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ORIGINAL ARTICLE

Impact of a product-specific reference standard for the measurement of a PEGylated rFVIII activity: the Swiss Multicentre Field Study

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Introduction: Measuring factor VIII (FVIII) activity can be challenging when it has been modified, such as when FVIII is pegylated to increase its circulating half-life. Use of a product-specific reference standard may help avoid this issue. **Aim:** Evaluate the impact of using a product-specific reference standard for measuring the FVIII activity of BAX 855 – a pegylated FVIII – in eight of Switzerland's main laboratories. **Methods:** Factor VIII-deficient plasma, spiked with five different concentrations of BAX 855, plus a control FVIII sample, was sent to the participating laboratories. They measured FVIII activity by using either with a one-stage (OSA) or the chromogenic assay (CA) against their local or a product-specific reference standard. **Results:** When using a local reference standard, there was an overestimation of BAX 855 activity compared to the target concentrations, both with the OSA and CA. The use of a product-specific reference standard reduced this effect: mean recovery ranged from 127.7% to 213.5% using the OSA with local reference standards, compared to 110% to 183.8% with a product-specific reference standard, and from 146.3% to 182.4% using the CA with local reference standards compared to 72.7% to 103.7% with a product-specific reference standard. **Conclusion:** In this *in vitro* study, the type of reference standard had a major impact on the measurement of BAX 855 activity. Evaluation was more accurate and precise when using a product-specific reference standard.

Keywords: blood coagulation, blood coagulation tests, drug monitoring, factor VIII procoagulant activity, haemophilia A, *in vitro*

Introduction

Precise measurement of factor VIII (FVIII) activity is essential for monitoring therapies for haemophiliacs and, most importantly, in surgical settings [1]. FVIII levels are usually monitored using the one-stage clotting assay (OSA) calibrated against a human plasma pool standard. The two-stage (chromogenic) assay (CA) is less frequently used in coagulation laboratories because it may be more expensive [2] and perceived to be more complex and difficult to automate than the OSA [3]. However, the CA is associated with less interlaboratory variability and it detects some mild haemophilia variants better than the OSA does [3]. Measuring FVIII activity using both the OSA and CA may give divergent results, such as when the FVIII is modified (e.g. with deletion or truncation of the B domain) [4], leading many laboratories to use a product-specific reference standard (SS) to measure

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postinfusion B domain-deleted FVIII plasma samples [5].

Other modifications to FVIII have been evaluated with the goal of increasing its circulating half-life. Attaching polyethylene glycol (PEG) is considered an effective method of prolonging the half-life of recombinant proteins [6], but it makes the determination of FVIII activity even more challenging [7]. BAX 855 (Baxalta, now part of Shire, Vienna, Austria) is a PEGylated full-length rFVIII product and its measurement in plasma was recently investigated in a multicentre field study [8]. In this study, FVIII-deficient plasma spiked with three different concentrations of BAX 855 was evaluated in 55 laboratories performing the OSA; 11 of them also performed the CA. The majority of the participating laboratories (53 of 55) calibrated their method using a commercially available human plasma reference standard. They found that the mean recovery from the three target concentrations ranged from 101% to 124.3% with the OSA and from 95.4% to 124% with the CA, with an upper limit of the 95% confidence interval (CI) at 137.9% for a concentration of 20 IU dL^{-1} tested using the CA. The present study aimed to evaluate the impact of an SS on the measurement of BAX 855's FVIII activity in eight of Switzerland's main laboratories.

Materials and methods

This field study included all ($n = 8$) the laboratories represented in the Swiss Society of Haematology's Working Group on Haemostasis. They all fulfil either the ISO15189 or ISO17025 standards for measuring FVIII activity, agreed to participate in the study and answered a questionnaire collecting information on the assay methods, analysers and reagents they used to evaluate FVIII activity.

Sample preparation and assay design

Study samples were prepared using a commercially available non-lyophilized FVIII-deficient plasma (Siemens, Marburg, Germany, lot number 503250). Factor levels of the FVIII-deficient plasma were FVIII < 1 IU dL^{-1}, FV = 94 IU dL^{-1}, FIX = 87 IU dL^{-1}, FXI = 83 IU dL^{-1}, FXII = 89 IU dL^{-1} and vWF activity = 87 IU dL^{-1}. In the organizing laboratory (Geneva), FVIII-deficient plasma was spiked with BAX 855 (Adynovate, Baxalta, now part of Shire, lot number LE 18Q513AF, containing 262 IU of FVIII as provided by the manufacturer) to achieve a variety of target concentrations (3, 10, 30, 50 and 80 IU dL^{-1}). Positive controls were FVIII-deficient plasma samples spiked with standard human plasma (SHP, Siemens) at concentrations of 5, 10, 30, 50 and 80 IU dL^{-1} (control-FVIII). Master batches of each preparation were then distributed in aliquots blinded for FVIII concentration and immediately frozen at −80°C. Sample preparation was performed within 3 h of BAX 855 reconstitution and FVIII-deficient plasma thawing. The SS was prepared using FVIII-deficient plasma spiked with BAX 855 to achieve a target FVIII activity of 100 IU dL^{-1}. Control-FVIII and BAX 855 samples, in addition to the SS, were sent to each laboratory, frozen on dry ice.

Each laboratory received identical sets of aliquots. Each sample was run using both the OSA and CA with the batch of assay kit and reagent currently in use and with the local reference standard (LS) used in each laboratory or the SS provided by the organizing laboratory. Each evaluation of FVIII activity was performed in triplicate for each concentration, except for the lowest, intermediate and highest concentrations where the evaluation was performed 10 times per laboratory to ensure a reliable calculation of the intralaboratory coefficient of variation (CV) [9].

Statistical analyses

Differences between FVIII activity and target concentrations were described using dot plots. We used box plots as a visual representation for describing and assessing the FVIII activity distribution under each condition. As the distribution was normal, no logarithmic transformation was applied. We used a linear mixed-effect model with a random effect on laboratory for each possible condition and including all sample values to estimate the mean value, 95% CI and the precision of the measurements, which was defined as being within an interval containing 95% of results (mean ± 1.96 SD). These results were subsequently expressed as a percentage of the target concentration (recovery). Values in each laboratory were averaged to calculate an interlaboratory CV. Four unrelated, individual test results were considered as outliers as they were greater than three standard deviations from the laboratory sample mean and were not included in the data analysis.

Results and discussion

All eight laboratories performed the OSA and six also performed the CA. All the laboratories used silica/kaolin-based aPTT reagents (Siemens, ref OQGS35, $n = 4$; Instrumentation Laboratory, Bradford, USA, ref 20006300, $n = 2$; or Stago, Asnières, France, ref 00597, $n = 1$) except one which used ellagic acid reagent (Siemens, ref B42219). FVIII-deficient plasma was from Siemens (ref OTXW17, $n = 5$), Instrumentation Laboratory (ref 20011800, $n = 2$) or Stago, ref 00728, $n = 1$). The clot detection method was either mechanical ($n = 2$) or optical ($n = 6$). All the laboratories used commercially available LS (Siemens, $n = 5$; Instrumentation Laboratory, $n = 2$; Stago, $n = 1$). Chromogenic assays were from Siemens ($n = 3$, ref

B4238-40), Instrumentation Laboratory (n = 2, ref 49730503) and Hyphen Biomed, Neuville-sur-Oise, France (n = 1, ref 221402).

Control-FVIII activity measurement

The differences between control-FVIII activity and target concentrations, as evaluated using the OSA and CA, are depicted in Fig 1a and 1b, respectively. Differences are distributed around zero for each concentration with both assays, showing an increased variability with higher control-FVIII concentrations. Mean recoveries as a percentage of target control-FVIII activity are detailed in Table 1. Mean target recovery ranged from 100% to 128.2% for the OSA and from 61.1% to 105.7% for the CA. Intralaboratory CV and interlaboratory CV are shown in Table 2. Overall, intralaboratory CV was below 10% with all control-FVIII concentrations, using both the OSA and CA, except at the lowest concentration with the CA. Interlaboratory CV followed the same pattern, although with somewhat higher values than intralaboratory CV.

Table 1. Recovery of control-FVIII or BAX 855 activity measured using the one-stage clotting assay (OSA) or the two-stage (chromogenic) assay (CA).

			Reference standard	
		Target FVIII activity (IU dL^{-1})	LS Mean recovery (95% CI), % of target	SS
FVIII	Assay			
Ctrl-FVIII	OSA	5	128.2 (87.5–168.8)	–
		10	114.9 (92.3–137.4)	–
		30	109.9 (89.3–130.5)	–
		50	105.8 (87.7–123.9)	–
		80	100 (86.8–113.3)	–
	CA	5	61.1 (0–122.1)	–
		10	64.1 (8.3–119.9)	–
		30	104.6 (89.3–119.9)	–
		50	105.7 (89.9–121.6)	–
		80	99.7 (85.5–113.9)	–
BAX 855	OSA	3	213.5 (165.3–261.7)	183.8 (117.4–250.3)
		10	146.6 (118.9–174.3)	127.2 (95.8–158.5)
		30	132.2 (111.3–153.2)	110.0 (92.2–127.9)
		50	139.3 (120–158.6)	114.3 (102–126.5)
		80	127.7 (96.8–158.6)	111.0 (102.3–119.8)
	CA	3	146.3 (2.9–289.7)	78.8 (−3.3–160.9)
		10	172.1 (133–211.2)	72.7 (28.9–116.5)
		30	169.3 (147.4–191.2)	96 (83.1–108.8)
		50	173.7 (146.9–200.4)	102.8 (97.7–108)
		80	182.4 (143.7–221.2)	103.7 (99.7–107.7)

Ctrl-FVIII, control-FVIII; LS, local reference standard; SS, product-specific reference standard. N = 80 or 79 for OSA and n = 60 or 59 for CA.

BAX 855 activity measurement

Differences between BAX 855 activity and target concentrations according to assay type and reference standard are depicted in Fig. 1c–f. Inspection of the graph suggests that using LS overestimates FVIII activity in comparison with SS, especially in the CA (Fig. 1d), with variability increasing along with the BAX 855 concentration. The reference standard's impact (LS or SS) on the precision and accuracy of the measurement is more pronounced when using the CA (Fig. 1d and 1f) than OSA (Fig. 1c and 1e). Figure 2 depicts the results of BAX 855 activity measurements according

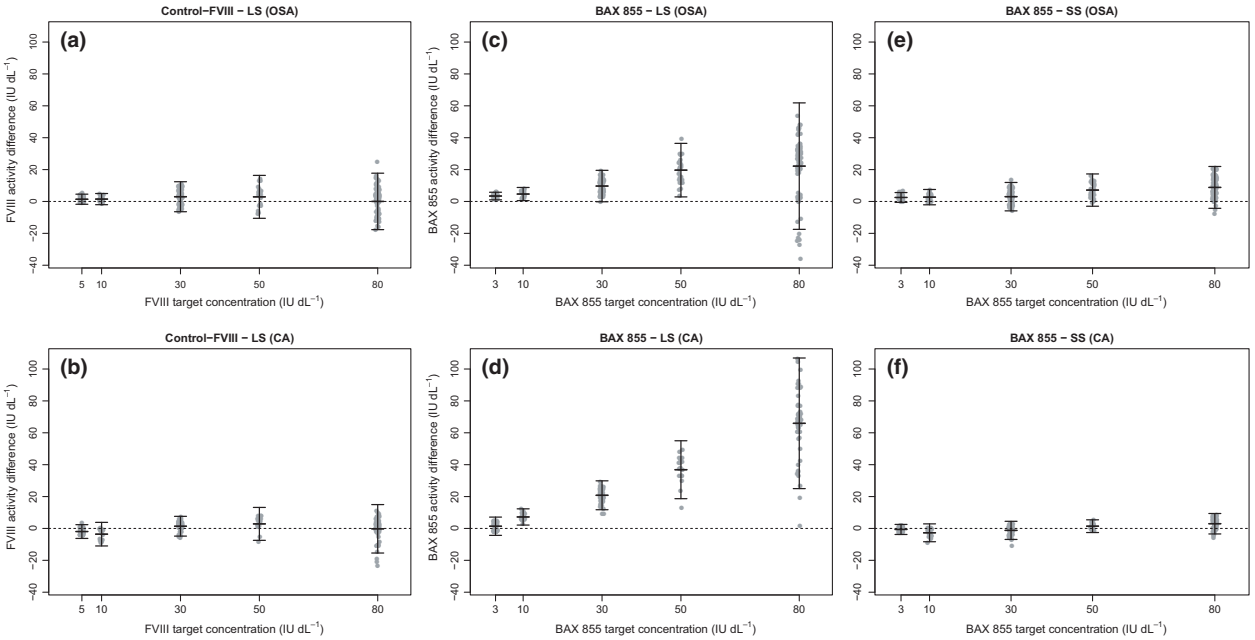

Fig. 1. Differences between control-FVIII or BAX 855 activity and target concentrations according to assay type and reference standard. OSA, one-stage clotting assay; LS, local reference standard; SS, product-specific reference standard; CA, two-stage (chromogenic) assay.

Table 2. Intra- and interlaboratory coefficients of variation (CV) for control-FVIII or BAX 855 activity measured using the one-stage clotting assay (OSA) or the two-stage (chromogenic) assay (CA).

FVIII	Assay	Target FVIII activity (IU dL^{-1})	Intralaboratory CV (%) Reference standard		Interlaboratory CV (%) Reference standard	
			LS	SS	LS	SS
Ctrl-FVIII	OSA	5	7.2	–	23.4	–
		30	4.4	–	13.8	–
		80	5.2	–	9.8	–
	CA	5	29.4	–	63.8	–
		30	4.1	–	9.3	–
		80	3.5	–	9.1	–
BAX 855	OSA	3	8.3	8.7	16.6	26.6
		30	4.6	6.3	11.7	12.0
		80	7.3	4.2	17.8	5.8
	CA	3	22.5	18.4	62.5	66.4
		30	3.8	4.6	8.2	8.5
		80	4.4	3.0	13.6	2.4

Ctrl-FVIII, control-FVIII; LS, local reference standard; SS, product-specific reference standard. N = 80 or 79 for OSA and n = 60 or 59 for CA.

to assay type and reference standards. Using the OSA, mean recovery ranged from 127.7% to 213.5% with the LS and from 111.0% to 183.8% with the SS. Using the CA, however, mean recovery ranged from 146.3% to 182.4% with LS compared to 72.7% to 103.7% with the SS (Table 1). Intra- and interlaboratory CV are shown in Table 2. Whereas intralaboratory CV was below 10% for all BAX 855 concentrations with the OSA and irrespective of the reference standard, the CA yielded higher CV at low BAX 855 concentrations. The same applied for the interlaboratory CV, with higher values at low BAX 855 concentrations, reflecting the more pronounced impact of small absolute changes around low mean values.

The most significant result of this *in vitro* study is that the type of reference standard has a major impact on the measurement of BAX 855 activity, with a more accurate and precise evaluation using SS than LS. This impact was even more pronounced when using the CA (Fig. 1d vs 1f) rather than the OSA (Fig. 1c vs 1e). In the present study, the analytical conditions associated with the most accurate and precise result were the CA combined with the SS (Fig. 1f). The evaluation of BAX 855′s FVIII activity using an SS is consistent with the 'like versus like' principle recommended by the International Society on Thrombosis and Haemostasis' dedicated scientific subcommittee, and this particular method may provide greater reliability for the critical determination of FVIII activity (e.g. in clinical monitoring) [3]. Indeed, product-specific standards are currently under evaluation for another PEGylated rFVIII (N8-GP) [10,11] and the selective measurement of functional BAX 855 has also recently been described [12].

The present study's mean recovery of target level BAX 855 differed from a previous field study using three different BAX 855 concentrations [8]. The discrepancy is most obvious at the lowest concentration tested in both studies and using the OSA. The causes of these differences are not clear. Turecek *et al.* used FVIII-deficient plasma from haemophiliac patients [9],

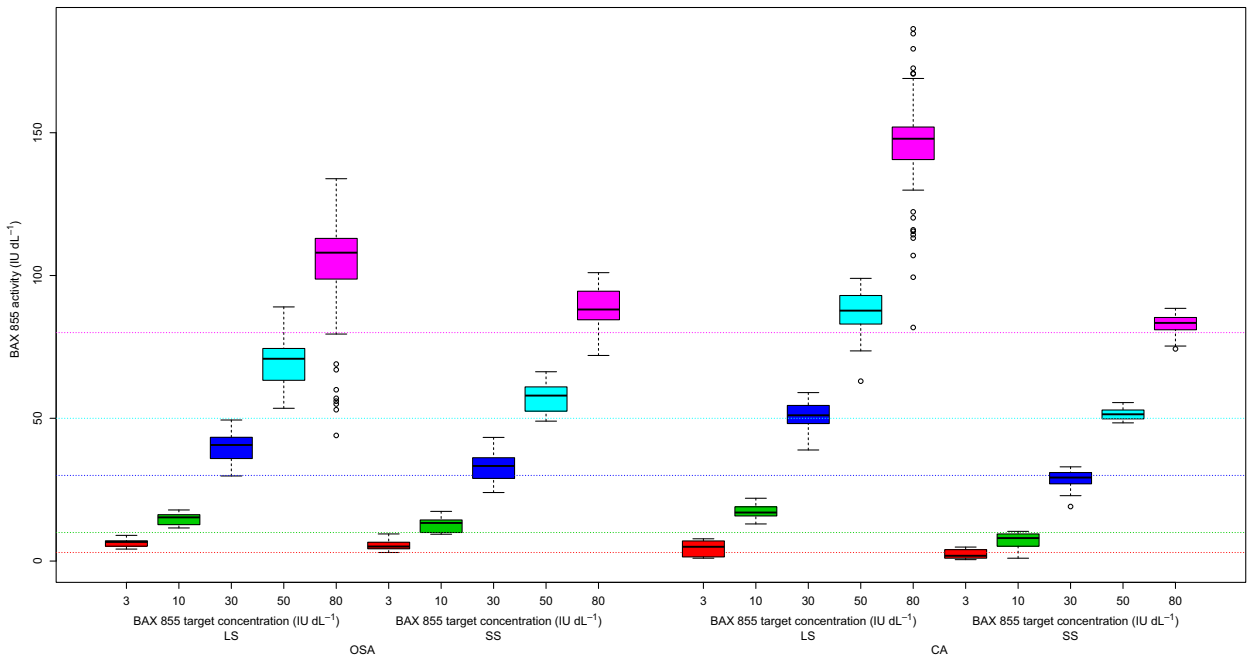

Fig. 2. BAX 855 activity according to assay type and reference standard. Dashed lines represent target concentrations. LS, local reference standard; OSA, one-stage clotting assay; SS, product-specific reference standard; CA, two-stage (chromogenic) assay.

whereas we prepared samples using FVIII-deficient plasma produced by immunoadsorption from normal plasma (Siemens). However, coagulation factor levels, including von Willebrand factor, were above 80%, as recommended [13]. Differences in reagents may have accounted for this difference, with 71% of the laboratories in the first study using nine different brands of silica-based aPTT [8], whereas most laboratories (five of eight) in our study used the same Siemens reagents.

The present study's main limitations are that it was an *in vitro* evaluation and the samples were not from haemophiliac patients treated with BAX 855. However, a similar study with patient samples would need a quite large amount of plasma and would be difficult to be conducted. Another limitation is that the evaluation of different coagulation instruments and reagents was limited due to the relatively small number of laboratories.

Conclusion

In conclusion, this study suggests that the use of a product-specific reference standard may increase the accuracy and precision of FVIII activity measurement in patients treated with BAX 855, especially when using the CA. The magnitude of the benefit of using SS seems clinically relevant. This should prompt an evaluation of LS vs SS in patients switching to BAX 855.

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Authors' contributions

O. Bulla, A. Poncet and P. Fontana designed the research study, performed the analyses, analysed the data and drafted the manuscript. L. Alberio, L. M. Asmis, A. Gähler, L. Graf, M. Nagler, J.-D. Studt and D. A. Tsakiris performed the research, analysed the data and critically revised the intellectual content of the manuscript.

Disclosures

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