Intra-subject biological variation and reference change value data made available to clinicians: a step toward the interpretation of patient test results

Anna Carobene, Eleonora Sabetta, Elena Monteverde, Annunziata Spina, Massimo Locatelli
Laboratory Medicine, IRCCS San Raffaele Scientific Institute, Milan, Italy

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To the Editor,

the within-subject (CV_I) biological variation (BV) is defined as the random fluctuation of a measurand in a steady state condition in a biological fluid around a homeostatic set point (1). CV_I has several clinical implications including the setting of analytical performance specifications (APS), and the reference change value (RCV, also called critical difference, CD) (2,3).

The European Biological Variation Study (EuBIVAS) was established by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group on BV, to deliver reliable BV estimates for a list of measurands (4,5). Briefly, the EuBIVAS involved six European laboratories from Italy (Milan and Padua), Norway, Spain, the Netherlands, and Turkey, resulting in the recruitment of 91 healthy volunteers (38 males, 43 pre-menopausal and 10 post-menopausal females; age 21-69 years) (4).

Fasting blood samples were drawn by each laboratory by venipuncture weekly for 10 consecutive weeks. The samples collected, stored at -80 °C, were shipped on dry ice to the IRCCS San Raffaele Hospital, Milan, Italy (OSR), where they were stored in a dedicated freezer at -80 °C until the analysis (4,5).

The analytical approach used by EuBIVAS, valid for measurands that are stable in samples stored, was not possible for the measurands of the complete blood count (CBC), given the requirement for fresh whole blood. With this aim, an Italian study was recently established to deliver BV estimates for CBC parameters based on the collection of fresh blood samples in a single center (6-8). The project was conducted in line with the EFLM checklist (9) and the EuBIVAS protocol (4) to assure the derivation of the highest-quality BV estimates.

Reliable data are essential for the application of the BV of measurands not only for defining APS used in laboratory for internal quality control and for external quality assurance, but also in clinical practices through the use of RCVs values. These latter, in fact, may be used as a tool for monitoring patients in assessing what changes between two measurements can be explained by biological and analytical variation (2).

However, the information collected by a recent survey promoted with the aim to evaluate the clinicians' knowledge concerning BV, and to investigate if clinicians use BV in the interpretation of test results, pointed out that clinicians do not use BV data or tools derived from BV such as RCV, to interpret test results (10). However, it was reported that 79.4% of clinicians accepted that even if the results of the two consecutive measurements are both within the reference interval, the difference between these two measurements may be clinically significant (10).

Even though the survey was organized in Turkey, the lack of knowledge about BV concept on the part of clinicians is undoubtedly generalizable to other Countries.

To fill this gap, CV_I and RCV values for a list of measurands (Table 1) were made available on line in the OSR (a large tertiary care academic hospital in Milan, northern Italy, with 1295 beds and more than 45 000 recoveries/year) web site (11).

As reported in Table 1, most CV_I values (82 out of 93) are EuBIVAS based (4-5), while the 11 values related to the complete blood count (CBC) are derived from the Italian hematological project carried out at the hospital Papa Giovanni XXIII, in Bergamo, northern Italy (6-8). The analytical variation (CV_A) values, reported in the Table 1, represent the typical long-term analytical variability of OSR specific analyzers.

The RCVs were estimated using the formula:

$$RCV = \sqrt{2} * Z * \sqrt{(CV_A^2 + CV_I^2)}$$

Corrispondenza a: Anna Carobene, Laboratory Medicine, IRCCS San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy, Telephone +39 02 26432850, E-mail carobene.anna@hsr.it

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Table 1

Analytical variation (CV_A), within subject (CV_I) biological variation (BV) and reference change values (RCV) estimates for 93 measurands, reported on the laboratory cards available on line (https://medicinadilaboratorio.hsr.it/schede/index.html). RCVs are obtained combining the typical long-term local variability (CV_A) and the CV_I estimates (1-82 based on the EuBIVAS population (4,5), 83-93 based on the Italian hematological BV study (6-8)).

	Measurand, Unit	Measurand	CV_A%	CV_I%	RCV%
1	ALT, U/L	Alanine Amino Transferase	4.0	9.3	28.0
2	AST, U/L	Aspartate Amino Transferase	4.0	9.5	28.6
3	GGT, U/L	Gamma-Glutamyl Transferase	3.5	8.9	26.5
4	ALP, U/L	Phosphatase Alkaline	5.0	5.3	20.2
5	LDH, U/L	Lactate dehydrogenase	3.0	5.2	16.6
6	CK, U/L	Creatine Kinase	5.0	14.5	42.5
7	AMY, U/L	Total amylase	1.9	6.8	19.6
8	PAMY, U/L	Pancreatic amylase	1.7	6.3	18.1
9	LIP, U/L	Lipase	4.0	7.7	24.0
10	Na, mmol/L	Sodium	0.8	0.5	2.7
11	K, mmol/L	Potassium	1.2	3.9	11.4
12	Cl, mmol/L	Chloride	4.0	1.0	11.4
13	Ca, mg/dL	Calcium	3.0	1.8	9.7
14	Mg, mg/dL	Magnesium	3.0	2.9	11.5
15	PHOS, mmol/L	Inorganic Phosphate	4.0	7.7	24.0
16	COL, mg/dL	Total Cholesterol	2.0	5.2	15.4
17	HDL Chol, mg/dL	HDL-Cholesterol	4.0	5.7	19.2
18	LDL Chol, mg/dL	LDL-Cholesterol	4.0	8.5	25.9
19	NonHDL Chol, mg/dL	Non HDL-Cholesterol	4.0	6.9	22.0
20	Trig, mg/dL	Triglycerides	2.0	19.8	55.1
21	Glu, mg/dL	Glucose	2.8	4.7	5.2
22	Urea, mg/dL	Urea	3.0	14.1	39.9
23	ACUR, mg/dL	Uric acid	2.0	8.3	23.7
24	TP, g/L	Total Protein	2.0	2.6	9.1
25	T Bil, mg/dL	Total Bilirubin	5.0	20.9	59.5
26	D Bil, mg/dL	Direct Bilirubin	5.0	20.9	59.5
27	Crea, mg/dL	Creatinine	2.5	4.4	14.0
29	NSE, µg/L	Neuron-specific enolase	2.0	10.9	30.7
30	S100, µg/L	S100-β protein	10.0	10.2	39.6
31	A1AT, g/L	α1-antitrypsin	6.0	3.8	19.7
32	MUCO, g/L	α1 acid glycoprotein	8.0	6.8	29.1
33	Alb, g/L	Albumin	2.0	2.5	8.9
34	B2MICR, mg/L	β 2-Microglobulin	8.0	4.0	29.8
35	C3, g/L	Complement Component 3	5.0	4.6	18.8
36	C4, g/L	Complement Component 4	8.0	6.9	29.3
37	Cer, g/L	Ceruloplasmin	5.7	4.9	20.8
38	CistC, mg/L	Cystatin C	8.0	3.9	24.7
39	APTG, g/L	Haptoglobin	5.4	7.4	25.4

Table 1
Continues...

	Measurand, Unit	Measurand	CV_A%	CV_I %	RCV%
40	IgA, g/L	Immunoglobulin A	3.0	3.3	12.4
41	IgG, g/L	Immunoglobulin G	6.0	2.8	18.3
42	IgM, g/L	Immunoglobulin M	6.0	3.9	19.8
43	Trf, g/L	Transferrin	2.4	3.4	11.5
44	sTfr, mg/L	Soluble Transferrin Receptor	3.9	6.9	22.0
46	Lpa, g/L	Lipoprotein(a)	7.2	8.9	31.7
47	ApoA, g/L	Apolipoprotein A-I	4.6	4.8	18.4
48	ApoB, g/L	Apolipoprotein B	5.0	6.7	23.2
51	PARAT, pg/mL	Biointact parathyroid hormone	6.0	14.7	44.0
52	GLA, ng/mL	Osteocalcin	11.0	8.9	39.2
55	CTX, µg/L	C-terminal telopeptides of type I collagen	2.8	15.1	42.5
56	FGF23, pg/mL	Intact Fibroblast growth factor 23	3.4	13.9	39.6
57	Cort, ng/mL	Cortisol	7.0	15.5	47.1
58	INS, mUI/L	Insulin	7.0	25.3	72.7
59	TSH, µU/mL	Thyroid Stimulating Hormone	3.5	18.9	53.2
60	FT3, pg/mL	Free triiodothyronine	4.5	5.0	18.6
61	FT4, ng/dL	Free thyroxine	4.5	4.8	18.2
62	CT, pg/mL	Calcitonin	4.2	13.0	37.8
63	TG, ng/mL	Thyroglobulin	4.9	10.3	31.6
64	TPSA, ng/mL	Total Prostate-Specific Antigen	5.0	6.8	23.4
65	fPSA, ng/mL	Free Prostate-Specific Antigen	5.0	7.1	24.1
68	Ca15-3, IU/mL	Cancer antigen 15-3	5.0	4.4	18.4
69	CEA, ng/mL	Carcinoembryonic Antigen	5.0	6.3	22.3
70	Ca19-9, IU/mL	Cancer antigen 19-9	5.0	4.0	17.7
71	Cyfra 21-1, µg/L	Cytokeratin fragment 21-1	2.7	19.7	55.1
72	HE4, pmol/L	Human epididymis protein 4	2.1	6.7	19.4
73	AFP, ng/mL	Alpha fetoprotein	2.7	4.1	13.6
74	Ca125, IU/mL	Cancer antigen 125	5.0	8.6	27.6
75	APTT, ratio	Activated Partial Thromboplastin Time	2.1	2.9	9.9
76	PT, ratio	Prothrombin time	2.2	2.6	9.4
77	AT, %	Antithrombin	4.8	3.5	16.5
78	FVIII, %	Factor VIII	7.7	8.3	31.4
79	Fib, mg/dL	Fibrinogen	4.5	10.2	30.9
81	Prot C, %	Protein C	6.3	5.4	23.0
82	Free prot S, %	Free protein S	4.4	4.0	16.5
83	WBC, 10^{*9}/L	Leukocytes	1.9	11.1	31.2
84	RBC, 10^{*12}/L	Erythrocytes	0.9	1.8	5.6
85	HBG, g/dL	Haemoglobin	0.7	2.0	5.9
86	HCT, %	Hematocrit	1.8	2.4	8.3
88	PLT, 10^{*9}/L	Platelets	1.8	7.2	20.5

Table 1
Continues...

	Measurand, Unit	Measurand	CV_A%	CV_I %	RCV%
89	NE, 10^9/L	Neutrophils	3.0	14.6	41.3
90	LY, 10^9/L	Lymphocytes	3.6	11.0	32.1
91	MO, 10^9/L	Monocytes	6.3	13.4	41.0
92	EO, 10^9/L	Eosinophils	7.9	15.6	48.4
93	BA, 10^9/L	Basophils	3.1	12.8	36.5

Table 2
Serum glucose test laboratory card available on line (https://medicinadilaboratorio.hsr.it/schede/index.html). It contains all the information relating to the laboratory test

Test	Glucose - serum
Test acronym:	GLU
Availability on patients:	Inpatients YES – Outpatients YES - In emergency YES
Test agreed with the national health system	YES
Regional Code:	90271
Grouping:	06 – Clinical chemistry
Analytical method:	Esochinase-G6PD
Typical analytical variation (CVA)	2.80%
Intra–subject biological variation (CVI)	4.7%
Critical difference (DC or RCV)	15.2%
Clinical meaning:	Fasting values between 100 and 125 mg/dL indicate a "pre-diabetes" (IFG impaired fasting glucose). Values equal or higher than 126 mg/dL. if confirmed more than once. lead to the diagnosis of diabetes.
Analytical instrument:	COBAS Roche C 8 000
Unit of measurement:	mg/dL
Reference range:	60 - 100
Execution laboratory:	Automation Laboratory
Internal phone number:	6499
Sample matrix:	Serum
Minimum sample volume:	3 mL
Plug Tube/container:	Yellow top. serum separator tube
Tube Volume/container:	4 mL
Dosing days:	Every day from Monday to Sunday
Response times: (in working days)	1
Storage and transport methods (only for external centers):	The sample. if collected in tube with separator. capped and centrifuged within 1-2 hours. can be stored for 48 h and transported at 2-8 °C. For longer storage time. the serum sample have to be frozen at -20 °C and transported in dry ice.
Additional information:	For a test requested in an URGENCY regime (only for Inpatient and Emergency Room patients) the response times are as follows: EMERGENCIES: 1 Hour URGENCIES: 3 Hours
Synonyms:	Glycaemia

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Where Z used was 1.96 at the probability level of significant change set at 95%.

Data analysis was performed using Excel 2010.

The CV_A values here reported (Table 1) are overall higher than those reported in the publications used to derive CV_I estimates, in which CV_A estimates were based on duplicate analysis of all study samples performed in a single analytical run, as recommended (1,9). RCVs estimates here presented cannot be considered as universal values. In fact, each laboratory should calculate its own RCVs using relevant CV_A estimates reflecting relevant sampling intervals and settings (2,3).

CV_I and RCVs estimates listed in Table 1, have been used to update the medicine laboratory page available on the OSR website (11). Particularly, the specific card for each single measurand has been enriched by the insertion of both CV_I and RCV estimates. The example of the serum glucose test card is reported in Table 2.

As advocated by Emre HO et al., laboratory specialists should be instructors and consultants, providing guidance to clinicians on laboratory-related information. Improvement in collaborations between laboratory specialists and clinicians may result in a widespread clinical use of BV (10).

The entry in the OSR webpage of these new information, was accompanied by an email to all clinicians with the aim to explain the use of RCV values in clinical practice, and the laboratory availability for advising in using the data. The activity of the EFLM to critically appraise published studies of BV for obtaining new reliable BV estimates, has pointed out that differences in study population (i.e. age pediatrics or elderly; healthy or non-healthy status) or in study design (within-one day studies, weekly/monthly sampling intervals), for particular measurands led to significant differences in BV estimates (12-14). The use of online BV data without "clinical consultation" by a laboratory specialist may lead to errors in the clinical evaluation of the patients avoiding, for example the inconvenience to use the same RCV for different age groups and pathological situations.

According to the ISO 15189:2003 (paragraph 5.5.2), the laboratory "should define biological reference intervals or clinical decision limits, document their rationale and communicate this information to the users" (15), and, as a consequence, some accredited laboratories might have already provided BV information online. However, to the best of our knowledge, with the exception of one hospital that in any case has reported obsolete data (data not shown), OSR is the only Italian hospital that reports online BV data, thus making them easily accessible to the clinicians.

This small step towards BV related data better use evocates future studies to evaluate the effective use of the information provided and to study its influence on the clinical interpretation of the patient results.

CONFLICT OF INTEREST

None.

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