction using monoclonal anti-FVIII camelid nanobodies. After washing, the FVIII is eluted, heat denatured and trypsin digested. Finally, a specific peptide sequence from the A3 active domain of the FVIII molecule is selected as a surrogate for quantification by mass spectrometry. This signature peptide has a sequence that is unique for FVIII proteins, both endogenous and exogenous, and the sequence is not present in other human proteins. The method had a mean precision (%CV) within-run of 6.8% and between-run of 7.2%, a mean accuracy (bias%) of -2.6%, a linearity range 1-500 ng/mL and 1 ng/mL was the lower limit of quantification.

### 2.4 | Determinants and patient characteristics

Patient-, disease- and treatment characteristics, such as gender, age, weight, exogenous FVIII products, anti-FVIII antibodies (also termed inhibitors) and comedication interacting with the coagulation cascade, were identified as potential determinants for differences between the two methods. Anti-FVIII antibodies were measured with the Bethesda assay (Nijmegen modification) for which the clinical cut-off  $\geq 0.6$  Bethesda Units (BU) per millilitre was used.<sup>17</sup> Samples were carefully checked for presence of exogenous FVIII products by (a) all comments linked to the assay orders, (b) data on active medication obtained from the Research Data Platform (also taking drug half-life into account) and (c) multiple measurements of activity and inhibitors per patient in time. Based on this information, the samples

were labelled as “exposed” or “unexposed” to exogenous FVIII product, independent of the presence of endogenous FVIII or procoagulant comedication. The information regarding the determinants was obtained from medical records using the Utrecht Patient Oriented Database, a local data platform.<sup>18</sup>

### 2.5 | Data analysis

To enable statistical analyses on laboratory results expressed in different units and with categories of different interval size, the correlation between FVIII activity and plasma concentration was evaluated with linear regression and a Bland-Altman analysis. The potential determinants of relative differences were analysed with linear regression analysis. The differences in the Bland-Altman analysis and in the linear regression of the determinants were expressed as relative differences not only to compare the two parameters of different units but also to compare the differences over the complete activity range (as an absolute difference in the lower range would weigh less compared to an absolute difference in the higher activity category). For the interpretation of the latter argument, an ln transformation of the linear regression is performed. Relative differences were calculated as follows:

$$\text{Relative difference (\%)} = \frac{(\text{plasma concentration} - \text{activity})}{((\text{plasma concentration} + \text{activity}) / 2)} \times 100\%$$

As the units of both parameters are different, the values of the relative differences can only be used for the statistical interpretation of the result and cannot be interpreted clinically. Univariable linear regression analysis was used to assess the correlation of relative difference with various FVIII products (using nonexposed samples as a reference), patient characteristics (age, weight, presence of anti-FVIII antibodies) and comedication. In the multivariable regression analysis, differences associated with exogenous FVIII products were adjusted for anti-FVIII antibodies and vice versa. The application IBM SPSS Statistics for Windows (version 25.0. IBM Corp.) was the software used for statistical analysis.

## 3 | RESULTS

In the study, 87 samples were included, from 70 patients (54 patients with one sample, 15 patients with two samples and one patient with three samples) as patients were allowed to be included in multiple categories. The study population consisted primarily of men (98%) with a mean age of 37 years and a mean body mass index of 23 kg/m<sup>2</sup>. Of the included samples, 6% had anti-FVIII antibodies  $\geq 0.6$  BU/mL and 67% had exogenous FVIII product present (see Table 1 for more patient characteristics). Fifty-eight samples contained one or two FVIII products: mostly one FVIII product, four samples contained two FVIII products and two samples contained an unspecified FVIII product.

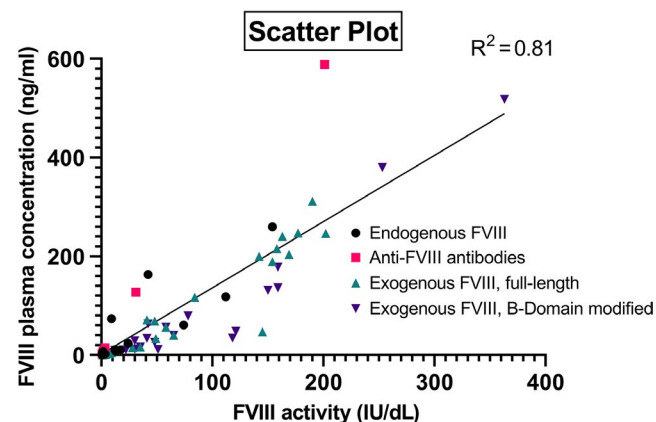
**TABLE 1** Patient characteristics

	Number	Percentage of total
Total	n = 87	100%
Gender, male	n = 85	98%
Anti-FVIII antibodies ( $\geq 0.6$ BU/mL)	n = 5	6% (0.8–5.3 BU/mL)
Samples “exposed” to exogenous FVIII products	n = 58	67%
Samples “unexposed” to exogenous FVIII products	n = 29	33%
Plasma-derived FVIII, full-length		
Aafact®	n = 7	8%
Recombinant FVIII, full-length	n = 26	30%
Octocog alfa, Advate®	n = 8	9%
Octocog alfa, Helixate®/Kogenate®	n = 18	21%
Recombinant FVIII, B-domain modified	n = 27	31%
Turoctocog alfa, NovoEight®	n = 22	25%
Efmoroctocog alfa, Elocta®	n = 5	6%
	<b>Mean</b>	<b>SD</b>
Age (years)	37	22
Height (cm) (n = 72)	167	33
Body weight (kg) (n = 75)	69	31
Body mass index (kg/m <sup>2</sup> ) (n = 72)	23	6

Abbreviations: FVIII, factor VIII; BU, Bethesda Units.

The correlation between the FVIII activity and FVIII plasma concentration demonstrated an overall  $R^2$  of .81 (Figure 1). For better interpretation of the lower range, a linear regression figure with ln transformation was made available in Figure S1.

The Bland-Altman analysis showed an overall mean difference of  $-1\%$  between FVIII activity and plasma concentration, with an SD of 64% (limits of agreement are  $-127\%$  to  $125\%$ ) and more variability



**FIGURE 1** Scatter plot of linear regression between FVIII plasma concentration (measured with LC-MS/MS) compared to FVIII activity (measured with OSA).  $R^2$  of .81. Subgroups are illustrated with different symbols, see legend [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

in the lower measurement range. The relative differences were normally distributed. When cut-off at a mean of 40% (the upper activity limit for haemophilia A diagnosis), the mean difference was  $-13\%$  in the lower range (mean  $< 40\%$ ) and a mean difference of 20% was found in the upper range (mean  $> 40\%$ ). In Figure 2, Bland-Altman plots are demonstrated for four different subgroups, all with the overall mean and limits of agreement.

Linear regression analyses demonstrated the correlation between determinants and the relative differences in the results from LC-MS/MS and OSA. Relevant results are demonstrated in Table 2, and two significant results are illustrated in the boxplots of Figure 3. The relative differences in samples with anti-FVIII antibodies ( $\geq 0.6$  BU/mL,  $n = 5$ ) were 133% (95% CI: 81; 185%) higher compared to the relative differences in samples without anti-FVIII antibodies. The relative differences in samples with exogenous FVIII products ( $n = 58$ ), the “exposed” group, were  $-37\%$  (95% CI:  $-65$ ;  $-9\%$ ) when compared to samples without exogenous FVIII ( $n = 29$ ), the “unexposed” group. When multivariable linear regression was performed, the found correlation between differences and exogenous FVIII products was independent of anti-FVIII antibodies and vice versa (both  $P < .01$ ).

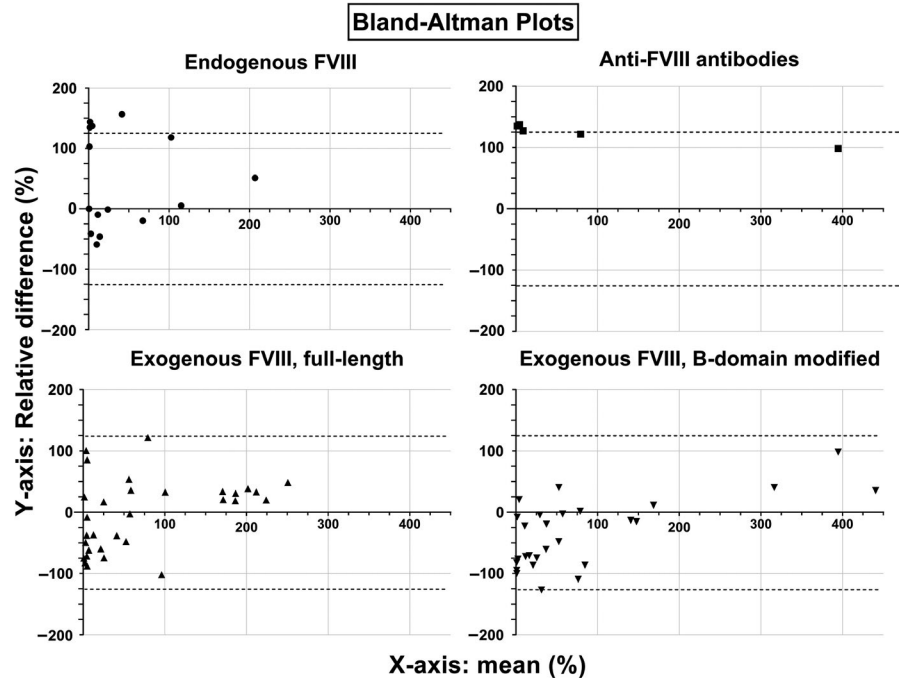
Also, specific FVIII product subgroups were compared to the unexposed group. Plasma-derived FVIII product ( $n = 7$ ) had a relative difference of  $-64\%$  (95% CI:  $-114$ ;  $-15\%$ ). The two B-domain-modified products included in this study were turoctocog alfa (NovoEight®  $n = 22$ ) and efmoroctocog alfa (Elocta®  $n = 5$ ). The relative differences in samples with B-domain-modified products ( $n = 27$ ) were  $-58\%$  (95% CI:  $-89$ ;  $-26\%$ ) lower compared to the relative differences in the unexposed group. Linear regression was also performed on turoctocog alfa and efmoroctocog alfa separately and significantly demonstrated their relative differences were  $-53\%$  (95% CI:  $-86$ ;  $-20\%$ ) and  $-77\%$  (95% CI:  $-136$ ;  $-17\%$ ) lower when compared to the relative differences in the unexposed samples. In general, all exogenous FVIII products resulted in negative relative differences (Table 2), meaning that the FVIII plasma concentration is lower compared to the FVIII activity.

No significant differences were found for age, body weight, the subgroup full-length FVIII products ( $n = 32$ ), octocog alfa (recombinant full-length FVIII: Advate®  $n = 8$ ; Helixate®/Kogenate®  $n = 18$ ), comedications desmopressin ( $n = 6$ ), heparin and low-molecular-weight heparins ( $n = 16$ ), tranexamic acid ( $n = 10$ ) and comorbidities heart disease ( $n = 6$ ) or hepatitis B and C ( $n = 19$ ). The determinants gender, other comedications (such as oral anticoagulants, antiplatelet drugs, immunosuppressive drugs and corticosteroids) and other comorbidities (such as HIV, lung disease, hypertension) could not be studied because of low variability and numbers.

## 4 | DISCUSSION

This study demonstrates that regardless of an overall strong correlation, there is a large variability between FVIII activity measured

**FIGURE 2** Bland-Altman plots of relative difference compared to mean (FVIII plasma concentration – FVIII activity)/2). Subgroups are illustrated per panel and with different symbols, see subtitles. Overall mean of –1%, limits of agreement are –127% to 125%



by OSA and the FVIII plasma concentration measured with LC-MS/MS. Significant differences between the two methods were independently correlated with the presence of anti-FVIII antibodies or use of exogenous FVIII products.

#### 4.1 | Correlation and variability

To our knowledge, this is the first clinically established comparison between a FVIII activity assay and a FVIII plasma concentration method using LC-MS/MS. A strong correlation was expected; however, we did not expect this large variability. Explanations for the large variability could be (a) the influences of the determinants and (b) the OSA result variability. We found that the FVIII plasma concentration measurements are evidently higher compared to the FVIII activity in the high measurement range (from >40). As only haemophilia A patients were included, activity results >40 IU/dL (cut-off for diagnosis) could be indicative for the presence of FVIII product. When hypothesizing, a potential false estimate of FVIII activity in this range could result in a misleading drug half-life, which might be interesting to investigate with efficacy outcome measurements in new upcoming research.<sup>8</sup> Another explanation for a higher plasma concentration than activity is that LC-MS/MS method may measure dysfunctional FVIII, with no activity, in so-called cross-reacting material (CRM)-positive patients.<sup>19</sup> In contrast, in the range of <1-40 IU/dL FVIII activity, most measurement points were under the regression line or relative difference line (indicating a higher activity than plasma concentration). In other studies, OSA demonstrated a significantly higher activity than CSA in approximately 30% of the moderate and mild patients with haemophilia A.<sup>13,20,21</sup> To prevent misclassification in

the diagnostic phase, but especially to prevent underestimation of the bleeding risk, it is preferred to use more than one OSA activity measurement, to combine OSA with CSA or to identify the FVIII gene mutation.<sup>10,17</sup> Whether a FVIII plasma concentration measurement is a better representation of the clinical effect than the FVIII biological coagulation activity cannot be demonstrated with the results of this exploratory study. The presented data support the supposed overestimation of OSA in selected samples, which might indicate that LC-MS/MS could be a useful predictor for classification of disease severity and therapeutic monitoring in these specific patients, but this needs to be confirmed with further research.

#### 4.2 | Determinants

Currently, the major complication in haemophilia A treatment is the development of neutralising anti-FVIII antibodies, rendering endogenous and exogenous FVIII ineffective. Anti-FVIII antibodies neutralise the FVIII activity by forming FVIII-antibody complexes that accelerate clearance of FVIII or by sterically hindering the interaction of FVIII with other coagulation factors.<sup>22,23</sup> For the samples with anti-FVIII antibodies (in the range 0.8-5.3 BU/mL, n = 5) present, we found a substantial higher FVIII plasma concentration than activity. Due to the small sample size of anti-FVIII antibodies in this study, the proportional correlation between antibody level and relative difference could not be confirmed. This result might indicate that for some patients the specific FVIII-antibody complexes are still present in the circulation, not being cleared by the immune system, and that nonfunctional FVIII is still being measured by the LC-MS/MS method. This phenomenon deserves

**TABLE 2** Linear regression analysis on the correlation between the determinant and relative differences

Univariable linear regression analysis	B% (95% CI)	p-value
Age	-0.3 (-0.9; 0.3)	.372
Weight	-0.2 (-0.7; 0.3)	.498
Anti-FVIII antibodies ( $\geq 0.6$ BU/mL)	132.6 (80.7; 184.5)	<.001
Exogenous FVIII products (exposed) <sup>a</sup>	-36.7 (-64.8; -8.5)	.011
Full-length FVIII products <sup>a</sup>	-29.3 (-60.0; 1.3)	.060
pdFVIII		
rFVIII		
pdFVIII, Aafact® <sup>a</sup>	-64.1 (-113.8; -14.5)	.013
Full-length rFVIII <sup>a</sup>	-21.2 (-53.4; 11.1)	.194
Octocog alfa, Advate®		
Octocog alfa, Helixate®/Kogenate®		
B-domain-modified rFVIII products <sup>a</sup>	-57.5 (-88.7; -26.4)	<.001
Turoctocog alfa, NovoEight®		
Efmoroctocog alfa, Elocta®		
Efmoroctocog alfa, Elocta® <sup>a</sup>	-76.7 (-136.1; -17.3)	.013
Turoctocog alfa, NovoEight® <sup>a</sup>	-53.2 (-86.2; -20.2)	.002
Comedication		
Desmopressin	9.3 (-45.1; 63.7)	.735
Tranexamic acid	-8.9 (-52.1; 34.3)	.683
Heparin	24.9 (-10.3; 60.1)	.163
Comorbidities		
Any heart disease	24.9 (-29.2; 79.1)	.363
Hepatitis B or C	-8.9 (-42.2; 24.4)	.597

Abbreviations ;B, the influence of determinant (slope in linear regression); 95%-CI, 95%-confidence interval; FVIII, factor VIII; pd/rFVIII, plasma-derived/recombinant factor VIII.

<sup>a</sup>Reference group is "unexposed to exogenous FVIII products (n = 29)".

further clinical exploration, but could not be included in the present study as bleeding data were unavailable for this retrospective study on remnant material.

Another essential finding of this study is the trend seen in exogenous FVIII products of a higher FVIII activity than plasma concentration. This result is consistent with the study of Barrowcliffe et al<sup>24</sup> mentioning an overestimation of FVIII activity by unbound FVIII, as FVIII may become activated during sample collection. Other reasons for overestimation of FVIII activity are that OSA is simply not validated for innovative new products such as shortened (turoctocog alfa) or deleted (efmoroctocog alfa) B-domain-modified products or

Fc-fusion (also efmoroctocog alfa) products, and also the discrepancy seen in mild and moderate patients with haemophilia A.<sup>5,12,21</sup> The LC-MS/MS method is less affected by the new types of FVIII products as the camelid antibodies have a high affinity for the specific FVIII epitope. It should be mentioned that the full-length FVIII product group was on borderline of significance, which might make this group interesting as well to investigate in a study with more power.

### 4.3 | Limitations and strengths

The study was limited by the retrospective design and the lack of an appropriate gold standard to compare to the new LC-MS/MS method. Although OSA is a functional assay with result variability, OSA was used as a reference in this study, as it is the most used method worldwide, and corrected for by using relative differences in this study.<sup>24</sup> Bias might have been introduced by including the same patient in another category, but a sensitivity analysis demonstrated no differences in results. We performed this study as a proof-of-principle experiment to commence validated further clinical research on FVIII quantified with mass spectrometry, therefore undoubtedly making the strength of this study its first clinical contribution.

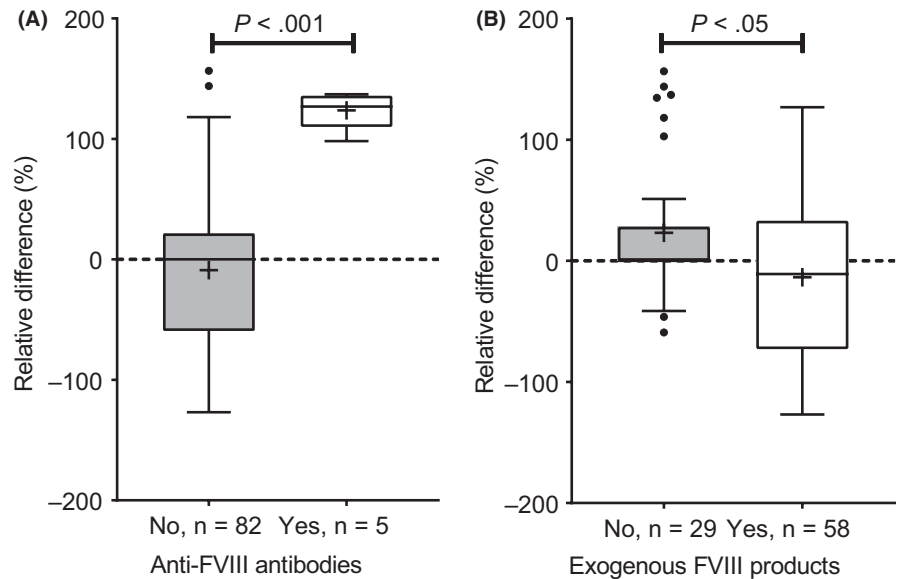
### 4.4 | Recommendations

Further research regarding the quantification of FVIII with OSA, CSA and LC-MS/MS is indicated and is expected to start at our centre to make a well-founded recommendation for the potential correcting steps per different subgroups of patients and products and for correcting steps to simplify the interpretation of the FVIII plasma concentration. A first recommendation for future research would be to conduct a prospective study where the FVIII activity (OSA and CSA) and FVIII plasma concentration (LC-MS/MS) are measured and compared in multiple samples over time, in a curve, after administering FVIII product to a haemophilia A patient, or after a high-stress test in a volunteer. This would allow investigating the inpatient variability, potential loss of activity over time and mutated FVIII molecules. Another recommendation for future research is to conduct a study with different types of FVIII products and different batches per FVIII product that are reconstituted, not only with FVIII-deficient plasma (as human material might contain FVIII fragments) but also with solvents (eg NaCl 0.9%) to compare OSA, CSA and LC-MS/MS results over time. As the LC-MS/MS method was developed with one FVIII product, the method would benefit from studying multiple product types and batches, for example to compare different activation rates.

Major advantages of LC-MS/MS measuring are the possibility to elucidate the discrepancies between OSA and CSA in different patient populations, to distinguish between neutralizing and clearing



**FIGURE 3** Boxplots comparing the relative differences of the determinant group with the nondeterminant group. The “+” illustrates the mean. Boxplot 3A, determinant is anti-FVIII antibodies  $\geq 0.6$  BU/mL with a mean relative difference 133% (95% CI: 81, 185%). Boxplot 3B, determinant is the exposed group compared to unexposed group with a mean relative difference  $-37\%$  (95% CI:  $-65, -9\%$ )



anti-FVIII antibodies and to determine the FVIII concentration variation in the normal population. Other advantages with more clinical implications for the future are that LC-MS/MS offers the opportunity to patient-friendly telemonitoring (with dried blood spot) facilitating blood sampling from home and a patient-friendly sampling volume, which could be used in neonatal diagnostic screening as  $50\mu\text{L}$  would suffice. In the future, the possibility of mass spectrometry-based techniques to measure multiple samples in one run could be exploited by the combined measurement of multiple clotting and anticlotting factors, thus constructing a personal haemostasis profile and bleeding score.<sup>25</sup>

## 5 | CONCLUSION

Despite a strong overall correlation between the two methods, the relative differences between measured FVIII activity and FVIII plasma concentration in individual samples were large, especially in case of the presence of anti-FVIII antibodies or use of exogenous FVIII products. These differences may have impact on clinical decision-making regarding diagnosing disease severity and monitoring the treatment of FVIII products. Further research is needed to determine the value of FVIII plasma concentration measurements in comparison with the FVIII activity measurements.

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## CONFLICT OF INTERESTS


The authors have declared that no competing interests exist.

## AUTHOR CONTRIBUTIONS

AD, EM, CR and TE have designed the study, retrieved data and drafted the manuscript. AD, YW, TE and MA performed

statistical and analytical analysis. All authors assisted in data analysis and interpretation, critically reviewed and approved of the final manuscript.

## ORCID

Anouk A. M. T. Donners  <https://orcid.org/0000-0002-8147-013X>

Albert Huisman  <https://orcid.org/0000-0002-2291-2487>

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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