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# Biological variation data and analytical specification goal estimates of the thrombin generation assay with and without thrombomodulin in healthy individuals

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## Abstract

**Background:** Evaluation of an individual's thrombin-generating capacity enables a global assessment of the coagulation cascade and is therefore thought to better reflect the clotting function of blood. However, the lack of standardization still hampers the use in routine clinical practice.

**Methods:** Nineteen healthy subjects were sampled once a week for 5 consecutive weeks. Thrombin generation assay (TGA) was performed in duplicate by calibrated automated thrombogram (CAT) on platelet poor plasma with and without thrombomodulin. After exclusion of outliers, a nested analysis of variance (ANOVA) was performed to evaluate the biological variability (BV) results. Analytical variation ($CV_A$), within-individual variation ($CV_I$), between-individual variation ($CV_G$), index of individuality (II), and reference change value (RCV) were calculated.

**Results:** All parameters taken together, the $CV_{A,}$ $CV_I$, and $CV_G$ without TM, ranged from 2.8% to 6.5%, from 4.1% to 13.3% and from 10.4% to 28.4%, respectively. For TG with TM, $CV_I$ and $CV_G$ were higher and ranged from 5.0% to 18.1% and from 14.9% to 35.3%, respectively. For endogenous thrombin potential (ETP), a $CV_I$ of 4.1% and $CV_G$ of 10.4% were obtained without addition of thrombomodulin (TM). With addition of TM, both $CV_I$ and $CV_G$ were higher: 14.0% and 34.8%, respectively. The II was low and the RCV ranged from 17.2% to 50.4%.

**Conclusion:** CAT parameters are highly individualized and population-based reference values could be called into question. The assessment of BV and RCV for thrombin generation assays could optimize interpretation of serial patient results and guide setting of analytical specification goals.

KEYWORDS

biological variation, calibrated automated thrombogram, platelet poor plasma, reference change value, thrombin generation assay

## 1 | INTRODUCTION

Routine coagulation assays such as prothrombin time (PT) and activated partial thromboplastin time (APTT) reach reading end-point when only 5% of the total amount of thrombin is generated. This means that 95% of the thrombin generated is not reflected by these tests.[1] In addition, PT and APTT respond mainly to procoagulant, but much less to anticoagulant drivers. As a consequence, there may be discrepancies between the physiological (in vivo) blood clotting process and the commonly used blood coagulation assays (in vitro). Initially described in 1953, thrombin generation assays (TGA) offer the potential to better reflect the physiological clotting process but still suffer from a lack of standardization.[1]

The amount of thrombin generated in plasma can be used to predict the risk of thrombosis or bleeding in an individual patient.[1] Indeed, it has been shown that high peak thrombin generation and elevated endogenous thrombin potential (ETP) are predictive of thrombosis in high-risk subjects.[1] On the other hand, in patients with a coagulation factor deficiency, like hemophilia, thrombin generation is correlated with clinical bleeding.[2] In trauma patients, TGA can be used to appreciate the patient's hemostatic potential in order to guide transfusion.[3] Beside these applications, TGA has also helped to gain more insight into complex acquired hemostatic alterations like chronic liver disease, where the unsteady balance between pro- and anticoagulant factors can lead to bleeding as well as thrombosis.[4]

Recently, it has been suggested that TGA needs to be performed in the presence and absence of thrombomodulin (TM) to allow detection of a possible state of hypercoagulability.[5] This approach has for example elucidated that patients with cirrhosis have some degree of hypercoagulability.[6] Exploring the thrombomodulin-protein C pathway could also be of interest in numerous other indications.

Although the calibrated automated thrombogram (CAT) method has the potential to enter our arsenal of assays for the diagnosis or the monitoring of coagulation disorders, the current lack of standardization still hampers its widespread use. Indeed, considerable inter-laboratory variability exists due to different origin and concentration of reagents used: tissue factor (TF), phospholipids and thrombomodulin (TM).[7,8] As a matter of fact, the amount as well as the origin (recombinant or animal) of TF and TM used significantly impacts the TGA results.[7,9]

In order to solve these issues, several standardization initiatives of preanalytical and analytical conditions have been initiated.[8,10] Among them, Perrin et al introduced external reference plasma to normalize TGA results and demonstrated a significant reduction in inter-laboratory variation.[7]

In parallel with these efforts, studies on biological variation should be mandatory to make this type of test an integral part of our routine. This kind of studies could allow laboratories to generate reference change values, therefore helping the interpretation of serial measurement results. An approach based on biological variation (BV) data is also considered to be the optimal method to establish the maximum analytical variation allowed for hemostasis tests.[11] Laboratory results of individuals may vary over time, depending on three main factors: (a) the influence of the preanalytical phase, (b) the analytical variation (precision and bias), and (c) finally, the BV. The latter comprises the within-individual variation ($CV_I$; random fluctuation around a homeostatic setting point) and the between-individual variation ($CV_G$; the variation in homeostatic setting points of different subjects).[12] Currently, a reference database exists for BV data (The "European Federation for Laboratory Medicine (EFLM) Biological Variation Database") but data still lack for several coagulation tests, including TGA.[13] Taking these elements into consideration, the aim of the following study was to calculate the biological variation of the TGA with and without TM.

## 2 | MATERIALS AND METHODS

This study was realized at the clinical hemostasis laboratory of Cliniques universitaires Saint-Luc, Brussels, Belgium. Informed consent was obtained from each participant, and the study protocol was approved by the local Ethics Committee (2019/29JUL/340).

### 2.1 | Analyte/measurand

Blood samples were collected under standardized conditions to minimize preanalytical variation. Samples were collected either on Wednesday or Thursday during the morning between 9 and 11 AM for each participant. The same phlebotomist collected blood from all participants throughout the 5 weeks of the study from October 2019 to November 2019. The collection of samples was done in accordance with CLSI H3-H6 guidelines for the collection of blood by antecubital venipuncture. A discard tube was systematically drawn before the collection of citrate tubes (Monovette plastic 3.0 mL, 3.2% citrate, 106 mmol/L (Sarstedt); 10:1 blood: citrate ratio).[14] Samples were drawn while seated and homogenized immediately after collection. Platelet poor plasma (PPP) was obtained following two centrifugation steps (2 × 15 minutes at 2500 *g*) within 30 minutes after venipuncture: After the first centrifugation, plasma was aliquoted and centrifuged a second time. The aliquots were then stored at −80°C. All samples were thawed at 37°C for 15 minutes before analysis 3 month after freezing.

TGA parameters analyzed were lag time (LT), thrombin peak height (Peak), endogenous thrombin potential (ETP), and velocity index (VI). LT represents the time between the addition of the trigger and the initiation of thrombin generation. Peak shows the highest concentration of thrombin that can be generated, ETP represents the total amount of thrombin produced under the action of pro- and anticoagulants drivers, while VI is a composite index that includes the peak, the time to reach the peak, and the lag time (VI = peak/(time to reach the peak − lag time)).

### 2.2 | Subject

Healthy subjects were included in the study following the inclusion and exclusion criteria according to Carobene et al.[11] Every participant completed a medical questionnaire.

Participants were required to be over 18 years, nonsmokers and should have limited alcohol consumption (<10 g of ethanol/d). Participants were excluded from the study if at least one of these criteria was met: (a) diabetes and prescribed oral or insulin therapy, (b) history of chronic liver or kidney disease, (c) a family history of thalassemia syndrome and other hemoglobinopathies, (d) severe acute or chronic diseases (cancerous, cardiovascular or neurological), (e) known Hepatitis B virus (HBV), Hepatitis C virus (HCV), and/or Human Immunodeficiency Virus (HIV) infection, (f) pregnancy, nursing, or recent delivery (within 1 year).

During the study, all participants maintained their usual lifestyle and every drugs/substance taken were inventoried. In order to closely monitor every participant's health status, a short questionnaire, assessing drugs taken, exercise, and health status, was completed prior to each blood draw.

## 2.3 | Measurement procedure

Thrombin generation was measured with the CAT according to Hemker et al.[15] Briefly, 80 μL of PPP was mixed with 20 μL of STG-Thromboscreen (Stago, Asnières-sur-seine) with and without thrombomodulin and 20 μL of fluorogenic substrate in buffer containing $CaCl_2$ (FluCa kit, Stago). The STG-Thromboscreen reagent also contains a mixture of phospholipids and an intermediate concentration of TF. The exact TF concentration and phospholipid composition were not disclosed by the manufacturer. Calibration was performed with Thrombin Calibrator (Stago) for each duplicate analysis. Assays were performed on the same automate by the same operator and with the same calibrator and reagent lots. All of the analyses of the same healthy volunteer (HV; plasma specimens collected over 5 weeks) were performed in the same analysis run. The thrombin generation curve and parameters were calculated with the Thrombinoscope software version 5.0.0.742 (Stago). For each run, CRYOcheck Pooled Normal Plasma (Cryopep®; Lot A1236), for which reference values had been established, was included as internal quality control.

## 2.4 | Statistical analysis

### 2.4.1 | Outlier, normality, and subgroup analysis

Three statistical tests to exclude outliers using the statistical software XLSTAT (version 2019.2.2 by Addinsoft) were performed. Cochran's C test was used to identify outliers in variance values within the replicates of individuals tested (a). The distribution of the variance of each individual ($S^2_{I+A}$) was examined to see whether any individual's dispersion was smaller or larger compared with the whole group (b). Reed's criteria were used to benchmark the mean values of each individual (c).[12] At each step, the outliers were excluded from the analysis.[16,17] Normality was evaluated using the Shapiro-Wilk test, while Student *t* test was used to compare $CV_I$ and $CV_G$ values between subgroups.

**TABLE 1** Characteristics of the study population

| Variable | Study population (n = 19)[a] |
|---|---|
| Age (y) | 36 (22-60) |
| Females | 11 (58%) |
| Smokers | 0 (0%) |
| Oral contraceptive group | 4 (21%) |
| Lipid lowering medication | 1 (5%) |
| Analgesics (paracetamol and ibuprofen) | 4 (21%) |

[a]Values are given as n (%) for categorical variables and as means (range) for age.

### 2.4.2 | Analysis of variance

As recommended by Braga et al,[16] nested analysis of variance (ANOVA) was performed to assess the components of biological variation using statistical software XLSTAT and JMP Pro (JMP, version 14.3.0. SAS Institute Inc.). Sources of variability were calculated using a nested model with 3 factors (individuals, sampling occasions, and replicates). In this way, analytical, within-individual, and between-individual variances were obtained to calculate $CV_A$, $CV_I$, and $CV_G$.

### 2.4.3 | Analytical performance specifications

Index of individuality (II) was calculated according to the following formula: $(CV_A^2 + CV_I^2)^{1/2}/CV_G$. Reference change value (RCV) was determined using the formula: $2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$, where a Z value of 1.96 represents a probability of 95% of significant change between two results. Finally, optimal, desirable, and minimum analytical goals for imprecision, bias, and total error were calculated from BV components.[18]

## 3 | RESULTS

Our group was composed of 19 HV, comprising 8 males (25-59 years, median age: 33.5 years ± 10.8 years) and 11 females (22-60 years, median age: 39 years ± 11.4 years) with a median age of 33.5 years. Characteristics of HV are detailed in Table 1. Analgesics, including paracetamol and ibuprofen, were consumed sporadically by 4 participants. Only one patient was on lipid lowering medication. For both CAT, with and without TM, one subject was excluded by outlier analysis bringing the total on 18 subjects. Considering the procoagulant effects of oral contraceptives and their known effect on TGA tracing (significant higher peak and ETP values), we excluded the females

on oral contraceptive therapy (OC group). No significant difference between sexes was found after exclusion of the OC group.

### 3.1 | Within-individual and between-individual variation

$CV_{A,}$ $CV_I$, and $CV_G$ were obtained for both TGA with and without TM. The $CV_A$ ranged from 2.7% to 6.5%, $CV_I$ from 4.5% to 14.2% and $CV_G$ from 11.6% to 48.4%, for TGA without TM (details in Table 2). For TGA with TM, $CV_A$ ranged from 2.9% to 7.4%, $CV_I$ from 5.4% to 17% and $CV_G$ from 16.9% to 57.3%, (details in Table 2).

### 3.2 | Analytical specification goals

Optimal, desirable, and minimum analytical goals for imprecision, bias, and total error are depicted in Tables 3 and 4. II was low (0.24-0.62) for all parameters confirming that the CAT parameters are highly individualized and that population-based reference values are of limited relevance in result's interpretation. RCV values varied from 15.7% to 43% for CAT parameters without TM and from 17.2% to 54.1% for CAT parameters with TM. The $CV_A$ found in our study was lower than the maximum desirable performance goal $CV_A$ calculated for LT and peak and slightly higher for ETP and VI.

## 4 | DISCUSSION

Thrombin generation is a global hemostasis test that allows better understanding of the balance between procoagulant and anticoagulant factors. In cirrhotic patients, a simultaneous decrease in pro- and anti-hemostatic factors frequently occurs, resulting in a normal TGA pattern (or even hypercoagulable in the presence of TM).[19] This discovery had an impact on the management of these patients (allowing anticoagulant treatment for example) for which conventional coagulation assays poorly reflect the hemostatic state. Another possible application of TGA is the investigation of the recurrence risk of venous thromboembolism.[20-22] Indeed, for the selection and duration of anticoagulant treatment, most expert groups recommend consideration of the individual risk/benefit ratio. Therefore, the monitoring of the TGA pattern of patients under anticoagulant treatment could allow individual adjustment of their treatment. This indication remains however controversial and further investigations are mandatory.[23,24] The reasons for these discordances are unclear but the lack of standardization plays probably a role. In addition to inter-laboratory standardization, knowledge of physiological TGA variation over time could help clinicians monitor their patients. In our study, a higher $CV_I$ and $CV_G$ were seen after addition of TM compared to TGA without TM. It could be hypothesized that a variation in protein C concentrations, as well as a variable susceptibility to TM between subjects, could have an impact on TGA tests with TM. De Maat et al showed a $CV_G$ of 19.5% and a $CV_I$ of 7.6% for chromogenic protein C (U/mL).[25] More importantly, a difference in affinity of protein C for FVa and FVIIIa between patients could also explain the higher $CV_G$ seen in the TGA assay with TM. Calzavarini et al also found a higher inter-individual variability with TM in 123 patients at one time point.[26]

Oral contraceptives are well known to induce a significant procoagulant effect. Moreover, several authors showed a hypercoagulable thrombin generation profile after the addition of TM or activated protein C in this population.[20,27-29] These findings were also observed in our subjects taking OC, with higher ETP and peak values following TM addition.[26] Therefore, and as expected, the $CV_G$ was substantially lower after exclusion of the OC group.

As mentioned in the result section, II was low for all studied parameters. Since population-based reference values are recommended when the II is greater than 1.4, clinicians should base their clinical interpretation on the sequential evolution of an individual's results rather than on a reference interval.[30] Comparison of serial results can be realized using the reference change value (RCV). This latter is an objective tool able to assess whether a

**TABLE 2** Components of variation for CAT 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 | All (n = 18) | 2.6 | 2.8 | 3.9 | 3.5 | 6.0 | 5.4 | 19.0 | 16.9 |
| | $^w$/o OC group (n = 14)[a] | 2.7 | 2.9 | 3.8 | 3.7 | 5.9 | 5.0 | 16.5 | 14.9 |
| Peak | All (n = 18) | 274.0 | 274.0 | 2.7 | 2.9 | 8.1 | 10.0 | 22.7 | 34.8 |
| | $^w$/o OC group (n = 14)[a] | 250.6 | 184.0 | 2.8 | 4.2 | 8.2 | 13.5 | 12.8 | 27.4 |
| ETP | All (n = 18) | 1587.5 | 948.1 | 3.6 | 6.1 | 4.5 | 11.5 | 11.6 | 40.9 |
| | $^w$/o OC group [c] (n = 14)[a] | 1538.1 | 818.1 | 3.9 | 6.3 | 4.1 | 14.0 | 10.4 | 34.8 |
| VI | All (n = 18) | 102.5 | 75.3 | 6.3 | 6.7 | 14.2 | 17.0 | 48.4 | 51.3 |
| | $^w$/o OC group (n = 14)[a] | 86.2 | 62.7 | 6.5 | 7.4 | 13.3 | 18.1 | 28.4 | 35.3 |

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

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

**TABLE 3** Index of individuality, reference change values, and analytical goal specifications for CAT without thrombomodulin

| | | | | Analytical goals | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Imprecision (CV, %) | | | Bias (CV, %) | | | Total error (CV, %) | |
| Parameter | Group (n) | II | RCV (%) | Minimal | Desirable | Optimal | Minimal | Desirable | Optimal | Minimal | Desirable | Optimal |
| LT | All (18) | 0.32 | 19.8 | ≤±4.5 | ≤±3.0 | ≤±1.5 | ≤±7.5 | ≤±5.0 | ≤±2.5 | ≤±12.4 | ≤±9.9 | ≤±7.4 |
| | w/o OC group (14)[a] | 0.36 | 19.5 | ≤±4.4 | ≤±2.9 | ≤±1.5 | ≤±6.6 | ≤±4.4 | ≤±2.2 | ≤±11.4 | ≤±9.2 | ≤±7.1 |
| Peak | All (18) | 0.36 | 23.7 | ≤±6.1 | ≤±4.0 | ≤±2.0 | ≤±9.0 | ≤±6.0 | ≤±3.0 | ≤±15.7 | ≤±12.7 | ≤±9.7 |
| | w/o OC group (14)[a] | 0.64 | 24 | ≤±6.2 | ≤±4.1 | ≤±2.1 | ≤±5.7 | ≤±3.8 | ≤±1.9 | ≤±12.5 | ≤±10.6 | ≤±8.7 |
| ETP | All (18) | 0.39 | 16 | ≤±3.4 | ≤±2.3 | ≤±1.1 | ≤±4.6 | ≤±3.1 | ≤±1.5 | ≤±8.3 | ≤±6.8 | ≤±5.3 |
| | w/o OC group (14)[a] | 0.39 | 15.7 | ≤±3.1 | ≤±2.0 | ≤±1.0 | ≤±4.2 | ≤±2.8 | ≤±1.4 | ≤±7.6 | ≤±6.2 | ≤±4.8 |
| VI | All (18) | 0.29 | 43.1 | ≤10.7 | ≤±7.1 | ≤±3.6 | ≤±18.9 | ≤±12.6 | ≤±6.3 | ≤±30.6 | ≤±24.3 | ≤±18.0 |
| | w/o OC group (14)[a] | 0.47 | 41 | ≤±9.9 | ≤±6.6 | ≤±3.3 | ≤±11.8 | ≤±7.8 | ≤±3.9 | ≤±22.7 | ≤±18.8 | ≤±14.9 |

Abbreviations: CV, coefficient of variation; ETP, endogenous thrombin potential; II, index of individuality; LT, lag time; RCV, reference change value; Peak, thrombin peak height; VI, velocity index.

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

difference between two serial measurement is statistically significant. Therefore, RCV could allow better interpretation of TGA results when monitoring patients for thromboembolic or hemorrhagic risk.

In 2003, Hemker et al already showed that the inter-individual variation ($CV_G$ = 15%) was much larger than the daily intra-individual variation ($CV_I$ < 5%) without TM.[31] They monitored only four individuals but their results were quite comparable to our data. Besides, Rudež et al also published on this subject in 2009. They used 1 and 5 PM of tissue factor in their protocol but did not investigate the thrombomodulin-protein C pathway.[32] Compared with our study, they found higher $CV_I$, $CV_G$, and II but some methodological issues have to be underlined since duplicate measurements were not performed. An advantage of the present study is the addition of TM, which is indispensable in the evaluation of thromboembolic risk and could also be of great interest in other thrombin generation applications. Interestingly, Rudez et al followed their subjects for a whole year which allowed them to identify a seasonal change in CAT parameters.[32]

Studies aimed to define analytical goals for TGA with the CAT-method are still scarce in literature. Hence, biological variation studies give the opportunity to easily determine the analytical performance specifications for an appropriate routine use. Given the low $CV_I$ in TGA without TM, the desirable criteria for imprecision, bias, and total error are likely too strict and minimal criteria can be used.

Despite the strict adherence to our study protocol, some limitations can be pointed out in our work. Firstly, as the samples were collected in the morning, we were unable to study if TGA has circadian fluctuations. Indeed, several studies have demonstrated significant circadian variations in human fibrinolytic or coagulation factors.[33,34] We included a relatively young study population (median age = 36 years), whether an older population shows distinct biological variation data should require further study. Thrombin generation results were not normalized with reference plasma as is the case for the ST-genesia® (Stago). This could minimize variability between laboratories and measurement runs. Finally, due to practical considerations and according to the guidelines from Ricos et al, the collection was carried out over only 5 weeks.[35] Therefore, we were not able to confirm a possible seasonal effect as was shown in the study of Rudez et al.[32] Purified rabbit lung TM was used for the TGA assay with thrombomodulin. Rabbit TM is a more efficient inhibitor of thrombin procoagulant activity than human TM.[36] However, the manufacturer established the concentration of a suitable soluble TM preparation that would cause half maximum inhibition (50%) of thrombin generation in normal plasma.

In addition to these limitations inherent to the study, it should be emphasized that TGA has still some practical limitations: lack of reference values for specific conditions (these are expected to vary according to the type and concentration of the triggering agent), variability in the use of a contact inhibitory factor like corn trypsin inhibitor, or also the need of technical expertise and the difficulty

**TABLE 4** Index of individuality, reference change values, and analytical goal specifications for CAT with thrombomodulin

| | | | | Analytical goals | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Imprecision (CV, %) | | | Bias (CV, %) | | | Total error (CV, %) | | |
| Parameter | Group (n) | II | RCV (%) | Minimal | Desirable | Optimal | Minimal | Desirable | Optimal | Minimal | Desirable | Optimal |
| LT TM | All (19) | 0.32 | 18.1 | ±≤4.0 | ±≤2.7 | ±≤1.3 | ±≤6.7 | ±≤4.4 | ±≤2.2 | ±≤11.2 | ±≤9.0 | ±≤6.8 |
| | w/o OC group (14)[a] | 0.33 | 17.2 | ±≤3.7 | ±≤2.5 | ±≤1.2 | ±≤5.9 | ±≤3.9 | ±≤2.0 | ±≤10.0 | ±≤8.1 | ±≤6.1 |
| Peak TM | All (18) | 0.29 | 28.9 | ±≤7.5 | ±≤5.0 | ±≤2.5 | ±≤13.6 | ±≤9.1 | ±≤4.5 | ±≤21.8 | ±≤17.3 | ±≤12.8 |
| | w/o OC group (14)[a] | 0.49 | 39.2 | ±≤10.1 | ±≤6.8 | ±≤3.4 | ±≤11.5 | ±≤7.6 | ±≤3.8 | ±≤22.6 | ±≤18.8 | ±≤15.0 |
| ETP TM | All (18) | 0.28 | 36.1 | ±≤8.6 | ±≤5.8 | ±≤2.9 | ±≤15.9 | ±≤10.6 | ±≤5.3 | ±≤25.4 | ±≤20.1 | ±≤14.8 |
| | w/o OC group (14)[a] | 0.40 | 42.6 | ±≤10.5 | ±≤7.0 | ±≤3.5 | ±≤14.1 | ±≤9.4 | ±≤4.7 | ±≤25.6 | ±≤20.9 | ±≤16.2 |
| VI TM | All (17) | 0.33 | 50.6 | ±≤12.8 | ±≤8.5 | ±≤4.3 | ±≤20.3 | ±≤13.5 | ±≤6.8 | ±≤34.3 | ±≤27.5 | ±≤20.8 |
| | w/o OC group (14)[a] | 0.51 | 54.1 | ±≤13.6 | ±≤9.1 | ±≤4.5 | ±≤14.9 | ±≤9.9 | ±≤5.0 | ±≤29.8 | ±≤24.8 | ±≤19.9 |

Abbreviations: CV, coefficient of variation; ETP, endogenous thrombin potential; II, index of individuality; LT, lag time; RCV, reference change value; Peak, thrombin peak height; VI, velocity Index.

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

to interpret the results. This last point has nevertheless evolved since the introduction of automated platforms (ST-genesia® (Stago), Ceveron®alpha TGA (Technoclone)).

In conclusion, the biological variation seems to be an important contribution in TGA variability and the use of RCV could therefore be of added value to monitor patients in daily clinical practice. Biological variation was higher for TGA with thrombomodulin and patient's results should be interpreted accordingly.

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## CONFLICT OF INTEREST

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or nonfinancial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript. On behalf of the various authors: Dr.Antoine Mairesse.

## AUTHOR CONTRIBUTION

Mairesse Antoine and Marie-Astrid van Dievoet conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis, and wrote the paper. Jean-Louis Bayart conceived and designed the analysis, collected the data, and contributed data or analysis tools. Sandrine Desmet, Pascale Saussoy, and Helder Lopes Dos Santos collected the data and contributed data or analysis tools. Jean-Philippe Defour and Stephane Eeckhoudt conceived and designed the analysis and collected the data.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, MA, upon reasonable request.

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