Intrinsic activated thrombin generation for efficacy and monitoring of emicizumab

One test to measure them all!

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Background
Traditionally, severe hemophilia A (SHA) patients are treated with intravenous prophylactic Factor (F)VIII replacement concentrates to prevent spontaneous bleeding. Various extended half-life FVIII and non-factor products , such as emicizumab, were introduced as alternatives. However, traditional laboratory tests are not able to monitor the effects of these products on hemostasis, especially not in acute situations like bleeding or during surgery. The Thrombin Generation Assay (TGA) is a potentially useful tool for monitoring the efficacy of various FVIII and non-FVIII replacement products in blood. However, the commonly used extrinsically activated TGA, with Tissue Factor (TF) as a reagent, is not suitable to investigate this specific part of the coagulation pathway. For this purpose, an intrinsically activated TGA was developed.

Aim
To investigate the applicability of intrinsically activated TGA in comparison to the commonly used extrinsically activated TGA in monitoring FVIII and non-factor replacement therapy in SHA.

Methods
The CAT assay was optimized and validated for the new PPP Reagent INT (contact activator, 4 μM phospholipids) for measuring octocog alfa and emicizumab.

Results
TG in normal plasma triggered by PPP Reagent INT (36 replicates) was characterized by a lag time of 5.39±0.22 min, an endogenous thrombin potential (ETP) of 1451±38 nM.min, a peak height of 420±9 nM and a velocity index of 254±25 nM/min. The overall variability of the assay was <10% coefficient of variation for all parameters, with a within-run and between-day variation <5%. PPP Reagent INT failed to induce TG in plasmas deficient for FXII, FXI, FIX, or FVIII, whereas normal profiles were obtained for FVII deficient plasma. Addition of octocog alfa or emicizumab dose dependently increased TG when triggered with PPP Reagent INT. Both PPP Reagent LOW (low tissue factor) and PPP Reagent (mid tissue factor) displayed poor sensitivity for the two replacement products. No dose dependent correlation was found between plasma emicizumab levels and extrinsic triggered TG in plasma from SHA subjects (n=58) treated with emicizumab. However, plasma emicizumab levels showed a good correlation with maximum thrombin (peak height) in the intrinsically activated TGA.

Conclusion
Intrinsically activated thrombin generation showed a dose dependent correlation with FVIII and the non-factor replacement product Emicuzmab and might be suitable as a universal tool for monitoring different Hemophilia A replacement therapies. Future applicability of this PPP Reagent INT TGA in monitoring SHA patients requires further study.

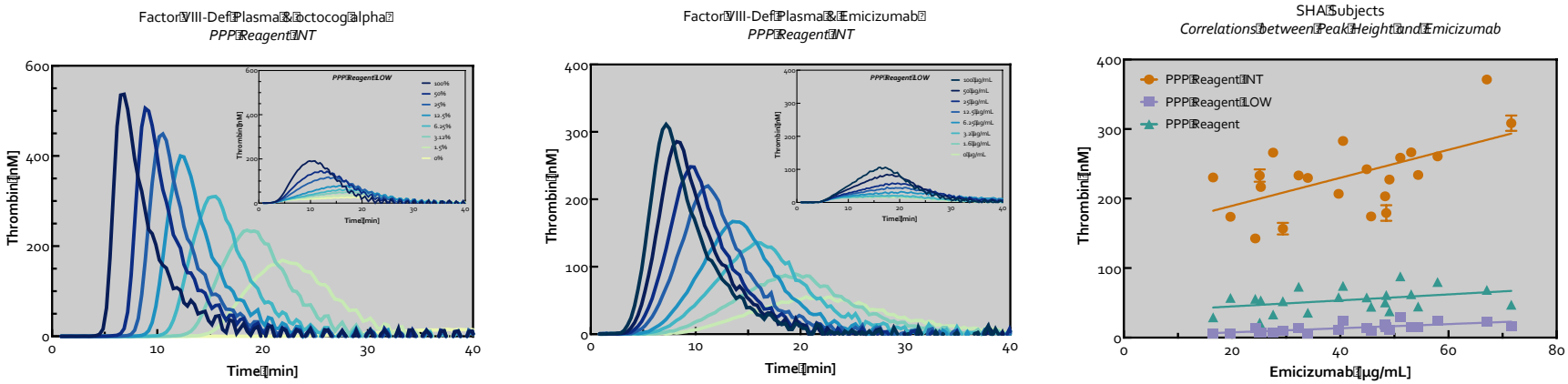

Figure 1A-C. Dose response curves and correlations of octocog alpha and emicizumab in PPP Reagent INT, compared to PPP reagent (mid TF) and PPP reagent LOW (low TF), showing a dose dependent correlation in the PPP Reagent INT and not in the PPP reagent and PPP reagent LOW