Letter to the Editor

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Short-term biological variation of coagulation and fibrinolytic measurands

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To the Editor,

Laboratory tests for evaluation of haemostasis-related measurands reflecting coagulation and fibrinolytic activity are used in the diagnostic work-up of bleeding and thrombotic conditions and for monitoring treatment of these disorders. Accurate and precise quantification is necessary for correct diagnosis and subsequent treatment of patients, and robust estimates of biological variation (BV) are important to be able to establish analytical performance specifications, reference change values (RCVs) and personalized reference intervals. Previously, we performed a systematic review and meta-analysis for BV data for haemostasis-related measurands, where we included publications with study periods from more than 1 week up to 3 years [1]. However, coagulation tests are often performed more frequently and within a shorter time frame, especially in critically ill patients. The focus of this study was therefore to perform a systematic review focusing on short-term BV studies defined as study duration up to 7 days and to assess if short-term BV estimates differed from previously published long-term estimates [1].

A literature search performed in PubMed as previously described [1], now with cut-date June 2024, identified six publications where the study periods were up to 7 days in healthy individuals. Out of these publications, one included results from three different study cohorts [2] and another from two different sampling intervals [3], making up nine different sets of results altogether to be assessed. Detailed information about the study subjects, sampling protocol, pre-analytical handling and analytical methods were recorded and the studies were appraised by the Biological Variation Data Critical Appraisal Checklist (BIVAC) [4]. This checklist includes 14 quality items (QI), which were scored with an A to D (A: fully compliant and D: not fit for purpose). The QI with the lowest scoring determines the overall grading of the study. Three independent assessors amongst the authors reviewed and scored the papers. Differences were discussed and a joint score was achieved. If two or more publications reported data for the same measurand, a meta-analysis was performed. BV data for thrombin time and factor V were excluded from one study because in this study, the within-subject BV estimate (CV_I) was stated to be "0" for these measurands [5]. Publications presenting no overall BV estimate, but multiple BV estimates (i.e. for men and women with and without use of oral contraceptives (OCs) [2] or for multiple time frames [3]) were not included in the meta-analysis, but shown as single results (Table 1).

In the six publications included in this review, BV data were reported for 28 different measurands. One publication reported BV data for 1 day, 5 days and 6 weeks (the data for 1 and 5 days are included in this study) [3] and another publication reported BV data separately for men and for women

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Table 1: Short-term within-subject (CV_I) and between-subject (CV_G) estimates with 95 % confidence intervals (CIs) of coagulation and fibrinolytic measurands. For measurands in bold and underlined, meta-analysis results are shown.

Measurands	Meta-analysis (number of included studies)	References	Study duration (number of days)	Sampling (number of samples per subject)	Short-term estimates (study period≤7 days)		Long-term estimates (literature data [1])	
					CV_I, CI %	CV_G, CI %	CV_I, CI %	CV_G, CI %
APTT, seconds	2	[5, 6]			**3.5 (3.4–3.5)**		2.8 (1.7–6.8)	7.2 (4.9–8.9)
		[5]	1	4	3.4 (1.3–4.3)			
		[6]	5	9	3.5 (3.1–3.9)	10.4 (8.3–13.9)		
APTT, ratio		[6]	5	9	3.4 (2.9–3.8)	10.4 (8.3–13.9)		
Antithrombin act	2	[5, 7]			**4.9 (4.6–5.2)**		3.4 (1.1–7.0)	7.8 (2.6–25.2)
		[5]	1	4	5.2 (4.5–5.9)			
		[7]	5	9	4.6 (3.9–5.2)	6.4 (4.9–9.1)		
dRVVT (screen, ratio)		[7]	5	9	7.1 (6.3–8.0)	10.0 (7.6–14.1)		
dRVVT (screen, seconds)		[7]	5	9	7.1 (6.3–8.0)	10.0 (7.6–14.1)		
dRVVT (screen ratio/confirm ratio)		[7]	5	9	4.6 (4.0–5.2)	6.8 (5.2–9.6)		
Factor II		[6]	5	9	6.7 (5.9–7.5)	8.9 (7.0–12.1)	5.8 (5.7–5.9)	9.7 (7.0–15.4)
Factor V[a]		[6]	5	9	6.7 (6.0–7.4)	16.8 (13.3–22.5)	5.3 (3.6–6.6)	18.7 (14.1–27.5)
Factor VII		[6]	5	9	7.7 (7.0–8.5)	12.8 (10.1–17.2)	8.2 (6.9–14.2)	17.8 (16.7–19.4)
Factor VIII		[6]	5	9	11.4 (10.3–12.6)	24.0 (19.0–32.2)	8.7 (4.9–16.0)	22.5 (15.5–31.4)
Factor IX		[6]	5	9	6.0 (4.7–6.9)	10.7 (8.4–14.4)	6.9 (5.8–9.1)	16.3 (15.7–18.2)
Factor X	2	[5, 6]			**5.1 (4.8–5.3)**		5.9 (4.6–8.5)	11.4 (8.2–18.2)
		[5]	1	4	4.8 (4.1–5.5)			
		[6]	5	9	5.3 (4.8–5.8)	12.2 (9.7–16.5)		
Factor XI		[6]	5	9	5.2 (4.0–5.9)	11.0 (8.7–14.8)	5.1 (4.2–6.3)	11.5 (8.5–17.5)
Factor XII		[6]	5	9	5.2 (4.7–5.8)	23.7 (18.9–31.8)	4.0 (3.0–5.1)	23.3 (17.6–34.5)
Fibrinogen (Clauss)[b]	2	[5, 6]			**4.8 (3.5–5.3)**		10.2 (9.3–11.9)	17.1 (8.5–17.3)
		[5]	1	4	3.5 (1.9–4.3)			
		[6]	5	9	5.3 (4.8–5.8)	16.9 (13.4–22.6)		
		[3]	1	5	10.7 (8.5–12.5)			
		[3]	5	5	14.2 (11.9–16.5)			
		[2] – non-OC	3	2	3.3 (2.0–4.6)	17.8 (15.2–21.3)		
		[2] – OC	3	2	4.9 (3.6–6.5)	18.9 (15.8–23.4)		
		[2] – men	5	3	6.3 (5.5–7.2)	16.9 (14.7–19.8)		
FDP		[6]	5	9	10.0 (8.7–11.1)	13.9 (10.9–18.8)		
Plasmin inhibitor		[5]	1	4	6.6 (5.9–7.4)		5.8 (4.8–5.8)	7.1 (5.2–10.8)
Plasminogen		[5]	1	4	3.8 (3.1–4.4)		5.7 (4.2–7.7)	10.5 (7.8–15.8)
PC-Act		[7]	5	9	5.6 (4.8–6.3)	14.3 (11.1–20.0)	5.5 (5.3–7.9)	16.9 (9.1–55.2)
PS-Act		[7]	5	9	7.5 (6.6–8.4)	16.8 (13.0–23.5)	7.3 (7.1–8.1)	20.3 (18.8–23.8)
Prothrombin time, sec		[6]	5	9	2.5 (2.3–2.8)	4.6 (3.6–6.2)	2.6 (2.4–5.8)	5.1 (2.8–5.7)
Prothrombin time, %		[6]	5	9	4.6 (4.2–5.1)	8.1 (6.4–10.9)		
Prothrombin time, INR		[6]	5	9	2.4 (2.2–2.7)	4.5 (3.5–6.0)	2.5 (2.3–3.0)	4.6 (2.9–6.8)
Thrombin time[a]		[6]	5	9	2.1 (1.8–2.3)	3.9 (3.1–5.2)		
t-PA Act[b]		[2] – non-OC	3	2	31.3 (26.9–37.4)	42.1 (33.9–52.3)	32.0 (27.6–37.4)	
		[2] – OC	3	2	26.1 (21.9–32.2)	23.6 (15.6–32.1)		
		[2] – men	5	3	37.7 (34.3–41.8)	48.3 (41.0–57.5)		
t-PA Ag[b]		[2] – non-OC	3	2	13.0 (10.5–16.1)	35.4 (30.0–42.7)	13.3 (11.0–30.9)	38.1 (23.9–191.1)

Table 1: (continued)

Measurands	Meta-analysis (number of included studies)	References	Study duration (number of days)	Sampling (number of samples per subject)	Short-term estimates (study period≤7 days) CV_I, CI %	Short-term estimates (study period≤7 days) CV_G, CI %	Long-term estimates (literature data [1]) CV_I, CI %	Long-term estimates (literature data [1]) CV_G, CI %
		[2] – OC	3	2	15.7 (12.7–19.9)	40.0 (33.1–49.8)		
		[2] – men	5	3	14.1 (12.5–16.0)	29.8 (25.8–35.0)		
PAI-1 Ag[b]		[2] – non-OC	3	2	40.0 (34.3–47.8)	60.9 (49.9–75.0)	48.6 (35.6–55.0)	59.8 (26.0–90.0)
		[2] – OC	3	2	51.8 (43.6–63.6)	63.5 (48.4–82.4)		
		[2] – men	5	3	37.9 (34.4–42.1)	70.0 (60.6–82.2)		
VWF Ag	2	[7, 8]			**5.7 (5.0–5.8)**	**23.2 (18.0–31.7)**	12.7 (11.1–19.4)	29.9 (22.6–31.6)
		[7]	5	9	5.8 (5.2–6.6)	31.7 (24.7–44.2)		
		[8]	7	3	5 (0–8.5)	18 (13.7–25.5)		

[a]One study was excluded because $CV_I = 0$ [5]. [b]Abbreviation included in column with references including separate studies for: women without or with oral contraceptive (respectively; non-OC and OC) and men [2]. APTT, activated partial thromboplastin time; Act, activity; Ag, antigen; CI, confidence interval; dRVVT, dilute Russell's viper venom time; FDP, fibrin degradation products; INR, international normalized ratio; PC, protein C; PS, protein S; t-PA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1; VWF, von Willebrand factor.

with and without use of hormonal OC [2]. The highest number of BV estimates were identified for fibrinogen (Clauss), as was also previously observed [1]. All six publications were awarded a BIVAC grade C, due to the lack of outlier analysis (QI8), variance homogeneity testing (QI10), and not reporting the number of results excluded following analysis of outliers and variance homogeneity (QI13). A probable cause for these omissions was the low number of samples per individual (2–9 samples) in these studies. In all studies, samplings were performed during standard working hours. Thus, especially for studies where all the samples were drawn in the same day, this data represents daytime variation, which is relevant for the majority of requested tests in standard laboratories.

No short-term BV studies were identified for the following haemostasis-related measurands; reptilase time, factor XIII and activated clotting time. Short-term BV data were identified for three measurands (dilute russel viper venom time [7], fibrin degradation products [6] and thrombin time [6]) for which there are no long-term BV data available (Table 1) and thus at time of study were not included in the EFLM BV Database.

For most of the measurands where short-term data were identified, meta-analysis could not be performed as only single studies were available. Meta-analyses were performed for five measurands (activated partial thromboplastin time, antithrombin activity, coagulation factor X, fibrinogen (Clauss) and von Willebrand factor (VWF) antigen), each based on two studies (Table 1). In general, the short-term BV estimates were comparable to long-term estimates as previously published [1], for most measurands. Exceptions were seen for fibrinogen (Clauss) and VWF antigen where the long-term CV_I estimate was significantly higher than the short-term CV_I ($p<0.001$, Mann–Whitney U-test). Both these measurands are acute-phase reactants. In the long-term studies, it was reported that only healthy individuals were included [9, 10], however, it is possible that mild or subclinical acute phase episodes may have gone undetected. In light of these differences, it may be argued that more stringent APS are needed for settings where monitoring is more frequent e.g. before and after substitution therapy, in stable patients. However, most laboratories will typically serve both types of populations and in this case, the more stringent APS should, preferably, be fulfilled. One study on fibrinogen (Clauss) reported CV_I estimates for one single day (CV_I=10.7 %, range: 8.5–12.5 %), 5 days (CV_I=14.2 %, range: 11.9–16.5 %) and 6-weeks (CV_I=17.8 %, range: 16.0–19.8 %) [3]. Although a significantly higher CV_I was found for the 6-weeks study period compared to the 1 and 5 days study periods, the short-term BV estimates (1 day and 5 days) were similar to the long-term CV_I for fibrinogen (Clauss) identified in our previous meta-analysis [1], while the CV_I for the 6-weeks study period was considerably higher. Considering how these data differ from data reported from other studies with similar sampling intervals and study duration, it is difficult to draw any conclusions. Furthermore, the single day study consisted of five repeated samples per individual around the same timepoint, so this represents the variation between five replicates [3]. The major advantage of the BV with a long study period is, that in addition to the influence of variation in a short period (such as diurnal rhythm), also seasonal effects are included.

One study reporting BV data for different populations was identified. This assessed gender-related differences, including in females using OC and not using OC, for fibrinogen (Clauss), t-PA activity, t-PA antigen and PAI-1 antigen. A higher CV_I estimate was reported for fibrinogen (Clauss) in men than in women not using OC, while CV_I estimates for t-PA activity and PAI-antigen were higher in men than in OC-using women. However, 95 % confidence intervals overlapped, except for PAI-antigen between men and women using OC. Further studies should be performed to investigate gender-related differences, as these have been demonstrated for other haemostasis-related measurands in studies with weekly sampling [11]. Further knowledge is also needed about BV in women using OC, because OC use is characterized by increased clotting factor levels, a reduction in coagulation inhibitors and changes in the fibrinolytic system [12], which could influence BV of related measurands.

In conclusion; based on our study, BV estimates for most haemostatic measurands derived from short- and long-term study designs are similar, with the exception for VWF and fibrinogen (Clauss). However, the short-term BV estimates reported in our study are derived from grade C quality studies and for most measurands, only single studies were identified. This is a limitation when assessing differences between short-term and long-term BV estimates and must also be considered when using these short-term BV data to inform on APS and for other BV applications. Further high-quality BV studies are therefore very welcome, especially for measurands where BV data are presently lacking.

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