Thrombin generation assay triggered with tissue factor or factor XIa in patients with severe haemophilia A

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Hemostasis tests and assays

Introduction

•Haemophilia A (HA) is an hereditary X-linked disorder with a frequency of 1 in 10000 per capita in the population caused by a deficiency of coagulation factor VIII (FVIII) (Khanum et al., 2014).

•A patient with severe HA is at risk of developing spontaneous haemorrhages and requires FVIII replacement.

•Once a patient is diagnosed with HA, disease severity is assessed through precise measurements of FVIII activity which are then used to guide FVIII replacement.

•However, it is known there are discrepancies between individuals with similar FVIII levels and tendencies for bleeding (Dargaud et al., 2005; Beltran-Miranda et al., 2005).

•TG assays may be better suited to predicting clinical phenotype and response to treatments (Shima et al., 2008).

•Recently, it has been demonstrated that activated factor XI (FXIa) can be used as a trigger in TG assay in FVIII and FIX deficient plasma spiked with different preparations of factor replacement therapeutic products (Waters et al., 2015). However, this has yet to be performed using samples from patients with HA or HB.

•This study aimed to investigate the usefulness of a FXIa triggered TG assay in severe HA patients; does it correlate with current assays and are they a better marker of bleeding tendency than currently used assays?

Methods

•Citrated blood samples (Becton Dickinson, Plymouth, UK) were collected from severe HA patients (n=17; median age 23 years and no inhibitors).

•Venous trough and post FVIII infusion samples, with and without corn trypsin inhibitor (CTI (contact pathway inhibitor), final concentration 33ug/ml) were taken for TG analysis.

•Platelet poor plasma was isolated and a calibrated automated thrombogram (CAT, Thrombinoscope BV, Maastricht, The Netherlands) was used to measure TG parameters.

•Tissue factor (TF, final concentration 1 pM and 4 µM procoagulant phospholipids) or FXIa (Enzyme Research Laboratories, South Bend, IN, USA) diluted in micro-particle reagent (containing 4 µM procoagulant phospholipids (Thrombinoscope BV, Maastricht, The Netherlands) final FXIa activity 0.008 units/ml) were used as a trigger and comparability was analysed.

•Four parameters were derived from the TG curves: lag time, endogenous thrombin potential (ETP) time to peak height and peak height.

•FVIII levels were measured using a chromogenic assay (Sysmex UK, CS5100) and used as a comparison.

•Statistical difference was estimated with the paired Student's t-test and (differences were assumed to be statistically significant at $p < 0.05$).

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Results

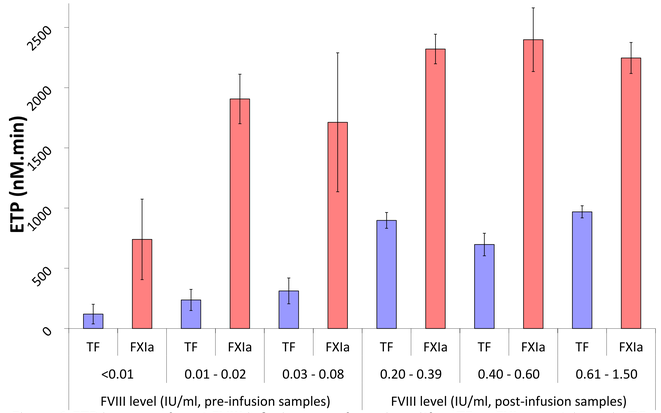

Figure 1.ETP in pre and post FVIII infusion samples using either TF or FXIa as a trigger in TG assay and relative FVIII concentration level. ETP values are expressed as means ± S.E.M.

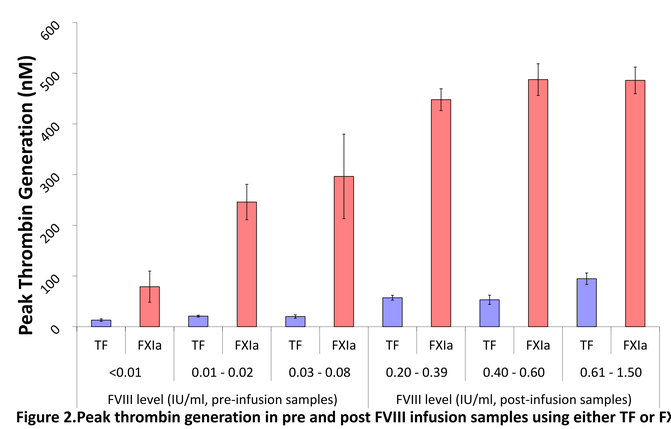

Figure 2.Peak thrombin generation in pre and post FVIII infusion samples using either TF or FXIa as a trigger in TG assay and relative FVIII concentration level. Peak thrombin generation values are expressed as means ± S.E.M.

•Pre-infusion ETP (nM.min) was significantly lower in TF triggered assays =213.3±217.7 [mean±SD] vs FXIa triggered ETP=1449.1 ±931.4 (p=<0.01); as was peak thrombin (nM) in TF triggered assays 17.93±6.63 vs FXIa triggered 198.93±137.58 (p=<0.01).

•Post-infusion ETP was significantly lower in TF triggered=848.0±204.3 vs FXIa triggered=2326.5±429.0 (p=<0.01) as was peak thrombin (fig. 1 & 2).

•FXIa triggered ETP and peak TG with and without CTI showed good correlation (R^2=0.93 & R^2=0.99, respectively, data not shown).

Conclusions

•Thrombin is the terminal enzyme in the blood coagulation cascade making it an ideal marker of bleeding tendency.

•This study assessed a method for using FXIa triggered TG assays for the first time in HA patient blood samples.

•A statistical difference in peak TG and ETP when comparing TF and FXIa as triggers in the assay (in both pre and post infusion samples) was demonstrated.

•We also note that addition of CTI has no effect on FXIa triggered TG.

•FXIa triggered TG offers a simple alternate method for assessing TG potential.

•Final activity of FXIa trigger and run time of assay may need adjusting to increase sensitivity of the TG assay.

•More work needs to be done with patients with mild and moderate disease and haemophilia B to determine TG.

•The correlation between bleeding tendency and ETP needs to be further studied.

•Full TG assays requires much more time than conventional FVIII estimation; its clinical utility for assessing factor concentrate replacement in severe HA patients will be assessed in future work.

References

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- Dargaud et al. Thrombosis and Haemostasis 2005, 93:475-480.
- Khanum et al. Haemophilia 2014, 20:287-293.
- Shima et al. Haemophilia 2008, 14:83-92.
- Waters et al. Journal of Thrombosis and Haemostasis 2015, 13:2041-2052.