ERRATA

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Biological Variation in Tests of Hemostasis

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ABSTRACT

The two components of biological variability are interindividual variability, which is the variability due to the heterogeneity of physiologic influences among subjects, and intraindividual variability, which is due to the variability in the same individual over time. Analysis of biological variation is crucial for estimating the critical difference, which corresponds with a threshold suggestive of a statistically significant difference between two consecutive results of a laboratory parameter in the same subject and is therefore unlikely attributable to casual (random) oscillation of values. Studies on biological variation of tests of hemostasis are outdated, and the published results should be confirmed by using modern, fully automated methods. Biological variation for coagulation screening tests (prothrombin time and activated partial thromboplastin time) is low and comparable with the values registered for hematologic parameters. However, the index of individuality (ratio between intraindividual and interindividual variability) suggests that the usual preoperative screening for coagulation disorders is influenced by the between-subjects variability. Thrombin time is very constant within and between subjects. Proteins such as fibrinogen, clotting factors, and antithrombin show a low biological variability. In contrast, fibrinolytic parameters, such as plasminogen activator inhibitor 1 and fibrinopeptide A, show very high variability, and their interpretation in the clinical setting must take this into consideration.

KEYWORDS: Coagulation tests, biological variation, critical difference, individuality index

The two main components of biological variability (BV) are interindividual variability, which is the variability due to the heterogeneity of physiologic influences among individuals, and intraindividual variability, which is due to the variability in the same individual over time. The biological variability of a laboratory parameter includes the variability of cyclical biological rhythms and the fluctuation beyond a homeostatic value. The amplitude of the fluctuation, which is independent from preanalytical phase, corresponds with the intraindividual variability, reported as a coefficient of variation (CV_i). The variability of the homeostatic value within a group of different individuals corresponds with the interindividual variability (CV_g). The total variability (V_t) is the sum of analytical (V_a) and biological variability (V_b). In general, the V_a is low in automated coagulation testing; thus, V_t is highly dependent on V_b. The traditional analytical goals for laboratory parameters consider $V_a = ½V_b$. The V_a and V_b of coagulation parameters (screening tests) are also typically low, fulfilling the analytical goals, but these values are higher for specific and esoteric tests. Fraser has combined the analytical precision (CV_a) and the average within-subject biological variation (CV_i) from which a critical difference (CD) can be calculated for a predetermined probability.[1]

The CD corresponds with the value indicating that a difference between two consecutive results in the

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same person is statistically significant and it is therefore unlikely attributable to random oscillation of values. In general, difference in results characterized by a probability greater than 95% of representing a true change in a person is described as $CD^{95} = 2.77 \times (CV_a^2 + CV_i^2)^{1/2}$. The factor 2.77 is equal to $\sqrt{2}$ times the z score for the difference. CD, in other words, identifies whether an external factor (acute disease, therapy, physical exercise, diet, etc.) can really impact on results of a given parameter and if the modification is independent from instrumental or biological variabilities.

The ratio between intraindividual and interindividual variability is referred to as individuality index ($II = CV_i/CV_g$). The II provides information about the biological individuality of a given laboratory parameter and, primarily, about the usefulness of the reference ranges calculated on a population of apparently healthy individuals. The reference interval is traditionally appropriate when II is > 1.4; when the II is < 1.0, the reference interval is inappropriate, and when the II is < 0.6, the reference interval should not be used.[2]

There are only a few studies on the biological variability of coagulation parameters, and they are generally outdated. Thus, results from these articles are biased by obsolete methods and technologies, as a continuous development and diffusion of automated devices has occurred over recent years, leading to a substantial improvement of precision and throughput. In a data bank of biological variability of laboratory parameters published 11 years ago and based on 107 publications from 1951 until 1996, only one study included prothrombin time (PT) and activated partial thromboplastin time (APTT).[3] Although the current review has the limit of heterogeneity of both sources and methods used for measuring coagulation parameters, the data reported, extrapolated from different epidemiologic or clinical studies, are however mostly concordant, and they could be of practical value for routine and research laboratories.

SCREENING TESTS

In a population of 39 subjects (19 women, 20 men; age range, 20 to 49) followed at monthly intervals for a period of 9 months, the intraindividual biological variation of PT, calculated as a mean CV, was 2.3%, and the interindividual biological variation was 6.8%. The corresponding values for the APTT were 2.1% (intraindividual) and 8.9% (interindividual), respectively.[4] The authors[4] also calculated the median intraindividual CV for the two parameters, and the values were lower than mean data: 1.7% for PT and actually 0.0% for APTT. These values differ broadly from those originally quoted by Costongs et al,[5] (5.8% for PT and 6.8% for APTT), which are however not considered in the current review. In a companion paper, Costongs et al[6] actually reported values very similar to those described by Dot et al[4]: 0.9% (median intra-individual CVs) for PT, and 3.4% for APTT in long-term (six months) evaluation. In a study performed on 17 Japanese subjects[7] (8 males, 9 females; age range: 20–60 years) the CV_i values for PT and APTT were lower than those reported by Costongs et al[6] and more consistent with those reported by Dot et al[4]: the values were 2.4% for PT and 3.3% for APTT. The authors[7] highlighted that analytical techniques were changed and improved since 1985 when Costongs et al[6] published their values, which were also derived from a population including smokers and women taking oral contraceptives, major differences that could explain the discrepancies between the two studies. Wada et al[7] described CV_g of 4.0% for PT and 7.1% for APTT. It is interesting to note that the CVs calculated for APTT reported as percentage or for international normalized ratio (INR) were higher than the CVs calculated for the respective clotting times (seconds): CV_i of 5.6% and CV_g of 9.4% for percentage values, CV_i of 3.0% and CV_g of 4.0% for INR.

A value of CV_i of 4% for prothrombin time is reported in a database.[8] This value was used by Kouri et al[9] for a comparison with the combined preanalytical and analytical uncertainty of measurement, which they observed to be higher (5.6%) than the CV_i. In 32 patients taking anticoagulants, the CV_i of the PT, evaluated on samples taken every 5 weeks during a period of 6 months, was 10.1%, thus higher than that reported in healthy subjects.[10] The BV data of PT and APTT could be compared with those reported for platelet count, a parameter used as first-line test for diagnosing hemostasis disorders.[11] A CV_i of 9% and a CV_g of 23% were described for platelet count.[3]

Dot et al[4] also calculated the CD: 19% for PT and 16% for APTT. The II was 0.96 for PT and 0.68 for APTT, concluding that reference values for these tests derived from general populations are more appropriate for longitudinal comparison than for diagnosing diseases. This topic is particularly interesting and of practical value, because the usual preoperative screening for coagulation disorders based on PT and APTT is largely influenced by the interindividual variability.

FIBRINOGEN

Costongs et al[6] described a median intraindividual CV of 3.5% during a short-term evaluation, based on 1-day calculation in 60 individuals. The CD was 13.2%. When the calculation of variability was performed by the same authors on a group of 274 individuals during a long-term period (6 months), the median CV_i was 10% and the CD was 29.6%. The data reported by Sakkinen et al[12] on 26 subjects observed during a period of 6 months are similar: 18.6% and 20.2% for intraindividual and

Table 1 Biological Variation of Various Hemostasis Parameters: Part 1

Parameter	Time Span Range	Number of Examined Subjects			Reference
	1 d	60 (23 M, 37 F)	Median CV_i	Median CV_g	6*
Thrombin time			0.0		
Antithrombin III			5.2		
α_2-antiplasmin			6.6		
Plasminogen			3.8		
	6 mo	274 (148 M, 126 F)	Median CV_i	Median CV_g	5*
Thrombin time			0.0		
Antithrombin III			3.1		
α_2-antiplasmin			6.6		
Plasminogen			3.8		
	3–6 wk	39 (16 M, 23 F)	CV_i†		22
Fibrinopeptide A‡			80.3		
D-dimer‡			17.4		
β-thromboglobulin‡			9.5		
Platelet factor 4‡			12.5		
Tissue plasminogen activator‡			11.1		
Plasminogen activator inhibitor 1‡			53		
Protein S§			2.9		
	6 mo	20 (10 M, 10 F)	Mean CV_i	Mean CV_g	13
Tissue plasminogen activator‡				97	
	3 mo	34 F (postmenopausal)	Mean CV_i	Mean CV_g	21
Tissue plasminogen activator‡			11		
Plasminogen activator inhibitor 1‡			55		
Plasmin–α_2-antiplasmin complex‡			17		
Thrombin-antithrombin complex‡			11		
	24 wk	26 (10 M, 16 F)	Mean CV_i	Mean CV_g	II (Ref. 12)
Fibrinopeptide A‡			82.7	45.8	1.77
D-dimer‡			56.4	89.5	0.63
Tissue plasminogen activator‡			15.0	45.6	0.33
Plasminogen activator inhibitor 1‡			47.2	70.9	0.67
Tissue plasminogen activator–plasminogen activator inhibitor 1 complex‡			22.4	59.3	0.38
Plasmin–α_2-antiplasmin complex‡			19.9	29.3	0.68
Prothrombin fragment F1–2‡			24.2	28.3	0.85
Thrombin-antithrombin complex‡			25.0	60.5	0.41
	12 mo	17 (8 M, 9 F)	Mean CV_i	Mean CV_g	7
α_2-antiplasmin inhibitor§			4.8	7.1	
Plasminogen§			4.2	10.5	
Thrombin-antithrombin complex‡			26.0	20.0	
Plasmin–α_2-antiplasmin complex‡			18.0	26.0	
Thrombomodulin‡			11.4	16.5	
Tissue plasminogen activator–plasminogen activator inhibitor 1 complex‡			15.9	31.2	
Protein S§			7.6	22.3	
Protein C§			7.9	17.5	

*The measured values and the kind of measures are not specified.
†It is not specified if the CV is a median or a mean.
‡Quantity measured as mass concentration.
§Quantity measured as percentage.
d, day; wk, week; mo, month; M, male; F, female; CV, coefficient of variation (%); II, index of individuality.

interindividual variability, respectively. In a further study on 59 healthy individuals, the CV_g was 15%,[13] whereas in a longitudinal study performed over 1 year on 26 subjects (13 men, 13 women; age range, 25 to 52 years), the CV_i was 13.5% and the CV_g was 16.2%.[14] In a study including 17 subjects (8 males, 9 females; age range, 20 to 60 years), the CV_i was 8.7% and the CV_g was 14.8%.[7] In synthesis, all the studies are concordant on the values of the biological variability of fibrinogen. The variation of fibrinogen during a 1-year longitudinal study performed on 206 Finnish subjects aged 50 to 60 years was not linked to genetic polymorphisms, namely *Taq*I and *Bcl*I mapped to the 3′ end of the α- and the β-fibrinogen genes, and *Hind*III, based on the presence of either C or T base at position −148 upstream from the start of the transcription site of the β-fibrinogen gene.[15] Genetic influence explains 39% of variation; however, no quantitative differences of genetic influence on hemostatic levels were observed between males and females, as reported in a study involving 93 monozygotic twin pairs (44 males and 49 females) and 116 dizygotic twin pairs (36 males, 40 females, 40 opposite sex).[16]

The values reported for fibrinogen, which is typically increased during acute phase and atherosclerosis, are similar to those found for other parameters (cholesterol, triglycerides, blood pressure) traditionally used for assessing cardiovascular risk.[17,18] Fibrinogen concentrations are correlated with cardiovascular risk factors, but modifiable lifestyle characteristics (smoking, alcohol consumption, physical activity, obesity) only explain a few percent of the total variation, as reported in a meta-analysis of 31 prospective studies, including 154,211 adults.[19] Fibrinogen CV_i was also not increased in patients with underlying disease compared with healthy controls.[13] It is noteworthy that the association with body mass index was twice as strong in women as in men.[19]

COAGULATION FACTORS

The median intraindividual variation in a short-term evaluation (within 1 day) was nil (0.0%) for factor V and 4.8% for factor X; the corresponding CDs were 9.9% and 14.9%.[6] A higher value for factor V (CV_i 3.6%) is, however, reported in a database.[8] Long-term evaluation, over a 6-month period, yielded an intraindividual variability of 3.6% for factor V and 5.9% for factor X, resulting in a CD of 14.2% and 17.8%, respectively.[5] The intraindividual variability of factor VIII, as evaluated during a pharmacokinetic study, was 8.6%.[20] The within-subject mean CV% for factor VII in 34 postmenopausal women followed during a period of 3 months was 8.5%: the proportion on the total variance of the intraindividual variability was 5%. The corresponding values for factor VIII were 16% and 19%.[21]

ADDITIONAL PARAMETERS

The biological variations of various hemostasis parameters are summarized in Tables 1 and 2. Low values of biological variability were described for antithrombin, plasminogen, and α_2-antiplasmin. Identical CV_i has been reported for tissue plasminogen activator (tPA) in two different studies. A high variability characterizes thrombin-antithrombin complex, plasmin–α_2-antiplasmin complex, prothrombin fragment 1 + 2 (F1–2), whereas that of plasminogen activator inhibitor 1 (PAI-1) and fibrinopeptide A is even higher. These data suggest that an accurate and appropriate interpretation of laboratory results should be advisable when these data are used for establishing either a diagnosis or the prognosis.

EVALUATION OF THE PROPORTION OF INTRAINDIVIDUAL AND INTERINDIVIDUAL VARIABILITY

In some studies, the intraindividual and interindividual biological variability was reported as a proportion of the total variability; in others[18,23,24] the intraindividual variability is included in the analytical variability. In the study of Thompson et al,[24] the sum of CV_i and CV_g is not equal to 100 because the authors also included a between-batch variation, due to analytical phase, representing the difference between total variation and the sum of between-subject and within-subject variations. Only for fibrinogen was the between-batch variation equal to 0%. Discrepant results were observed only for tissue plasminogen activator: two studies are concordant, whereas a third one showed very high CVi. A very high CVi for fibrinogen was reported in one paper only (Table 2).

GENDER AND AGE

The median intraindividual variability of plasminogen showed a significant difference in a 1-day short-term evaluation between males ($n = 23$; $CV = 3\%$) and females ($n = 37$; $CV = 4.3\%$). Females who used oral contraceptives had higher values (11.5%) than did nonusers (7.3%).[6] This difference could not be confirmed in a population of 473 subjects (208 males and 265 females) after a 3-year follow-up. The proportion of intraindividual variance of the total variance for plasminogen was 51% in males and 55% in women.[18] Although a large study on a general population reported higher levels of von Willebrand factor (VWF) in women when compared with men,[25] this difference could not be confirmed in a study on a wide series of twins.[16] It should be outlined, however, that the influence of genetic factors on VWF concentrations is high (72%)[16] and VWF levels of blood group O individuals are 25% lower than those in non-O individuals.[26] A gender difference has been reported for

Table 2 Biological Variation of Various Hemostasis Parameters: Part 2

Parameters	CV_i (%)	CV_g (%)	Time Span Range	Number of Subjects Studied and Reference
Tissue plasminogen activator*	49	51	1 y	16 M, age 20–30 y, 79 observations[23]
Tissue plasminogen activator*	3	97	6 mo	20 (10 M, 10 F), 9 observations[13]
Tissue plasminogen activator*	4	96	3 mo	34 F (postmenopausal)[21]
Factor II†	32	72	3 y	14 (6 M, 8 F), 20 observations[24]
Factor II†	11	89	3 mo	34 F (postmenopausal)[21]
Factor VII	42	58	1 y	16 M, age 20–30 y, 79 observations[23]
Factor VII:c	37	63	3 y	208 M, age 45–64 y, 2 observations[18]
Factor VII:c†	38	62	3 y	265 F, age 45–64 y, 2 observations[18]
Factor VII†	5	95	3 mo	34 F (postmenopausal)[21]
Factor VII	57	43	3 y	208 M, age 45–64 y, 2 observations[18]
Factor VII:Ag	58	42	3 y	265 F, age 45–64 y, 2 observations[18]
Factor VIII†	34	57	3 y	14 (6 M, 8 F), 20 observations[24]
Factor VIII†	19	81	3 mo	34 F (postmenopausal)[21]
Factor X†	35	47	3 y	14 (6 M, 8 F), 20 observations[24]
Von Willebrand factor–related antigen†	28	71	3 y	14 (6 M, 8 F), 20 observations[24]
Antithrombin III†	48	13	3 y	14 (6 M, 8 F), 20 observations[24]
Fibrinogen*	67	33	1 y	16 M, age 20–30 y, 79 observations[23]
Fibrinogen*	33	67	3 y	208 M, age 45–64 y, 2 observations[18]
Fibrinogen*	39	61	3 y	265 W, age 45–64 y, 2 observations[18]
Fibrinogen*	13	87	6 mo	20 (10 M, 10 F, 9 observations)[13]
Fibrinogen*	28	72	3 y	14 (6 M, 8 F), 20 observations[24]
Plasminogen†	51	49	3 y	208 M, age 45–64 y, 2 observations[18]
Plasminogen†	55	45	3 y	265 F, age 45–64 y, 2 observations,[18]
Plasminogen activator inhibitor 1*	9	91	3 mo	34 F (postmenopausal)[21]
Plasmin–α_2-antiplasmin complex*	17	83	3 mo	34 women (postmenopausal)[21]
Thrombin-antithrombin complex*	20	80	3 mo	34 women (postmenopausal)[21]

*Quantity measured as mass concentration.
†Quantity measured as percentage.
mo, month; y, year.

activated protein C (APC) resistance on a population of 104 women and 119 men ($p < 0.01$).[27] Gender variability on thrombophilia-associated markers has been occasionally described. Thus, a small but statistically significant effect on protein S and antithrombin was described in one study, with females displaying slightly lower levels of the former and slightly higher levels of the latter.[28] Antithrombin and protein C levels have been reported as higher in males than in females,[29–31] whereas in other studies higher values were reported in females.[25,32] Age has also a small but significant effect on thrombophilia-associated markers. Protein C and protein S both increase in the elderly, whereas antithrombin decreases in both genders,[28,31] although an increase of antithrombin has also been reported in women.[29] Plasma PAI-1 activity is the main determinant of whole blood fibrinolytic activity in females, whereas tPA and PAI-1 activities equally contribute in males.[33] The association of the tPA activity with gender and age was confirmed by Stegnar,[34] although the correlation was not confirmed in a more recent paper from the same group.[35] A positive correlation between increased concentration and/or activity and increasing age has been described for fibrinogen,[19] VWF,[16] tPA,[16,34,35] PAI-1,[36] and D-dimer.[34] For thrombin-antithrombin complex, a negative correlation was reported.[34]

CORRELATION WITH BODY MASS INDEX

A strong association between fibrinogen levels and body mass index (BMI) has been reported in women, but not in men.[19,37] Factors VII, VIII, XII, and F1–2 correlate with BMI.[37] There are conflicting data on VWF: Bowles et al[37] did not observe a significant correlation, but this finding was disputed by Conlan et al.[25] A similar scenario was depicted for D-dimer: Bowles et al[37] did not show a significant correlation between D-dimer and BMI, whereas Cushman et al did.[38] Increasing BMI was also associated with an impaired fibrinolytic activity: tPA activity fell significantly and PAI-1 rose as BMI increased.[34,37] A rise of antithrombin activity and protein C was related to high

BMI, reflecting a compensatory response to activated coagulation and impaired fibrinolysis.[37]

CIRCADIAN RHYTHMS

Haus et al[39] observed lower PT values at night, whereas a peak was recorded in the afternoon. APTT, on the contrary, showed a peak in the evening or at night, although the magnitude of variation was small (< 10%).[39] A nocturnal peak was reported for thrombin time,[39] whereas a morning peak and nocturnal lower levels have been described for fibrinogen.[39,41] A morning peak and a trough in the evening or at night have been described for factor VIII activity.[39,40] A lack of circadian rhythm was demonstrated for antithrombin activity,[39,40] factor V, and plasminogen.[39] A marked circadian rhythm was instead observed for tPA, which is characterized by an early morning fall and a peak in the afternoon, with a peak-trough difference of nearly 150%.[41] The circadian variation of PAI-1 showed a time course opposite to that of tPA, showing an early morning peak and a trough in the afternoon, with a variation of nearly 200%.[41] No circadian rhythm was observed for plasminogen.[41] A circannual rhythm was reported for antithrombin, which is increased in healthy volunteers during the summer, whereas fibrinolytic activity is increased at low temperature.[42] Fibrinogen concentrations exhibited a significant high amplitude yearly variation (peak-trough is close to 28%). Peak values were observed in the period February–June and in December, whereas lower values occurred in January and the period August–September.[14] In diabetic patients, the peak of vWF was found in the morning.[39]

CONCLUSION

Biological variability for coagulation screening tests (PT and APTT) is generally low and comparable with the values reported for traditional hematologic parameters. However, the index of individuality (ratio between intraindividual and interindividual variability) suggests that the usual preoperative screening for coagulation disorders might be substantially influenced by the between-subjects variability. Thrombin time is very constant within and between subjects. Proteins, such as fibrinogen, clotting factors, and antithrombin, display a low biological variability. In contrast, PAI-1 and fibrinopeptide A show very high variability, and their interpretation in the clinical setting requires caution. The data reported in this article were collected from various sources, often outdated, and are heterogeneous for the high variability of methods, number of subjects studied, and interpretation of results. All these variables should be taken into consideration when using these data for clinical purposes. Accordingly, multicenter studies, enrolling a heterogeneous population and using modern analytical methods, are needed to definitively assess the impact of biological variability on hemostasis tests.

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