USE OF THE THROMBIN GENERATION ASSAY IN PATIENTS WITH COAGULATION FACTOR DEFICIENCIES

INTRODUCTION

Factor assays, based on the prothrombin time (PT) and activated partial thromboplastin time (aPTT), see Figure 1, are used to diagnose and monitor bleeding disorders resulting from a deficiency of specific coagulation factors. These assays provide useful clinical information, however they are not wholly representative of in vivo clot formation. It is therefore not surprising that clinical heterogeneity exists among patients with bleeding disorders who have the same degree of factor deficiency.

The thrombin generation assay (TGA) is a method of assessing global haemostasis, thought to be useful in predicting the bleeding tendency in these patients regardless of factor assay result. Research suggests it can distinguish milder phenotypes of severe haemophilia (Santagostino et al., 2010). Factor XI deficiency can pose a significant clinical challenge as there is very limited correlation between factor level and bleeding tendency; however research using TGA found that severe bleeders had a dramatically impaired thrombin generation (Ruger et al., 2010, Mumford et al., 2014).

The primary aim is to evaluate the correlation between thrombin generation, factor assays and clinical bleeding phenotype, to identify the most appropriate laboratory test.

MATERIALS AND METHODS

Sample Collection

Samples obtained as part of routine patient follow-up

Whole blood collected into 3.2% sodium citrate

PT, aPTT & Factor Assays

IL-TOPS 700 coagulometer using Werfen Reagents

UKAS accredited assays under ISO15189:2012

Thrombin Generation Assay

Plasma-poor plasma (PPP) assayed using Fluoroskan Ascent Flurospectrometer. Thrombinscope software and reagents

RESULTS

A. The thrombin generation assay is sensitive to absence of Factors II, V, VII, VIII, IX and X.

Figure 2: Thrombin Generation Profiles of Werfen factor deficient plasma compared to the mean reference plasma. Almost absent thrombin generation was seen in Factor II, V and X deficient plasma. The thrombin generation in Factor VII, VIII and IX deficient plasma was reduced. Factor XI and Factor XI deficient plasma showed normal thrombin generation, consistent with the understanding that Factor XI level does not correlate with coagulation and Factor XII deficiency is not associated with a bleeding tendency.

B. There was a statistically significant difference in TGA parameters between patients with Haemophilia A and B and the reference plasma.

Figure 3: Box and whisker plots summarizing the TGA in patients with Haemophilia A (n=10) and B (n=5) compared to 20 reference plasma samples. In patients with Haemophilia B, in both Haemophilia A and B there was a statistically significant difference between patients and the reference plasma in three TGA parameters; peak thrombin, time to peak and velocity. Patients with Haemophilia A had reduced ETP compared to the reference plasma.

C. The Thrombin Generation Assay may be useful to identify the bleeding tendency in patients with Factor XI Deficiency

Figure 4: Thrombin Generation Profiles in Factor XI Deficiency. Nine of these Factor XI deficient patients had no bleeding tendency. One patient, Patient J, reported bruising after minor injuries. The Factor XI levels are shown in brackets in Figure 4 alongside each patient.

Figure 5: Comparison of TGA in patients with Factor XI Deficiency and Reference Plasma

Box and whisker plots comparing the TGA parameters between the patients with Factor XI deficiency and the reference plasma samples. There was no statistically significant difference between the two groups however an obvious outlier can be seen in the big time and time to peak.

DISCUSSION AND CONCLUSION

In this research, patients with haemophilia A had reduced ETP, peak thrombin and velocity compared to the reference plasma and increased time to peak thrombin. Furthermore, in the patients with Haemophilia B, there was a statistically significant difference between patients and the reference plasma in three TGA parameters; peak thrombin, time to peak and velocity. There was no statistically significant difference in lag time in patients with Haemophilia A or B, consistent with other research, and explained by the fact that Factor VIII and Factor IX play a minor role in determining lag time.

Our initial results provide further evidence that the TGA may be a useful assay to identify the bleeding tendency in patients with Factor XI Deficiency, although more patients with a bleeding tendency would need to be evaluated prior to introduction into clinical practice (Ruger et al., 2010; Livnat et al., 2013).

A limitation of this research is the use of PPP as opposed to plasma containing platelets. Dunn et al performed TGA on patients with severe haemophilia A patients and found that the association between bleeding and lag time was lost when PPP was used compared to platelet-rich plasma, demonstrating the critical role of platelets (Dunn et al., 2017). Additionally, the sensitivity of the assay could be increased by reducing tissue factor concentration.