P0158

Thrombin generation measurement using the automated ST Genesia Thrombin Generation System in healthy adults and some adult patients

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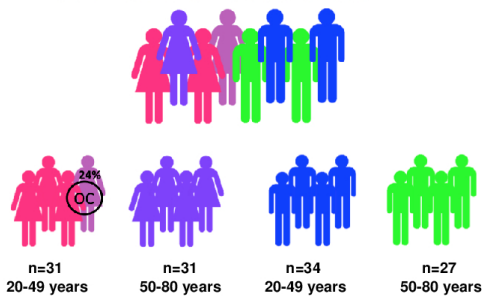
INTRODUCTION & AIM

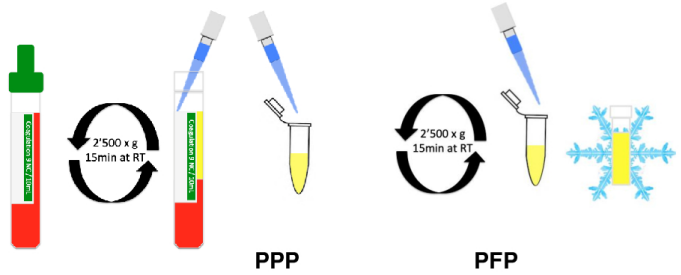
Thrombin generation (TG) assays evaluate the balance between pro- and anticoagulant forces, aiming to better assess bleeding and thrombotic risks. Although TG readouts obtained with the calibrated automated thrombin generation have been investigated in multiple clinical conditions, TG still needs standardization and clinical validation. The new automated TG instrument ST Genesia® (STG) provides a normalization of each TG parameters based on a reference plasma for each test aiming at reducing intra-/inter-laboratory variability.

In this study we evaluated the accuracy of the new TG analyzer and established the normal reference intervals for all TG parameters to provide a clear view on the inter-individual TG variability.

STUDY GROUP



SAMPLE PREPARATION



3mL PPP from 10 mL WB collected in the middle of the tube

2.7mL PFP collected and stored at -80°C

STG ANALYSIS

Bleed screen (BLS) assay composition:
Human TF at low picomolar level with procoagulant phospholipids.

Thrombo screen (TS+/-TM) assay composition:
Human TF at medium picomolar level with procoagulant phospholipids and with/without thrombomodulin.

STG REAGENTS

REFERENCE PLASMA

Parameter	Value	BLS	TS-no TM
Lag time	Absolute (min)	2.5±0.1,2.6	2.3±0.0,2.0
Peak	Absolute (nM)	163±18,**11**	248±30,**12**
Time to peak	Absolute (min)	5.4±0.2,2.8	4.5±0.1,2.1
ETP	Absolute (nM*min)	1091±114,**11**	1312±142,**11**
Velocity index	Absolute (nM/min)	74±9.7,**13**	149±14,9.4

Data are expressed as mean, standard deviation and coefficient of variation (%).

QUALITY CONTROLS

Assay	Parameter	Value	STG-QC Low	STG-QC Normal	STG-QC High
BLS	Lag time	Absolute (min)	2.8±0.1,4.0	1.9±0.0,2.4	NA
		Normalized (ratio)	1.2±0.1,4.3	0.8±0.0,2.7	NA
	Peak	Absolute (nM)	51±4.9,9.6	123±9.5,7.7	NA
		Normalized (%)	37±3.3,8.9	88±6.6,7.6	NA
	Time to peak	Absolute (min)	7.2±0.2,2.1	5.1±0.1,2.8	NA
		Normalized (ratio)	1.3±0.0,3.1	0.9±0.0,3.2	NA
	ETP	Absolute (nM*min)	552±53,9.6	1010±91,9.0	NA
		Normalized (%)	54±2.5,4.6	99±4.0,4.1	NA
	Velocity index	Absolute (nM/min)	18.3±1.6, 8.6	52.8±3.6, 6.8	NA
		Normalized (%)	33.3±4.3,**12.9**	95.7±11.2,**11.7**	NA
TS-noTM	Lag time	Absolute (min)	2.6±0.1,2.5	1.9±0.1,4.1	1.4±0.0,2.2
		Normalized (ratio)	1.2±0.0,2.5	0.8±0.0,3.8	0.7±0.0,2.7
	Peak	Absolute (nM)	101±8.7,8.6	216±20,9.2	496±43,8.7
		Normalized (%)	38.2±2.5,6.5	82±4.4,5.4	188±8.8,4.7
	Time to peak	Absolute (min)	5.8±0.2,4.0	4.3±0.1,2.4	2.3±0.0,2.0
		Normalized (ratio)	1.3±0.0,3.4	1.0±0.0,2.6	0.5±0.0,2.6
	ETP	Absolute (nM*min)	654±60,9.2	1216±120,9.9	1577±214,**14**
		Normalized (%)	46±1.9,4.1	87±3.8,4.4	112±5.0,4.5
	Velocity index	Absolute (nM/min)	39±4.5,**12**	123±9.6,7.8	881±89,10
		Normalized (%)	26±2.8,**11**	84±7.6,9.0	598±57,9.5
TS+TM	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)	62±7.4,**12**	181±17,9.4	484±16,3.4
	Time to peak	Absolute (min)	5.0±0.2,3.4	3.9±0.1,2.5	2.4±0.0,1.9
	ETP	Absolute (nM*min)	320±35,**11**	816±61,7.5	1491±73,4.9
	ETP inhibition	Ratio (%)	51±4.3,8.6	32±3.6,**11**	5.4±0.6,**13**
	Velocity index	Absolute (nM/min)	38±5.5,**11.5**	127±15,**11**	855±59,6.9

Data are expressed as mean, standard deviation (SD) and coefficient of variation(%). STG-QC Low, ST Genesia quality control for low values; STG-QC Normal,ST Genesia quality control for normal values; STG-QC High, ST Genesia quality control for high values; CV coefficient of variation;ETP, endogenous thrombin potential; TM, thrombomodulin; NA not available.

PLASMA SAMPLE

INTRA-ASSAY

Assay	Parameter	Value	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
BLS	Peak	Absolute (nM)	157±8.5,5.5	105±11, 10	109±15, **14**	117±8.6,7.4
		Normalized (%)	127±6.9,5.4	77±7.8,10	87±12,**14**	93±6.9,7.4
	ETP	Absolute (nM*min)	1029±37,3.6	865±61,7.0	805±106,**13**	960±57,5.9
		Normalized (%)	109±3.9,3.5	88±6.1,7.0	84±11,**13**	100±6.0,5.9
TS-no TM	Peak	Absolute (nM)	218±11,4.9	173±6.4,3.7	157±16,9.8	136±4.6,3.4
		Normalized (%)	85±4.1,4.9	65±2.4,3.7	60±5.9,9.8	51±1.7,3.4
	ETP	Absolute (nM*min)	1086±41,3.7	1101±34 ,3.1	908±58,6.4	889±22,2.4
		Normalized (%)	81±3.0,3.7	78.6±2.4,3.1	64±4.1,6.4	62±1.5,2.4
TS+TM	Peak	Absolute (nM)	134±16,**12**	73.2±3.2,4.3	87±8.2,9.5	94±2.9,3.1
	ETP	Absolute (nM*min)	512±64,**13**	3032±10,3.3	330±26,7.9	406±13,3.1
	ETP inhibition	Ratio (%)	52±6.1, **13**	72.5±0.9, 1.2	64±1.5,2.4	54±1.3,2.3

Data are expressed as mean, standard deviation and coefficient of variation (%);ETP, endogenous thrombin potential; TM, thrombomodulin

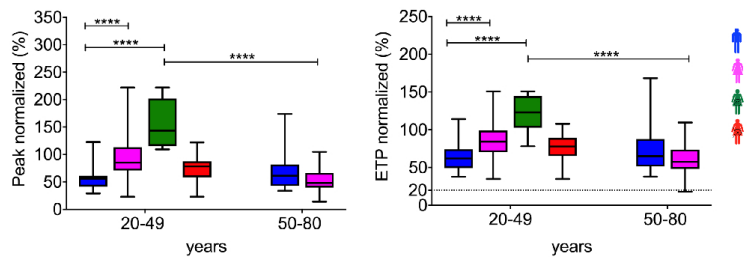
INTER-ASSAYS

Assay	Parameter	Value	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
BLS	Peak	Absolute (nM)	174±20,**12**	114±16,**14**	94±18,**19**	87.0±23,**27**
		Normalized (%)	130±13,9.7	85±11,**13**	70±13,**19**	63.9±17,**26**
	ETP	Absolute (nM*min)	1099±100,9.1	953±125,**13**	677±113,**17**	698.8±134,**19**
		Normalized (%)	110±8.9,8.1	95±11,**12**	68±12,**17**	69.8±13,**19**
TS-no TM	Peak	Absolute (nM)	235±15,6.4	177±11,6.3	169±12,6.9	148.0±14,9.6
		Normalized (%)	90±7.8,8.7	68±4.8,7.1	64±4.5,7.0	56.3±5.3, 9.5
	ETP	Absolute (nM*min)	1140±512,4.5	1138±48,4.2	958±48,5.1	937±45,4.8
		Normalized (%)	83±4.4,5.3	83±4.0,4.9	69±4.3,6.2	67±4.0,6.0
TS+TM	Peak	Absolute (nM)	152±21,**14**	83±7.4,8.9	93±8.8,9.4	95±12,**13**
	ETP	Absolute (nM*min)	574±81,**14**	346±31,8.9	349±29,8.3	408±52,**13**
	ETP inhibition	Ratio(%)	50±5.8,**12**	70±2.3,3.2	65±4.6,7.2	56±5.0,8.9

Data are expressed as mean, standard deviation and coefficient of variation (%) ETP, endogenous thrombin potential; TM, thrombomodulin

NORMAL REFERENCE VALUES

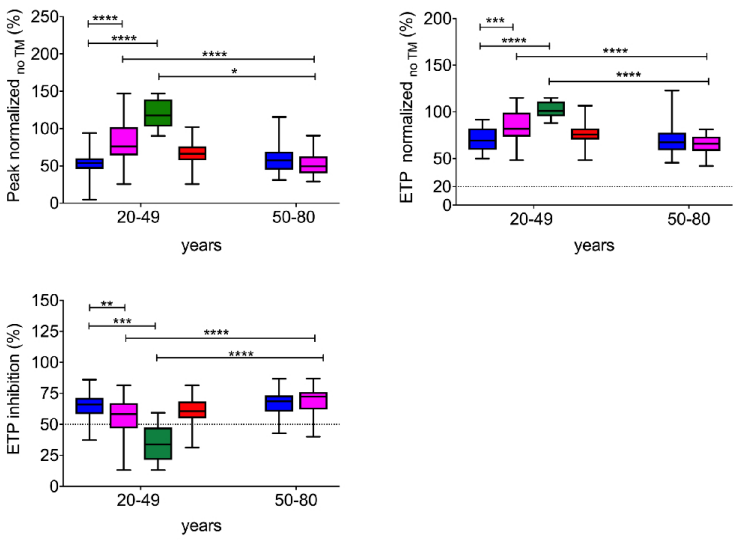
BLEEDSCREEN ASSAY



Categories	Lag time	Peak	Time to peak	ETP	Velocity index
Males (20-80 y)	1.1 (1.0-1.3)	57 (42-72)	1.1 (1.0-1.2)	64 (51-79)	58 (44-76)
Females (20-49 y) with OC	1.0 (0.9-1.0)	143 (115-202)	0.9 (0.8-1.0)	123 (103-145)	174 (144-322)
Females (20-49 y) without OC	1.0 (0.9-1.1)	78 (59-88)	1.0 (0.9-1.1)	78 (65-90)	82 (68-105)
Females (50-80 y)	1.2 (1.0-1.3)	48 (40-67)	1.2 (1.0-1.3)	57 (48-74)	47 (35-67)

Data are expressed as median and interquartile range (25-75% percentile). OC, oral contraception

THROMBOSCREEN ASSAY



Categories	Lag time	Peak	Time to peak	ETP	Velocity index	ETP Inhibition
Males (20-80 y)	1.2 (1.1-1.3)	55 (45-66)	1.2 (1.2-1.3)	68 (59-80)	49 (36-57)	67 (60-73)
Females (20-49 y) without OC	1.1 (1.0-1.1)	66 (58-76)	1.2 (1.0-1.2)	76 (70-82)	61 (53-77)	61 (55-68)
Females (20-49 y) with OC	0.9 (0.9-1.1)	118 (103-139)	0.9 (0.8-1.1)	101 (95-111)	149 (95-214)	33 (21-48)
Females (50-80 y)	1.3 (1.1-1.4)	49 (40-63)	1.4 (1.2-1.5)	66 (58-74)	40 (31-50)	72 (62-76)

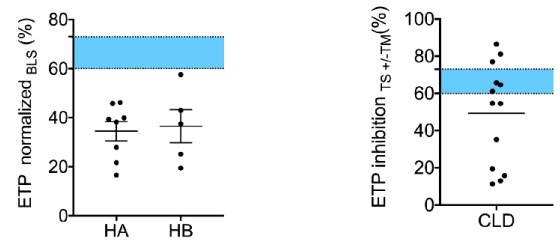
Data are expressed as median and interquartile range (25-75% percentile). Normalizd Lag time, Peak, Time to Peak and ETP values hae been obtained using Thromboscreen assay in the absence of thrombomodulin. Differently the ETP inhibition parameter has been calculated from ETP values obtained in presence and in absence of thrombomodulin. OC, oral contraception

CONCLUSIONS

STG appears to be suitable for the accurate measurement of TG in healthy adults. The use of a reference plasma for data normalization is effective in reducing the inter-assay variability of quality controls (CV<15%), but it has a little impact on PFP samples variability especially for Bleedscreen assay (CV <30%).

Oral contraception possibly influenced TG parameters, resulting in a higher median and a broader reference interval for peak height and ETP in females aged 20 to 49 years than in all other sex- and age-categories.
Therefore, we propose the following reference interval categories: males, females <50 years not using OC, females <50 using OC, females ≥50 years.

PATHOLOGIC SAMPLES: PRELIMINARY DATA



ETP were outside the reference intervals (blue) in severe hemaophilic patients. ETP inhibition showed a wide distribution in samples from patient affected by chronic liver disease (CLD): for some of them this parameter was 2-10%, highlighting a procoagulant imbalance.

FOR MORE INFO

Calzavarini et al. «**Thrombin generation measurement using the ST Genesia Thrombin Generation System in a cohort of healthy adults: normal values and variability»,** accepted for publication on RPTH 2019

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