Impact of FVIII source in the Bethesda-Nijmegen inhibitor test result. Difference between FVIII/VWF complex concentrates and concentrates of isolated FVIII

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INTRODUCTION AND OBJECTIVES

Quantification of inhibitory antibodies against infused factor VIII (FVIII) has an important role in the management of patients with hemophilia A. The Bethesda and Bethesda-Nijmegen (Nijmegen) assay are widely used to monitor the development and progression of FVIII inhibitors. The Nijmegen assay quantifies Factor VIII (FVIII) activity that remains after inhibitor patient's plasma is mixed with normal plasma that provides FVIII activity in the test. However, several reports describe significant differences in inhibitory reactivity among FVIII concentrates.

In this study we replaced normal plasma with FVIII-deficient plasma spiked with different types of FVIII concentrates and tested in parallel to the standard Nijmegen assay.

METHODS

The titer of a pool of plasmas from haemophilic patients with inhibitors was measured with the standard Nijmegen assay (Peerschke EI, et al. Am J Clin Pathol 2009;131:552): Inhibitor patient's plasma (or dilutions in VWF containing FVIII-deficient buffered plasma) is mixed with an equal volume of normal plasma as FVIII source.

For the modified Nijmegen assay, normal plasma was replaced by FVIII concentrates, from the different sources, diluted to 1 IU FVIII with FVIII-deficient plasma FVIII. Plasma deficient FVIII controls, without plasma with inhibitor, were assayed in parallel. Mixture samples were incubated for 2 hours at 37ºC (see Figure 1).

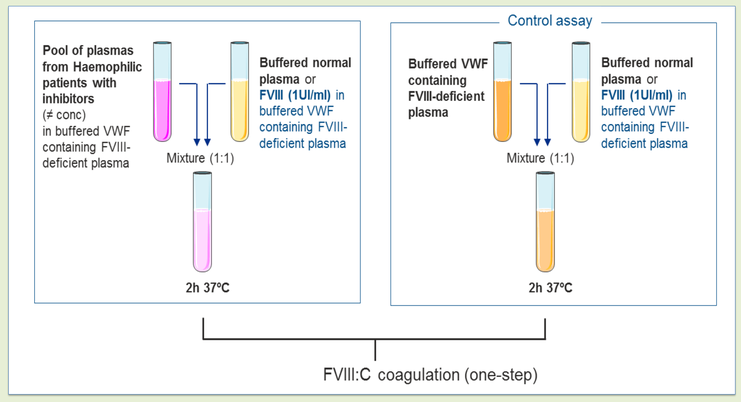

FIGURE 1. Standard Nijmegen assay *vs* control assay
(Text in blue: modified Nijmegen assay).

After incubation, residual FVIII activity (FVIII:C) was assayed by the one-stage clotting assay with a START-4 coagulometer (Diagnostica Stago, Île de France, France). A standard curve was plotted using a secondary standard calibrated against human coagulation FVIII concentrate Pharmacopea Europea BRP Batch 4. Differences in the inhibitor titer data were analyzed using a one way ANOVA, parametric test, Dunnet's test was used for multiple comparisons *versus* plasma.

The following biologicals were used:

- Pool of hemophilic plasmas with inhibitors (Technoclone, Vienna, Austria). This pool contains antibodies reacting against Heavy and Light Chain of FVIII.
- As FVIII source:
  - Normal pooled plasma (Diagnostic Grifols, Parets del Vallès, Spain)
  - Therapeutic concentrates: pdFVIII/VWF (plasma-derived FVIII/VWF complex); rFVIII (full-length recombinant FVIII); BDD-rFVIII (B-domain-deleted recombinant FVIII).
  - A BDD-rFVIII lab reagent (ProSpec, Ness-Ziona, Israel).

RESULTS

Similar inhibitor titres were obtained when using natural FVIII/VWF complex and normal plasma (17.8±3.5 BU/ml and 17.5±1.2 BU/ml, respectively; n=3-4). In contrast, much higher inhibitor titres were obtained with concentrates of isolated FVIII: 23.7±1.7 BU/ml with rFVIII (n=3), 35.9±6.1 BU/ml with BDD-FVIII therapeutic concentrate (n=3) and 34.8±1.8 BU/ml with BDD-FVIII lab reagent (n=3) (see Table 1, Figure 2).

TABLE 1. Inhibitor titers against different FVIII sources, and ratio with respect to plasma

FVIII Source	Inhibitor titers (n= 3-4)	
	BU/ml (mean ± SD)	Ratio FVIII product /plasma
Plasma	17.5 ±1.2	---
pdFVIII/VWF	17.8 ±3.5	1.02
rFVIII	23.7 ± 1.7	1.35
BDD-FVIII	35.9 ± 6.1	2.05
BDD-FVIII lab reagent	34.8 ± 1.8	1.99

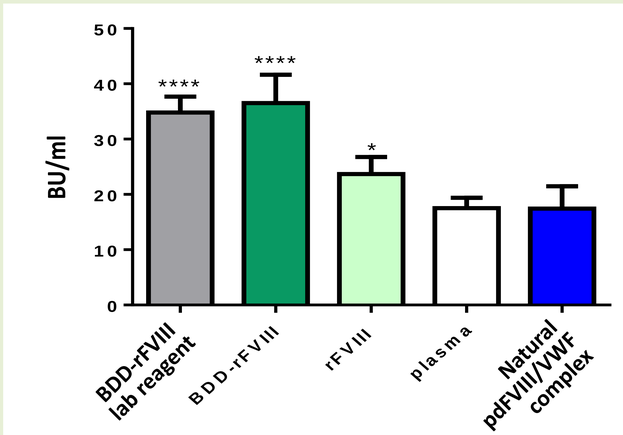

FIGURE 2. Inhibitor titers against different FVIII sources
One way ANOVA, parametric test. Dunnet's multiple comparisons test vs plasma (*p <0.05, **p <0.01 ***p <0.001 ****p <0.0001)

CONCLUSIONS

Nijmegen assay is greatly influenced by FVIII source:

- **Natural pdFVIII/VWF complex concentrates showed titres comparable to the normal plasma widely used in this test.**
- **Concentrates of isolated FVIII showed much higher titres, despite the presence of VWF in the FVIII deficient plasma used in the assay.**

Although in vivo FVIII:C recovery results in a FVIII KO mice model are in good agreement with these in vitro results, correlation of these results with the FVIII:C in vivo recovery in haemophilic patients with inhibitors is pending to be evaluated.

Due to the relevance in clinical practice, inhibitor titration should be done using the FVIII concentrates considered for treatment of a patient with inhibitor.

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