

Assessment of two chromogenic FVIII assays in haemophilia A patients infused with B-domain truncated or deleted products: results from a French multicentric study

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Introduction

The measurement of FVIII may be achieved by one-stage clotting assay (OSA) or chromogenic substrate assay (CSA), which is the reference method for the European Medicine Agency.

The aim of this multicentric study was to compare FVIII activity on controls and haemophilia patients treated by known and new recombinant FVIII B-truncated products: ReFacto AF® (Pfizer), NovoEight® (NovoNordisk), Nuwiq® (Octapharma), using OSA with one specific APTT reagent and CSA with 2 chromogenic kits and performed on Sysmex/Siemens Healthineers analysers.

Methods

Factor VIII activity was performed in 4 centers, using BCS-XP (Montpellier and Rouen), CS2100i (Dijon) and CS2500 (Lille)

Controls and patients:

- 25 control samples were recruited and tested in each center
- 142 samples from haemophilia patients were recruited by 10 haemophilia centers (Bordeaux, Kremlin Bicêtre, Le Mans, Marseille, Nantes, Paris-Necker, Reims, Rouen, Tours, Versailles) between March and July 2016, anonymized and randomly distributed to the 4 centers for analysis. Patients were treated with Refacto AF® (n=53), NovoEight® (n=40) and Nuwiq® (N=49).

Factor VIII measurement:

- One-stage APTT-based clotting assay (the centers used one's own FVIII deficient plasma and the APTT reagent Actin FS® from Siemens Healthineers)
 - Chromogenic assay with 2 kits: Chromogenic FVIII® Siemens Healthineers and Biophen FVIII® Hyphen BioMed.
- (statistics were produced with a wilcoxon signed-rank test and passing and bablok regression)

Results

Calibration curves were performed according to manufacturers. A low range curve was used for samples < 20 IU/dL with Biophen FVIII. As calibration and internal controls showed good agreement, results were pooled according Sysmex and Siemens Healthineers instrument (CS group and BCS group).

The bias as the difference between CSA and OSA level according to the limit of agreement (LoA) were calculated for each product and each instrument. Results are presented in the table: mean, SD, range and bias between Actin FS FVIII and Chromogenic FVIII and between Actin FS FVIII and Biophen FVIII

- For controls (concentration < 150 IU/dL), a good correlation was observed between the 3 methods in the CS group and BCS group.

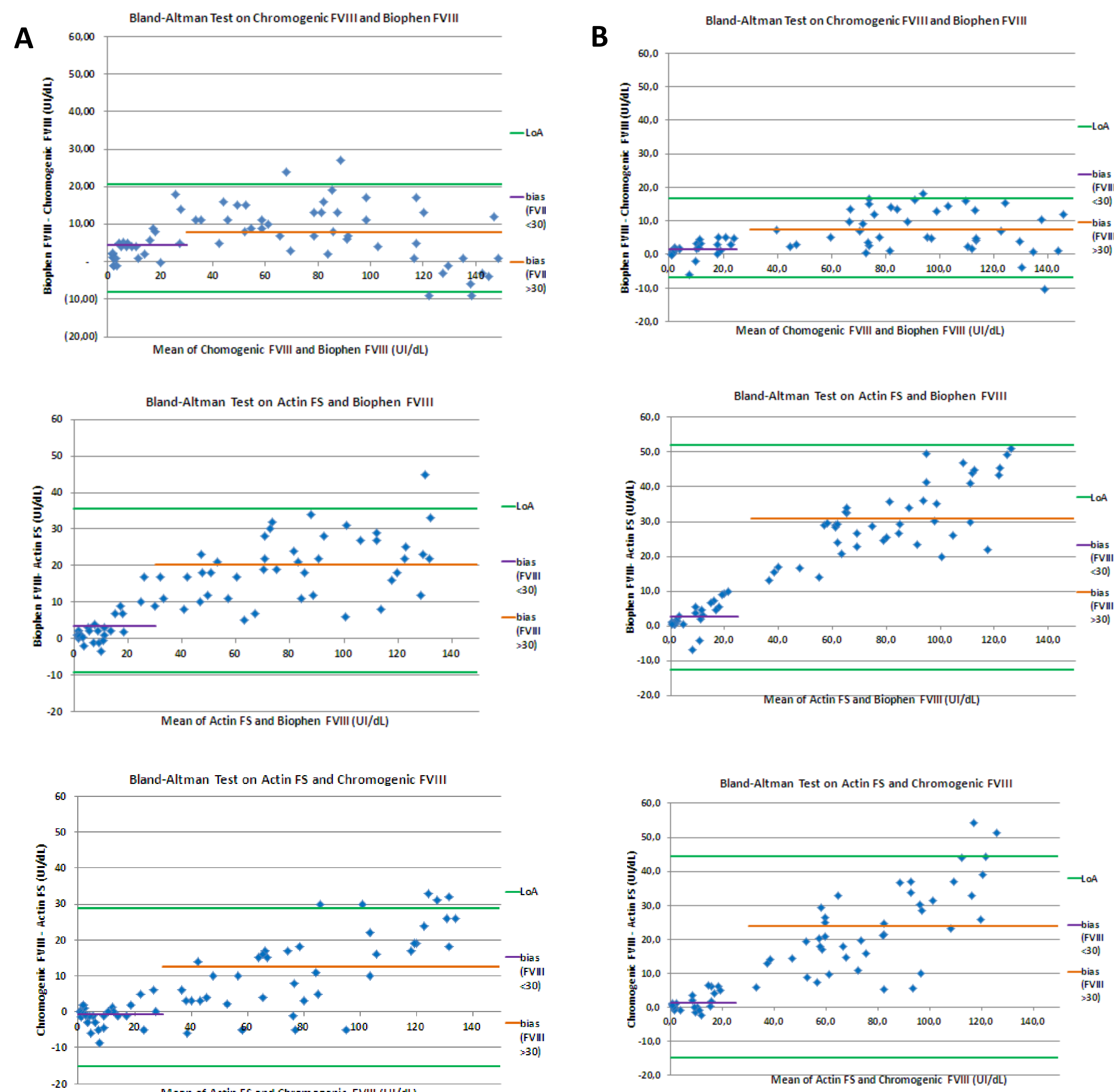
- As expected for haemophilia patients, levels were significantly lower with clotting assay than with both chromogenic assays; This is more pronounced for a concentration higher than 30 IU/dL (Bland & Altman graphs). Differences were less important in BCS group and with Chromogenic factor VIII as it is illustrated with mountain plot (comparison of both chromogenic assays with clotting assay in abscissa, the peak representing 50% of the values). When both chromogenic assays are compared, no significant difference was observed (p = 0,064 in BCS group and p = 0,748 in CS group). Values are better correlated in BCS group (systematic error of only 4 IU/dL) than in CS group (proportional error). No specific effect was observed for each product (RefactoAF®, NovoEight® and Nuwiq®).

Conclusion

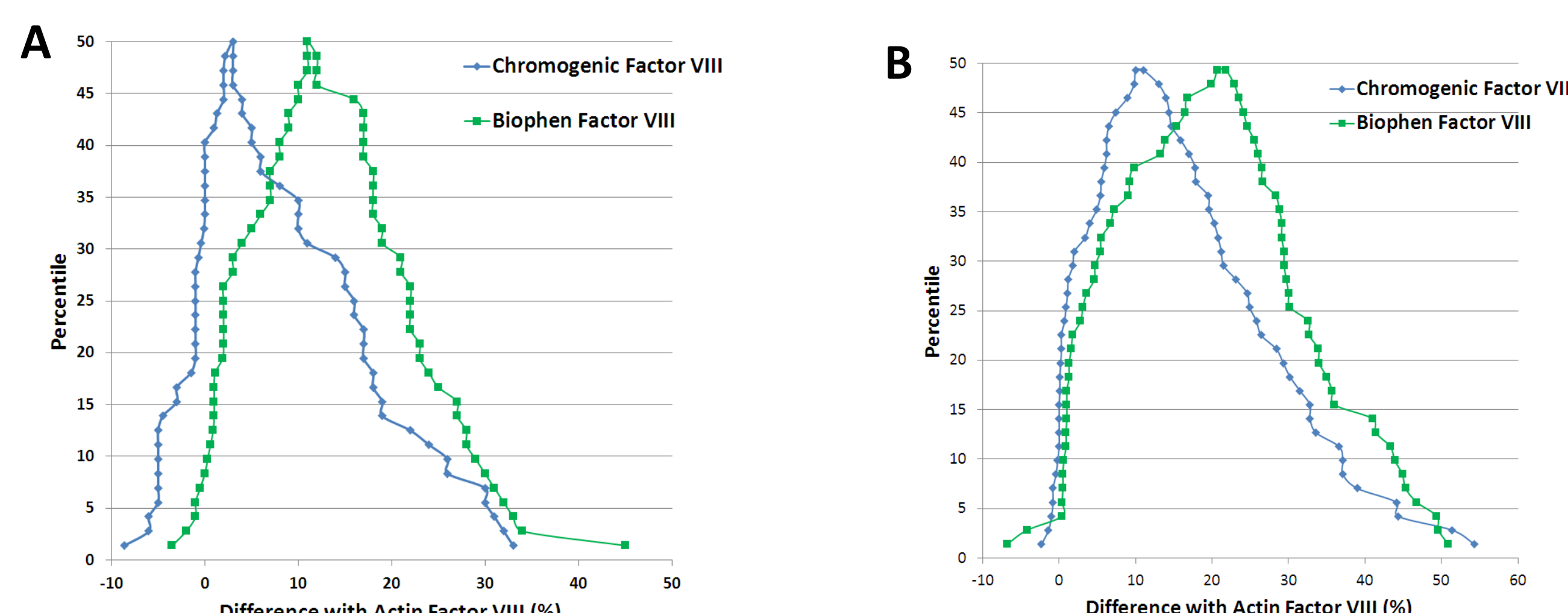
A discrepancy between CSA and OSA is confirmed as expected but seems to be less important in BCS group and with Chromogenic factor VIII Siemens Healthineers. Differences between both chromogenic assays may be acceptable in the Siemens Healthineers configuration (CS and BCS groups).

CS Instruments					BCS-XP Instruments				
		Actin FS	Chromo Siemens	Chromo Hyphen			Actin FS	Chromo Siemens	Chromo Hyphen
ReFacto AF (n=36)	Mean	62.3	82.9	88	ReFacto AF (n=35)	Mean	64.4	70.6	79.8
	(SD)	(42.3)	(61.6)	(63.1)		(SD)	(50)	(56.3)	(56.5)
Novo Eight (n=21)	Range	0.6-137	1.5-194	1.3-192	Novo Eight (n=23)	Range	4-153	1.9-160	6.9-184.5
	Mean	49.5	63.8	69.5		Mean	49.9	53.8	57.3
Nuwiq (n=24)	(SD)	(38.8)	(53.1)	(53.9)	Nuwiq (n=25)	(SD)	(47.7)	(56.1)	(52.8)
	Range	0.4-132	1.5-166	1.4-175		Range	0.5-133	0.3-149	1.8-141
Normal plasmas (n=70)	Mean	41.4	58.8	62.7	Normal plasmas (n=60)	Mean	60.2	71.2	77.3
	(SD)	(37.8)	(52.7)	(55.1)		(SD)	(42.2)	(52)	(50.7)
	Range	1.2-101	1.5-144	1.8-152		Range	1.1-121	0.8-148	2.5-149
	Mean	112	113	117		Mean	113	111	105
	(SD)	(57.8)	(62.1)	50.6		(SD)	(34)	(39)	(36)
	Range	45-364	45-371	52-305		Range	64-285	49-298	43-295

Results for each product in controls and haemophilia patients with BCS and CS instruments



Comparison with BCS (A) and CS (B) instruments (Bland and Altman)



Mountain plot with BCS (A) and CS (B) instruments