Preliminary in vitro results from the PredicTGA study: thrombin generation assay (TGA) as a test of hemostatic effectiveness of factor VIII concentrates in patients with Hemophilia A and inhibitors

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Topic: Inhibitors

OBJECTIVES

A longitudinal, observational, prospective, multicenter cohort study was started to verify whether or not TGA may predict effectiveness of different FVIII concentrates class (devoid or rich of von Willebrand factor [VWF]) in patients affected by severe or moderately severe hemophilia A with FVIII inhibitors (high or low responders).Other goals were to assess the inhibitor reactivity against the different FVIII concentrates, to evaluate the protective role of VWF on FVIII and its correlations with epitope specificity.

METHODS

Patients underwent a laboratory assessment after 72-h wash-out period followed by a prospective observation of 12 months (prophylaxis or on demand treatment) and a maximum of 33 months (immune tolerance induction, ITI). Centralized baseline laboratory assessment included in vitro spiking experiments mixing patient plasma with each of the 3 products (plasma-derived FVIII/VWF; full-length recombinant FVIII [rFVIII] and B-domain-deleted recombinant FVIII [BDD-rFVIII]) at a concentration equivalent to FVIII doses used in clinical practice (50-200 IU/kg); TGA (CTA Thrombinoscope. Maastricht, NL) (Figure 1) and inhibitor testing by Nijmegen-Bethesda assay were measured. Baseline plasma samples for epitope mapping were also collected. Epitope mapping and TGA before and after FVIII infusions were periodically repeated during follow-up.

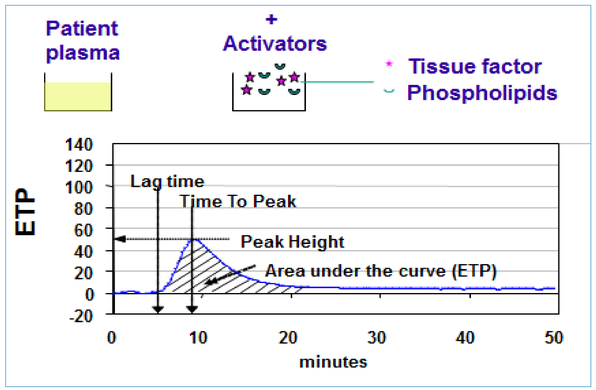

FIGURE 1. Thrombin generation assay (TGA)

RESULTS

Eighteen Italian centers enrolled 41 patients aged 1-72 years . The median inhibitor titer at enrollment was 12 BU (0-670); 5 patients were low responders and 36 high responders. Statistically significant differences between-concentrate in TGA parameters were shown in baseline spiking experiments (N=39); e.g. median peak-thrombin was highest after spiking with plasma-derived FVIII/VWF, 80 (2-288) nM and lowest with full-length rFVIII, 44 (0-206) nM, p<0.001 (Figure 1). These results paralleled the inhibitor titer when measured against each of the 3 concentrates (See Figure 3 and Table 1).

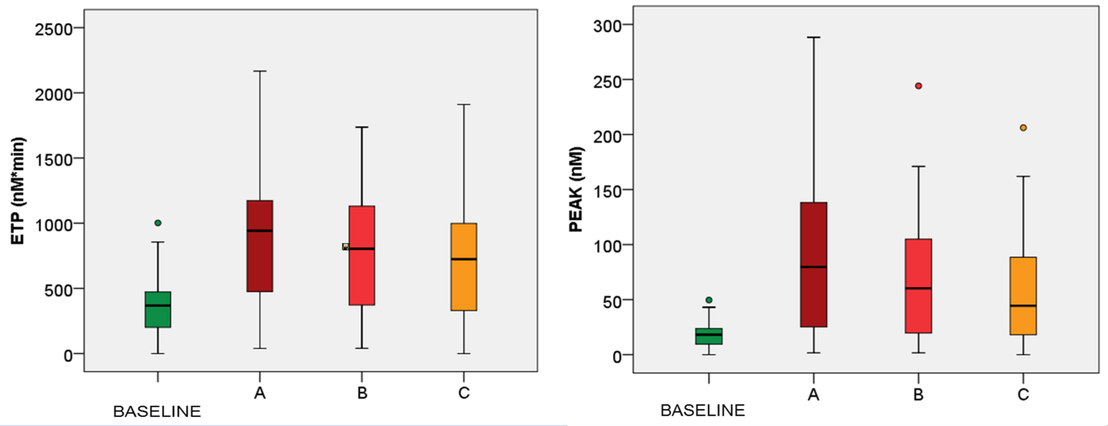

FIGURE 2.
Thrombin generation assay (TGA) at baseline and after spiking with FVIII/VWF, rFVIII and BDD-rFVIII.
Left panel: Endogenous Thrombin Potential (ETP). Right panel: Peak

TABLE 1. Inhibitor titers (BU/ml) against different FVIII concentrates compared to fresh-frozen plasma.

	BU/ml		P value
	Median	Max.	vs PLASMA
Plasma	6.00	1800.00	---
A	5.00	2200.00	**0.032***
B	6.00	2500.00	**0.649**
C	10.00	4000.00	**<0.001****

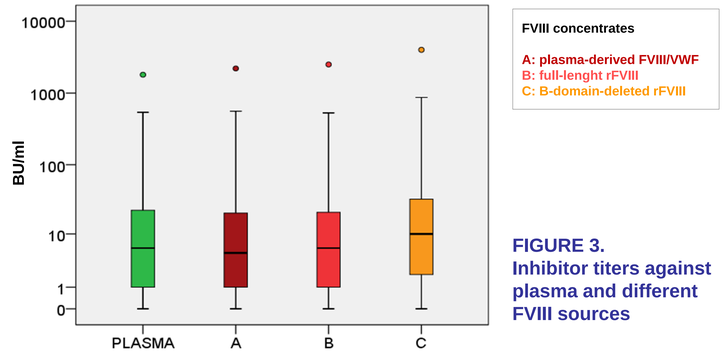

FIGURE 3.
Inhibitor titers against plasma and different FVIII sources

CONCLUSIONS

Baseline TGA and inhibitor cross-reactivity tests showed a between-concentrate difference and the plasma-derived FVIII/VWF concentrate (Alphanate®, GRIFOLS) resulted as the least reactive against inhibitors and the most efficient in generating thrombin. Clinical and laboratory follow-up is on-going in 23 patients who will provide in vivo evidence about the clinical meaning of in vitro results.

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