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Opinion Paper

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A checklist for critical appraisal of studies of biological variation

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Abstract: Data on biological variation are used for many purposes in laboratory medicine but concern exists over the validity of the data reported in some studies. A critical appraisal checklist has been produced by a working group established by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) to enable standardised assessment of existing and future publications of biological variation data. The checklist identifies key elements to be reported in studies to enable safe accurate and effective transport of biological variation data sets across healthcare systems. The checklist is mapped to the domains of a minimum data set required to enable this process.

Keywords: biological variation; checklist; critical appraisal; reference values.

Introduction

Biological variation data have many applications in laboratory medicine [1]. Those include setting of analytical performance specifications based on components of biological variation [2]. Models attempt to minimise the ratio of "analytical noise" to the biological signal within clinical laboratory measurements and to ensure that population-based reference intervals are transferable over time and geography. If analytical goals are achieved then this implies that there is no advantage in further improvement of the method in terms of the derived quality standard. The valid use of biological variation data (BVD) in this and other applications requires that they are robust and have characteristics concordant with those of the population to which the measurement procedure is to be applied. This requires that BVD are appropriately quantified, well defined, characterised and understood to enable their translation into safe and effective applications

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and transportability across populations and health care systems.

There are parallels to be drawn between the production and use of BVD and production and use of reference values [3–8]. The requirements for delivery and characterisation of the latter have been clearly identified by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and more recently in guidance issued by the Clinical and Laboratory Standards Institute (CLSI) [9]. The approach identifies need for characterisation of populations studied, methods for production of data, and the statistical treatment of data. A need for this degree of definition is accepted in the context of population-based reference values and is as important in the context of BVD. There are currently no recognised international standards for the production and reporting of BVD.

Review of the literature relating to biological variation (BV) identifies a significant volume of work stretching back over 40 years. The papers published are of varying quality in terms of study designs and presentation. This delivers a high degree of uncertainty around published estimates of BV [10–12]. The heterogeneity in quality of BVD and the use of non-standardised terminology to describe the data in publications are also problematic [13] and provide further complexity for the user. Attempts to make BVD accessible to laboratory medicine specialists have resulted in the delivery of a biological variation data base by Ricós and colleagues which is currently hosted online [14–16]. The criteria they used to construct the database have been published recently [17]. The authors recognised that there is a need to further develop criteria to better characterise BVD and enable selection of BVD from publications for inclusion in their database. In the absence of such criteria compiled data collections are readily available in accessible formats that potentially enable an uncritical application of often poorly characterised data sets.

Work has been undertaken to develop the critical appraisal checklist presented here by the Biological Variation Working Group (BVWG) [18] established by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). The checklist is similar to that published as part of the Standards for Reporting of Diagnostic Accuracy guideline (STARD) which aimed to raise the quality of publications in that area [19, 20]. The checklist proposed here, by the BVWG, is a tool that will assist laboratory medicine professionals to generate and publish high quality BVD accompanied by relevant metadata to enable safe accurate and effective clinical applications [21]. It provides a framework for end users of BVD to critically appraise existing publications, and for reviewers of future BVD publications to assure a standard of reporting that enables valid clinical application of new BVD studies by those same end users. Studies and publications that are compliant with the checklist will allow effective transportability of appropriately derived and characterised BVD across health care systems as reference data. It follows that valid application of BVD by laboratory medicine specialists at other locations or times requires recording and transmission of key metadata [22]. Those metadata describe and give information concerning the key attributes of BVD that impact on transportability (e.g., demographics of the population from which they were derived, description of the analytical methods used etc). The metadata can be further grouped into a defined "data archetype" to enable consistency and constancy of transmission of BVD through information and knowledge management systems as reference data. It has been proposed that definition of BVD as transportable reference data requires that key metadata forming the archetype can be clearly identified within six domains (e.g., study characteristics, population characteristics, and data characteristics) [21, 23]. The key metadata from each of those domains delivers a minimum data set (MDS) that can be used to define the data archetype as part of a future health informatics standard for onward transmission of BVD.

Adherence to STARD guidelines is required by many journals for studies on diagnostic accuracy providing an important checklist of items to be included in publications. The positive impact of the STARD guidelines has been acknowledged by a Consortium of Laboratory Medicine Journal Editors [24]. The importance of the detail required in publications to enable the insight of readers into the value of research is recognised. The Biological Variation Data Reporting critical appraisal Checklist (BioVarC) is proposed to deliver a similar approach and benefit. It stands as a precursor initiative to production of any formalised standards for delivery and reporting of BV studies, being based on an evaluation of current best practice and the need to ensure incorporation of key metadata into publications that impact upon the utility of BVD. Such standards may enable delivery of a need identified in 1989 by Fraser and Harris to be able to ensure comparability of data by use of common study design and analysis of data [25].

Materials and methods

The BVWG established by the EFLM consisted of laboratory medicine specialist with a remit to establish a critical appraisal checklist for publication of biological variation data. The group has studied existing BV literature and databases and undertaken discussions to enable

construction of a critical appraisal checklist applicable to existing and future publications of BVD. The group have further identified a MDS required by users to enable transportability of BVD into local clinical practice.

Results

The checklist is shown in Table 1. It is based on the same structure as the STARD table and identifies six main items for focus with a number of sub items. The sub items have been additionally mapped to minimum data set domains (Table 2; MDS: A–F) previously identified by the BVWG [21, 23]. Domain (F), which relates to a data rating concept, is not included in the checklist at this time as this is a quality measure that requires further development. The attributes identified in MDS domains A–E are identified as describing key metadata to enable safe, accurate and effective use of BVD by third party users. Domains E and F will provide further sources of information to support a users decision-making processes in the context of their clinical practice and support delivery of data through media such as online databases.

Table 1: Biological Variation Data Reporting Checklist (BiVarC).

Section and Topic	Item # (MDS Domain Mapping: A-F)[a]		Evidenced
Title/abstract/keywords	1	The title should indicate that the content relates to a study of biological variation, the subject of the study, the sample matrix, and the population studied. Analyte (component being measured), the measurand/s (the quantity or quantities to be measured, see Section 1.1), and state of well-being of the subjects under study should be clearly and unambiguously identified. *Relevant coding systems might be employed*, (e.g., LOINC [27], SNOMED [28], C-NPU [29])	
Abstract	1.1	As a minimum it should contain the headline biological variation data, the major characteristics of the population studied (numbers of subjects with demographics), clearly identify the analyte and measurand/s studied [the analyte quantities studied in a particular sample matrix, (e.g., concentration of glucose in plasma)], the statistical approach taken, the duration of the study and the geographical location of the study.	
Introduction	2	Introduction should clearly identify the context and aims of the study and cite any previous relevant studies of biological variability of the target analyte. Recommended terminology to be adopted re description of variability [13].	
Methods	3	Described in enough detail to facilitate transportability of the derived data across populations and health care systems. The biological variation data produced are effectively reference data and their applicability requires delivery of appropriately described metadata to enable their use as such.	
Analyte/measurand	3.1 (A)	The described study should clearly identify the target analyte and measurand/s. Where available internationally agreed terminology and codings should be utilised.	
Subjects	3.2 (B)	The description of the subjects and population studied should be detailed enough to enable transportability of the biological variation data. Minimum data set should be present [21–23]. This should include number of subjects studied, age, gender, and state of well-being.	
Measurement procedure	3.3 (A)	A clear description of the analytical methodology used should form part of the metadata. This may be made available via an appropriate reference or be presented within the publication. Deviation from standard operating procedures, use of adaptations of published methods, and deviation from manufacturers recommended methods in the case of commercially available systems should be documented. Standardisation and traceability should be clearly identified.	

(Table 1: Continued)

Section and Topic	Item # (MDS Domain Mapping: A-F)[a]	Evidenced
Length of study	3.4 (C)	Length of the study periods should be clearly identified.
Sampling	3.5 (C)	Sampling protocols (e.g., subject preparation, sampling conditions) that minimise pre-analytical variation should be adequately described to enable transportability of the data [25]. Numbers of samples taken should be sufficient to deliver the required power to the study [25, 26].
Samples	3.6 (C)	Recorded details should include the beginning and end date of the study and timings of sampling. Sampling conditions and sample type should be described in detail. Pre-analytical storage conditions of samples should be described.
Conditions for analysis of samples	3.7 (C)	A description of conditions under which the samples were analysed. Analytical protocols should be designed to minimise sources of analytical variation (Optimal Conditions Precision) [24].
Data analysis	4	Data analysis techniques should be described. The power of the study to identify indices of biological variation should be calculated and presented[b] [26].
Outlier analysis	4.1 (C)	Outliers should be excluded from the final analysis of the data. Test for outliers should be applied to all levels of data (between replicate analysis, between samples within subject, between subjects) [25]. The numbers of outliers and reasons for their exclusion must be given.
Heterogeneity of variance	4.2 (C)	Subjects with outlying within subject variance should be rejected from calculations used to determine an estimate of common true variance. The numbers of outliers and reasons for their exclusion must be given[b].
Statistical methods described and appropriate	4.3 (C)	Statistical methods used should be appropriately identified, fit for purpose and referenced. Data that do not conform to a normal distribution should be appropriately transformed [25].
Results	5	Unified terminology [13] should be used and appropriately defined metadata clearly presented to enable understanding and transportation of the data through time and across health care systems.
Terminology	5.1 (D)	Terms and symbols should be used to describe biological variation should conform standards identified by Simundic et al. [13].
Results clearly presented and managed	5.2 (D)	Biological variation data, with derived indices, should be tabulated in a format that enables extraction of the key data unambiguously associated with a minimum data set to enable transportability of the data. Power of the study and confidence limits around estimates of biological variation should be presented [26]. The results section should clearly identify the results of outlier analysis undertaken and confirm homogeneity of the data sets. If data are stratified the variables used to enable this should be clearly characterised.
Discussion	6	The discussion of the data should clearly include a focus on factors that impact on the transportability of the data to other settings. Limitations and strengths of the study should be addressed. If the data are used to set analytical performance specifications, derive reference change values and study individuality, the recommendations of Simundic et al. should be followed [13].

[a]MDS domains defined as A–F as shown in Table 2; [b]Tests to determine the power of a study to identify heteroscedasticity need to be developed. If variances are not homogenous derived estimates of biological variation cannot be trusted, and are not representative for the population in which it is examined.

Table 2: Description of the minimum data set, classified in domains, required to enable safe, accurate and effective transportability of biological variation data across health care systems.

Domain	Area for application	Attributes
(A)	Checklist & database	Target – definition of analyte and measurand/s, method characteristics.
(B)	Checklist & database	Population characteristics – demographics, state of well-being, physical/physiological characteristics, medication.
(C)	Checklist & database	Study characteristics – study duration and design, statistical power of study to detect BV, model assumptions, statistical approach.
(D)	Checklist & database	Data characteristics – estimates of biological variability, confidence intervals, tests for model assumptions.
(E)	For database	Publication details – links to the original publication.
(F)	For database	Data rating – new concept to be developed to indicate the quality of the BV data against a set of key criteria.

Discussion

There are currently no clearly defined internationally recognised standards for production, reporting and transmission of BVD. If BVD are considered to be reference data it follows that they should be characterised and described with sufficient key metadata to enable valid applications in clinical settings. If they are to be used to set quality standards then users of the data must have confidence that the data are the product of appropriately designed and delivered studies and further aware of the confidence limits around the estimates of the variability which they are about to use. Delivery of confidence in both senses will allow appropriate contextual application of BVD sets in clinical settings across populations and health care systems (transportability).

Reviews of BVD available for a range of analytical targets highlight many issues in study designs and reporting [10–12]. This provides a major challenge to users trying to translate the content of individual publications into practice and to those attempting to collate valid data sets into databases for use by multiple users [17]. The problems associated with, and the needs for standardisation of, terminology used in publications of BV studies have also been highlighted by Simundic et al. [13].

The critical appraisal checklist presented here follows an approach that has been shown to raise standards of reporting of studies in other settings (STARD). The BVWG have attempted to identify major items, sub items to be considered in the design, delivery and reporting of BV studies. It should apply equally to laboratory based measurements and quantitative physiological measurements (e.g., blood pressure). Compliance with the checklist will enable authors, reviewers and journal editors to assure that studies are fit for purpose, appropriately powered [26], share common terminology [13] and deliver estimates of BV accompanied by key metadata required to enable valid application of the BVD described [20–22]. Use of BV estimates accompanied by an MDS outlined in Table 2, delivers key metadata to enable transportability of data and further enable compilation of a database of BVD for use in setting of quality standards and other applications. Metadata could include the use of recognised coding systems to enable ease of transmission of relevant detail. Logical observation identifiers names and codes (LOINC) [27], the systemised nomenclature of medicine (SNOMED) [28] and the nomenclature, properties and units coding system (C-NPU) [29] provide examples of such. The MDS provides the foundation for construction of a data archetype to enable consistency and constancy of transmission of BVD through information and knowledge management systems as reference data. This is an important concept. Transportation of poorly defined and characterised BVD to populations that do not share characteristics may not only lead to setting of erroneous quality standards, but may also deliver patient safety issues. As an example of the latter if BVD are used to set reference change values, significance of change may be misidentified in the target population if they do not exhibit the same biological variation as the population from which they were derived.

The concept of scoring publications containing BVD has been described by Perich et al. [17]. It is proposed by the BVWG that a more sophisticated score should be included in the MDS to accompany BVD as a quality measure to further aid to users as a quality measure [18]. This concept needs to be further developed and has parallels with the scoring of medical evidence.

The checklist described here is based on expert opinion and provides an interim framework that may be used prospectively to improve future reporting of BVD and

retrospectively to enable critical appraisal of existing publications. It will benefit from future iterations and develop in the event of delivery of defined and agreed standards for generation and reporting of BVD. Development of specific standards for the generation, reporting and transmission of BVD should also be considered by appropriate bodies [21]. Until such are available the detailed supporting information could be supplied by a series of publications similar to those developed by the IFCC and applying to reference values [3–8].

The practical application of this current checklist will be aided by current and future developments. Currently delivery of a pro forma set of focused questions by the BVWG and others will enable users to deliver a practical objective assessment of compliance of BVD studies with the high level checklist. This tool is being developed for future publication and to be made available online. In the future standardised approaches to data management might be greatly aided by the creation of a bespoke statistical package that supports appropriate design of studies and data analysis. Availability and internet-based access to such tools and supporting information should increase understanding of factors that impact upon the utility of BVD, enable valid application of the data and drive up the quality of future published studies.

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