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LETTER TO THE EDITOR

Biological variation of thrombin generation on ST Genesia

Dear Editors,

We previously reported on the biological variation of the thrombin generation assay (TGA) with and without thrombomodulin (TM) using a calibrated automated thrombogram (CAT) system.[1] Recently, the ST Genesia (Diagnostica Stago, Asnières sur Seine, France), a fully automated thrombin generation system, was launched with the aim of standardizing TGA by reducing inter-experiment and inter-laboratory variation. This study therefore aimed to determine the biological variation of TGA on this automated platform.

Important information on the whole coagulation process is not provided in routine hemostasis tests based on optical or mechanical detection of clot formation, (i.e., prothrombin time and activated partial thromboplastin time) but deserves to be investigated to fine-tune the identification of coagulopathies and hemorrhagic status.[2] Thrombin generation depicts thrombin generation and inactivation using a fluorogenic substrate. In adding soluble TM to the testing mixture, the endogenous protein C pathway is also investigated. Thrombin generation has been largely used in the research setting but has gained interest in a clinical context, like in hemophilia A, or to assess inherited or acquired coagulopathies.[3,4]

Three different determinants are responsible for the total variability of a parameter in an individual over time: (i) pre-analytical variation, (ii) analytical variation, and (iii) biological variation. Biological variation consists of both within-subject variation (CV_I; fluctuation of a value around a subject homeostatic setting point) and between-subject variation (CV_G; difference in homeostatic setting points between different subjects).

Few studies report on the biological variation of thrombin generation. This type of study helps laboratories to define reference change values (RCV) which may support the interpretation of serial measurements at the individual level. In addition, biological variation of a parameter can aid in setting analytical specification goals, now recommended by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM).[5]

To study biological variation, 19 healthy adults were followed for 5–6 weeks with a once weekly blood draw. Samples were analyzed on the ST Genesia using the STG-ThromboScreen reagent. After outlier exclusion, components of variation were obtained by nested analysis of variance (ANOVA). Analytical variation (CV_A), within-individual variation (CV_I), between-individual variation (CV_G), index of individuality (II) and RCV were calculated. Informed consent was obtained from each participant, and the study protocol was approved by the local Ethics Committee (2019/29JUL/340).

Nineteen apparently healthy volunteers were included with a median age of 33.5 (range 22–60 years), 11 (61%) were female. Figure 1 represents the mean and absolute range for each individual for all parameters without and with TM. Due to the known effect on hemostasis, results of healthy volunteers taking oral contraceptives (OC; $n = 4$) were compared with results of healthy volunteers excluding healthy volunteers on OC. A significant difference was seen between subjects on OC and the group of subjects not taking OC for the mean of all parameters. Both subgroups were therefore studied separately. From the remaining group of 15 healthy volunteers, 2 were excluded for all parameters and 1 for lag time (LT) and time-to-peak (TTP) only. No difference between sexes was found after exclusion of the OC group. For both TGA without and with TM, CV_A, CV_I, and CV_G are represented in Table 1. For the non-OC group, CV_A ranged from 1.6% to 8.1%, CV_I from 3.9% to 16.0% and CV_G from 8.1% to 30.6% for parameters without TM. For TGA in presence of TM, CV_A varied from 1.9% to 4.7%, CV_I from 3.3% to 16.6% and CV_G from 7.2% to 44.6%. Details on the OC group can be found in Table 1. II and RCV are shown in Table 2 for parameters without and with TM. For all TGA parameters (±TM) II was low (<1) in the non-OC group. For the OC subgroup, the index was high for TTP, peak, ETP, and velocity index. In the non-OC group, RCV values ranged from 13.5% to 49.8% and from 10.4% to 48% without and with TM respectively. Similar RCV's were seen for the OC-group, except for peak and ETP with TM. For all parameters, no significant difference in mean was seen between men and women without OCs. This is in line with the article by Ninivaggi et al.[6] In this paper 117 healthy donors were analyzed by ST Genesia to establish reference values. Calvarazini et al. reported different normalized reference ranges according to sex and age with ST Genesia.[7] After exclusion of women on OC, these differences were eliminated except for the differences seen between women <50 years and women ≥50 years. They proposed to use four separate reference interval categories for routine laboratories: men, women aged <50 years not using OC, women aged <50 years on OC and women aged ≥50 years. Kristensen et al. only saw a minor effect of age.[8] For women aged ≥50 years, the use of hormonal replacement therapy may also lead to change in hemostasis which may require an additional category of subjects.

Like our previous study on TGA with CAT, CV_I and especially CV_G were higher in TGA with TM compared to TGA without TM for LT, peak, ETP and velocity index.[1] As already mentioned in the previous study, this could be due to a difference in resistance to the TM/protein C axis or a difference in protein C or protein S concentration. Between-individual variation (CV_G) was 9.1% for protein C and

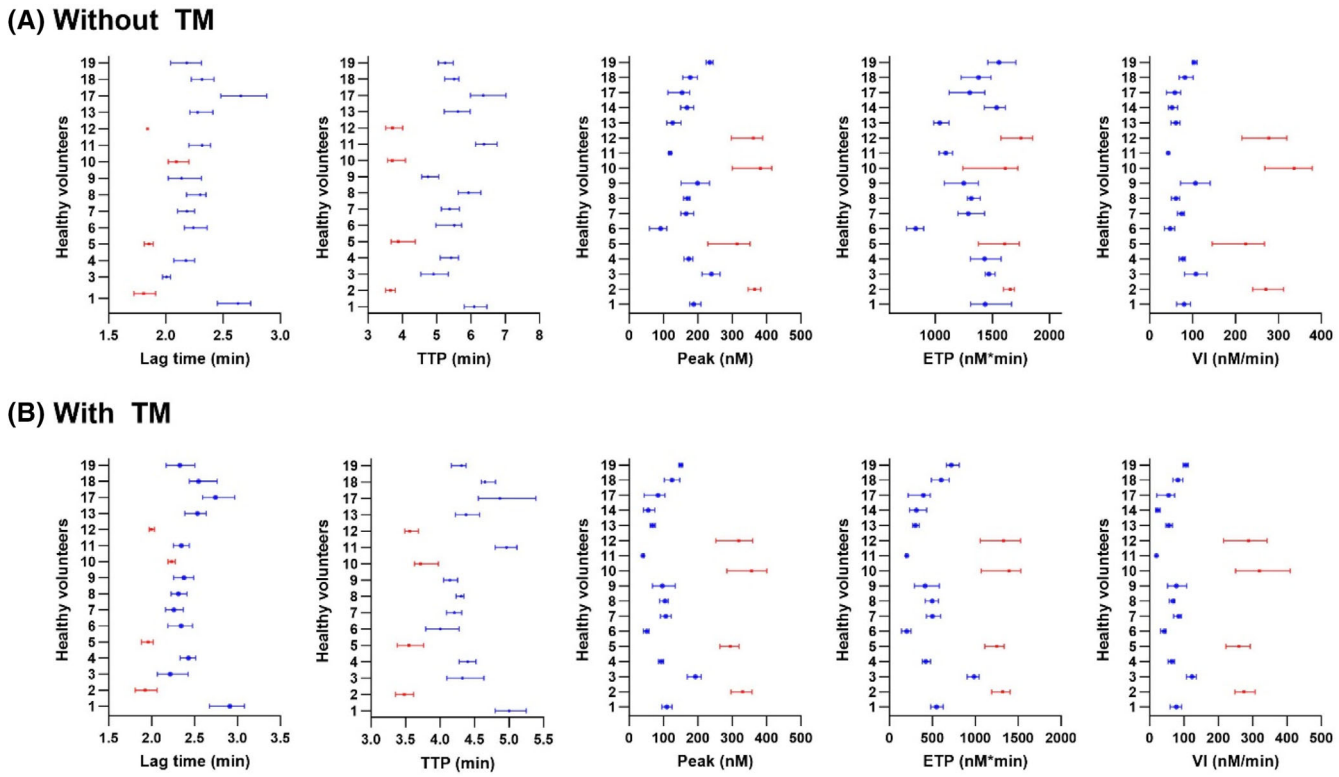

FIGURE 1 Representation of individual mean and absolute range (min–max) of values for each parameter without thrombomodulin (A; Without TM) and with thrombomodulin (B; With TM). Blue: healthy volunteers not on oral contraceptives. Red: healthy volunteers on oral contraceptives. The outlying results are excluded from the figure.

TABLE 1 Components of variation for ST Genesia with and without thrombomodulin.

		Mean		CV_A (%)		CV_I (%)		CV_G (%)	
Parameter	Group	w/o TM	TM	w/o TM	TM	w/o TM	TM	w/o TM	TM
LT	w/o OC group ($n = 12$)[a]	2.28	2.45	3.00	1.93	3.85	4.65	8.06	8.18
	OC ($n = 4$)[b]	1.91	2.04	2.38	1.78	2.67	3.20	6.65	7.13
TTP	w/o OC group ($n = 12$)[a]	5.59	4.47	1.64	1.86	4.67	3.26	9.07	7.24
	OC ($n = 4$)[b]	3.73	3.58	1.67	1.25	5.54	3.33	0.59	2.33
Peak	w/o OC group ($n = 13$)[a]	170.76	98.25	3.08	3.74	9.94	14.23	24.10	41.38
	OC ($n = 4$)[b]	359.79	326.33	1.84	1.63	9.18	11.72	3.23	5.47
ETP	w/o OC group ($n = 13$)[a]	1316.89	494.9	2.54	4.52	7.01	14.11	15.29	44.63
	OC ($n = 4$)[b]	1664.17	1320.61	2.71	2.52	6.98	8.95	2.02	3.41
VI	w/o OC group ($n = 13$)[a]	72.81	68.83	8.13	4.73	16.03	16.66	30.57	41.97
	OC ($n = 4$)[b]	268.98	281.38	7.33	3.56	15.70	18.22	11.71	8.35

Abbreviations: CV_A, analytical coefficient of variation; CV_G, between-individual variation; CV_I, within-individual variation; ETP, endogenous thrombin potential; LT, lag time; Peak, thrombin peak height; TM, CAT with thrombomodulin; TTP, time-to-peak; VI, velocity index; w/o, TM CAT without thrombomodulin.

[a]w/o OC group: healthy volunteers without subjects on oral contraceptive therapy.

[b]OC group: healthy volunteers on oral contraceptive therapy.

24.1% for protein S in this patient group which could support our hypothesis.[9] Absolute values are also lower in TG with TM for peak, ETP and velocity index, which could explain a higher CV in percentage. When comparing groups not taking OC between these studies, CV_A was comparable for parameters without TM (peak and velocity index a little higher with ST Genesia) and CV_A was lower on ST Genesia for all parameters with TM. This would be expected since the ST Genesia is more standardized and less operator dependent, although performance was very similar with CAT in the present study. Comparable imprecision between ST Genesia and CAT was also seen by

TABLE 2 Index of individuality and reference change values for CAT without and with thrombomodulin.

Parameter	Group	II		RCV (%)	
		−TM	+TM	−TM	+TM
LT	w/o OC group ($n = 12$)[a]	0.61	0.62	13.53	13.96
	OC ($n = 4$)[b]	0.54	0.51	9.91	10.15
TTP	w/o OC group ($n = 12$)[a]	0.55	0.52	13.71	10.40
	OC ($n = 4$)[b]	9.84	1.52	16.04	9.85
Peak	w/o OC group ($n = 13$)[a]	0.43	0.36	28.84	40.78
	OC ($n = 4$)	2.90	2.16	25.95	32.80
ETP	w/o OC group ($n = 13$)[a]	0.49	0.33	20.67	41.07
	OC ($n = 4$)[b]	3.71	2.73	20.75	25.77
VI	w/o OC group ($n = 13$)[a]	0.59	0.41	49.82	48.00
	OC ($n = 4$)[b]	1.48	2.22	48.03	51.46

Abbreviations: ETP, endogenous thrombin potential; II, index of individuality; LT, lag time; RCV, reference change value; Peak, thrombin peak height; TM, thrombomodulin; TTP, time-to-peak; VI, velocity index.
[a]w/o OC group: healthy volunteers without females on oral contraceptive therapy.
[b]OC group: healthy volunteers on oral contraceptive therapy.

Kristensen et al.[8] CV_I was comparable for most parameters (except for ETP without TM: 4.1% with CAT vs. 7.0% with ST Genesia and LT without TM: 5.9% with CAT vs. 3.9% with ST Genesia). Oddly, more important differences in CV_G were observed between the two methods.

RCVs, based on CV_A and CV_I were very similar between the OC and non-OC subgroups, except for peak and ETP with TM. A low II, reflecting a larger CV_G compared to CV_I, was seen for all parameters in the subgroup without OC. This was already reported by Hemker et al. in 2003.[10] Interestingly, II was high for all parameters, except for LT, in the OC subgroup. Although it is difficult to draw conclusions in this very small ($n = 4$) subgroup, this high index suggests an impact of the OC treatment on the individual's homeostatic setting point. OC is well known to induce a hypercoagulable TGA profile and resistance to inactivation by TM or activated protein C.[11] CV_G was a lot smaller in the OC group compared to the subgroup without OC. A fixed reference interval could be of value in this latter group. For the non-OC group, clinicians could better rely on sequential follow-up rather than reference intervals but this needs to be confirmed in a larger cohort.

Some limitations can be pointed out in our work. One major limitation is the low subject size. As stated before, the subgroup on OC was very small. Although the subgroup not on OC is still within limits of the article by Ricos et al.[12] subgroup analysis could be hampered by this low number of subjects. We did not evaluate normalized results. Kristensen et al.[8] did not see an improvement of analytical variation after normalization of results, but normalization might improve within and between subject variation by lowering batch-to-batch variation. However, as these samples were analyzed with the same batch of reagent, it is unlikely that normalization would improve the CVs. Also, since healthy volunteers were followed for 5 weeks, we can only comment on short term biological variation. A relatively young group of healthy volunteers was included (median age: 36 years) and the impact of age on biological variation requires further study.

In conclusion, we assessed the short-term biological variation of TGA parameters with STG-ThromboScreen on ST Genesia, a fully automated TGA platform. CV_I (%) and CV_G (%) for thrombin generation with TM were higher than for thrombin generation without TM for most parameters. II and RCV were calculated for every TGA parameter. In general, parameters were highly individualized in healthy volunteers not on OC. The opposite was seen for healthy volunteers on OC for TTP, peak, ETP, and velocity index, suggesting a different approach for interpreting thrombin generation results in these subjects but this deserves further investigations in a larger cohort of subjects.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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