Clin Chem Lab Med 2012;50(2):189–190 © 2012 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/CCLM.2011.751

Editorial

Biological variation and reference change values: an essential piece of the puzzle of laboratory testing

Mario Plebani and Giuseppe Lippi

The biological variability of analytes assessed in clinical laboratories is pivotal to understand several issues related to the quality of laboratory information throughout all steps of the testing process (Figure 1). In the pre-analytical phase, the knowledge of predictable biological cyclical biorhythms is required for appropriate collection of specimens at times pertinent to the clinical questions. Daily, monthly and seasonal rhythms have been broadly described and all play a critical role for addressing proper sample collection but also for interpreting test results (1, 2). This is the typical case of serum cortisol in Cushing's disease, as it is widely recognized that the 24 h cortisol secretory pattern is characterized by a lack of normal circadian variation (3).

In the analytical phase, appreciation of biological variation represents precious information for setting reliable quality specifications (4, 5). This principle, originally proposed by Cotlove et al. (6), has been thoroughly investigated by Fraser and Harris (7) and finally endorsed by the 1999 International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the International Union of Pure and Applied Chemistry (IUPAC) and the World Health Organization (WHO) "*Stockholm Conference Hierarchy*" (8). Although quality specifications based on how analytical quality affects medical decision-making are at the top of the hierarchy, this approach is difficult to apply because few tests are used in single, well-defined clinical situations and with standardized medical strategies that are directly related to the test results. Conversely, data on biological variation and related analytical quality specifications are available for a large number of laboratory tests (9). Finally, in the post-analytical phase, knowledge of underlying biological variation of quantities examined in medical laboratories plays a central role in understanding the proper generation and application of traditional population-based reference values (10). In a seminal article published in 2004 in this *Journal*, Fraser (11) emphasized how the awareness of the biological changes that occur over the life span is a necessary prerequisite for deciding whether stratification of reference values according to age, gender, race and other demographical variables is likely to be necessary. Moreover, studies carried out over the last three decades on inherent random biological variation have provided significant insights into serious intrinsic problems associated with conventional population-based reference values, thus supporting the need for revising the concept of reference values (12).

Quantitative data on within- and between-subject components of biological variation have revealed that most quantities of interest in laboratory medicine are characterized by a marked individuality and a quantitative measure is the index of individuality (II), originally proposed by Harris (13) in 1981. Individuality provides an indisputable argument for the more appropriate use of individual-specific reference values, especially when monitoring individuals over time. The acknowledgment that changes in serial results of an individual may originate from clinical improvement or deterioration of health status, but might also be due to the three inherent sources of variation (pre-analytical, analytical and within-subject biological variation), led Harris and Yasaka (14) to develop and introduce the concept of reference change values (RCV). In this issue, we are delighted to publish an article on reference change values, written by the most widely recognized expert in this field, Callum G. Fraser (15).

Although the concept of RCV is simple and its calculation extremely straightforward because clinical laboratories are aware of their analytical coefficient of variation (CVA) and within-subject biological variation (CVI) estimates are available for a large number of analytes, some drawbacks and problems have been raised by Cooper et al. (4) to explain the unwarranted delay in its application in clinical laboratory practice. The great value of this paper, along with an outstanding ability to summarize and ease the comprehension of the body of knowledge gathered so far on this

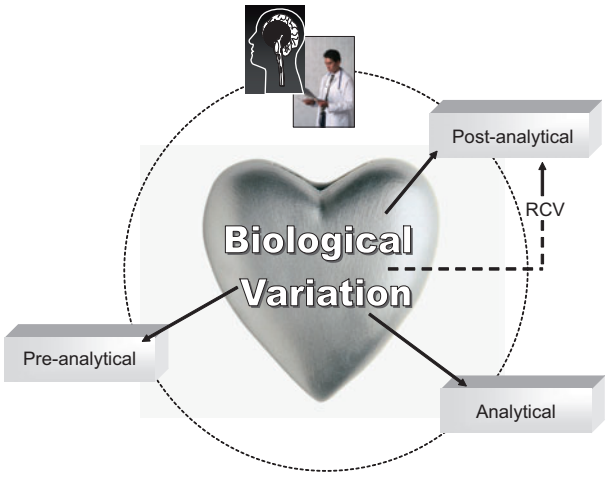

Figure 1 Biological variation, RCV and total testing process.

topic, is to provide new insights on a variety of interesting means of improvement of the RCV calculation, such as the use of log-normal transformation to obtain relevant unidirectional RCV as well as the increasing need to measure it as contained in recent clinical practice guidelines for a large number of pathologies. Some recent applications of the RCV further highlight the importance of closing the gap between the theoretical basis and the application of this concept in practice. The first paradigmatic example is the use of RCV for interpreting results of high-sensitive cardiac troponin (hs-cTn) results, particularly in patients in whom hs-cTn values increase from low concentrations (16). Other important examples include the use of RCV in athletes for establishing reliable thresholds in the never-ending fight against doping (17), for assessing the metabolic response to the therapy in diabetics (18) or for defining pharmacokinetic, pharmacogenetic and pharmacodynamic individual properties in therapeutic drug monitoring (19).

The renewed focus on RCV and biological variation still represents a crucial issue for improving the effectiveness of laboratory information, the clinical decision-making process and, ultimately, the clinical outcomes. In particular, the RCV can thus be considered an objective tool for evaluating the clinical significance of differences in serial results from an individual. It does not represent, however, the final curtain, but just an essential piece of the challenging puzzle that is laboratory diagnostics.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Haeckel R, Wosniok W. A new concept to derive permissible limits for analytical imprecision and bias considering diagnostic requirements and technical state-of-the-art. Clin Chem Lab Med 2011;49:623–35.
2. Banfi G, Del Fabbro M. Biological variation in tests of hemostasis. Semin Thromb Hemost 2009;35:119–26.
3. Alexandraki KI, Grossman AB. Novel insights in the diagnosis of Cushing's syndrome. Neuroendocrinology 2010;92(Suppl 1): 35–43.
4. Cooper G, DeJonge N, Ehrmeyer S, Yundt-Pacheco J, Jansen R, Ricós C, et al. Collective opinion paper on findings of the 2010 convocation of experts on laboratory quality. Clin Chem Lab Med 2011;49:793–802.
5. Lippi G, Plebani M. Athlete's biological passport: to test or not to test? Clin Chem Lab Med 2011;49:1393–5.
6. Cotlove E, Harris EK, Williams GZ. Biological and analytic components of variation in long-term studies of serum constituents in normal subjects. 3. Physiological and medical implications. Clin Chem 1970;16:1028–32.
7. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27:409–37.
8. Fraser CG, Kallner A, Kenny D, Petersen PH. Introduction: strategies to set global quality specifications in laboratory medicine. Scand J Clin Lab Invest 1999;59:477–8.
9. Ricós C, Alvarez V, Cava F, García-Lario JV, Hernández A, Jiménez CV, et al. Current databases on biological variation: pros, cons and progress. Scand J Clin Lab Invest 1999;59:491–500.
10. Plebani M, Lippi G. Improving the post-analytical phase. Clin Chem Lab Med 2010;48:435–6.
11. Fraser CG. Inherent biological variation and reference values. Clin Chem Lab Med 2004;42:758–64.
12. Henny J, Peticlerc C, Fuentes-Arderiu X, Hyltoft Petersen P, Queralto JM, Schiele F, et al. Need for revising the concept of reference values. Clin Chem Lab Med 2000;38:589–95.
13. Harris EK. Statistical aspects of reference values in clinical pathology. Prog Clin Pathol 1981;8:45–66.
14. Harris EK, Yasaka T. On the calculation of a "reference change" for comparing two consecutive measurements. Clin Chem 1983;29:25–30.
15. Fraser CG. Reference change values. Clin Chem Lab Med 2011 [Epub ahead of print].
16. Christ M, Bertsch T, Popp S, Bahrmann P, Heppner HJ, Müller C. High-sensitivity troponin assays in the evaluation of patients with acute chest pain in the emergency department. Clin Chem Lab Med 2011 [Epub ahead of print].
17. Zorzoli M. The Athlete Biological Passport from the perspective of an anti-doping organization. Clin Chem Lab Med 2011;49:1423–5.
18. Lippi G, Targher G. Glycated hemoglobin (HbA1c): old dogmas, a new perspective? Clin Chem Lab Med 2010;48:609–14.
19. Wallemacq PE. Therapeutic monitoring of immunosuppressant drugs. Where are we? Clin Chem Lab Med 2004;42:1204–11.

Mario Plebani[1,*]
Giuseppe Lippi[2]
[1]Department of Laboratory Medicine, University-Hospital, Padova, Italy
[2]U.O. Diagnostica Ematochimica, Dipartimento di Patologia e Medicina di Laboratorio, Azienda Ospedaliero-Universitaria di Parma, Italy

*Corresponding author: Professor Mario Plebani, Department of Laboratory Medicine, University-Hospital of Padova, Via Giustiniani 2, 35128, Padova, Italy
Phone: +39 49 8212792, Fax: +39 49 663240,
E-mail: mario.plebani@unipd.it