Comparison of the behavior of different factor VIII products measured with a chromogenic and a Factor VIII one stage assay

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Introduction and objectives

Factor VIII levels can be meaured with either a factor VIII one stage clotting assay (FVIII:C) as well as a FVIII chromogenic assay (FVIIICS). The use of both assays is recommended as the FVIII clotting assay is not sensitive for factor VIII mutations affecting the stability of factor VIII. Morever, it has been suggested to analyze plasma FVIII levels with the FVIII chromogenic assay or with the FVIII clotting assay calibrated with a ReFacto standard if patients are treated with ReFacto AF®. In our study we compared the effect of different factor VIII compounds after suppletion therapy measured with both assays either or not in combination with a ReFacto calibrator.

Materials and methods

The **APTT** based FVIII:C assay was determined with deficient FVIII plasma (HRF Inc.), a polyphenolactivator (STAGO) and 33 mmol/L calcium (homemade) to start the reaction. The calibration curve was made with normal pooled plasma (homemade) and buffer. If the factor VIII activity was below 3% a different calibration curve made with PTT-LA (silica activator, STAGO) was used to measure the sample.

We compared the FVIII:C assay with the FVIIICS assay (Biophen FVIII:C assay, HYPHEN BioMed). The calibration curve was made with normal pooled plasma (homemade) and factor VIII deficient plasma (HRF Inc.). Together with Factor IXa, phospholipids and calcium, thrombin activated Factor VIII forms an enzymatic complex, which activates factor X to factor Xa, This activity is directly related to the amount of factor VIII, the limiting factor Generated factor Xa is then measured by its activity on a specific factor Xa chromogenic substrate. If the factor VIII activity with the chromogenic assay was below 3% a low calibration curve was used to analyse the sample.

One hundred twenty samples of 70 haemophilia patients treated with FVIII products or DDAVP (carrier: n=1, mild: N=14, moderate: N=2, severe: N=53) were analyzed. The slope, intercept and correlation coefficient are determined.

Results

Graph 1: Factor VIII one stage clotting assay vs factor VIII chromogenic assay

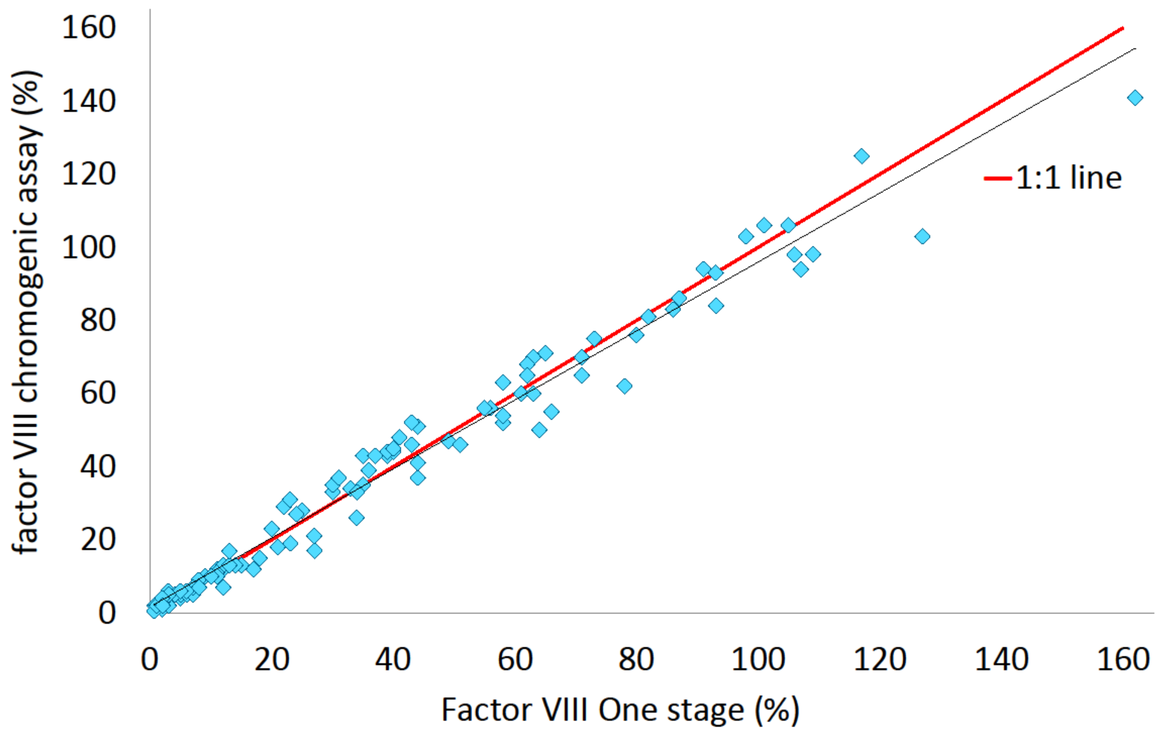

Table 1: Different FVIII products measured with the FVIII:C assay and the FVIII chromogenic assay calibrated with normal pooled plasma

Product	n	Slope		Intercept		Correlation
DDAVP	**3**	1.063	0.076	-8.727	4.024	0.997
Aafact	**4**	1.018	0.117	-1.162	5.104	0.987
Advate	**30**	0.966	0.021	0.565	0.867	0.993
Helixate	**25**	0.974	0.039	-0.837	2.067	0.982
Haemate P	**1**	-		-		-
Kogenate	**39**	1.019	0.016	0.073	0.751	0.996
ReFacto	**18**	1.101	0.044	**4.948**	**1.406***	0.987
Total group	**120**	0.992	0.015	0.900	0.685	0,986

*Significant from perfect line

Table 2: Refacto treated patients measured on a calibration curve made with normal pooled plasma vs calibration curve made with ReFacto standard.

Product	n	Slope		Intercept		Correlation
FVIII:C calibrated with NPP VS ReFacto std.	18	**1.114**	**0.021***	**-1.287** **0.663***		0.997
FVIII:C calibrated with ReFacto std. vs FVIIICS calibrated with NPP	18	0.987	0.054	**6.247**	**1.876***	0.977

*Significant from perfect line

Conclusion

The FVIII activity measured with the chromogenic assay is comparable with the FVIII activity measured with the FVIII one stage clotting assay and is able to measure different FVIII products in patient samples. Furthermore, we showed that there is a significant difference between the FVIII:C assay and FVIII activity with chromogenic substrates in plasma of ReFacto treated patients. These results confirm previous reports that it is recommended to analyse ReFacto suppletion with the FVIII chromogenic assay. Similar assays are assumed to be required for the new factor VIII products with longer half-life.

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