Review

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Biological variation: recent development and future challenges

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Abstract: Biological variation (BV) data have many applications in laboratory medicine. However, these depend on the availability of relevant and robust BV data fit for purpose. BV data can be obtained through different study designs, both by experimental studies and studies utilizing previously analysed routine results derived from laboratory databases. The different BV applications include using BV data for setting analytical performance specifications, to calculate reference change values, to define the index of individuality and to establish personalized reference intervals. In this review, major achievements in the area of BV from last decade will be presented and discussed. These range from new models and approaches to derive BV data, the delivery of high-quality BV data by the highly powered European Biological Variation Study (EuBIVAS), the Biological Variation Data Critical Appraisal Checklist (BIVAC) and other standards for deriving and reporting BV data, the EFLM Biological Variation Database and new applications of BV data including personalized reference intervals and measurement uncertainty.

Keywords: biological variation; BIVAC; EuBIVAS; personalized reference intervals (prRI); reference change value.

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Background

Biological variation (BV) describes the variation observed in the concentration or activity of different constituents in a person, reflecting the regulation by homeostatic processes in the body [1]. In a steady state setting, the concentration of most measurands is characterized by random variation around a homeostatic set point, whereas the concentration of some measurands is also influenced by different life phases or predictable cyclic variation. The within-subject variation BV (CV_I) denotes the variation of the concentration/activity of a measurand around a homeostatic set point within a single individual in steady state, whereas the between-subject BV (CV_G) denotes the variation between the homeostatic set points of different individuals.

BV data have many different applications in laboratory medicine. A major use of BV data is for setting analytical performance specifications (APS) for imprecision, bias, total error and measurement uncertainty. These and other characteristics can be established on estimates of within- and between-subject BV, utilizing different formulae. The utility of the conventional population-based reference intervals can be assessed by the index of individuality (II), which usually is calculated as the ratio between CV_I and CV_G. Estimates of CV_I and analytical imprecision can also be used to calculate reference change values (RCVs) to assess the probability that a difference between two consecutive results in an individual can be explained by analytical and within-subject biological variation [1]. Also, a model for calculating personalized reference intervals (prRI) based on BV data has recently been published [2]. This utilizes previous test results of a subject in a steady-state condition and

estimates of the individual's BV, derived either for the relevant population or for the individual.

Different sources of BV data have been available in the last decades [1]. In the 1st European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Strategic Conference in 2014, it was, however, recognised that much of the available BV data were compromised because of uncertainty around the estimates or other factors affecting fitness for purpose. Thus, this limited the utility of these data for the many different BV applications. Furthermore, it highlighted the need for critical appraisal of existing BV data as well as for new studies to generate high-quality data [3]. As a result of the Strategic Conference, different EFLM Working Groups and Task Groups set in motion initiatives to improve on the availability of quality-assessed BV data and their applications [4]. In this review, an update on the achievements and developments driven by the EFLM Working Group for Biological Variation (WG-BV) [5], the EFLM Task Group for the Biological Variation Database (TG-BVD) [6] and other key players in the field of BV the last 10 years will be presented, with suggestions for future developments, focussing on the following:

1. New mathematical models to calculate BV estimates.
2. Delivery of high-quality BV data from the highly powered European Biological Variation Study (EuBIVAS).
3. The Biological Variation Data Critical Appraisal Checklist (BIVAC), a standard for evaluating BV publications.
4. The EFLM Biological Variation Database.
5. Standards for reporting of BV data and BV terminology.
6. New applications of BV data.

New mathematical models to calculate BV estimates

BV studies have historically been undertaken as prospective experimental studies. The methodology for this was first described by Fraser and Harris [7]. In essence, a group of reference individuals is selected and sampled at regular time intervals, with a strictly controlled preanalytical phase and then analysed under standardized conditions. Duplicate analysis of the samples is recommended to estimate the CV_A component directly. The resulting data are examined for clinical events, trends and statistical outliers as well as homogeneity. The CV_A, CV_I, and CV_G estimates are thereafter derived by traditional statistical approaches such as e.g. ANOVA. More details about this study design and traditional calculations can be found in [8].

The last decade, three new approaches have been developed to estimate BV components. The two first models, CV-ANOVA [9] and the Bayesian approach [10], are based on data derived from experimental studies, in principle using the study design of Fraser and Harris [8] to estimate BV components. The third model uses retrospectively collected data, extracted from laboratory databases, for data mining studies [11–13].

The CV-ANOVA approach to calculate BV estimates

Different ANOVA methods have over time been used to derive BV data, historically mostly standard ANOVA and ln-ANOVA. The standard ANOVA, based on a nested ANOVA approach, is performed on the raw data, after exclusion of outliers, ensuring a steady state situation by trend analysis and assessment of variance homogeneity. The resulting within-subject standard deviation (SD_I) estimate is divided by the total mean to derive the CV_I estimate. In the ln-ANOVA method, data are first ln-transformed and the nested ANOVA is performed on the transformed results. The estimated components from the ANOVA are then transformed back and become CV values on the original scale. A new ANOVA-method, a CV-ANOVA method – the Røraas method – was published in 2016 [9] and has since then been widely used. It is based on CV transformation with normalization/standardisation of each person's data by dividing by that person's mean value, and then performing ANOVA on these results, which provides estimates of analytical and within-subject variation in the form of CV values. However, this does not provide an estimate of the CV_G, since each individual has a mean value of 1. Thus, by this approach, the CV_G must be estimated by the standard or alternatively by the ln-ANOVA approach, if data are not normally distributed. These three different methods, standard ANOVA, ln-ANOVA and CV-ANOVA have been compared in computer simulations for different distributions of raw data [9], i.e. normal distribution, ln-normal distribution and mirrored ln-normal distribution. The CV-ANOVA method performed well for all types of distributions, as opposed to the other ANOVA methods.

The Bayesian approach of calculating BV estimates

An approach utilizing Bayesian statistics to derive BV data, based on the same experimental study design as that recommended by Fraser and Harris [7], was published by Røraas in 2019 [10]. This Bayesian model disregards the assumption of normality and is more robust to extreme

observations by using adaptive Student-*t* distributions instead of normal distributions. The advantage over traditional methods such as ANOVA is that laborious statistical operations, associated with possible subjectivity in data trimming to achieve homogeneity and exclusion of outliers, are not required. Furthermore, the Bayesian model delivers individual within-person BV estimates (CV_P) that can be used to explore heterogeneity of data, asses if the data can be generalised for the whole population, assess relevant subgroups or to identify individuals not belonging to the group. It is then also possible to assess correlations between the CV_P and e.g. age or homeostatic set points and to calculate personalized reference intervals, prRI, directly, as described later. The model utilizes, if available, prior knowledge to make more precise inference from previous performed studies. This is particularly valuable if previous data on BV for the measurand, or related measurands, are available. Some studies that have applied both ANOVA and Bayes methods on the same data set have reported obtaining similar BV results with both methods, however, this may depend on distribution of the data [10, 12].

Using previously analysed data derived from laboratory information management systems to calculate BV estimates

This model uses a different approach for the generation of BV data, unlike the two previous models, as it utilizes already available data, analysed as part of routine follow-up of patients, held in laboratory databases. This is achieved by extracting results from patient cohorts from laboratory information management systems (LIMS) consisting of a large number of individuals, where two or more samples routinely have been analysed for the same measurand. Assessment of such data collected for diagnostic or monitoring purposes also provides an opportunity to assess subgroups including different states of health, the effect of time between sampling, or other factors, without the efforts of prospectively collecting large data sets. For many analytes requested for a patient, the concentrations of the analytes are not impacted by non-relevant pathology and may represent values obtained for the healthy population. This "big data" or "data mining" approach is particularly useful if the measurand in question is not present in apparently healthy subjects (e.g. unusual proteins found in myeloma), if the concentration of the measurand is significantly different from what is found in healthy individuals (e.g. HbA_{1c} in diabetes mellitus) or if it is unacceptable or unethical to collect specimens from individuals, for example children. Studies detailing utilizing data derived from laboratory databases for estimating e.g. CV_I and RCVs for e.g. routine chemistry, endocrine and haematology tests have reported results more or less equivalent to those delivered by standard, prospective methods [11, 12, 14, 15]. However, most such studies do not report measures of uncertainty, which is a great limitation. However, one recently published study has now proposed how this can be done [15]. Further work is required to identify the strengths and limitations of this retrospective, data mining approach to deliver BV data and to meet challenges in measurand data distributions (e.g. skewed data), and other aspects allowing to fine-tuning this approach further.

Delivery of high-quality BV estimates – the European Biological Variation Study (EuBIVAS)

The EFLM WG-BV decided in 2014 to design and establish the EuBIVA with the aim of providing updated high-quality BV estimates for many measurands, derived from a highly powered and rigorously executed BV study [16]. Briefly, EuBIVAS involved six European laboratories (Milan, Italy; Bergen, Norway; Madrid, Spain; Padua, Italy; Istanbul, Turkey; Assen, The Netherlands). Following a detailed screening of enrolled participants, 91 healthy volunteers (38 males and 53 females; age range, 21–69 years) were included in the study. All involved laboratories followed the same protocol for the pre-analytical phase, with all participants compiling an enrolment questionnaire to verify their health status and to collect information regarding their lifestyle. Fasting blood samples were drawn for 10 consecutive weeks at each participating laboratory. The samples were stored at −80 °C until shipped on dry-ice and analysed in duplicate for a high number of measurands at the San Raffaele Hospital in Milan [16].

BV estimates for all participants, males, females, and if considered relevant for the specific measurands for female subgroups (above and below 50 years, in reflection of menopausal status), were for most EuBIVAS measurands estimated by the CV-ANOVA method [9]. The EuBIVAS approach presents a number of benefits. Firstly, it is sufficiently powered to enable subgroup analysis. This has allowed gender specific data and for some measurands also menopausal age specific data [17–24] which until this point have been unavailable for many measurands. Secondly, the BV estimates have been obtained based on current best-practice recommendations for study design [25, 26]. EuBIVAS has so far delivered BV estimates for 81 different

measurands; with data for two target measurands (cardiac troponin and serum creatinine) obtained using two different analytical methods [20, 27]. The results of most of the studies are summarized in [28]. For 10 of these measurands, no previous BV studies had been carried out and further studies for measurands for where there as of yet are no available BV data, are also on the way.

For haematology measurands, given the requirement for fresh whole blood, the analytical approach used by EuBIVAS was not possible. Two different projects, in Italy [29–31] and Turkey [32], have been carried out to deliver high-quality BV data for complete blood count, utilizing fresh samples. While the analytical approach necessarily varies from that employed within EuBIVAS, all other elements of these studies were similar. In addition to updating the BV estimates of complete blood count parameters currently in use, these studies also provided BV data for some parameters where this was lacking.

The EuBIVAS study might be described as an exemplar of the classical approach to delivery and reporting for BV data. It has delivered BV estimates for many measurands that are lower than those previously reported. This is probably due to tight control of pre-analytical factors, the use of modern examination methodology, and the critically important application of correct statistical approach to data handling with e.g. assessment of outliers, variance homogeneity and trend, according to best practice recommendations. The absence of clear differences between the subject cohorts from Turkey, Norway, The Netherlands, Spain, and Italy, confirms that the obtained data are transportable across health care systems indicating that they may be used to deliver APS for systems to be used internationally. It is reassuring that this consistency was also demonstrated using principal component analysis, an unsupervised machine learning approach [33]. It should be noted that a possible disadvantage of the meticulously generated EuBIVAS data could be that they are “too good”, having been delivered under rigorously controlled conditions that do not reflect routine practice. The EuBIVAS estimates may thus be more useful for setting APS than for RCV calculations or to calculate personalized reference intervals. Instead, data mining from results in LIMS might deliver BV data that more accurately reflect the “real life” situation that can be used for e.g. RCV [14] and personalized reference intervals. Furthermore, there is still a great lack of studies on different age groups and states of health, even though high-quality studies for population subgroups such athletes and pregnant women have also been published in the last 10 years [34–39]. For many states of health, however, it may be that using the approach of extracting data from LIMS is a more pragmatic approach to delivery of required data sets, rather than highly powered experimental studies like the EuBIVAS.

The Biological Variation Data Critical Appraisal Checklist (BIVAC)

A high number of BV studies has been published in the last four decades. Though historical BV studies were typically designed according to that time’s standard, many do not to fulfil today’s standard for study design and execution, or they utilized analytical methods that are now considered obsolete. Thus, much of the historical BV data may be compromised with uncertainty or considered unfit for use today. Following the 1st Strategic Conference of EFLM defining APS in November 2014, the EFLM TG-BVD was established within the WG-BV, with the objective to appraise the quality of BV data that is publicly available. The result of this work was, among others, the Biological Variation Data Critical Appraisal Checklist (BIVAC), a standard for evaluating BV studies [25]. The BIVAC is designed to assess the quality of BV publications by addressing essential elements that may impact upon veracity and utility of the BV estimates. It consists of 14 quality items and focuses on the effect of study design, the measurement procedure and statistical handling of data on BV estimates. The individual quality items can be awarded scores A, B, C and for some essential items, also D, indicating decreasing compliance with the checklist. The lowest score obtained for any quality item decides the overall grade. A BIVAC grade A indicates that the publication is fully compliant with all BIVAC quality items. If the lowest score for any quality item is a B, then the overall grade is a B and similarly C or D if the lowest score is a C or D, respectively. Studies receiving a D grade are not considered fit for purpose. Systematic reviews of BV studies for many different measurands have shown that the majority of historical studies receive a BIVAC grade C, mostly related to statistical items such as analysis of variance homogeneity and outliers [23, 40–48]. Since the publication of BIVAC in 2017, however, an increasing number of BV studies fulfil the criteria for a high-quality study and are fully BIVAC compliant, as illustrated by the papers included in the Clinical Chemistry and Laboratory Medicine special issue on BV in 2022 [4, 49]. The BIVAC also provides, in combination with the Biological Variation Data Reporting Checklist [26], a framework that may help those planning BV studies both to perform and publish their study in an appropriate manner.

The EFLM Biological Variation Database

Historically, different sources of BV data have been available, also online. After the Strategic Conference, one of the main objectives of the WG-BV and TG-BVD was to establish a new database with quality-assessed BV data, available to users worldwide. The "EFLM Biological Variation Database" was launched during the EuroMedLab in Barcelona in May 2019 and is available at www.biologicalvariation.eu. To populate the database, systematic literature searches for BV studies for relevant measurands have been performed and identified publications appraised by the BIVAC. Both the BIVAC scores as well as a BV minimum data set, encompassing around 30 descriptive items are published in the database for all included measurands, thus offering a detailed and updated source of quality-assessed data. Also, a meta-analysis approach to pool estimates from BIVAC compliant studies with similar study design to provide global BV estimates was developed [25]. In the EFLM Biological Variation Database, meta-analyses are automatically performed for studies of acceptable study design (BIVAC grade A–C), when performed in healthy adults with more than two samples collected per individual and with biweekly to monthly samplings. The meta-analysis report estimates for CV_I and CV_G with confidence intervals (CI), based on a weighted median approach where the study design as well as the BIVAC grade is taken into account [25]. As of December 2022, global BV results derived by meta-analysis have been published for 139 measurands, as well as several thousand detailed BV data sets and more than 560 BV studies (Figure 1). In addition to presenting BV estimates for each evaluated paper and the summary BV estimates derived by meta-analysis, the database also provides automatic calculation of RCV and the most common APS used in laboratory practice. These applications are available when accessing the global meta-analysis BV estimates (Figure 1) [8].

In today's database, global BV estimates are published based on data derived from healthy adults with biweekly to monthly sampling. However, data from studies performed in different states of health, other age groups and sampling intervals are also included in the database. In the future, meta-analyses will also be provided for these different settings and states of health. As of yet, BV data derived from data mining of retrospectively collected data have not been included in the meta-analysis, as these are, with one very recent exception [15], not accompanied by CI, which is one of the prerequisites to be included. Furthermore, a standard for assessing the quality of such kind of studies is lacking. Such a standard will be developed, prior to including these types of studies in the meta-analysis that provides global BV estimates in the database.

Reporting of BV data and BV terminology

The Biological Variation Data Reporting Checklist

There have historically been no internationally recognized standards for production, reporting and transmission of BV data. The WG-BV published in 2015 the Biological Variation Data Reporting Checklist [26], which identifies key elements required in published BV studies. The reporting checklist is based on the same structure as the STARD [50] and identifies six main items for focus with a number of sub items including 1) title/abstract/keywords, 2) introduction, 3) methods, 4) data analysis, 5) results and 6) discussion. The sub items have been additionally mapped to a minimum data set domains previously identified by the WG.

Studies complying with this checklist and the BIVAC can be considered fit for purpose, include essential statistical analyses such as outlier and variance homogeneity testing, use recommended terminology and report BV estimates accompanied by key metadata. This is essential as these BV data are reference data; they need to be applied with care, with understanding of their provenance and intrinsic characteristics if they are to be transported safely and effectively into clinical practice across health care systems.

The EFLM BV groups have developed further materials to support the delivery and reporting of studies. This work has initially focused on the "classical" prospective experimental approach to delivery of BV data. Access is available online in the form of an interactive mind map [51] and an overview of the structure is presented in Figure 2. The content includes embedded links and documents that can be used to assess the veracity of data from existing BV studies and to enable the design of new BV experiments. It reflects what is believed to be the current state of the art and is in a format that draws parallels with that of STARD to be used as a possible publication structure to guide researchers (Figure 2). This represents an initial iteration as there is a degree of complexity now arising in the subject area as a consequence of the emergence of new approaches to the generation of data sets as described in this review. However,

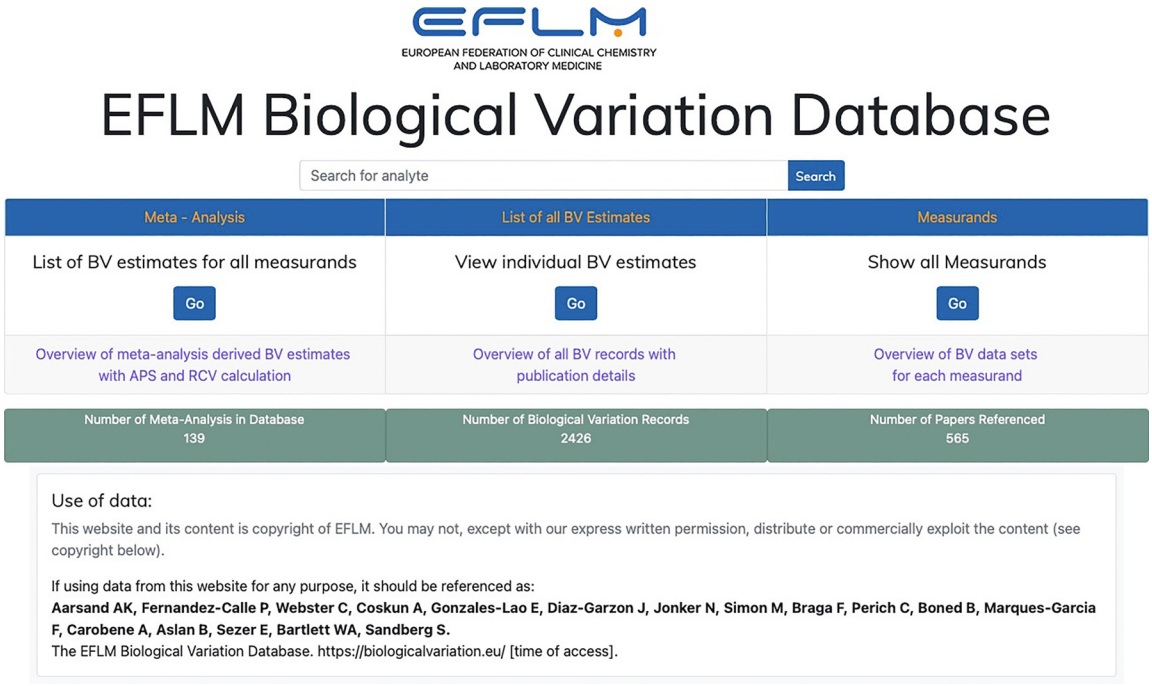

Figure 1: Screenshot of the front page of the online EFLM Biological Variation Database [8]. In this database, detailed information for biological variation data sets derived from critically appraised biological variation studies, as well global CV_I and CV_G from meta-analysis are published and automatically updated whenever new data are added. For each measurand analytical performance specifications (APS) and reference change values (RCV) are also automatically calculated.

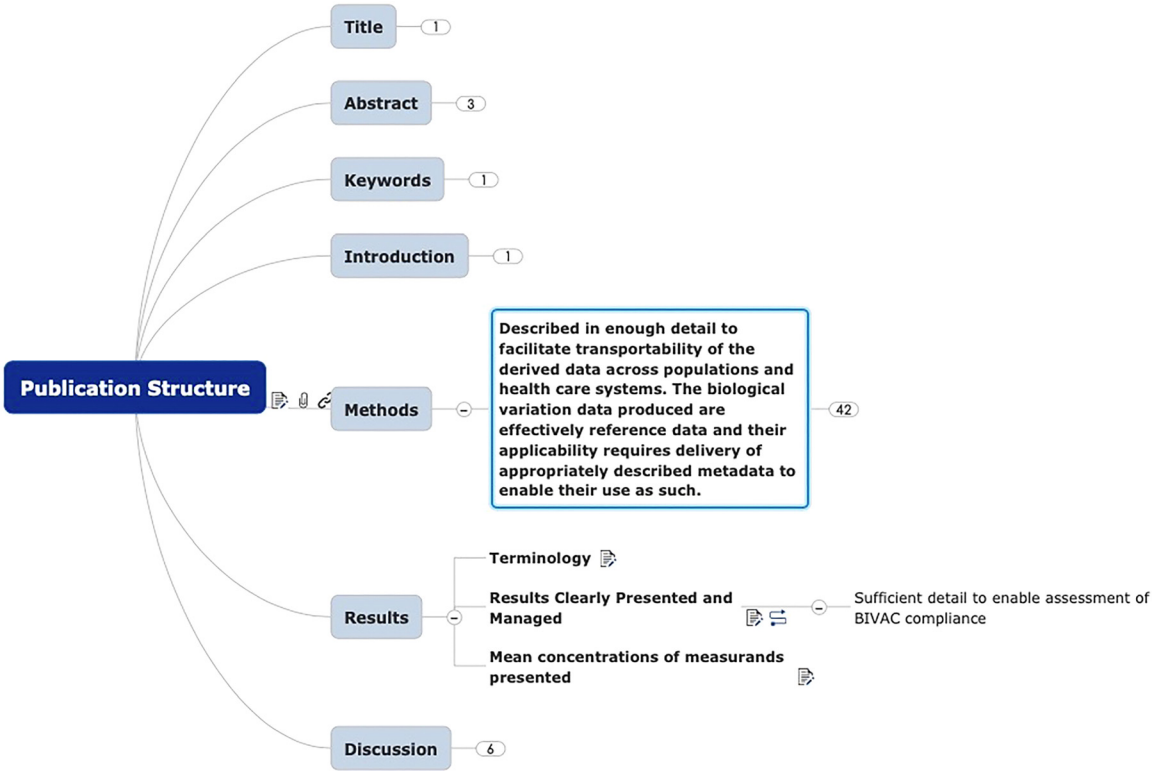

Figure 2: Screenshot of the online proposed publication structure for studies of biological variation data. The published version [51] which is a further development of [26] allows the topics in the topic boxes to be expanded to reveal further subtopics with notes key reference sources embedded as links. In addition to the The Biological Data Critical Appraisal Checklist (BIVAC) is also embedded with scoring criteria for the different quality items [25].

through time the map can be adapted dynamically to address emergent developments, thus enabling assurance of quality of newer data sets. One of the challenges faced by those assessing (e.g. BIVAC scoring) publications of BV data is that key metadata required to enable the process are missing or poorly described. In practice, this means well executed studies deliver data that are devalued and non-translatable as a consequence of deficiencies in reporting. The mind map will enable users to check that existing and new studies are compliant with a proposed structure, reporting a considered minimum data set that includes the embedded BIVAC. Further development needs to include consideration of data mining approaches and differing data management.

Terminology for components of BV

Over years, a wide range of terms and symbols have been used to describe the components of BV in published literature. In 2015, a recommendation on harmonized use of terminology in the field of BV was published [52] and these definitions and abbreviations, as used in this review, are recommended the EFLM WG-BV and TG-BVD as well as by Clinical Chemistry and Laboratory Medicine [53]. Additionally, in 2016, the EFLM WG-BV proposed and got accepted the establishment of a Medical Subject Heading (MeSH) term for "Biological Variation" in the National Library of Medicine. This MeSH term has been available from December 2017, thus facilitating systematic searches for BV publications for current publications.

New application of BV data

Reference change values and index of individuality

Most BV application have been published several decades ago, as reviewed in [1]. This includes the use of the index of individuality, which is used to assess the value of using population-based reference interval or RCV for monitoring of an individual. The RCV enumerates the value that the difference between two test results in the same individual can be, with a certain probability, explained by analytical and within-subject biological variation. The RCV has earlier been calculated as a symmetrical value, whereas it should in fact be calculated with asymmetrical limits derived from a ln RCV method [1]. In the EFLM Biological Variation Database [8], the published RCVs are based on the asymmetrical approach.

Analytical performance specifications

In the consensus document from the 1st Strategic Conference of the EFLM in 2014 it was agreed that APS can be established using three different models [54]. *Model 1* is based on the effect of analytical performance on clinical outcomes. *Model 2* is based on components of BV and *Model 3* is based on state of the art. In the Biological Variation Database, model 2 based APS utilizing the global BV estimates are automatically calculated for imprecision, bias, total error and measurement uncertainty, presented as minimum, desirable and optimal [8]. These first three are presented in the database [6] with the pro and con for each [8] and are also described in detail elsewhere [1]. In addition, APS for maximum allowable standard measurement uncertainty (MAu) is included. When using MAu bias should, in principle, be eliminated, and all the remaining sources of variation added linearly as variances. Accordingly, the MAu can be set as $0.5 \times CV_I$, and the maximum expanded allowable measurement uncertainty (MAU) as $k \times 0.5 \times CV_I$. The "k" is the coverage factor, for example, 2 or 3, to obtain a certain confidence level (95 or 99). The most used coverage factor is 2. Thus, MAU can be calculated as MAU<$2 \times 0.5 * CV_I$.

However, when calculating APS, we also must consider for which situations or scenario we are going to use them. Different APS will probably be relevant if these are applied in e.g. internal quality control, in External Quality Assurance (EQA) schemes, to evaluate lot to lot variations, in clinical guidelines. To set correct APS is an ongoing and difficult process. The Cutting Edge of Laboratory Medicine (CELME) conference, to be arranged in 2023 [55], will have the title "Analytical performance specifications: Moving from models to practical recommendations" and aims to take this area forward.

Personalized reference intervals

When using laboratory tests for the diagnosis and monitoring of patients, a reliable reference to which the results can be compared is required. Today, most reference data are derived from the population, derived either by experimental reference interval studies or by utilizing laboratory test results stored in LIMS. However, such reference data have limitations when used as the reference for an individual, especially for measurands with a low index of individuality. Thus, patients' test results preferably should be compared with their own, individualized reference intervals, i.e. a personalized RI (prRI). Recently, a new model for calculating prRI has been developed, utilizing BV data [2, 56–60]. The model is based on the homeostatic set point (HSP) and the

total variation around the HSP (TVset) of the analytes. To derive the prRI, firstly the HSP of the measurand for the individual being assessed, must be estimated. This is achieved by calculating the mean of previous test results obtained in a steady state situation. Thereafter, the prRI can be constructed by prediction intervals in two ways: 1) using the person's own within-person biological variation estimate (SD_P) or 2) using the within-subject biological variation estimate (SD_I) derived from a population similar to the person being monitored.

The prRI can thereafter be calculated as

$$\text{prRI} = \text{HSP} \pm k \times \sqrt{\frac{(n+1)}{n}\left(SD_A^2 + SD_B^2\right)}$$

where HSP is the homeostatic set point; k is a constant depending on the type of distribution (normal distribution (z) in case of population-based SD_I and t distribution in case of SD_P) and the probability, n is the number of previous test results and the SD_B refers to either the SD_P or the SD_I and the SD_A the analytical variation. To get a reliable estimate of the SD_P, more than five previous test results from a steady state situation for an individual is required. This will often be a limitation, and it may therefore be easier to construct the prRI using the SD_I. In this setting, at least three samples are required to estimate the HSP [2]. The two models will give rather similar prRIs if the presuppositions of the models are fulfilled.

It is well known that clinicians use the limits of a population-based reference intervals to act on patients' results, although these limits are not the same as clinical decision/action limits [61]. It is possible to estimate prRIs for all measurands where there are repeated measurements from a steady state situation and as such, these may represent personalized action limits. The calculation of prRI can be integrated in laboratory information systems and has the potential to be useful in diagnosing and follow up of patients. However, there are unresolved questions considering the clinical use of prRIs e.g., no studies have demonstrated their actual benefit to patients. Furthermore, clinical decision/action limits are set independently of reference intervals, and they are, unlike reference limits, similar for all kinds of measurement procedures. A further question that remains is if clinical decision/action limits should be personalized, such as for example each person having their own diagnostic cut off for a diabetes mellitus diagnosis or for being treated for hypercholesterolemia. This will probably be dependent if the measurand itself is part of the pathology or if it is only a marker of pathology.

Concluding remarks

In the later years, there has been a great progress in the methodology of deriving, calculating, estimating and reporting BV data and derived parameters. The results of many of these initiatives have been incorporated in the EFLM Biological Variation Database, which provides quality-assessed BV data, with automatically calculated BV applications, freely available for users worldwide. Furthermore, new applications for use of BV data, such as prRI and MAU have been developed. *Clinical Chemistry and Laboratory Medicine* has been a key partner for the EFLM BV groups and others in driving the area of BV forward, including by publishing a Special Issue on Biological Variation in 2022. This special issue contains 21 articles on BV and related aspects [4, 49]. We indeed congratulate *Clinical Chemistry and Laboratory Medicine* with its 60th anniversary.

For the future, it is important to take into account the fact that there is a lack of robust and high-quality BV for many measurands, population groups and settings, and furthermore, it is important to be aware that some BV data may be associated with a large uncertainty that should be considered whenever these data are used for different BV application. Importantly, we must in the future address how BV data can best benefit screening, diagnosing and monitoring of patients and thereby improve patient outcomes.

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