Received: 30 May 2018 | Revised: 6 July 2018 | Accepted: 12 July 2018

DOI: 10.1111/ijlh.12910

ORIGINAL ARTICLE

WILEY ISLH International Journal of Laboratory Hematology

Biological variation estimates of prothrombin time, activated partial thromboplastin time, and fibrinogen in 28 healthy individuals

Mesude Falay[1] | Mehmet Senes[2] | Selcuk Korkmaz[3] | Turan Turhan[4] | Murat Okay[5] | Berna Afacan Öztürk[6] | Doğan Yücel[2] | Gulsum Ozet[1]

[1]Hematology, Ankara Numune Training and Research Hospital, Ankara, Turkey

[2]Biochemistry, Saglik Bakanligi Ankara Egitim ve Arastirma Hastanesi, Ankara, Turkey

[3]Biostatistics, Trakya Universitesi Tip Fakultesi, Edirne, Turkey

[4]Biochemistry, TC Saglik Bakanligi Ankara Numune Egitim ve Arastirma Hastanesi, Ankara, Turkey

[5]Biochemistry, Ordu Devlet Hastanesi, Ordu, Turkey

[6]Hematology, Diskapi Yildirim Beyazit Egitim ve Arastirma Hastanesi, Ankara, Turkey

Correspondence: Mesude Falay, Hematology, Ankara Numune Training and Research Hospital, Ankara, Turkey (mesudey@gmail.com).

Abstract

Background: Although tests of global hemostasis prothrombin time (PT) and activated partial thromboplastin time (aPTT) should not be used for prediction of bleeding risk, these tests are often used by many clinicians in daily practice particularly as a preoperative screening test. Robust biological variation (BV) data are needed for safe clinical applications of these tests. In this study, a stringent protocol was followed to estimate the BV's for PT, aPTT, and fibrinogen levels.

Methods: Weekly blood samples were obtained from 28 healthy individuals (18 females, 10 males) during 10 weeks study period. All measurements were performed with Stago STA-R coagulation analyzer. Prior to coefficient of variation (CV)-analysis of variance (ANOVA), the data were assessed for normality, trends, outliers, and variance homogeneity. Sex-stratified within-individual (CV_I) and between-individual (CV_G) BV estimates were determined for PT, aPTT, and fibrinogen tests.

Results: No difference was found between male and female estimates of BV. The observed CV_I and CV_G estimates were found to be lower than those previously published. Only for fibrinogen, CV_I was higher than CV_G.

Conclusion: Following a meticulous protocol, our study results provide up-to-date and more stringent BV estimates of global hemostasis tests.

KEYWORDS

aPTT, biological variation, coagulation, fibrinogen, hematology, preanalytical phase, PT

1 | INTRODUCTION

Optimal approach for establishing analytical performance criteria for secondary hemostasis tests is still under progress, because different manufacturers produce prothrombin time (PT) and activated partial thromboplastin time (aPTT) reagents with different sensitivities for coagulation factors. Establishing reliable criteria for safe and valid interpretation of the results of these tests is a very important purpose for the laboratories. To aid in safe and valid clinical interpretation of the test results, objective analytical performance specifications (APS) are needed, and the biological variations (BV) for these tests should be well known. Two components of BV are the between-individual variation, which occurs due to heterogeneity of the physiological effects between individuals, and the within-individual variation, which is the result of the biological variability in the same individual over time. Most of the limited number of published studies on BV of coagulation parameters used older devices for measurement. There are very few studies that have been published recently on this subject. However, these studies were performed using frozen samples, and no sex

stratification was applied. There have been concerns regarding the quality of the earlier studies on BV,[1,2] and consequently, the reliability of the BV estimates reported in these studies as well as the online 2014 BV database.[3]

In this study, we aimed to determine BV estimates for PT, aPTT, and fibrinogen tests by analyzing fresh plasma samples following 1 hour of sampling. To assure derivation of highest quality BV estimates for calculation of APS's and other BV-related applications, stringent preanalytical and analytical protocols were followed and a rigorous statistical approach was applied.[4]

2 | MATERIALS AND METHODS

This study was conducted at the Hematology Laboratories of Ankara Numune Training and Research Hospital, Ankara, Turkey. A total of 28 subjects (10 males, 18 females) with a mean age of 35 years (range 22-54) were recruited to estimate the BVs for PT, aPTT, and fibrinogen. The participants were checked to be in healthy status and did not take any medications or herbal supplements. Individuals having at least one of the following conditions were not included in the study: (a) concomitant autoimmune or autoinflammatory disease; (b) acute or chronic infection; (c) malignancy; (d) systemic diseases such as diabetes mellitus or heart failure; and (e) pregnancy or postpartum 6 months. During the study period, the participants maintained their normal life styles. Further exclusion criteria were verified by laboratory tests (alanine aminotransferase, creatine kinase, triglycerides, and C-reactive protein measured in serum samples from weeks 1 to 10). We followed-up the health status of all enrolled subjects. Blood collection was performed under standardized conditions to minimize preanalytical variation. The specimens were collected after 10 minutes of rest in a seated position. Fasting venous blood samples were collected by the same phlebotomist between 8 and 10 AM on the same day for 10 consecutive weeks from April 2017 to June 2017. The blood samples were collected after 12 hours of fasting into tubes containing 0.109 mol/L sodium citrate (1 vol./9 vol.) (Greiner Bio-one, Kremsmünster, Austria) as anticoagulant. All blood samples were transported to the laboratory under identical conditions in terms of temperature and elapsed time. Plasma was obtained by centrifugation at 2200 *g* and +18°C for 15 minutes, within 1 hour after sampling and immediately analyzed. The following reagents were used with STA-R (Diagnostica Stago, Asniéres, France) coagulation analyzer: STA-Cephascreen for aPTT; STA-Neoplastine for PT; and STA-Liquid Fib for fibrinogen. All assays used commercial reference plasma (Unicalibrator, Stago or Normal reference plasma, Precision Biologic, Kordia) that was standardized against the WHO standard by the manufacturer. All samples of each individual were measured in duplicate in the same analytical run. Since we aimed to analyze analytical variation, no criteria were defined for reanalysis on the basis of bad duplicates. Each analytical run included a normal and abnormal QC sample. All study participants' samples were measured with the same batch of reagents. For proficiency testing, our laboratory participated in the United Kingdom National External Quality Assessment Scheme (UK NEQASLI, Sheffield, UK). The study protocol was approved by the Ethical Committee of Ankara Numune Training and Research Hospital. All participants signed informed consent.

2.1 | Statistics

2.1.1 | Identification of the outliers

Prior to analysis of data, outlier values were checked in three steps: (a) presence of outliers between the replicate measurements was checked with Cochran test; (b) to determine whether an individual's distribution was greater or smaller than those of the group as a whole, variance distributions of each individual were examined, and the outlying individuals were identified using Cochran test; (c) whether a mean value of any individual was significantly different compared to other individuals was investigated using Reed criteria.[5] In each outlier identification step, those individuals that were observed to have outlier value were excluded from the analysis[6,7]

2.1.2 | Normality assessment

For each analyte, normality assessment was performed separately for each individual using Shapiro-Wilk test.[8] In case more than 50% of the individuals did not meet normal distribution, logarithmic transformation was applied to all data. Normality was checked again with Shapiro-Wilk test after logarithmic transformation. Additionally, whether the arithmetic means of the individuals were distributed normally was checked with Shapiro-Wilk test. After checking for assumption of normal distribution, it was found that more than 50% of the individuals showed normal distribution for every analyte. Thus, as recommended by Bartlett and Braga,[9] it was decided to continue the analyses with ANOVA.

2.1.3 | Comparison for gender

In this step, Student t test was used to examine whether there was a significant difference between the two sexes regarding each analyte. In addition, homogeneity of variances was examined with Bartlett test.

2.1.4 | Analysis of variance

Data were analyzed using analysis of variation (ANOVA)-coefficient of variation (CV), a type of ANOVA in which data are first subjected to CV-transformation.[10] Data from males and females were analyzed separately. The difference between male and female CVI's, and between-individual BV (CVG) estimates was calculated as described by Burdick and Graybill, and in consideration of the overlap between 95% confidence intervals (CI).[11] When there was no overlap between the 95% CI's of male and female mean values, the lowest of the two CVG estimates was used for calculating APS. In case there

was no significant difference between males and females, CVI and CVG values were reported for all individuals, and these estimates were used in application of BV data.

2.1.5 | Analytical performance specifications and other applications

CVI and CVG data were used for calculating the number of samples required to estimate performance specifications desired for imprecision (CVAPS) and bias (BiasAPS), individuality index (II), reference change value (RCV) and homeostatic set points (NHSP). The following equations were used for this purpose: CVA refers to the analytical variation,[12] and D refers to the allowable percent deviation from the true homeostatic set point; *Z* is 1.96 (for *P* value <0.05).

3 | RESULTS

The mean ages of the females and males were 38 (range, 22-56) and 40 (range 25-54) years, respectively. A total of five subjects were excluded from analysis according to the outlier checks control (Cochrane's test and Reed's criteria) (for APTT: subject 22; for PT: subject 10; and for fibrinogen: subjects 1, 17 and 25). Data showed normal distribution after logarithmic transformation (normality percents are 81%, 89% and 52% for APTT, PT, and fibrinogen, respectively). All individuals were assumed to be in a stable state throughout the study period. aPTT, PT, and fibrinogen levels showed similar distribution between both sexes ($P = 0.42$, $P = 0.27$ and $P = 0.90$, respectively).

3.1 | Within-individual and between-individual variations

CV_I, CV_G, CV_A and II are shown in Table 1. For all parameters, CV_A value was below 2%. CV_I and CV_G estimates of PT, aPTT, and fibrinogen were found to be lower than the values listed in online 2014 BV database. Only the CV_I estimates were found to be significantly different in our study.

3.2 | Determination of reference change value or critical difference

RCV's of coagulation parameters were calculated (Table 1). RCV was used to determine whether there was a significant difference (increase or decrease) between two consequential test results of an individual. While 95% probability ($P < 0.05$) indicates presence of significant difference (probability of randomness is 5%), 99% ($P < 0.01$) probability represents highly significant difference (probability of randomness 1%). According to the results, RCV's associated with screening PT and aPTT tests were relatively lower; however, CV_I and CV_G values for fibrinogen were higher ($CV_I > CV_G$), and this resulted in higher RCV. In our study, we observed that estimates of the components of BVs of PT and aPTT were lower compared to

TABLE 1 Within-subject (CV_I) and between-subject (CV_G) biological variation estimates ±95% CIs, mean concentrations, the associated estimates of analytical variation (CV_A), the total number of subjects, and the average number of samples of aPTT, PT, and fibrinogen tests

Analyte	Sex	Number of subjects	Total number of results	Mean ± SD	Mean	CV_I (%)	CV_G (%)	CV_A (%)	II	RCV (%)	Online 2014 BV database[a]	
											CV_I	CV_G
aPTT (s)	F	17	340	31.46 ± 1.62	31.66 (31.51-31.80)	**2.26**	**4.9**	0.92	0.461	6.76	2.70	8.6
	M	10	200	31.98 ± 1.49								
PT (s)	F	17	340	11.93 ± 0.66	11.82 (11.77-11.88)	**2.78**	**5.07**	1.87	0.548	9.28	4.00	6.8
	M	10	200	11.65 ± 0.50								
Fibrinogen (mg/L)	F	15	300	296 ± 29.8	296.7 (293.2-300.2)	**10.41**	**8.53**	1.26	1.220	29.06	10.70	15.8
	M	10	200	298 ± 24.0								

Numbers in bold indicate CVGs used to calculate the analytical performance specification (APS)
aPTT, activated partial thromboplastin time; F, females; M, males; PT, prothrombin time; QFA, fibrinogen.
[a]Data obtained from online 2014 BV database, common estimate for both sexes.

those listed on the online 2014 BV database, and this resulted in a narrower range of APS and RCV. Additionally, while II values for PT and aPTT were lower than 0.60, II for fibrinogen was 1.220. Our quality specifications calculated in relation to BV are presented in Table 2.

We showed median values with range (minimum-maximum) of PT (A), APTT (B), and fibrinogen (C) counts for individuals based on weekly samplings for 10 weeks.

4 | DISCUSSION

In this study, we demonstrated that global coagulation tests could satisfy the criteria defined by BV approach in healthy individuals. Coagulation test results can be affected by various preanalytical factors such as the type of biological sample, participants' age, disease, medications etc. For this reason, to assure derivation of highest quality BV estimates, stringent preanalytical and analytical protocols were followed similar to the EFLM control list[12] and a rigorous statistical approach was applied.

Most of the published studies on BV of coagulation parameters are outdated, used older devices for measurement and no sex stratification was applied.[13,14] Very few studies have been conducted recently with new coagulation analyzers.[4,5] In this study, we tested the BV of coagulation parameters and compared our results to those listed in the BV database. As shown in Table 1, we found lower CV_I and CV_G values of the screening tests compared to the values presented in the database. Individuality index (II), which is calculated as CVI/CVG ratio, provides information regarding the biological individuality of a given laboratory parameter and primarily the utility of reference ranges calculated for a population made of apparently healthy individuals. Utilization of reference range is considered acceptable when II is higher than 1.4, and not appropriate when II is lower than 1.0.[5] Moreover, conventional population-based reference ranges are not useful for interpreting the results when II is lower than 0.6, because although the results are within the reference range, significant changes may have occurred in the level of markers in comparison with their usual levels.[14,15] As shown in Table 1, all II values were <1.0, which means that for these parameters, conventional reference ranges have little utility in deciding whether a change observed in an individual is clinically significant or not.

Our BV estimates for coagulation parameters were stable except fibrinogen activity. Qian Chen et al[15] examined within day and between days BV to determine short-term BV by obtaining blood samples on days 1, 3, and 5 at 8 AM, 12 PM, and 16 PM. They used frozen plasma samples and performed analyses on Sysmex CA7000 (Sysmex, Kobe, Japan) and ACL TOP 700 (Hemos, MA) coagulation analyzers with colorimetric method. They found stable BV for PT, aPTT, and fibrinogen. In our study, fibrinogen had higher CVI and CV_G values. This can be explained by the difference in assay method (mechanical assay), as well as performing analyses with fresh samples. Additionally, Qian Chen et al examined intraday and short-term BV, but they did not explain how they calculated BV in detail. Our study encompassed a longer period, and a rigorous statistical approach was employed as recommended by EFLM BV group. In another study, Moniek et al calculated BV estimates by obtaining 13 samples in 1 year from each of the 40 healthy individuals and using Stago Compact (Diagnostica Stago) analyzer, without applying a sex stratification.[16] They also used frozen samples. Additionally, there were smokers and oral contraceptive, lipid-lowering drug, and NSAID use among the volunteers. They found higher CVI and CVG values compared to our results. They used the same assay method and same PT and fibrinogen reagents as in our study, and only aPTT reagent was different. Our BV estimates were different for all parameters, and this can be explained by the fact that none of our cases used any medications, and none were smokers, and their health states were monitored weekly. Second, we used fresh blood samples in our study. Third, their sampling period was longer than our study. In addition, Moniek et al did not give a detailed description of the statistical methods that they used for estimating BV. Interestingly, we found higher CVI value than CVG value for fibrinogen in our study (10.41% vs 8.53%, respectively). In our review of the previous studies, we saw that only Markmann et al[17] found higher CVI value than CVG value for fibrinogen (67% vs 33%). However, that study used an outdated assay method. In our opinion, the fact that we used fresh samples may be the reason for finding higher CVI values. Another possible reason can be that most of our cases were female and none of them were postmenopausal. We

TABLE 2 Optimal, desirable, and minimum analytical goals for imprecision, bias, and total error calculated in relation to biological variability for aPTT, PT, and fibrinogen

	Quality specifications								
	Imprecision %			Bias %			Total error %		
Analit	Optimal	Desirable	Minimum	Optimal	Desirable	Minimum	Optimal	Desirable	Minimum
aPTT (n = 540)	<0.56	<1.13	<1.69	<1.38	<2.77	<4.15	<2.32	<4.63	<6.95
PT (n = 540)	<0.7	<1.39	<2.08	<1.76	<3.52	<5.29	<2.91	<5.82	<8.73
Fibrinogen (n = 500)	<2.6	<5.22	<7.81	<11.1	<22.2	<33.3	<15.39	<30.79	<46.18

aPTT, activated partial thromboplastin time; PT, prothrombin time.

think this high variation may have occurred due to menstruational changes.

We found higher RCV for fibrinogen in our study. RCV for PT and aPTT were 9.28% and 6.76%, respectively. If variations in two sequential measurements occur arise from the analysis, RCV can be used to decide whether there is a significant difference between the two test results of the same individual; thus, clinical prognosis of a disease or treatment efficacy can be evaluated. In other words, RCV can detect whether an external factor (acute disease, therapy, physical exercise, diet, etc.) can affect the results of a certain parameter, and whether the observed variation is independent of the instrumental or BV. It should be noted here that RCV calculated from BV in healthy individuals may not be the same that is observed in the presence of a disease.[17,18] As RCV varies according to the changes in analytical precision, RCV can be reduced by tightening the quality control of the testing process and reducing or minimizing the analytical variation.

5 | STUDY LIMITATION

One possible limitation of the present study is that all analyses were performed on a device from a single manufacturer and single reagent (Diagnostica Stago). As we know, there is no standard reagent for global hemostasis tests, and every reagent has different factor sensitivity, and therefore, different sensitivity. For that reason, we believe BV estimates should be calculated for all these reagents that have different factor sensitivities.

6 | CONCLUSIONS

In this study, stringent preanalytical protocols and appropriate statistical methods were employed to obtain reliable BV data for coagulation parameters. Blood samples were analyzed on the day of sampling. With replicate measurements, no variation in the results was observed between days except for fibrinogen. During the entire study period, all internal and external quality control results were kept within acceptable range, and no remarkable tendency was observed. We did not find difference between BV estimates of the two sexes, although the number of males could be a little higher in our study. We found stable weekly BV of coagulation parameters except for fibrinogen. As individuality is a characteristic feature of coagulation parameters, RCV can be used instead of reference range for monitoring patient.

CONFLICT OF INTERESTS

The authors state that they have no conflict of interest.

ORCID

Mesude Falay http://orcid.org/0000-0001-7846-3476

REFERENCES

1. Falay M, Senes M, Yücel D, Turhan T, Dagdaş S, Pekin M, et al. What should be the laboratory approach against isolated prolongation of a activated partial thromboplastin time? *J Clin Lab Anal*. 2018;32:e22415.
2. Ceriotti F, Fernandez-Calle P, Klee GG, et al. Criteria for assigning laboratory measurands to models for analytical performance specifications defined in the 1st EFLM Strategic Conference. *Clin Chem Lab Med*. 2017;55:189-194.
3. Minchinella J, Ricos C, Perich C, et al. Biological variation database and quality specifications for imprecision, bias and total error (desirable and minimum). The 2014 update. https://www.westgard.com/biodatabase-2014-update.htm. Accessed November 7, 2017
4. Sandberg S, Fraser CG, Horvath AR, et al. Defining analytical performance specifications: consensus statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine. *Clin Chem Lab Med*. 2015;53:833-835.
5. Fraser CG. *Biological Variation: from Principles to Practice*. Washington, DC: AACC Press; 2001.
6. Westgard JO, Barry PL, Hunt MR, Groth T. A multi-rule shewhart chart for quality control in clinical chemistry. *Clin Chem*. 1981;27:493-501.
7. Cochran WG. Human genetics. *Hum Genet*. 1941;11:47-52.
8. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika*. 1965;52:591-611.
9. Bartlett WA, Braga F, Prusa R, et al. A checklist for critical appraisal of studies of biological variation. *Clin Chem Lab Med*. 2015;53:879-885.
10. Dixon WJ. Processing data for outliers. *Biometrics*. 1953;9:74-89.
11. Burdick RK, Graybill F. *Confidence Intervals on Variance Components*, 1st edn. New York, NY: Marcel Dekker Inc; 1992.
12. Carobene A, Strollo M, Jonker N, et al. Sample collections from healthy volunteers for biological variation estimates' update: a new project undertaken by the Working Group on Biological Variation established by the European Federation of Clinical Chemistry and Laboratory Medicine. *Clin Chem Lab Med*. 2016;54:1599-1608.
13. Banfi G, Del Fabbro M. Biological variation in tests of hemostasis. *Semin Thromb Hemost*. 2008;34:635-641.
14. Banfi G, Del Fabbro M. Biological variation in tests of hemostasis. *Semin Thromb Hemost*. 2009;35:119-126.
15. Chen Q, Shou W, Wu W, et al. Biological and analytical variations of 16 parameters related to coagulation screening tests and the activity of coagulation factors. *Semin Thromb Hemost*. 2015;41:336-341.
16. Matt MPM, van Schie M, Kluft C, Leebeek FW, Meijer P. Biological variation of hemostasis variables in thrombosis and bleeding: consequences for performance specifications. *Clin Chem*. 2016;62(12):1639-1646.
17. Marckmann P, Sandström B, Jespersen J. The variability of and associations between measures of blood coagulation, fibrinolysis and blood lipids. *Atherosclerosis*. 1992;96(2):235-244.
18. Ricos C, Cava F, García-Lario JV, et al. The reference change value: a proposal to interpret laboratory reports in serial testing based on biological variation. *Scand J Clin Lab Invest*. 2004;64(3):175-184.

How to cite this article: Falay M, Senes M, Korkmaz S, et al. Biological variation estimates of prothrombin time, activated partial thromboplastin time, and fibrinogen in 28 healthy individuals. *Int J Lab Hem*. 2018;40:721–725.
https://doi.org/10.1111/ijlh.12910