

Abstract ID:
Category: Hemostasis, transfusion medicine, vascular, laboratory medicine

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INTRODUCTION

Global coagulation assays such as thrombin generation (TG) have been proposed to evaluate the balance between pro- and anti-coagulant forces to better reflect bleeding and thrombotic risks. Although TG readouts have been used for several clinical conditions, TG still need standardization and clinical validation¹. This is mainly due to the lack of a standard experimental protocol, reagents and analysers, responsible of a large interlaboratory variability and of the lack of large clinical studies in which the decision to treat is based on TG results. A new TG analyzer (STGenesis, STG, STAGO) has been recently released as the first fully automated TG analyser for clinical routine laboratories together with a set of reagents balanced for sensitivity to procoagulant and anticoagulant factor deficiencies.

OBJECTIVES

STG was evaluated in our laboratory for validation purpose in a large healthy population. Aims of our study were:

- to test the data variability of the new analyzer and its reagents (QCs and Ref Plasmas) and healthy frozen PFP samples,
- to establish accurate normal reference intervals for all TG parameters.

DONORS COHORT & BLOOD SAMPLES COLLECTION:

The institutional cantonal ethic commission of Berne, Switzerland approved the use of 120 human healthy volunteers for this study. (Table 1) Venous blood was collected into of 3.2% sodium citrate (10mL, S-Monovette, Sarstedt), using 21-gauge needle by experienced nurses of the Clinical Apheresis Unit. Samples for TGA were processed by double centrifugation (2500g x 15 min) in order to obtain platelet free plasma (PFP) according to guidelines recommendation of the SSC/ISTH subcommittee². PFP samples were frozen and store at -80°C.

TG ANALYSIS:

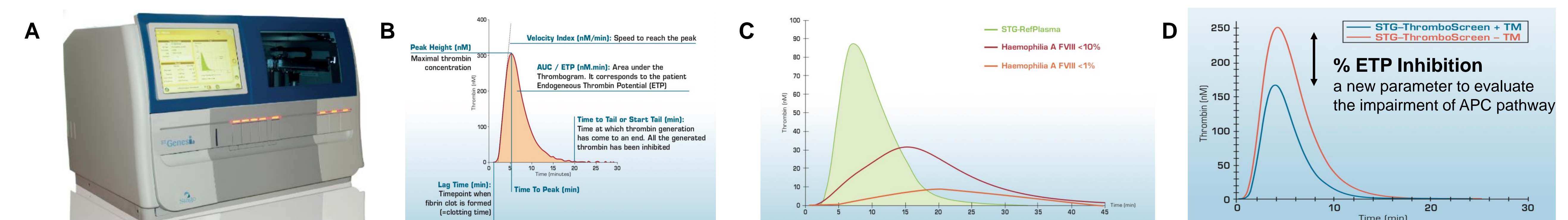
Quality controls (STG-QLs), reference plasmas (STG-RFs) and frozen PFP samples were measured with the same batch of STG-Bleedscreen (BLS) reagent, containing a low picomolar level of human tissue factor (hTF) and with the same batch of STG-Thromboscreen (TS) reagent, which contains a medium picomolar level of human TF with and without human Thrombomodulin (TM) on STG analyser.

Table 1: Demographic characteristic of the healthy donors population

Characteristic	n			
Gender:				
Male	59			
Female	61			
Therapy:				
Oral contraceptive (OC)	7			
Hormone Replacement Therapy (HRT)	5			
Neither OC or HRT	49			
Age (years):	All	Male	Female	No OC/HRT
20-39	40	19	21	14
40-59	40	20	20	18
60-80	40	20	20	17

METHODS

Figure 1: TG Analyzer STG (A), the six TG parameters provided by the new software from the thrombogram (B) and the thrombograms obtained using STG-Bleedscreen (C) of healthy donor sample in comparison to hemophilic patients and the STG-Thromboscreen in presence and in absence of TM (D) in healthy donor sample.



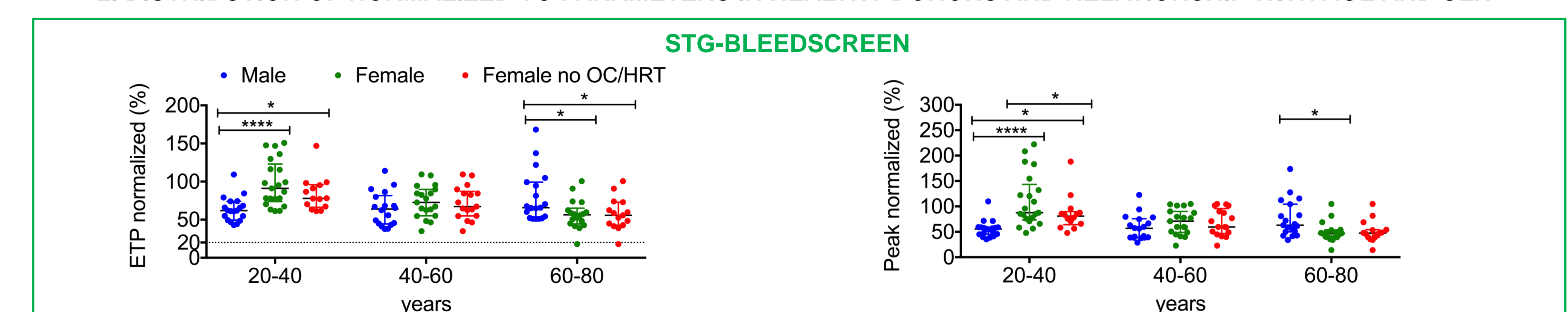
RESULTS

1. DAY TO DAY PRECISION IN QCs, REFERENCE PLASMAS, AND HEALTHY DONOR PFP FROZEN SAMPLE

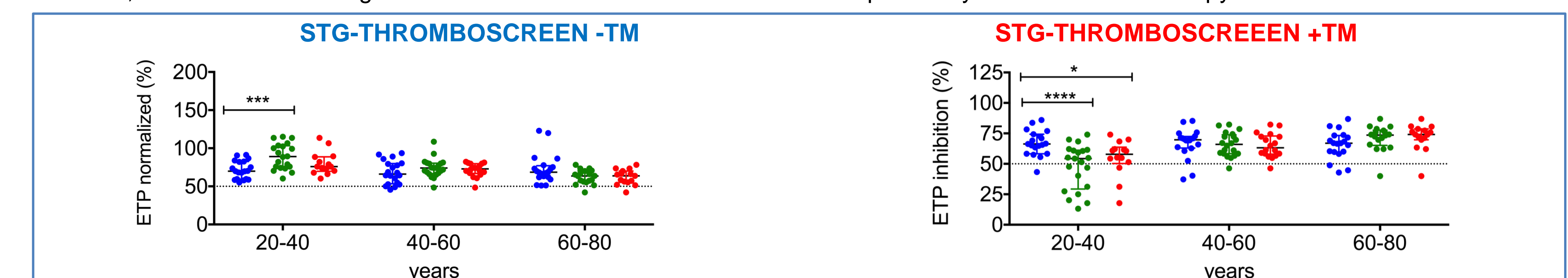
STG-BLS (n=33 tests)	STG-QC low	STG-QC norm	STG-REF Plasma
Parameters	Mean SD CV (%)	Mean SD CV (%)	Mean SD CV (%)
Lag time	absolute (min) 2.8 0.1 4.0	1.9 0.0 2.4	2.5 0.1 2.6
Peak	absolute (nM) 51.4 4.9 9.6	122.6 9.5 7.7	162.6 17.8 10.9
ttPeak	absolute (min) 7.2 0.2 2.1	5.1 0.1 2.8	5.4 0.2 2.8
ETP	absolute (nM·min) 551.6 53.1 9.6	1010.0 90.7 9.0	1091.0 114.3 10.5
Velocity Index	absolute (nM/min) 18.3 1.6 8.6	52.8 3.6 6.8	73.8 9.7 13.1

STG-BLS	donor 121 (n tests=5 indep, 24 meas)	donor 122 (n tests=5 indep, 24 meas)	donor 123 (n tests=5 indep, 22 meas)	donor 124 (n tests=5 indep, 20 meas)
Parameters	Mean SD CV (%)	Mean SD CV (%)	Mean SD CV (%)	Mean SD CV (%)
Lag time	absolute (min) 2.26 0.05 2.0	2.40 0.11 4.6	2.18 0.11 4.8	2.52 0.08 3.1
Peak	absolute (nM) 174.30 20.26 11.6	113.50 16.27 14.3	93.00 17.84 19.2	92.46 23.46 25.4
ttPeak	absolute (min) 5.02 0.20 3.9	5.77 0.22 3.8	5.13 0.19 3.7	5.55 0.12 2.6
ETP	absolute (nM·min) 1099.00 100.00 9.1	953.10 125.00 13.1	670.90 113.70 17.0	747.30 156.20 20.9
Velocity Index	absolute (nM/min) 85.99 14.50 16.7	46.75 7.81 16.7	44.10 9.43 21.4	45.75 14.49 31.7

2. DISTRIBUTION OF NORMALIZED TG PARAMETERS IN HEALTHY DONORS AND RELATIONSHIP WITH AGE AND SEX



Normalized ETP and Peak showed a different distribution for sex and age between young adults and elderly at low concentration of TF. In addition, TG increased with age in male while in female was decreased independently of the OC/HRT therapy.



Increasing TF concentration, the effects of age and sex were abolished and dependent by the presence of OC/HRT treated female in the 20-40 years group. Interesting, ETP inhibition in presence of TM showed an expanded distribution with values below 50% only in young female.

STG-TS + TM (n=45 tests)	STG-QC low	STG-QC Norm	STG-QC high	STG-REF Plasma
Parameters	Mean SD CV (%)	Mean SD CV (%)	Mean SD CV (%)	Mean SD CV (%)
Lag time	absolute (min) 2.9 0.1 3.4	2.0 0.1 3.1	1.5 0.0 2.3	
Peak	absolute (nM) 61.9 7.4 11.9	181.2 16.9 9.4	483.6 16.4 3.4	
ETP	absolute (nM·min) 5.0 0.2 3.4	3.9 0.1 2.5	2.4 0.0 1.9	
Velocity Index	absolute (nM/min) 320.3 34.9 10.9	815.6 61.3 7.5	1491.0 73.4 4.9	

STG-TS +TM	donor 121 (n tests=5 indep, 25 meas)	donor 122 (n tests=5 indep, 25 meas)	donor 123 (n tests=5 indep, 20 meas)	donor 124 (n tests=4 indep, 17 meas)
Parameters	Mean SD CV (%)	Mean SD CV (%)	Mean SD CV (%)	Mean SD CV (%)
Lag time	absolute (min) 2.39 0.08 3.4	2.60 0.08 3.2	2.34 0.11 4.9	2.78 0.08 2.8
Peak	absolute (nM) 152.30 21.25 14.0	83.01 7.41 8.9	93.03 8.69 9.3	93.30 12.00 12.8
ttPeak	absolute (min) 4.02 0.09 2.1	4.33 0.11 2.5	3.97 0.15 3.7	4.52 0.09 2.0
ETP	absolute (nM·min) 574.30 51.04 8.9	348.40 30.73 8.9	348.10 28.55 8.2	401.40 53.10 13.2
Velocity Index	absolute (nM/min) 49.71 5.81 11.7	69.59 2.25 3.2	64.40 4.50 7.0	57.00 5.30 9.3

STG-BLS assay: all TG parameters had an inter-variability (CV%) below 15% both for QCs and reference plasma. In healthy PFP samples, the variability increases and especially for ETP and Peak was $\geq 20\%$.

STG-TS assays: QCs and reference plasma presented an inter-variability below 15%, however, we noticed an increased CV for the ETP inhibition on QC with increased resistance to TM. Healthy PFP samples showed an inter-variability below 15% for all TG parameters.

For both assays the intra-variability of all the TG parameters in the 4 healthy PFP donor samples were below 10% (data not shown).

CONCLUSIONS: STG analyser provides overall a decreased variability for TG parameters compared to the data published on CAT³. However the variability is still too high using TF low assay in PFP frozen samples. Further analysis are needed to evaluate the effects of age and sex on TG parameters as well as of the TG determinants triggered with different TF concentrations (BLS vs TS assays).

REFERENCES: ¹Kintigh J et al. RPTH 2017; ²Dargaud Y et al JTH 2017; ³ Bloemen S. et al. AACC 2017

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