Letter to the Editor

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Biological variation of serum cholinesterase catalytic concentrations

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To the Editor,

Measurements of serum cholinesterase (CHE, also called pseudocholinesterase) catalytic concentrations are primarily used as a test of liver function and, less frequently, as an indicator of possible organic phosphorous insecticide poisoning in agriculture or organic chemical industry workers [1]. Preoperative screening of CHE activity has been also advocated to identify individuals bearing genetic causes of enzyme deficiency in whom some muscle relaxant drugs, such as suxamethonium, administrated to aid in endotracheal intubation in surgery, may not be hydrolysed by CHE rapidly enough and cause apnea by a prolonged paralysis of respiratory muscles [1]. Historically, many methods were proposed to measure CHE, using different acyl(thio)choline esters as substrates [2]. At present, however, all the most popular automated measuring systems use butyrylthiocholine for determining the CHE activity as this substrate provides the best reproducibility. To check the quality of CHE measurements it is essential to correctly define analytical performance specifications (APS). In 2014, the Strategic Conference organized by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) proposed models for establishing APS, recommending that the choice of the most appropriate model for a specific measurand should be based on its biological and clinical characteristics [3]. Allocation of CHE to the correct model to derive APS can be however not easy. Although this enzyme surely plays a role in monitoring the clinical conditions mentioned above, the measurand has not a defined role in the decision making of a specific disease and test results are not interpreted through established decision limits, so that outcome-based APS cannot be defined. On the other hand, its exact biological role is unknown, and this might be a limitation for applying the model based on biological variation (BV). As CHE demonstrated rather stable concentrations in healthy individuals, it appears however rational to use the BV model to derive APS [4]. This requires the availability of reliable BV data. Unfortunately, all the available studies evaluating CHE BV suffered important limitations, among those the collection of serial samples in enrolled individuals at a variable time distance, the derivation of the within-subject BV (CV_I) without subtracting analytical variation, the analysis of samples in different batches without considering the between-run analytical variation, or the wrong inclusion of the CHE seasonal variation in CV_I estimate. Table 1 summarizes the drawbacks of each published study evaluated on the basis of the compliance with the EFLM critical appraisal checklist quality items (BIVAC-QI) [5]. Maybe for these major limitations, the EFLM BV database (https://biologicalvariation.eu/) does not include CHE in the list of evaluated measurands. Therefore, we decided to perform a study assessing BV components of CHE catalytic concentration by adopting an accurately designed experimental protocol in accordance with previous remmendations for the BV data production [6].

We employed frozen (–80 °C) serum samples from 35 apparently healthy Caucasian volunteers (13 men, 12 pre-, and 10 post-menopausal women; ages 19–62 years) obtained for a previous BV study [7]. CHE activity in serum is stable for several years if stored at temperature lower than –20 °C [1]. All sample donors were free of any evident disease, had no history of chronic disease, and did not receive any medication, including hormonal contraceptives if women, previously shown to decrease CHE [8, 9]. Other criteria for inclusion were that the subjects should be within 80–120% of ideal body weight and maintain their

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Table 1: Evaluation of available studies on biological variation of serum cholinesterase catalytic activity according to the EFLM critical appraisal checklist quality items (BIVAC-QI).[a]

Study ref.	Substudy	Quality items														Overall grade[b]	CV_I	CV_G
		1. Scale	2. Subjects	3. Samples	4. Measurand	5. Pre-analytical procedures	6. Analytical variation	7. Steady state	8. Outliers	9. Normality test	10. Variance homogeneity	11. Statistical method	12. Confidence limits	13. Included results	14. Concentrations			
Sidell and Kaminskis. Clin Chem 1975;21:1961	Males (12 months)	A	D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	D_2	6.4%	NC
	Females (12 months)	A	D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	D_2	6.1%	NC
	Males (3 weeks)	A	A	A	A	C	B	B	C	B	C	C	A	C	B	$C_{5,8,10,11,13}$	3.9%	NC
Chu SY. Clin Biochem 1985;18:323	–	A	C	A	A	C	B	B	C	B	A	B	A	C	A	$C_{2;5,8,13}$	4.3%	NC
Moses et al. Clin Chem 1986;32:175.	–	A	A	A	A	A	B	B	C	B	C	A	A	C	A	$C_{8,10,13}$	6.8%	18.2%
Richter et al. Toxicol Lett 1986;33:25	Field workers	A	D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	D_2	9.7%	22.1%
	Kibbutzim residents	A	D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	D_2	8.0%	12.1%
Hölzel WGE. Clin Chem 1987;33:1133	Healthy subjects (males)	A	A	A	A	A	A	B	C	A	A	B	A	C	A	$C_{8,13}$	5.7%	NC
	Healthy subjects (females)	A	A	A	A	A	A	B	C	A	A	B	A	C	A	$C_{8,13}$	4.6%	NC
Mason and Lewis. J Soc Occup Med 1989;39:121	–	A	C	A	A	C	B	B	C	B	A	B	A	C	A	$C_{2,5,8,13}$	4.6%	NC
Brock A. J Clin Chem Clin Biochem 1990;28:851	–	A	A	D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	D_3	5.4%	NC
Brock and Brock. Scand J Clin Lab invest 1990;50:401	–	A	A	A	A	C	C	B	C	B	C	C	C	C	A	$C_{5,6,8,10-13}$	5.2%	NC

CV_I, mean within-subject biological variation; CV_G, mean between-subject biological variation; NA, not available; NC, not calculated. [a]When applying the BIVAC-QI, the study quality is rated according to 14 items, which are scored from A to D depending on the quality of information [5]. [b]An overall grade A indicates full compliance with all the 14 BIVAC-QIs; a grade B is awarded if the lowest QI score achieved is B. Similarly, the publication is graded C if the lowest QI score is C. D is the scoring option for QIs that are considered essential for estimate of biological variation to be reliable. In the BIVAC scoring system, the QIs associated with the grade are given as a subscript; for instance, a grade $C_{5,10}$ indicates that QI numbers 5 and 10 were scored as C.

usual lifestyle throughout the study. None of the subjects consumed substantial (>10 g of ethanol/day) quantities of alcohol or were smokers. We collected a total of four venous blood samples from each of study participants on the same day, every week for four consecutive weeks. All blood samples were obtained by standard venipuncture using a 20-G straight needle between 07:30 and 10:00 a.m. from seated subjects, who had fasted overnight and had not exercised that morning. Blood draw was performed by the same skilled phlebotomist with minimal stasis directly into 4 mL vacuum collection tubes with no anticoagulant and polymer gel for serum separation (Becton Dickinson Vacutainer, ref. no. 369032). Serum specimens were obtained by centrifugation and immediately aliquoted and stored at −80 °C until assayed. For measuring CHE, all specimens were thawed, mixed, centrifuged, and then analysed in a single run in duplicate in random order. To further minimizing analytical variation, a single analyst performed all measurements using a single lot of reagents. CHE activity was measured on the Alinity c platform (Abbott Diagnostics) by the proprietary kit (reagent code no. G91804R02; calibrator code no. G91855R02) using butyrylthiocholine as substrate for CHE and a colorimetric principle in which the thiocholine formed reduces yellow hexacyanoferrate(III) to colourless hexacyanoferrate(II) and the decrease of absorbance at 405 nm is directly proportional to the CHE activity in the sample. All measurements were performed according to the manufacturer's instructions, after checking the system alignment by using Alinity c Clinical Chemistry Control 1 and 2. The CV_I and between-subject BV (CV_G), with corresponding 95% confidence intervals (CI), were calculated using CV-ANOVA and standard ANOVA, respectively [10]. Outlier identification and homogeneity assessment were performed for replicates and within-subject variances by Cochran test. The steady-state situation of the enrolled population was verified by calculating the regression of the mean of the 70 duplicate analyses from every blood drawing versus the blood drawing number. Subject population is considered in steady state if the 95% CI of the slope of the regression equation included zero [11]. The Shapiro-Wilk test was applied separately to the set of normalized residuals for each individual and to subject means to check data distribution and validate the normality hypothesis. Student's unpaired t-test and F-test were used to compare CHE means and within-subject variances between groups, respectively.

The study involved a collection of 140 samples, each assayed in duplicate yielding 280 analytical results. No outliers among observations (Cochran's test value, 0.10) and within-subject variances (Cochran's test value, 0.19) were identified, showing data homogeneity. As the 95% CI of the slope of the regression equation included zero (−0.152 to 0.082), we confirmed that all enrolled subjects were in steady-state condition. The Shapiro-Wilk test accepted the hypothesis of normality (with $p>0.05$) for the distribution of most within-subject CHE values (i.e., 91%). Normal distribution was also confirmed for subject means ($p=0.249$). Supplemental Figure shows the individual mean and absolute range of CHE in evaluated subjects and Table 2 displays the derived results, including APS derived from BV data. As widely known [8, 9], CHE activity was significantly higher in men than in women, both in pre- ($p=0.0001$) and in post-menopause ($p=0.026$). In addition, in women CHE activity tended to increase in post-menopause ($p=0.053$ vs. pre-menopause). This sex and hormonal dependent differences were absent when CV_I were considered (for all comparison between groups, $p\geq0.23$). The gene on chromosome 3 controlling the

Table 2: Mean values, estimated average analytical (CV_A), within-subject (CV_I) and between-subject (CV_G) variability components, indices and analytical performance specifications derived from biological variation data for serum cholinesterase (CHE) activity in this study.

Group, n	Total number of samples	Mean CHE, kU/L (95% CI)	CV_A[a], %	CV_I, % (95% CI)	CV_G, % (95% CI)	II[b]	RCV for increasing/ decreasing values[c], %	n[d]	Analytical performance specifications			
									Standard measurement uncertainty[e],%		Allowable bias[f], %	
									M	D	M	D
All (35)	140	8.8 (8.1–9.4)	0.5	4.2 (3.7–4.9)	22.9 (18.5–30.1)	0.18	+10.4/−9.4	3	≤3.2	≤2.1	≤8.7	≤5.8
Men (13)	52	10.2 (9.3–11.2)		3.6 (2.9–4.6)	19.9 (14.2–33.0)							
PreM (12)	48	7.4 (6.8–7.9)		4.4 (3.6–5.7)	27.7 (19.5–47.1)							
PostM (10)	40	8.5 (7.5–9.5)		4.8 (3.8–6.4)	24.0 (16.4–44.0)							

CI, confidence interval; II, index of individuality; RCV, reference change value; n, number of blood specimens that should be collected to estimate the homeostatic set point of an individual within ±5%; M, minimum quality level; D, desirable quality level; PreM, pre-menopausal women; PostM, post-menopausal women. [a]From duplicate measurements of subject samples. [b]Calculated as (CV_{I+A}/CV_G). [c]Calculated by ln-RCV method according to Røraas et al. [10] as 100% (exp $(\pm Z\ 2^{1/2}\ (CV^2_{LnA} + CV^2_{LnI})^{1/2}) - 1)$, where Z is 1.64 (probability level 95%) and CV_{Ln} refers to ln-transformed data = $(\ln(1+CV^2))^{1/2}$. [d]Calculated as $[1.96^2\ (CV_A^2 + CV_I^2)/D^2]$ where D is 5. [e]Calculated as 0.75 CV_I (minimum) and 0.50 CV_I (desirable). [f]Calculated as 0.375 $(CV_I^2 + CV_G^2)^{0.5}$ (minimum) and 0.250 $(CV_I^2 + CV_G^2)^{0.5}$ (desirable).

synthesis of CHE can exist in many allelic forms that may influence the enzyme concentrations in blood [1]. This translates in a high biological individuality with a low index of individuality (0.18), indicating that conventional population-based reference intervals have little use in CHE interpretation. It is better to rely on longitudinal interpretation of the individual CHE variation: indeed, serial measurements of CHE have been promoted as an indication of prognosis in patients with liver failure and for monitoring liver function after liver transplantation [1]. By applying this approach, a reference change value of 10.4% (for increasing CHE values) and −9.4% (for decreasing values) can be employed.

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References

1. Panteghini M, Bais R. Serum enzymes. In: Rifai N, Horvath AR, Wittwer CT, editors. Tietz textbook of clinical chemistry and molecular diagnostics, 6th ed. St. Louis: Elsevier Saunders; 2018: 404–34 pp.
2. Panteghini M. Methods for serum cholinesterase assay and classification of genetic variants. Clin Chim Acta 1989;183:87–90.
3. Sandberg S, Fraser CG, Horvath AR, Jansen R, Jones G, Oosterhuis W, 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;53:833–5.
4. Ceriotti F, Fernandez-Calle P, Klee GG, Nordin G, Sandberg S, Streichert T, 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: 189–94.
5. Aarsand AK, Røraas T, Fernandez-Calle P, Ricos C, Dìaz-Garzòn J, Jonker N, et al. The biological variation data critical appraisal checklist: a standard for evaluating studies on biological variation. Clin Chem 2018;64:501–14.
6. Braga F, Panteghini M. Generation of data on within-subject biological variation in laboratory medicine: an update. Crit Rev Clin Lab Sci 2016;53:313–25.
7. Krintus M, Braga F, Kozinski M, Borille S, Kubica J, Sypniewska G, et al. A study of biological and lifestyle factors, including within-subject variation, affecting concentrations of growth differentiation factor 15 in serum. Clin Chem Lab Med 2019;57: 1035–43.
8. Sidell FR, Kaminskis A. Influence of age, sex, and oral contraceptives on human blood cholinesterase activity. Clin Chem 1975;21:1393–5.
9. Ledpage L, Schiele F, gueguen R, Siest G. Total cholinesterase in plasma: biological variations and reference limits. Clin Chem 1975;21:1393–5.
10. Røraas T, Støve B, Petersen PH, Sandberg S. Biological variation: the effect of different distributions on estimated within-person variation and reference change values. Clin Chem 2016;62: 725–36.
11. Aarsand AK, Díaz-Garzón J, Fernandez-Calle P, Guerra E, Locatelli M, Bartlett WA. The EuBIVAS: within- and between-subject biological variation data for electrolytes, lipids, urea, uric acid, total protein, total bilirubin, direct bilirubin, and glucose. Clin Chem 2018;64:1380–93.

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