Utility of Thrombin Generation Assay for inherited thrombophilia screening.

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Background

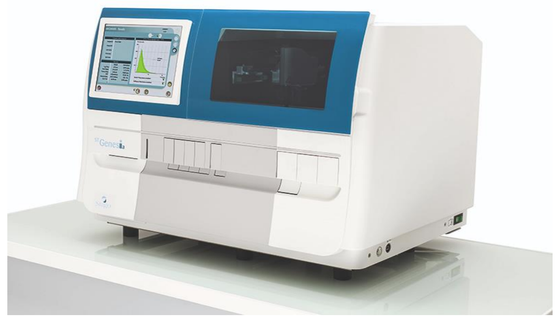
The thrombin generation assay (TGA) is a global dynamic test exploring the ability of a plasma sample to generate and inhibit thrombin over time. Various studies support the link between TGA results and the presence of laboratory thrombophilia risk factors. But these studies were conducted using analyzers in non-standardized conditions(1-2). The ST-Genesia® is a fully automated and standardized system for the measurement of thrombin generation. The STG®Thromboscreen kit is intended for the evaluation of thrombotic manifestations.

Objectives

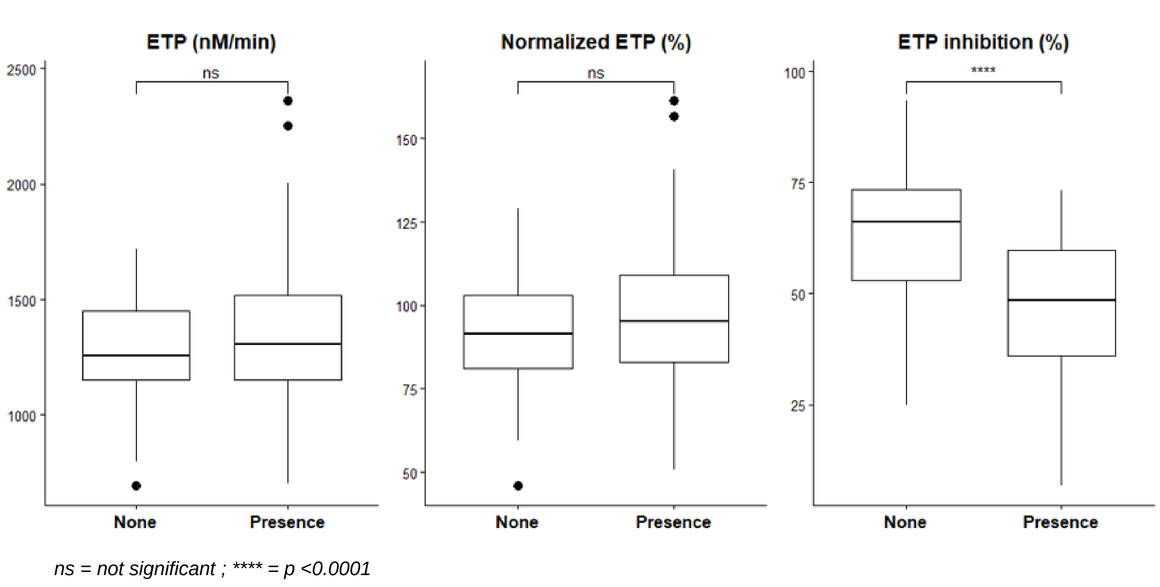
We aim at evaluating the STG®-ThromboScreen kit on ST-Genesia® as a global screening test for the main inherited thrombophilia risk factors : antithrombin (AT), protein C (PC) and protein S (PS) deficiencies genetically characterized, factor V Leiden or *F2*c,*97G>A variants.

Materiels and Methods

Platelet-poor citrated plasma samples of 90 patients with thrombophilia screening were studied. Several TGA parameters were studied including endogenous thrombin potentiel (ETP, nmol.min), normalized ETP (%) and ETP inhibition (%) (= (ETP without thrombomodulin – ETP with thrombomodulin)/ETP without thrombomodulin)). The wilcoxon rank-sum test was used to compare the TGA parameters in the different groups. The receiver operator characteristic (ROC) was used to determine the optimal threshold for thrombophilia laboratory risk factor detection.

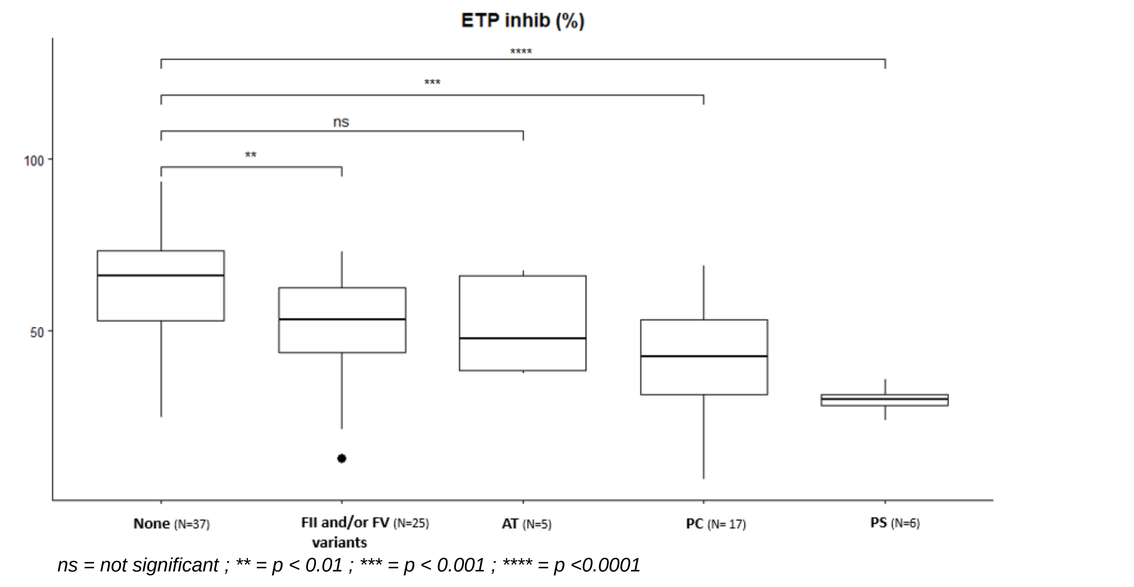


Results



*ns = not significant ; **** = p <0.0001*

Figure 1. Comparison of TGA parameters in the different groups : patients without risk factors (n=37) and patients with inherited thrombophilia (n=53)



*ns = not significant ; ** = p < 0.01 ; *** = p < 0.001 ; **** = p <0.0001*

Figure 2. Distribution of ETP inhibition values in patients without biological thrombophilia and with factor V Leiden and/or *F2*c,*97G>A variants, AT deficiency, PC deficiency and PS deficiency

ETP and normalized ETP were similar for patients with (n=53) and without (n=37) inherited thrombophilia (p≥ 0.05). There was neither statistically significant difference between ETP inhibition in the group with AT deficiency (n=5) and ETP inhibition in the group without risk factors. We found a different pattern for ETP inhibition with significantly lower values (p < 0.01) in the groups with FV Leiden and/or *F2*c,*97G>A variants (n=25), PC deficiency (n=17) or PS deficiency (n=6) in comparison with temoins and controls.

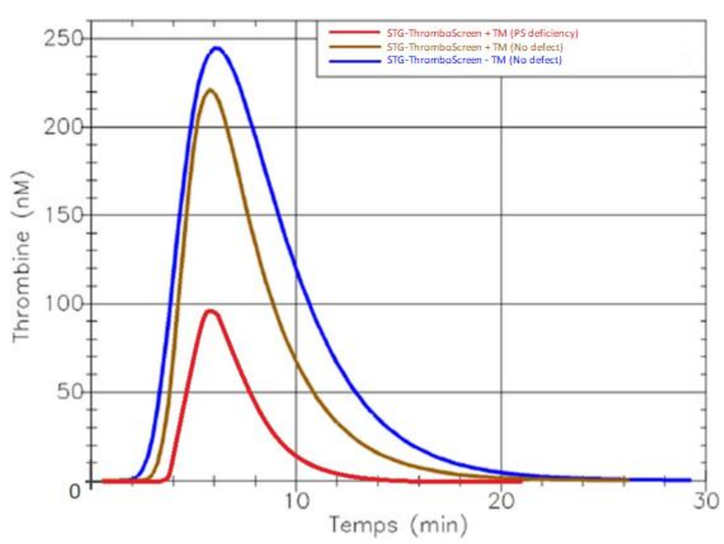


Figure 3. Examples of TGA results in thrombophilic disorders.

PS deficiency, ETP inhibition = 31% ; No defect, ETP inhibtion = 68%

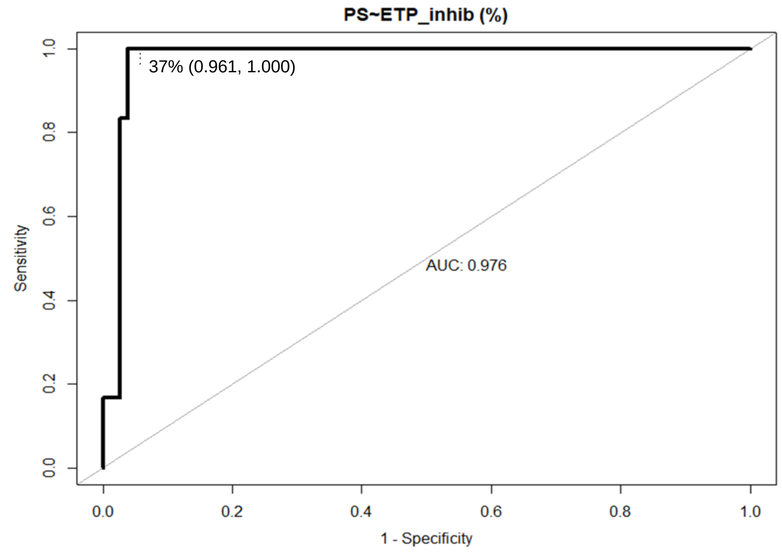


Figure 4. ROC curve in patients with PS deficiency.

The percentage of thrombomodulin-induced ETP inhibition is 100% sensitive for a cut-off of 37%.

An inherited PS deficiency can be excluded if ETP inhibition was > 37% (sensitivity 100%, specificity 96.1%, AUC = 0.976).

Conclusion

The STG®-Thromboscreen used on the ST Genesia® analyzer is able to discriminate patients with factor V Leiden and/or *F2*c,*97G>A variants, PC deficiency and PS deficiency from patients without principal inherited thrombophilia.
In particular thrombomodulin-induced ETP inhibition on the ST-Genesia® could be an additional tool before PROS 1 gene analysis for patients with moderate PS deficiency in the absence of relevant thrombosis history.

References :
(1) Dargaud Y, Trzeciak M, Bordet J, Ninet J, Negrier C. Use of calibrated automated thrombinography ± thrombomodulin to recognise the prothrombotic phenotype. Thrombosis and Haemostasis. 2006;96:562 7.
(2) Hézard N, Bouaziz-Borgi L, Remy MG, Nguyen P. Utility of Thrombin-Generation Assay in the Screening of Factor V G1691A (Leiden) and Prothrombin G20210A Mutations ans Protein S Deficiency. Clinical Chemistry 52:4 665-670 (2006)