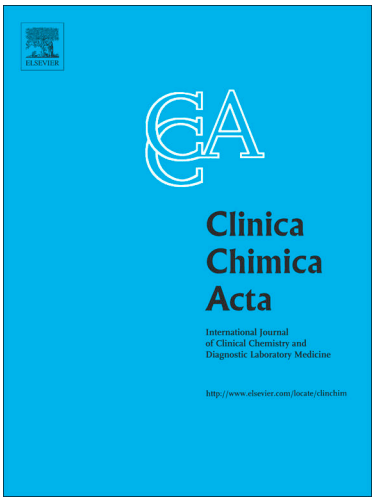
Journal Pre-proofs



Letter to the editor

Within-person biological variation estimates from the European Biological Variation Study (EuBIVAS) for serum potassium and creatinine used to obtain personalized reference intervals

Anna Carobene, Giuseppe Banfi, Massimo Locatelli, Matteo Vidali

PII: S0009-8981(21)00338-7
DOI: https://doi.org/10.1016/j.cca.2021.09.018
Reference: CCA 16724

To appear in: *Clinica Chimica Acta*

Received Date: 11 August 2021
Revised Date: 22 September 2021
Accepted Date: 22 September 2021

Please cite this article as: A. Carobene, G. Banfi, M. Locatelli, M. Vidali, Within-person biological variation estimates from the European Biological Variation Study (EuBIVAS) for serum potassium and creatinine used to obtain personalized reference intervals, *Clinica Chimica Acta* (2021), doi: https://doi.org/10.1016/j.cca.2021.09.018

Within-person biological variation estimates from the European Biological Variation Study (EuBIVAS) for serum potassium and creatinine used to obtain personalized reference intervals.

Anna Carobene[1*], Giuseppe Banfi[2,3], Massimo Locatelli[1], Matteo Vidali[4].

1. Laboratory Medicine, IRCCS San Raffaele Scientific Institute, Milan, Italy.
2. IRCCS Istituto Ortopedico Galeazzi, Milano
3. Università Vita e Salute San Raffaele, Milano
4. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

Keywords: biological variation, EuBIVAS, personalized reference intervals

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

To the editor,

The reference intervals (RI) defined for the measurands analyzed in clinical laboratories are commonly used to interpret the patient results and to take clinical decisions. As recommended by the IFCC (1), RIs should be locally established based on the population and on other factors (sex, age, physiological state, etc.); however, usually this does not represent a common practice. For the measurands with high individuality, where the intra-subject biological variation (CV_I) is small if compared to the between subject biological variation (CV_G) (defined as index of individuality (II), where $II=CV_I/CV_G$), for a correct interpretation of serial results, the use of the RIs should be replaced by the reference change value (RCV), based on CV_I. In such situations, the individual is clearly the best point of reference for assessing change, armed with the knowledge of the biological variation (BV) of the measurand, while the Ris are suitable for the assessment of measurands with a high II, particularly when it is >1.4 [2].

CV_I represents the natural fluctuation of one analyte within an individual around a homeostatic set point in a steady-state condition, and CV_G the variability between the homeostatic set points between subjects. CV_I and CV_G have several clinical implications including the setting of analytical performance specifications (APS) [3,4], and the RCV, that may be used as a tool for monitoring patients in assessing what changes between two measurements can be explained by biological and analytical variation [5]. 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 [6]

From these considerations, Coskun and coauthors recently proposed a new model based on analytical variation (CV_A) and CV_I estimates, using some previous results of an individual to obtain personalized reference intervals (prRI) [7].

Authors proposed the following formula:

$prRI = X_P \pm TVset$

Where X_P is the homeostatic set point of the single individual and TVset represents the prediction interval around X_P. TVset is obtained by the following:

$TVset = z * \sqrt{[(n+1)/n * (CV_I^2 + CV_A^2)]}$,

CV_I is taken from the literature, and CV_A is the analytical CV derived from the own routine laboratory. Although the authors agreed on that CV_I should be calculated in the single individual (CV_P), they suggested to use a not-personalized or common CV_I derived from high quality studies as a pragmatic solution [7].

The European Biological Variation Study (EuBIVAS) was undertaken by the EFLM working group on BV, to deliver high-quality BV data for a large set of measurands, using a multicenter approach (weekly sampling for ten consecutive weeks for 91 healthy subjects from six European labs) [8, 9]. All samples from the same participant were analyzed in duplicate within a single run, using an ADVIA 2400 Clinical Chemistry System (Siemens Healthineers) using Siemens reagents, calibrators, and control materials. The protocol was approved by the Institutional Ethical Board/Regional Ethics Committee.

EuBIVAS data of Potassium (K^+) [10] and Creatinine enzymatic method [11], two measurands with high individuality (IIs EuBIVAS based of 0.7 and 0.26 for K^+ and Creatinine respectively) were selected to verify the model proposed by Coskun et al. [7].

In particular, from 91 subjects 1716 data points (mean of 9.47 samples/subject and 1.98 replicates/sample) and 1706 data points (mean of 9.40 samples/subject and 1.99 replicates/sample) for Creatinine and K^+ were considered. Assessment of outliers, variance homogeneity, normality and steady state were performed as previously reported [12], with outlier identification and removal performed for replicates and samples. Homogeneity of CV_A (between-replicates) was examined using the Bartlett test and of CV_I by the Cochran test. To examine whether individuals were at steady-state, linear regressions on the 10 pooled mean group sample concentrations were performed for each measure. Participants were considered to be in steady-state if the 95% CIs of the slope of the regression line included zero. In addition, the steady state of each single participant was verified using the same procedure. If the 95% confidence interval of the slope of the regression did not include the zero, the data of the single participant was discarded.

CV_Ps for Creatinine and K^+ of each subject, to simulate the typical situation of the patient, were calculated considering 10 results (using only the first replicate of 10 collections) by computing the analytical variance (σ_A^2) calculated using the CV_A between replicates EuBIVAS based, using the following formula:

$SD_P=(\sigma_P^2-\sigma_A^2)^{1/2}$

$CV_P= (SD_P)/X_P*100$

Where:

σ_P^2 is the total biological variance for a single individual;

σ_A^2 is the analytical variance;

SD_P is intra-individual biological standard deviation;

X_P is the mean value for a single person;

Visual inspection of plots $\%CV_P$ vs concentration for both Creatinine (Figure1) and K^+ (Figure 2), as well as low Pearson correlation coefficients (Creatinine: -0.265, K: -0.04) indicate homogeneity of variances and therefore independency on concentration. The 91 CV_P values ranged from 1.3 to 7.03% for Creatinine (figure 1), and from 1.67 to 6.17% for K^+ (Figure 2), while the global CV_I were 4.4% and 3.92 % for Creatinine and K^+ respectively.

For Creatinine and K^+, TVset and the corresponding psRIs values for each subject, were calculated according to Coskun et al [7] using CV_I and CV_A from EUBIVAS [10, 11]. For each subject the single $TVset_P$, and the corresponding psRIs-2 (according to the new model here proposed CV_Ps based)were also calculated by substituting in the formula CV_I with CV_P.

From the EuBIVAS dataset, the frequency of data beyond the prRIs obtained according to Coskun (prRIs-1), and according to the new model (prRIs-2), were calculated.

Data analyses were performed using Microsoft Excel 2010 , the figures were obtained using python 3.7., and the linear regression for each single participant was performed by the R Language v.4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Three subjects were found not in steady state for creatinine measurement (CV_P ranging from 5.3 to 5.9%), and as a consequence discarded from the comparison. No subject was instead identified as not being in steady state for K^+.

Calculated $TVset_P$ ranged from 4.6 to 15.2 % and for Creatinine from 5.3 to 13.5% for K^+, while according to Coskun TVset were 10.1% and 9.01% for Creatinine and K^+ respectively.

38 Creatinine data (2.29%) resulted beyond the prRIs-1, while only 15 Creatinine (0.91%) resulted beyond the prRIs-2, and only 10 data were detected by both methods. 40 K^+ data (2.34%) resulted beyond the prRIs-1, while only 13 K^+ data (0.76%) resulted beyond the prRIs-2, with 6 data detected by both methods. EuBIVAS subjects are well defined, data were already cleaned of outliers for the homogeneity of the variances, and were demonstrated to be in steady state.

However, CV_Ps values showed surprisingly a wide range of values that do not depend on the concentration of the measurand. That means that most data detected by the method proposed by Coskun are false alarms, and rely on the individual CV_P which is much higher than the general estimate of CV_I. On the contrary, for the subjects with CV_P lower than CV_I, the method proposed by Coskun is not able to detect the data beyond the prRIs-2 leading to missed real alarms.

Both approaches, Coskun et al. and the new one here proposed, to obtain the prediction interval should include the uncertainties around the data and more efforts should be dedicated in this direction and this may raise concern in the use of prRIs. However we consider that the method proposed by Coskun represents an outstanding contribution toward the personalized medicine, with

the exception for individual CV_Ps far for the CV_I global estimate, for which the use of the individual CV_P is suggested.

Acknowledgments: The authors would like to thank the EFLM Working Group on BV for the use of data from the EuBIVAS. We thank Christian Uccheddu for his support in making the figures.

References

1. Horowitz GL. Establishment and use of reference value. In: CA Burtis, ER Ashood, DE Bruns, editors. Tietz text-book of clinical chemistry and molecular diagnostic. 5th Ed. St. Louis (MO): Elsevier; 2012.p.95-118.
2. Fraser CG. Biological variation: from principles to practice. Washington, DC: AACC Press; 2001.
3. Carobene A, Franzini C, Ceriotti F. Comparison of the results from two different External Quality Assessment Schemes supports the utility of robust quality specifications Clin Chem Lab Med 2011;49:1143–49.
4. Haeckel R, Wosniok W, Kratochvila J, et al. A pragmatic proposal for permissible limits in external quality assessment schemes with a compromise between biological variation and the state of the art. Clin Chem Lab Med. 2012;50:833-9.
5. Fraser CG. Reference change values. Clin Chem Lab Med 2011;50:807-12.
6. Emre HO, Karpuzoglu FH, Coskun C, et al. Utilization of biological variation data in the interpretation of laboratory test results - survey about clinicians' opinion and knowledge. Biochem Med (Zagreb). 2021;31:010705. doi: 10.11613/BM.2021.010705.
7. Coşkun A, Sandberg S, Unsal I, Cavusoglu C, Serteser M, Kilercik M, et al. Personalized Reference Intervals in Laboratory Medicine: A New Model Based on Within-Subject Biological Variation. Clin Chem. 2021 Jan 30;67(2):374-84.
8. Carobene A, Strollo M, Jonker N, Barla G, Bartlett WA, Sandberg S, 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;54:1599-608.
9. Carobene A, Aarsand AK, Bartlett WA, Coskun A, Diaz-Garzon J, Fernandez-Calle P, et al. The European Biological Variation Study (EuBIVAS): a summary report. Clin Chem Lab Med. 2021 May 31. doi: 10.1515/cclm-2021-0370. Epub ahead of print.
10. Aarsand AK, Díaz-Garzón J, Fernandez-Calle P, Guerra E, Locatelli M, Bartlett WA, et al. 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.

11. Carobene A, Marino I, Coŝkun A, Serteser M, Unsal I, Guerra E, et al. The EuBIVAS project: Within and between-subject biological variation data for serum creatinine using enzymatic and alkaline picrate methods and implications for monitoring. Clin Chem. 2017;63:1527-36.
12. Carobene A, Guerra E, Locatelli M, Cucchiara V, Briganti A, Aarsand AK, et al. Biological variation estimates for prostate specific antigen from the European Biological Variation Study; consequences for diagnosis and monitoring of prostate cancer. Clin Chim Acta. 2018;486:185-191.

Figure Captions

Figure 1

Correlation between the individual Creatinine CV_Ps values vs creatinine mean values (mg/dL) EUBIVAS based (10 sample/91 subjects). Linear regression equation: Y = -0.65*X + 4.79; the grey area represents the 95%CI around the equation. Pearson correlation coefficient (r) = -0.265.

Figure 2

Correlation between the individual Potassium CV_Ps values vs Potassium (K^+)mean values (mmol/L) EUBIVAS based (10 sample/91 subjects). Linear regression equation: Y = -0.26*X + 4.76; the grey area represents the 95%CI around the equation. Pearson correlation coefficient (r) = -0.04.