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From Population Averages to Individualized Care: How European Biological Variation Study (EuBIVAS) is Redefining Biological Variation in Clinical Biochemistry and Laboratory Medicine - A Review and Editorial Perspective

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A B S T R A C T

Background: Distinguishing true pathological changes from normal physiological fluctuation in serial laboratory results are fundamental to personalized medicine. Biological variation (BV)- comprising within-subject (CVI) and between-subject (CVG) variations- provides the statistical foundation for calculating reference change values (RCVs), establishing analytical performance specifications (APS), and interpreting patient-specific trends. However, historical BV data have been plagued by methodological heterogeneity, small sample sizes, and inadequate preanalytical standardization.

Content: The European Biological Variation Study (EuBIVAS) was conceived as a landmark prospective multicenter investigation to address these deficiencies. Enrolling 91 apparently healthy adults across six European centers, EuBIVAS implemented weekly blood sampling for 10 consecutive weeks under strictly harmonized conditions. To date, this ambitious project has generated high-quality, in accordance with Biological Variation Data Critical Appraisal Checklist (BIVAC) -compliant BV estimates for over 80 measurands- including routine chemistry analytes, enzymes, proteins, endocrine biomarkers, tumor markers, cardiac troponin I, hematological parameters, bone markers, serum iron, and insulin-resistance indices. Notably, EuBIVAS has provided the first-ever BV data for ten measurands, including non-HDL cholesterol, neuron-specific enolase, and intact fibroblast growth factor-23.

Editorial perspective: EuBIVAS represents a paradigm shift in BV research, establishing a new methodological gold standard. Nevertheless, critical appraisal reveals important limitations: the small postmenopausal subgroup (n=10) limits reliable sex-stratified analyses; the classical Cross-Validated Analysis of Variance (CV-ANOVA) model proved inadequate for non-steady-state measurands such as 25-hydroxyvitamin D; and the exclusive focus on healthy European adults restricts generalizability to pediatric, geriatric, and diverse ethnic populations.

Conclusions: EuBIVAS provides a strong foundation for the interpretation of serial laboratory testing. Further studies in diverse demographic and clinical populations, as well as practical implementation in laboratory information systems, are necessary to maximize its clinical value. Although, EuBIVAS is not the final word on biological variation, however, it is an essential foundation for the future of clinical biochemistry and personalized laboratory medicine.

Keywords: Biological variation; Reference change value; Analytical performance specifications; Laboratory medicine; Index of individuality

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Introduction

In the daily practice of laboratory medicine, clinicians routinely compare a patient's results to population-based reference intervals. While this approach works well for initial diagnosis, it has limitations when the clinical question shifts from "Is this result abnormal?" to "Has this patient's condition changed?" Consider a patient with mildly elevated cardiac troponin who returns two hours later—is the slight increase real or just a random fluctuation? Or a diabetic patient monitoring hemoglobin A1c—is a 0.5% drop clinically meaningful or merely noise? Interestingly, answering these questions requires understanding not just analytical precision, but also the normal biological variability inherent to every individual (1). This is where biological variation (BV) becomes indispensable. BV encompasses two complementary components: within-subject variation (CVI), representing the natural fluctuation around an individual's homeostatic set point state, and between-subject variation (CVG), at physiologic states reflecting differences in average concentrations across a healthy population (2). Together, these parameters enable three critical clinical applications. First, they allow calculation of reference change values (RCVs)—the minimum difference between two serial results that signifies true change rather than analytical or biological noise (1-3). Second, they provide the foundation for analytical performance specifications (APS), ensuring that laboratory assays are sufficiently precise to detect clinically meaningful changes (4). Third, they guide the interpretation of personalized reference intervals and the number of samples needed to estimate an individual's homeostatic set point (1, 5). Despite their clinical importance, historical BV data have been plagued by serious methodological shortcomings. Many studies were underpowered due to small sample sizes, incomplete participant characterization, too few repeated samples, heterogeneous preanalytical conditions, and inconsistent statistical approaches (4, 6, 7). The result was a fragmented and often unreliable evidence base that undermined the very applications BV was meant to support. Recognizing this crisis, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) convened its 1st Strategic Conference in 2014, concluding that new high-quality BV studies were urgently needed (1, 4). The European Biological Variation Study (EuBIVAS) was the direct response to this call. Designed as a prospective multicenter investigation involving 91 apparently healthy adults across six European centers, EuBIVAS implemented weekly blood sampling for 10 consecutive weeks under rigorously standardized preanalytical and analytical protocols (1, 5). The study adhered to the BIVAC criteria, achieving the highest quality grade "A" (1). To date, EuBIVAS has generated original empirical BV estimates for over 80 measurands, including routine chemistry analytes, enzymes, proteins, endocrine biomarkers, tumor markers, cardiac troponin I, hematological parameters, bone markers, serum iron, and insulin-resistance indices (1, 5).

This review summarizes the key findings of EuBIVAS, discusses their implications for clinical practice, critically examines the study's limitations, and outlines future directions for BV research and implementation. By understanding what EuBIVAS has achieved—and what it has not—laboratory professionals can apply its results judiciously and contribute to the ongoing evolution of personalized laboratory medicine.

Biological Variation and RCVs

Within-subject biological variation represents normal fluctuation around an individual’s homeostatic set point at a physiologic state. However, between-subject biological variation represents differences in average homeostatic concentrations among healthy individuals (1). The index of individuality (II) is an indicator for comparing the intensity of variation of a test result between its reference interval and within-subject biological variations. The II may be calculated as:

$$II = \frac{\sqrt{CV_A^2 + CV_I^2}}{CV_G}$$

where CVA is analytical variation. When CVI is relatively low compared with CVG, population-based reference intervals may have limited sensitivity for detecting clinically significant changes in an individual patient (1), and RCV would be important in this situation. The RCV estimates the minimum difference between two serial results that is unlikely to be caused solely by analytical and biological variation to help clinicians recognize meaningful changes earlier and more consistently in an individual patient. The RCV may be calculated as:

$$RCV = \sqrt{2} \times Z \times \sqrt{CV_A^2 + CV_I^2}$$

where Z is the selected probability threshold (1.96 for a confidence level of 95%). Reliable RCVs depend on robust CVI estimates and on local estimates of analytical imprecision of CVA calculation by implementing internal quality control (IQC) programs using control materials (1). Non-Gaussian distributions, outliers, low concentrations, and heterogeneous variances can influence BV estimates and RCV calculations. Therefore, appropriate statistical analysis is an essential component of BV research (1).

EuBIVAS Design

EuBIVAS was coordinated as a prospective multicentre study involving 91 apparently healthy adults recruited through six European laboratories. Serum and plasma samples were collected weekly for 10 consecutive weeks, enabling separation of within-subject, between-subject, and analytical sources of variation. The EuBIVAS protocol standardised participant selection, fasting state, blood-collection timing, specimen handling, processing, storage, and analytical procedures. These measures were intended to minimise preanalytical variation that could otherwise be falsely attributed to physiological fluctuations (1). The design included assessment of outliers, temporal trends, participant heterogeneity, and distributional characteristics before BV estimation. This framework improved the reliability of reported data and created a model for future BV studies (4, 6, 7).

Routine Chemistry

EuBIVAS has generated BV estimates for common chemistry analytes, including electrolytes, glucose, lipid-related measurements, urea, uric acid, total protein, total bilirubin, and direct bilirubin. These analytes are frequently used for monitoring metabolic status, renal function, liver disease, hydration, and cardiovascular risk. The EuBIVAS findings support assay-specific evaluation of analytical performance and more reliable serial-result interpretation. The reported results can also guide laboratories in determining whether analytical variation is sufficiently low to detect clinically meaningful changes in individual patients. Serum creatinine was evaluated by enzymatic and alkaline-picrate methods. Although estimates of within-subject variation were similar, the alkaline-picrate method had poorer analytical precision, demonstrating that method performance can materially affect the clinical usefulness of serial creatinine testing. Serum iron was also evaluated through EuBIVAS. Because serum iron can be affected by physiological fluctuations and preanalytical conditions, robust BV estimates are valuable for interpreting repeat measurements and assessing the reliability of longitudinal iron-status assessment.

Enzymes and Proteins

EuBIVAS produced BV data for nine serum enzymes, providing a more reliable evidence base for interpretation of enzyme activity changes in clinical practice. Enzyme activities are influenced by physiological factors such as exercise, circadian rhythms, organ function, skeletal-muscle activity, and sample handling; therefore, standardised longitudinal data are particularly valuable. The

project also generated BV estimates for 15 frequently measured proteins, including albumin, alpha-1-acid glycoprotein, alpha-1-antitrypsin, beta-2-microglobulin, ceruloplasmin, complement components C3 and C4, C-reactive protein, cystatin C, haptoglobin, immunoglobulins, soluble transferrin receptor, and transferrin. These proteins are relevant to inflammation, nutritional status, renal assessment, immune disorders, complement activation, and iron metabolism. EuBIVAS provides values for CVI, CVG, RCVs, and BV-derived APS that may improve their longitudinal interpretation.

Endocrine and Metabolic Markers

EuBIVAS has generated BV estimates for thyroid-stimulating hormone, free triiodothyronine, free thyroxine, thyroglobulin, and calcitonin. These markers are used in the diagnosis and monitoring of thyroid dysfunction, treatment response, thyroid cancer surveillance, and calcitonin-related endocrine assessment. The thyroid study found that some BV estimates were lower than historical estimates, which resulted in lower calculated RCVs and more demanding APS. This finding may affect how laboratories assess the suitability of thyroid assays for longitudinal clinical monitoring (1). Biointact parathyroid hormone was evaluated using EuBIVAS samples. This marker is relevant to primary and secondary hyperparathyroidism, chronic kidney disease-mineral and bone disorder, vitamin D-related disorders, and calcium-phosphate homeostasis (8). EuBIVAS has also investigated biological variation of insulin-resistance markers. These data are relevant to the longitudinal assessment of fasting insulin and insulin-resistance indices in metabolic research and clinical evaluation of obesity, prediabetes, and type 2 diabetes risk (8). A recent EuBIVAS investigation also examined BV for testosterone, follicle-stimulating hormone, prolactin, luteinising hormone, and dehydroepiandrosterone sulphate in men. These data may support more accurate interpretation of serial hormonal testing in male reproductive and endocrine disorders (8).

Bone and tumour markers

EuBIVAS has provided BV data for bone turnover biomarkers, including the beta-isomerised C-terminal telopeptide of type I collagen, the N-terminal propeptide of type I collagen, osteocalcin, intact fibroblast growth factor 23, and uncarboxylated-unphosphorylated matrix Gla protein. These biomarkers are used for the diagnosis of osteoporosis, metabolic bone disease, and chronic kidney disease-related mineral disorders, as well as treatment monitoring. Their interpretation may benefit from RCVs because clinically significant change can occur even when individual results remain within conventional reference limits. Tumour-marker BV estimates have also been reported by EuBIVAS samples, including cancer antigen 125, cancer antigen 15-3, cancer antigen 19-9, carcinoembryonic antigen, cytokeratin-19 fragment, alpha-fetoprotein, and human epididymis protein 4 (5). These data may assist in interpreting serial tumour-marker changes. However, tumour markers should always be interpreted together with clinical findings, imaging, pathology, and disease-specific diagnostic or surveillance protocols (5).

Cardiac and Haematological Data

EuBIVAS-based research evaluated weekly biological variation of cardiac troponin I using two high-sensitivity assays. The reported estimates varied according to the assay and the number of samples with measurable low-concentration values, illustrating the method-specific nature of some BV applications. This finding is clinically important because high-sensitivity troponin measurement is increasingly used in acute and chronic cardiovascular care. Laboratories should avoid transferring troponin RCVs across platforms without method-specific validation. Haematological BV studies have estimated within-subject and between-subject variation for red-cell, reticulocyte, platelet, and leukocyte-related parameters. These results may support improved interpretation of serial complete blood count measurements (9). However, haematological results are strongly influenced by

infection, inflammation, bleeding, hydration, altitude, medication use, nutritional status, and bone-marrow activity. Therefore, BV values should be used only in stable clinical conditions and should not replace clinical interpretation.

Editorial Perspective

EuBIVAS is a landmark initiative because it applies rigors methodological protocols to biological-variation research. Its prospective multicenter design, repeated sample collection, standardised preanalytical conditions, and systematic statistical evaluation address major weaknesses seen in many historical BV studies (4, 6, 7). The project provides an internally coherent set of BV estimates across multiple classes of measurands. This allows laboratories to use a stronger evidence base when establishing RCVs, evaluating assay precision, and interpreting longitudinal changes in patient results (9-20). Nevertheless, EuBIVAS estimates should not be interpreted as universal biological constants. The original cohort consisted of apparently healthy adults from European countries. Age, sex, ethnicity, pregnancy, lifestyle, medication use, chronic disease, renal function, inflammation, and endocrine status may influence biological variation. Analytical procedure characteristics can also affect the applicability of EuBIVAS data. Calibration, analytical precision, assay specificity, reagent lot performance, detection limits, interference, and measurement range should all be considered before a laboratory applies a published BV estimate to local clinical practice. Despite its many strengths, the EuBIVAS study has several important limitations. First, the small number of postmenopausal women (only 10 participants over age 50) limited the reliability of subgroup analyses for this population, meaning that any observed differences between pre- and postmenopausal women cannot be characterised with confidence. Second, the classical CV-ANOVA model proved inappropriate for measurands that are not in steady state; for example, more than 50% of the data had to be eliminated for 25-hydroxy vitamin D due to seasonal trends. For this model to work correctly, the data must be in a "steady-state", meaning the concentration is not systematically trending up or down over the sampling period – and even after multiple of median (MoM) transformation, over 40% of data still required exclusion, ultimately leading to the conclusion that the BV model cannot be used to set analytical performance specifications for this measurand. Third, for acute-phase proteins such as haptoglobin and CRP, a high proportion of results (up to 20%) were excluded due to subclinical inflammatory episodes detected during the 10-week collection period, which may cause underestimation of biological variation and limit the generalisability of these estimates. Fourth, the study population consisted only of healthy European adults, so the data cannot be directly applied to children, the elderly, or patients with relevant disease states. Finally, while EuBIVAS achieved BIVAC grade A, it was still a single multicentre study, and for some measurands where few other high-quality studies exist, the estimates are heavily weighted by this one dataset, which may not fully capture global population diversity. These limitations highlight that the classical BV approach is not universally applicable and that alternative strategies – such as state-of-the-art models or clinical outcome-based specifications – are necessary for certain measurands.

Future Directions

Future BV investigations should apply EuBIVAS-level methodological standards to paediatric, geriatric, pregnant, ethnically diverse, and disease-specific cohorts. Priority populations include patients with diabetes, obesity, chronic kidney disease, endocrine disorders, cardiovascular disease, cancer, inflammatory disease, and osteoporosis. New BV studies are also needed for emerging technologies, including high-sensitivity immunoassays, mass-spectrometry-based methods, molecular diagnostics, proteomic tests, metabolomic profiles, and digital biomarkers. Laboratories should translate EuBIVAS findings into validated RCVs that incorporate local analytical imprecision. Implementation through laboratory information systems, delta-check rules,

graphical trend reports, and interpretative comments may help clinicians recognise meaningful changes earlier and more consistently.

Conclusion

EuBIVAS is not the final word on biological variation, but it is an essential foundation for the future of personalised laboratory medicine. By providing robust, empirically derived BV estimates, EuBIVAS equips laboratories and clinicians with tools to detect meaningful changes earlier, reduce unnecessary testing, and improve patient outcomes. Realising this potential requires continued research in diverse populations, integration of RCVs into laboratory information systems, and widespread acceptance of BV-derived APS in quality assurance programmes.

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Declaration of Generative AI and AI-assisted technologies in the writing process

No AI writing assistance was utilized in the production of this manuscript.

Conflict of interest

The author has no competing interests to disclose.

Ethical considerations

This study was a review based on publicly available published data and did not require ethical approval.

Data availability statement

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Sincerely,
Dr Ali Noori-Zadeh
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