Biological variation data for lipid cardiovascular risk assessment biomarkers

Díaz-Garzón, Jorge; Fernández-Calle, Pilar; Minchinela, Joana; Aarsand, Aasne K.; Bartlett, William A.; Aslan, Berna

Accepted Manuscript

Biological variation data for lipid cardiovascular risk assessment biomarkers. A systematic review applying the biological variation data critical appraisal checklist (BIVAC)

Jorge Díaz–Garzón Marco, Pilar Fernández–Calle, Joana Minchinela, Aasne K. Aarsand, William A. Bartlett, Berna Aslan, Beatriz Boned, Federica Braga, Anna Carobene, Abdurrahman Coskun, Elisabet Gonzalez-Lao, Niels Jonker, Fernando Marques-Garcia, Carmen Perich, Carmen Ricos, Margarita Simón, Sverre Sandberg

BIOLOGICAL VARIATION DATA FOR LIPID CARDIOVASCULAR RISK ASSESSMENT BIOMARKERS. A SYSTEMATIC REVIEW APPLYING THE BIOLOGICAL VARIATION DATA CRITICAL APPRAISAL CHECKLIST (BIVAC)

Jorge Díaz–Garzón Marco[1,2], Pilar Fernández–Calle[1,2], Joana Minchinela[1,3], Aasne K. Aarsand[4], William A Bartlett[5], Berna Aslan[6], Beatriz Boned[17], Federica Braga[8], Anna Carobene[9], Abdurrahman Coskun[10], Elisabet Gonzalez-Lao[1,11], Niels Jonker[12], Fernando Marques-Garcia[1,13], Carmen Perich[1,14], Carmen Ricos[1], Margarita Simón[1,15], Sverre Sandberg[4,16,17],

on behalf of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group on Biological Variation and Task Group for the Biological Variation Database

[1] Analytical Quality Commission, Spanish Society of Laboratory Medicine (SEQC[ML])

[2] Department of Laboratory Medicine. La Paz University Hospital, Madrid, Spain

[3] Metropolitana Nord Clinical Laboratory (LCMN), Germans Trias I Pujol University Hospital, Badalona, Spain

[4] Norwegian Porphyria Centre, Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway

[5] School of Medicine, University of Dundee, Dundee, Scotland, UK.

[6] Institute for Quality Management in Healthcare (IQMH), Centre for Proficiency Testing, Toronto, Ontario, Canada

[7] Royo Villanova Hospital, Zaragoza, Spain

[8] Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan,Milan, Italy

[9] Servizio Medicina di Laboratorio, Ospedale San Raffaele, Milan, Italy

[10] Acibadem University, School of Medicine, Atasehir, Istanbul, Turkey

[11] Quality Healthcare Consulting. Grupo ACMS, Madrid, Spain

[12] Certe, Wilhelmina ZiekenhuisAssen, Assen, the Netherlands

[13] Department of Clinical Biochemistry, University Hospital of Salamanca, Spain

[14] Clinic Laboratory Hospital Valld'Hebron, Barcelona, Spain,

[15] Intercomarcal Laboratory Consortium of l'Alt Penedés, l'Anoia i el Garraf, Barcelona, Spain

[16] Department of Global Health and Primary Care, Faculty of Medicine, University of Bergen, Norway

[17] Norwegian organization for quality improvement of laboratory examinations, Noklus. Haraldsplass Deaconess Hospital, Bergen, Norway

Corresponding author: Pilar Fernandez-Calle
Email address: pfernandez.hulp@gmail.com (P.Fernandez-Calle)

Abstract

BACKGROUND: Biological variation (BV) data can be used to set analytical performance specifications (APS) for lipid assays. Poor performance will impact upon the efficacy of international guidelines for cardiovascular risk assessment (CVR) and relevant clinical decision limits. This systematic review applies the Biological Variation Data Critical Appraisal Checklist (BIVAC) to published studies of BV of CVR biomarkers enabling metanalysis of the data.

METHODS: Studies of BV of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and apolipoproteins A_1 and B, retrieved using a systematic literature search, were evaluated and graded using the BIVAC. Meta-analysis of CV_I and CV_G estimates were performed utilizing weightings based upon BIVAC grades and the width of the data confidence intervals.

RESULTS: Applying the BIVAC, ten publications were graded as D, 43 as C, 5 as B and 1 as A (fully compliant). A total of 196 CV_I and 87 CV_G estimates were available for the different lipid measurands. The meta-analysis-derived BV data estimates were generally concordant with those in the online 2014 BV database.

CONCLUSIONS: Application of BIVAC identifies BV data suitable for many important applications including setting APS. Additionally, this review identifies a need for new BIVAC compliant studies to deliver BV reference data in different subpopulations.

BIOLOGICAL VARIATION DATA FOR LIPID CARDIOVASCULAR RISK ASSESSMENT BIOMARKERS. A SYSTEMATIC REVIEW APPLYING THE BIOLOGICAL VARIATION DATA CRITICAL APPRAISAL CHECKLIST (BIVAC)

List of abbreviations

Apo A_1, Apolipoprotein A_1

Apo B, Apolipoprotein B

APS, Analytical Performance Specifications

B_{APS}, analytical performance specification for bias

BIVAC, Biological Variation Appraisal Checklist

BMI, Body Mass Index

BV, biological variation

CV_A, analytical variation

CV_{APS}, analytical performance specification for imprecision

CVD, cardiovascular diseases

CVR, Cardiovascular Risk assessment

CI, confidence intervals

CV_I, within-subject biological variation

CV_G, between-subject biological variation

EuBIVAS, European Biological Variation Study

EFLM, European Federation of Clinical Chemistry and Laboratory Medicine

HDLC, High Density Lipoprotein Cholesterol

IQC, Internal quality control

IFCC, International Federation of Clinical Chemistry and Laboratory Medicine

LDLC, Low Density Lipoprotein Cholesterol

QI, Quality Item

RCV, reference change values

$SEQC^{ML}$, Spanish Society of Laboratory Medicine

SD_A, analytical standard deviation

SD_I, within-subject standard deviation

TC, Total Cholesterol

TE_{APS}, analytical performance specification for total error

TFG-BVD, EFLM Task and Finish Group for the Biological Variation Database

TG, Triglycerides

WG-BV, EFLM Working Group on Biological Variation

Introduction

Cardiovascular diseases (CVD) are a major cause of death and morbidity in developed countries [1]. The economic impacts of CVD are significant. Therefore, there has been an international focus on CVD prevention, risk assessment, diagnosis and management [2, 3]. The adoption of international guidelines in these contexts has resulted in increasing volumes of requests for lipid measurements. Lipid tests are now amongst the most requested tests in laboratory medicine.

Accuracy and precision of lipid measurands, across and between healthcare systems, are required for delivery of equitable patient outcomes. Sub optimal analytical performance will impact on the efficacy of international guidelines where fixed clinical decision limits are based on a defined measurand value. Analytical performance specifications (APS) are required for such measurands. The European Federation of Laboratory Medicine (EFLM) 1st Strategic Conference Consensus Statement identified three models for assignation of APS: clinical outcomes, biological variation (BV) and state of the art *[4]*. For lipid measurands in the context of CVD, clinical outcome has been identified as the model of choice [5]. In the absence of relevant clinical outcome studies, laboratory professionals pragmatically apply the BV model. It follows, however, that application of incorrect estimates of biological variation to set (APS) will impact upon patient care. In addition to setting of APS, BV data have a number of other relevant and important applications; those include the derivation of method validation criteria for IVD providers, the monitoring of laboratory analytical performance and establishing reference change values to assess the significance of changes in serial results [6-8]. All these applications require access to relevant and well characterized BV data.

For many years, access to BV data has been enabled through the work of the Spanish Society of Laboratory Medicine (SEQC^{ML}). Their collated BV data set has been made widely available via an online database which was last updated in 2014 [9]. Criteria used to select data for inclusion in the database have been published [10]. Recently, however, concerns have been raised about the quality and current applicability of the BV estimates included; this has led to calls for new global BV estimates to be delivered using data from studies rigorously appraised against a new set of criteria [11,12]. In response, the Working Group on Biological Variation *[13]* and the EFLM Task Group for the Biological Variation Database have developed the recently published Biological Variation Data Critical Appraisal Checklist, (BIVAC) *[14]. BIVAC is* a tool that enables appraisal of the methodological quality of BV publications and of the reported BV data estimates. In addition, a meta-analysis method has been developed to enable delivery of robust global estimates of BV data, with confidence limits, from BIVAC compliant studies *[14]*. This approach makes available BV data estimates derived from quality assessed historical and contemporary studies.

The aims of the systematic review presented here are to use the BIVAC to critically appraise published studies of BV of biomarkers used for cardiovascular risk assessment. The review enables: (i) collation of data from BIVAC compliant studies for application of meta-analysis to identify point estimates of within-subject (CV$_I$) and

between-subject (CV_G) BV and (ii) a review of the effect of study design, differences in study population and sampling intervals on reported BV estimates.

Material and methods

All published studies of BV of total cholesterol (TC), HDL-cholesterol (HDLC), LDL-cholesterol (LDLC), triglycerides (TG) and apolipoproteins A_1 (Apo A_1) and B (Apo B) originally included in the online 2014 BV database [9] were retrieved for appraisal. Additional studies were identified employing a systematic literature search as previously described [14] using the cut-date of September 2018. Within this systematic review, all publications referred to from online 2014 BV database are denoted with the article number they had been allocated within that source; additionally a suffix letter has been assigned (a, b, c, etc.) to identify estimates from subgroup partitions (e.g. subjects stratified by age, gender, etc.) identified with the denoted publication.

The retrieved publications were evaluated by application of the BIVAC to verify compliance as evidenced by reporting of essential methodological elements for a study of BV [14]. The methodology of the reviewing process has been described previously [14]. Briefly, two independent assessors reviewed each publication. If there were discrepancies in the BIVAC scores, a third assessor did a full review of the paper and a decision was made. The BIVAC review process assesses and scores 14 quality items (QI); study population and design (QI2, QI3, QI5 and QI7), analytical methodology (QI1, QI4 and QI6), statistical analysis and reporting of data (QI8, QI9, QI10, QI11, QI12, QI13 and QI14) [14]. QI were scored as A, B, C or D, indicating decreasing compliance with the BIVAC. The lowest quality score achievedfor any of the QIs defines the overall BIVAC grade (A, B, C or D). Studies receiving even a single BIVAC QI scored as a D were considered lacking essential elements and excluded from the remainder of the study [14]. 95% confidence intervals (CI) for BV estimates were calculated as described by Røraas [15, 16] if the required data were provided,(i.e. (i) mean number of subjects, (ii) mean number of samples and (iii) estimates of analytical variation (CV_A)).

Estimates of BV, with 95% CI, from all studies and subgroups were plotted to facilitate visual inspection of the data and identification of aberrant data that may influencing the global BV estimates. We evaluated the influence of health status, age, study design (study duration, sampling interval, number of samples, etc.) and methodology used (analytical principle, analytical performance).

Meta-analysis methodology
Meta-analysis of CV_I and CV_G estimates were performed applying the weighted mean as previously described [14], by using the combined result of the inverse width of each estimate's confidence interval (CI) and the BIVAC quality grade (A papers weighted by 4, B papers by 2 and C papers by 1). When publications reported partitioned data and

recorded separate BV estimates for different subgroups, those estimates were combined to provide a common estimate by applying the weighted mean on the point estimates and corresponding CIs; this approach minimized the effect of widely differing results (e.g. from males and females) from the same study impacting adversely upon the common estimates. Percentile bootstrap with the weighted median performed on each of the resampled data sets were used for calculating the CI [17]. Only studies performed in healthy adults (age 18-75 years) were included in the meta-analysis. Studies receiving BIVAC grade D, studies with within-day samplings, studies with less than three samples per subject, and studies lacking the necessary information for the calculation of CI were excluded from the meta-analysis.

Results

In total 59 publications were collated reporting BV data estimates for any of the 7 measurands of interest. Data were available for both measured and calculated LDL-cholesterol (Table 1, Supplemental Table 1). Applying the BIVAC, ten of these publications were graded as D, 43 as C, 5 as B and 1 as A.

A total of 196 CV_I and 87 CV_G estimates were available for the various lipid measurands were available for objective assessment and analysis.

The BIVAC grade B was given to studies in most cases for non-compliance with statistically related quality indicators (QI 8: incomplete outliers testing, QI 9: normally distributed data and QI 11: statistical method). Papers graded most commonly graded C because of failure to report adequate information to the statistical approach used to deliver the BV estimates. Absence of data implied that some of the key statistical QIs were not addressed (e.g. use of appropriate statistics (QI 11), assessment of the data set for outliers, (QI 8) and examination for variance homogeneity (QI 10). A BIVAC grade D was most frequently the result of an insufficient description of the study population (QI 2) or use of obsolete analytical methods (QI 4) [14].

The initial review process identified discrepancies in the scoring of studies by the paired reviewers. This amounted to 40% (8 discrepancies in 20 evaluated papers), and occurred mostly because of lack of details on statistical procedure in historical papers. This required and promoted discussions between assessors to enable a consensus to be about the scoring criteria. Following that process, a second stage review of the larger number of paper, delivered an agreed BIVAC compliance grade in 95% of cases. Of the remaining 5% of the papers (2 discrepancies in 37 evaluated papers), discrepancies arose because of issues relating to QIs 8, 9, 10 and 11. Difficulties centered around the lack of explanation, details or clarity when describing outliers' detection and statistical methods in these publications were resolved by including a third reviewer.

Total cholesterol

Of the 59 reviewed studies, 57 reported results for TC (Table 1), ten of those were graded D, being non-compliant with QI 2 or QI 4 . Only one study achieved an A grade, the European Biological Variation Study (EuBIVAS) [*18*]. 36 papers, reported CV_I estimates for 55 subgroups and CV_G for 25 subgroups, fulfilled the criteria for inclusion in the meta-analysis. When estimates from subgroups from the same study were combined (e.g. men and women), this delivered a total of 34 CV_I estimates and 17 CV_G estimates for inclusion in the meta-analysis (Table 2). The meta-analysis delivered about similar point estimates as those available in the online 2014 BV database (Table 2), but with an added attribute in the form of CI.

On visual inspection of the TC data, no relationship was observed between BV estimates and the age of the subjects studied (Figure 1). Based on visual inspection and analysis of the data, CV_I results of the study identified as number 290, subgroup "a" for TC and HDLC were classified as outliers and excluded from the meta-analysis. Only a few of the included papers provided CV_G estimates, here estimates from non-healthy appeared higher than those based on studies in healthy subjects (Figure 2).

Triglycerides
Forty-one papers included results for TG. Twenty-seven of them were included in meta-analysis, with CV_I reported for 42 subgroups and CV_G reported for 17 subgroups. A total of 27 CV_I and 12 CV_G estimates were identified for inclusion in the meta-analysis (Table 2). A wide dispersion in CV_I estimates was observed for TG (Figure 3) with very variable confidence limits. Also, the CV_A estimate reported in paper number 53 which was higher than those of other studies. This was a C graded paper that included weekly sampling from 5 women following a controlled diet. Based on visual inspection of results, health status does not appear as a factor who influencing BV (Figure 3, Supplemental Figure 1).

HDL-cholesterol
Thirty-two papers reported BV data for HDLC and 21 of them met the criteria for inclusion in the meta-analysis, with estimates of CV_I and CV_G for 31 and 10 subgroups respectively, delivering a set of 20 data points for CV_I and 6 for CV_G (Table 2).

Figure 4 shows the CV_I estimates from the collated HDLC studies; the CV_I estimate from paper number 290, a study performed in Chinese healthy subjects, is an evident outlier for CV_I . The CV_I estimate was higher than that of other studies (Figure 4), however the CV_G estimate did not appear different (Supplemental Figure 2). Visual inspection of data identified that paper 255 delivers CV_G estimates for non-healthy and healthy sub-groups that are both higher than other estimates and exhibit a wide CI. This paper is a B graded paper; it reported on a study performed in 11 healthy Spanish subjects (8 females and 3 males, age range: 20-50). The measurement procedure, analytical variation and other assessed variables did not differ from the rest of studies.

Measured and calculated LDL-cholesterol, Apolipoprotein A_1 and Apolipoprotein B

Fourteen papers fulfilled the inclusion criteria for calculated LDLC, and 6 papers met these criteria for measured LDLC (Table 2). On visual inspection, there seems not to be any difference between measured and calculated LDLC CV_I estimates based on studies in healthy adult subjects (Figure 5, Supplemental Figure 3). Only a limited number of studies reported estimates for Apo A_1 and Apo B (Table 1, 2, Supplemental Figures 4-7).

Discussion

CVD presents many challenging healthcare issues to developed countries. Significant workloads accrue to clinical laboratories as a consequence of national and international guidelines applying to diagnosis and management of CVD that require lipid analysis. Laboratory measurements of lipids and lipoproteins are of utmost importance to ensure the correct classification of subjects in terms of cardiovascular risk, monitoring response to treatment and follow up [*19*]. The analytical performance of assay systems will impact diagnostic efficiencies, disease monitoring and management, delivering a requirement for defined APS.

It has been proposed that APS for the measurement of total cholesterol, HDLC and LDLC should be assigned using the clinical outcome model [*5*]. The rationale for this is based on the use of clinical cut-offs recommended by guidelines to classify patients into different groups for pharmacological treatments, other interventional strategies or combinations of both. Paradoxically, triglyceride measurement is recommended to be assigned to the alternative BV model to determine APS even though the result is used to calculate LDLC [5].

While the application of the outcome model may be the ideal for many CVD relevant lipid and lipoprotein measurands, the required outcome data are scarce. Also APS derived using the outcome model may not be appropriate to all clinical applications of an assay. This is important as the results of an analytical method deployed in a central laboratory may be applied in many clinical situations and in the context of mixed pathologies, thus applying APS criteria based on a single disease specific clinical outcome model, may not be generally valid. An alternative approach to setting valid APS using BV data may also prove challenging if the quality of the reference data is poor [13]. The latter can be addressed by application of BIVAC and use of meta-analysis which together provide approaches to deliver quality assessed BV data for such applications [14].

Our study shows that BV estimates for lipids delivered by meta-analysis of data from BIVAC compliant studies are of the same order as those made available in the online 2014 BV database [2]. All CVI and CV_G estimates from that source fall into the CI of the newer meta-analysis-derived estimates. This observation contrasts with that made of BV of enzymes where the meta-analysis estimates in general were lower [*14*, *21*]. A possible explanation of this difference is that analytical methods for enzymes have changed substantially over the last decades and because inadequate methods had

been used in historical publications to calculate BV data. The number of studies providing estimates using older methods included in the online 2014 BV database are proportionally higher for enzymes than for lipids [14].

The APS for lipids and lipoproteins generated from the new BV estimates presented here are not very different from those published in the online database. APS for imprecision is slightly stricter for HDLC (2.9% vs 3.7%) and Apo A_1 (2.3% vs 3.3%). In general, the APS for bias are wider. Triglycerides are an exception (27.8% vs 26.0%), and Apo A_1, where meta-analysis estimates provide more stringent APS (6.2% vs 9.1%).

Generally, the meta-analysis-derived BV estimates could be used as a resource for laboratory professionals when to set APS. Nevertheless, there may be situations for the different lipids where a different model should be considered.

In our study we have aimed to assess the effect of study design, differences in study population and sampling intervals on BV estimates. However, the large variations in BV estimates delivered by studies that appear similar makes it challenging to draw conclusions regarding the influence of these factors. This observation as exemplified in studies of triglycerides, where reported CV_I estimates based on studies with weekly or monthly samplings in healthy individuals (26 studies) vary from 12% to 32%. This necessitates caution in interpretation and application of our data given the heterogeneity of the historical data.

Total cholesterol

The 95% CI for the total cholesterol BV estimate derived from non-healthy subjects (dyslipidemia, diet, or pharmacological treatment, in a total of 9 subgroups) overlapped with the 95% CI observed for healthy subjects (55 subgroups). Our data therefore suggest that there are no differences between CV_I in non-healthy and "healthy" subjects (Fig 1) studied with sampling intervals of more than one day. In practice this is an important assumption because in most clinical laboratories treated and non-treated diseased patients are routinely mixed with healthy subjects in everyday practice. This means that the same APS could be validly applied to the assay for general application. Interestingly CV_I of TC seems to be lower in within-day studies, ranging from 2% to 5% (7 subgroups, Figure 1). This would indicate a requirement for a more stringent APS to be applied if several measurements are requested within a day.

In short-term conditions it has been reported that there may be some differences in the magnitude of BV [6]. In stable disease, the homeostatic set point may be reset, but the variation around that set-point is thought to be like that of the non-diseased. Use of an RCV derived for healthy subjects may therefore be valid in stable disease. Short term illness leading to an acute phase reaction impacts on a range of proteins and it is known that TC falls significantly after myocardial infarction [6]. This makes it difficult to use RCV in this situation. It is important to ensure that factors impacting BV are taken into account when evaluating a change in serial measurements in a particular individual by means of the RCV calculation [22].

When reviewing the studies that met the inclusion criteria for the meta-analysis, visual inspection indicated that subgroup "a" from paper 290 had a CV_I estimate that differed from other studies (Figure 1). Estimates in this study were based on 3 samples, at least a week apart, collected from 9 Chinese healthy men, with serum TC measured using an AU-5800 (Beckman Coulter). It was graded as C as testing of variance homogeneity was not described, but no other apparent factor apart from ethnicity could be identified to explain the unusual CV_I estimate. Ethnicity did not appear to be an issue in a study that comparing BV data from Caucasian subjects (study 291) with an Hispanic population where no differences were observed. More studies are needed across appropriate sampling periods to examine whether ethnicity may affect BV estimates.

Triglycerides
The point estimate from children (2 subgroups) and persons above the age of 75 years (4 subgroups) fell outside the CI of the meta-analysis estimate obtained in healthy adults below the age of 75, indicating that these age groups may have a different CV_I. This observation agrees with that of other authors *[23]* and should be taken into consideration by laboratories supporting elderly or paediatric care services.

On visual inspection it appears that within-day studies of TG may provide higher CV_I estimates (7 subgroups, Figure 3). A possible explanation for this is that temporal changes in blood TG levels related to food intake. It is interesting to note that the Joint Consensus Statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine recommends [*24*], in general, fasting is not a necessary pre-analytical requirement for lipid measurements except for TG.

HDL-cholesterol
On visual inspection it appears that CV_I estimates for HDLC in the pediatric population (2 subgroups) are slightly higher than those for adults. No obvious differences were observed between estimates of non-healthy (n = 10 subgroups) and healthy subjects (n = 31 subgroups).

Measured and calculated LDL-cholesterol, Apolipoprotein A1 and Apolipoprotein B
There were no significant differences observed between BV estimates obtained from the Friedewald formula (n = 35 subgroups) and direct LDLC measurement (n = 10 subgroups). The concordance of TG results obtained by the two methods has also been reported in one unique study (paper 255, BIVAC grade B). It was postulated in the report of that that in healthy subjects TG is usually less than 200 mg/dL, arrange where the Friedewald formula is valid and unaffected by TG [*25*]. There is only one paper where CV_I of measured LDLC is clearly different from calculated LDLC (study 103). This was scored against BIVAC as a C study. It included patients with type 2 diabetes treated with statins. The studied population was divided into 2 groups treated either with atorvastatin or simvastatin. BV was estimated using a protocol requiring samples

of ten blood samples taken at intervals of 4 days. The results showed that in those patients taking simvastatin the CV_I for calculated LDLC was 13.1% compared to 10.7% for measured LDLC. In those taking atorvastatin, the difference was much larger, with a CV_I for calculated LDL being 10.3% and <1% for measured LDLC. To our knowledge, no other paper has demonstrated a similar finding which raises the possibility that this finding is artefactual.

Conclusions

This study provides updated estimates of CV_I and CV_G for measurands important in the context of CVD. They have been derived utilizing meta-analysis of data from BIVAC compliant studies. The meta-analysis-derived BV estimates, with CI, were generally of similar magnitude to those cited in the online 2014 BV database. Therefore, to adjust the laboratory performance to the new APS derived from these new robust estimates will require only minor changes for clinical laboratories. Appraisal of the available studies of CVD relevant measurands with the BIVAC identified a small number of B papers and only one A paper. This observation indicates a requirement for delivery of new BIVAC compliant studies to make available BV data for the many important applications BV data such as identification and delivery of robust and valid APS and RCVs. Well characterized global BV estimates will in future be made available on-line within a new EFLM hosted website. Laboratory professionals should however be aware of the potential confounders to the application of BV data. There is for example a possibility that there may be differences in CV_I estimates between different populations, and in subgroup of populations, necessitating partitioning of data. Differences in BV associated with state of well-being and ethnicity identify a need for new BIVAC compliant studies to deliver appropriate BV data in some of these groups.

References

1. Mortality and Causes of Death: 2015 and 2030. World Health Organization. https://www.medigo.com/blog/infographics/mortality-causes-of-death-2015-2030/.Last accessed 23/12/2018

2. Robinson JG, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, et al. 2013 ACC / AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults. https://circ.ahajournals.org/content/early/2013/11/11/01.cir.0000437738.63853.7a (Accesed December 2017)

3. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias: The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Atherosclerosis. 2016 Oct; 253:281-344

4. Sandberg S, Fraser FG, Horvath AR, Jansen R, Jones G, Oosterhuis W, et al. Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem Lab Med 2015;53:833–5.

5. Ceriotti F, Fernandez-Calle P, Klee GG, Nordin G, Sandberg S, Streichert T, et al. Criteria for assigning laboratory measurands to models for analytical performance specifications defined in the 1st EFLM Strategic Conference. ClinChem Lab Med 2017; 55: 189–194.

6. Fraser CG. Biological variation: from theory to practice. AACC press 2001.

7. Ricós C, Álvarez V, Cava F, García-Lario JV, Hernández A, Jiménez CV, Minchinela J, Perich C, Simón M. Integration of data derived from biological variation into the quality managemet system. Clin Chim Acta 2004;346:13-18.

8. Ricós C, Álvarez V, Minchinela J, Fernández-Calle P, Perich C, Boned B, et al. Biologic Variation Approach to Daily Laboratory. Clin Lab Med 2017;37:47-56

9. Minchinela J, Ricós C, Perich C, Fernández-Calle P, Álvarez V, Doménech MV, Simón M, Biosca C, Boned B, Cava F, García-Laro JV, Fernández-Fernández MP. Biological variation database and quality specifications for imprecision, bias and total error (desirable and minimum). The 2014 update. http://www.westgard.com /biodatabase-2014-update.htm. Accessed Mars 8th 2018.

10. Perich C, Minchinela J, Ricós C, Fernández-Calle P, Álvarez V, Doménech MV, et al. Biological variation database: structure and criterion used for generation and update. ClinChem Lab Med 2015;53.299-305.

11. Carobene A, Braga F, Roraas T, Sandberg S, Bartlett WA. A systematic review of data on biological variation for alanine aminotransferase, aspartate

aminotransferase and γ-glutamyl transferase. ClinChem Lab Med 2013;51:1997-2007.

12. Braga F, Dolci A, Mosca A, Panteghini M. Biological variation of glycated hemoglobin. Clin Chim Acta 2010;411:21-2.

13. Bartlett WA, Braga F, Carobene A, Coşkun A, Prusa R, Fernandez-Calle P, et al. A checklist for critical appraisal of studies of biological variation. ClinChemLabMed 2015;53:879-85.

14. Aarsand AK, Roraas T, Fernandez-Calle P, Ricos C, Díaz-Garzón J, Jonker N, et al. The Biological Variation Data Critical Appraisal Checklist: A Standard for Evaluating Studies on Biological Variation. Clin Chem. 2018;64(3):501-14.

15. Roraas T, Steve B, Hyltoft P and Sandberg S. Biological variation: the effect of different distributions on estimated within-person variation and reference change values. Clin Chem 2016;62:725-736.

16. Roraas T, Petersen PH, Sandberg S. Confidence intervals and power calculations for within-subject biological variation: effect of analytical variation, number of replicates, number of samples and number of individuals. ClinChem 2012;58:1306–13.

17. Tu, D., & Shao, J. (1995). The Jackknife and bootstrap (1st ed.). New York: Springer Series in Statistics.

18. Aarsand A, Diaz Garzon J, Fernandez Calle P 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

19. Langlois MR, Descamps OS, van der Laarse A, Weykamp C, Baum H, Pulkki K, et al. Clinical impact of direct HDLC and LDLC method bias in hypertriglyceridemia. A simulation study of the EAS-EFLM Collaborative Project Group. Atherosclerosis 2014;233:83–90.

20. González-Lao E, Corte Z, Simón M, Ricós C, Coskun A, Braga F, Aarsand AK, Carobene A, Bartlett WA, Boned B, Asland B, Díaz-Garzón J, Marqués-García F, Minchinela J, Perich C, Fernández-Calle P, Roraas T, Fernández-Fernández P, Jonker N, Sandberg S; European Federation of Clinical Chemistry and Laboratory Medicine Working Group on Biological Variation and Task Group for the Biological Variation Database. Systematic review of the biological variation data for diabetes related analytes. Clin Chim Acta. 2019 Jan;488:61-67

21. Carobene A, Røraas T, Sølvik UØ, Sylte MS, Sandberg S, Guerra E, et al. European Biological Variation Study of the EFLM Working Group on Biological Variation.Biological Variation Estimates Obtained from 91 Healthy Study Participants for 9 Enzymes in Serum.Clin Chem 2017 63:1141-1150.

22. Fraser CG. Reference change values. Clin Chem Lab Med 2011;50:807-12

23. Carobene A, Graziani MS, Lo Cascio C, Tretti L, Cremonese E, Yabarek T, Gambaro G, Ceriotti F. Age dependence of within-subject biological variation of nine common clinical chemistry analytes.Clin Chem Lab Med. 2012 Jan 20;50(5):841-4

24. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E et al. Fasting Is Not Routinely Required for Determination of a Lipid Profile: Clinical and Laboratory Implications Including Flagging at Desirable Concentration Cutpoints-A Joint Consensus Statement from the European Atherosclerosis Society and

European Federation of Clinical Chemistry and Laboratory Medicine.Clin Chem. 2016;62:930-46

25. Corte Z, Obaya R. Variación biológica de lípidos y lipoproteínas séricas en individuos sanos y pacientes con enfermedad crónica renal terminal. Rev Lab Clin 2014;7:88-95

Table 1. Number of reviewed biological variation papers for lipids, the corresponding number of subgroups, and their Biological Variation Data Critical Appraisal Checklist (BIVAC) grade

Measurand (serum or plasma)	Nº papers	A	B	C	D	Nº subgroups*
Total Cholesterol	57	1	4	42	10	95
Triglycerides	41	1	3	33	4	79
HDL-Cholesterol	32	1	2	27	2	65
LDL-Cholesterol (measured)	9	-	2	5	2	12
LDL-Cholesterol (calculated)	19	1	2	16	-	37
Apolipoprotein A$_1$	12	-	1	11	-	21
Apolipoprotein B	10	-	1	9	-	17

*A paper may include two or more subgroups depending on the population or study design, for instance: different sex or age groups, sampling intervals, short or long term study duration, healthy or diseased population, etc..

16

Table 2. Numbers of CV_I and CV_G estimates for lipids included in and excluded from the meta-analysis and the CV_I and CV_G estimates derived from the meta-analysis calculation compared to the estimates reported in the online 2014 BV database. The last two columns show the desirable analytical performance specifications (APS) derived from the meta-analysis estimates.

Component	Included in Meta-analysis			Excluded from Meta-analysis			Meta-analysis estimates		Online 2014 BV database		Meta-analysis based desirable APS	
	Nº of papers	Nº of subgroups CV_I/CV_G	Nº of included estimates * CV_I/CV_G	Nº of papers	Nº of subgroups CV_I/CV_G	Nº of excluded estimates * CV_I/CV_G	CV_I % (95% CI)	CV_G % (95% CI)	CV_I %	CV_G %	CV_{APS} (%)	B_{APS} (%)
Total Cholesterol	36	55/25	34/17	21	40/13	21/10	5.7 (5.2-7.8)	17.3 (14.8-17.8)	5.9	15.3	2.9	9.3
Triglycerides	27	42/17	27/12	14	36/11	21/9	20.4 (19.5-25.7)	39.6 (32.8-47.3)	19.9	32.7	10.2	27.7
HDL-Cholesterol	21	31/10	20/6	11	35/10	18/6	5.7 (5.7-7.7)	24.8 (20.5-26.1)	7.3	21.2	2.9	11.1
LDL-Cholesterol (measured)	6	8/7	6/5	3	4/2	3/2	7.0 (5.1-10.7)	20.9 (17.4-38.0)	7.8	20.4	3.5	11.3
LDL-Cholesterol (calculated)	14	18/5	13/5	5	19/3	9/3	7.9 (6.2-8.7)	26.1 (18.5-29.1)	-	-	4.0	13.4
Apolipo protein A_1	6	7/3	6/3	6	13/4	7/3	4.5 (3.6-9.2)	8.6 (8.0-15.9)	6.5	13.4	2.3	6.2
Apolipo protein B	6	7/5	6/4	4	9/2	5/2	7.2 (4.2-12.5)	18.9 (10.7-25.1)	6.9	22.8	3.6	11.0

*Number of estimates from different subgroups included in the same study combined to provide a common estimate included in the meta-analysis.

CV_{APS}: 0.5 x CV_I

B_{APS} : 0.25 x $(CV_I^2 + CV_G^2)^{1/2}$

Figure 1. Total Cholesterol CV_I
Figure 2. Total Cholesterol CV_G
Figure 3. Triglycerides CV_I
Figure 4. HDL - Cholesterol CV_I
Figure 5. LDL - Cholesterol CV_I

Figure 1. CV$_I$ and 95% confidence interval estimates for total cholesterol. The different symbols indicate that estimates are derived from studies identified as: 1) BIVAC A, B and C graded studies performed in healthy adults, 2) the same in elderly and pediatric subjects, 3) those in non-healthy subjects, 4) estimates derived from within-day studies, 5) studies with CV$_A$ higher than desirable APS based on the online 2014 BV database and 6) studies with less than 3 samples per subject. Publications (X axis) are identified by the articles number given in the supplemental Table 1, with letters indicating subset of data (subgroups) derived from the same publication.
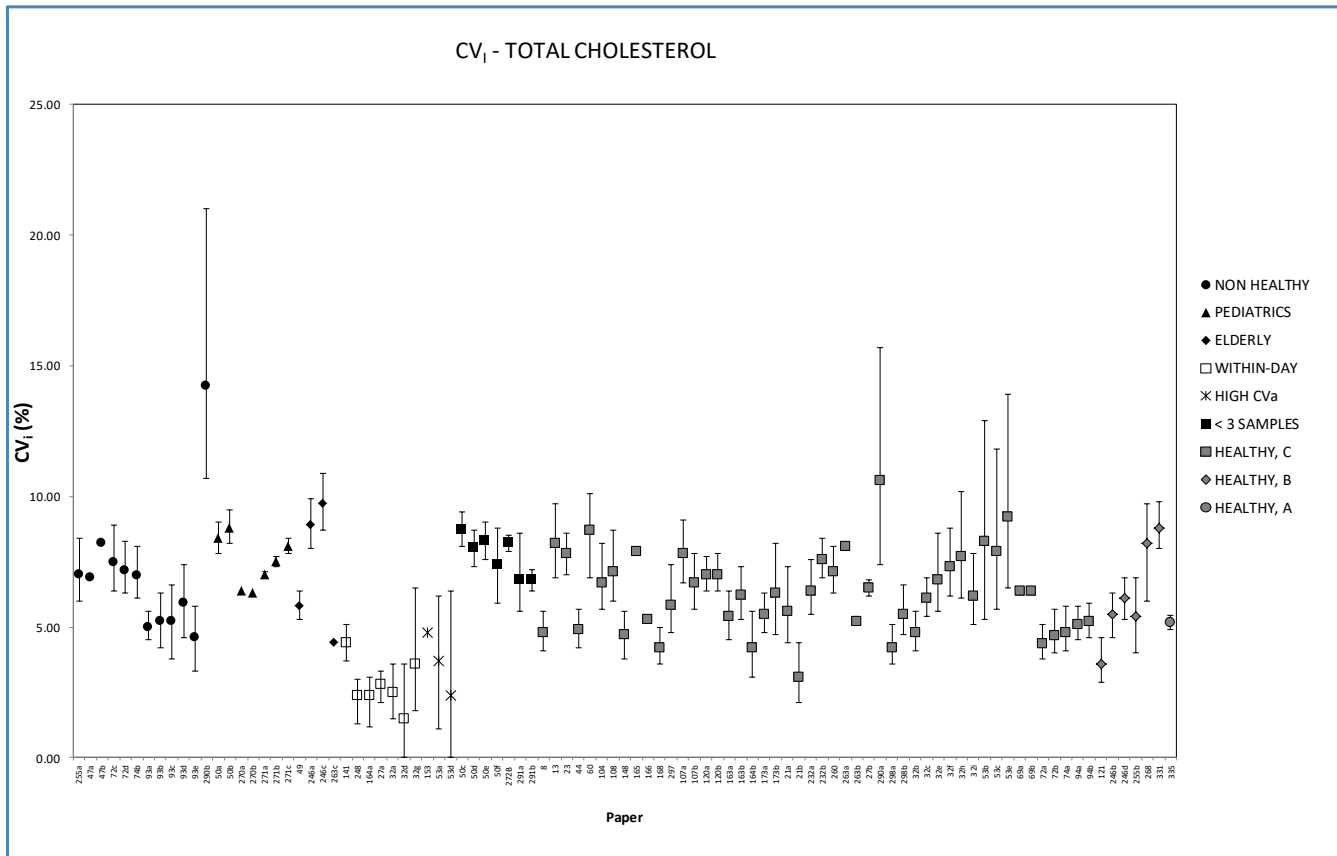
Figure 2. CV_G and 95% confidence interval estimates for total cholesterol. The different symbols indicate that estimates are derived from studies identified as: 1) BIVAC A, B and C graded studies performed in healthy adults, 2) the same in elderly and pediatric subjects, 3) those in non-healthy subjects and 4) estimates derived from within-day studies, 5) studies with CV_A higher than desirable APS based on the online 2014 BV database and 6) studies with less than 3 samples per subject. Publications (X axis) are identified by the articles number given in the supplemental Table 1, with letters indicating subset of data (subgroups) derived from the same publication.
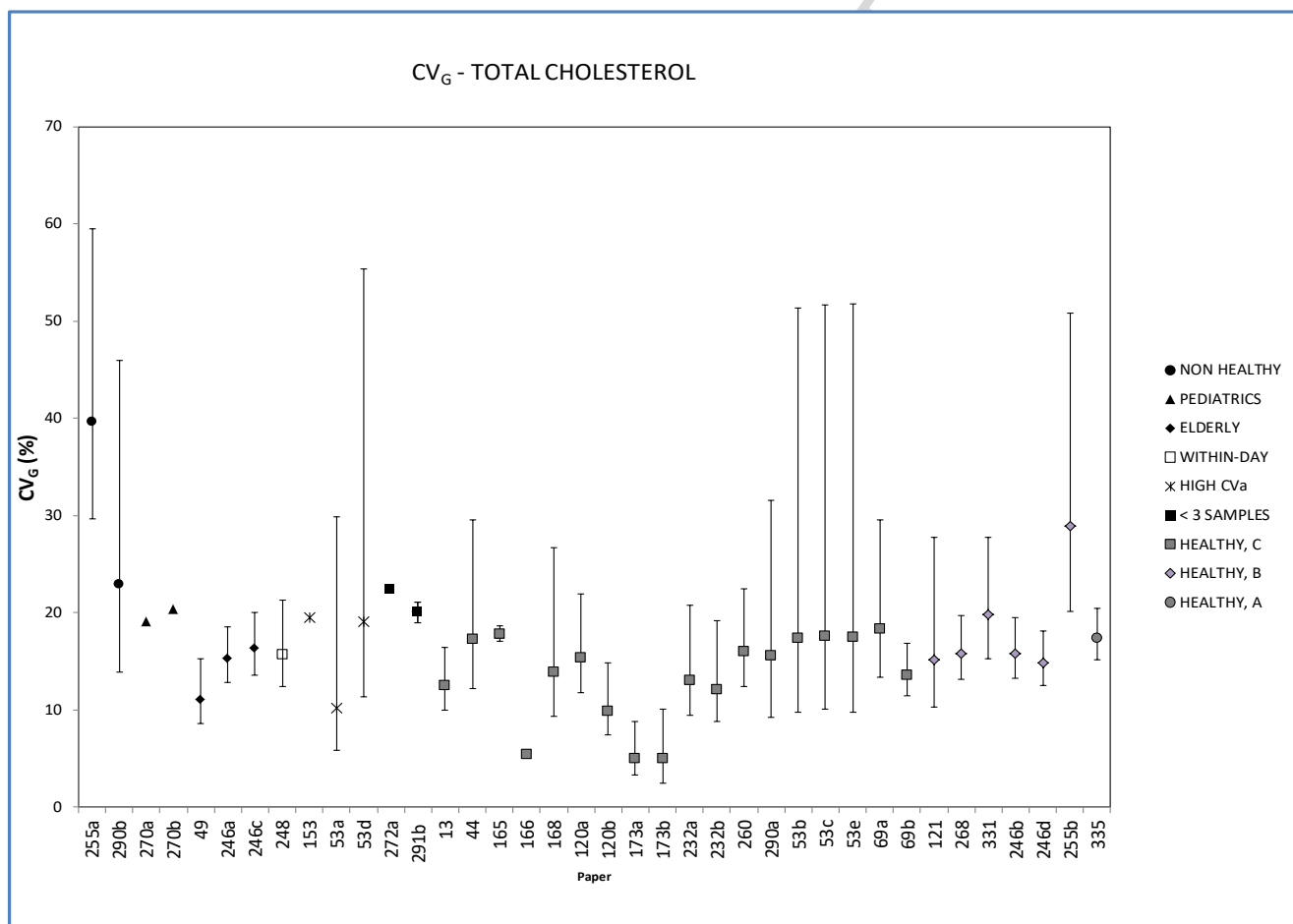
Figure 3. CV$_I$ and 95% confidence interval estimates for triglycerides. The different symbols indicate that estimates are derived from studies identified as: 1) BIVAC A, B and C graded studies performed in healthy adults, 2) the same in elderly and pediatric subjects, 3) those in non-healthy subjects and 4) estimates derived from within-day studies, 5) studies with CV$_A$ higher than desirable APS based on the online 2014 BV database and 6) studies with less than 3 samples per subject. Publications (X axis) are identified by the articles number given in the supplemental Table 1, with letters indicating subset of data (subgroups) derived from the same publication.
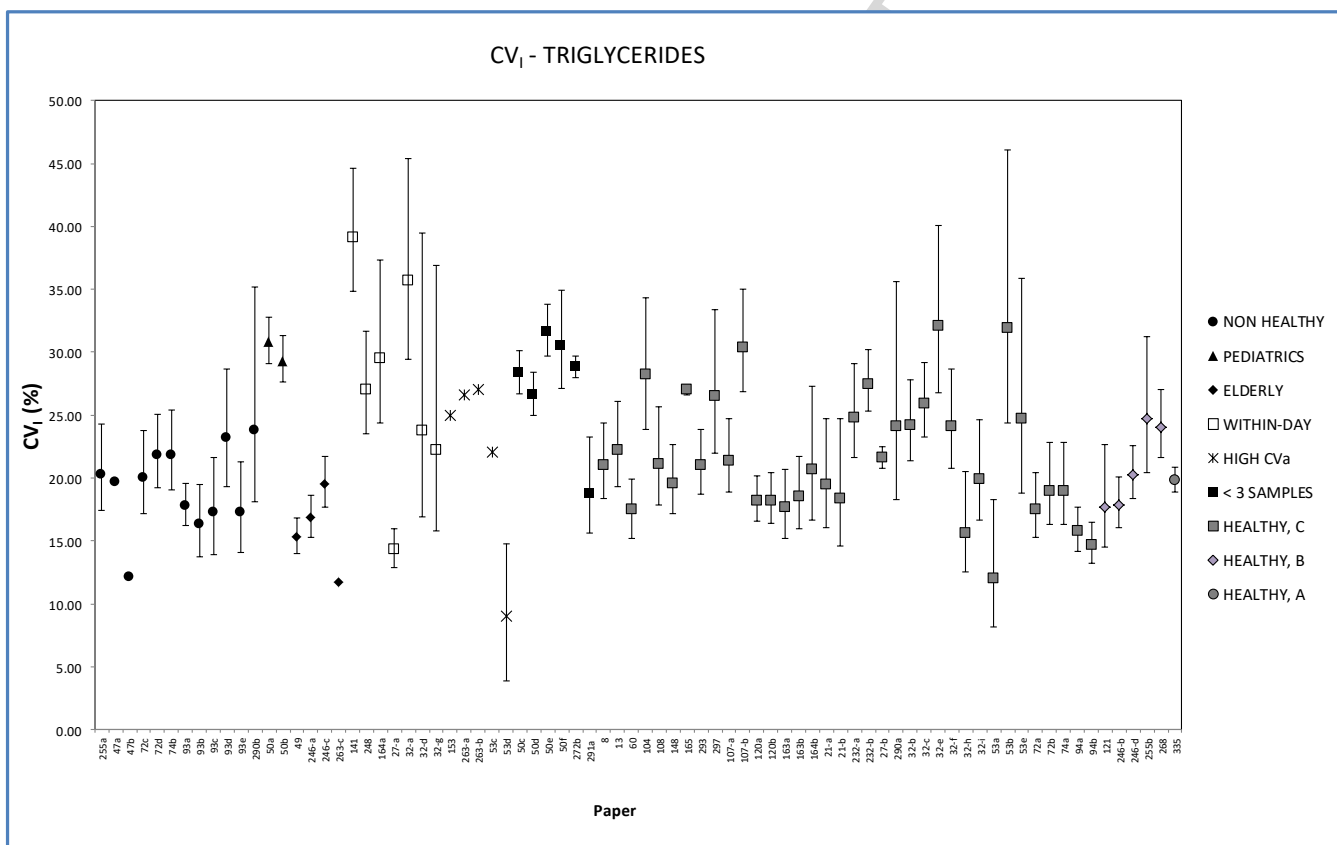
Figure 4. CV_I and 95% confidence interval estimates for HDL-cholesterol. The different symbols indicate that estimates are derived from studies identified as: 1) BIVAC A, B and C graded studies performed in healthy adults, 2) the same in elderly and pediatric subjects, 3) those in non-healthy subjects and 4) estimates derived from within-day studies, 5) studies with CV_A higher than desirable APS based on the online 2014 BV database and 6) studies with less than 3 samples per subject. Publications (X axis) are identified by the articles number given in the supplemental Table 1, with letters indicating subset of data (subgroups) derived from the same publication.
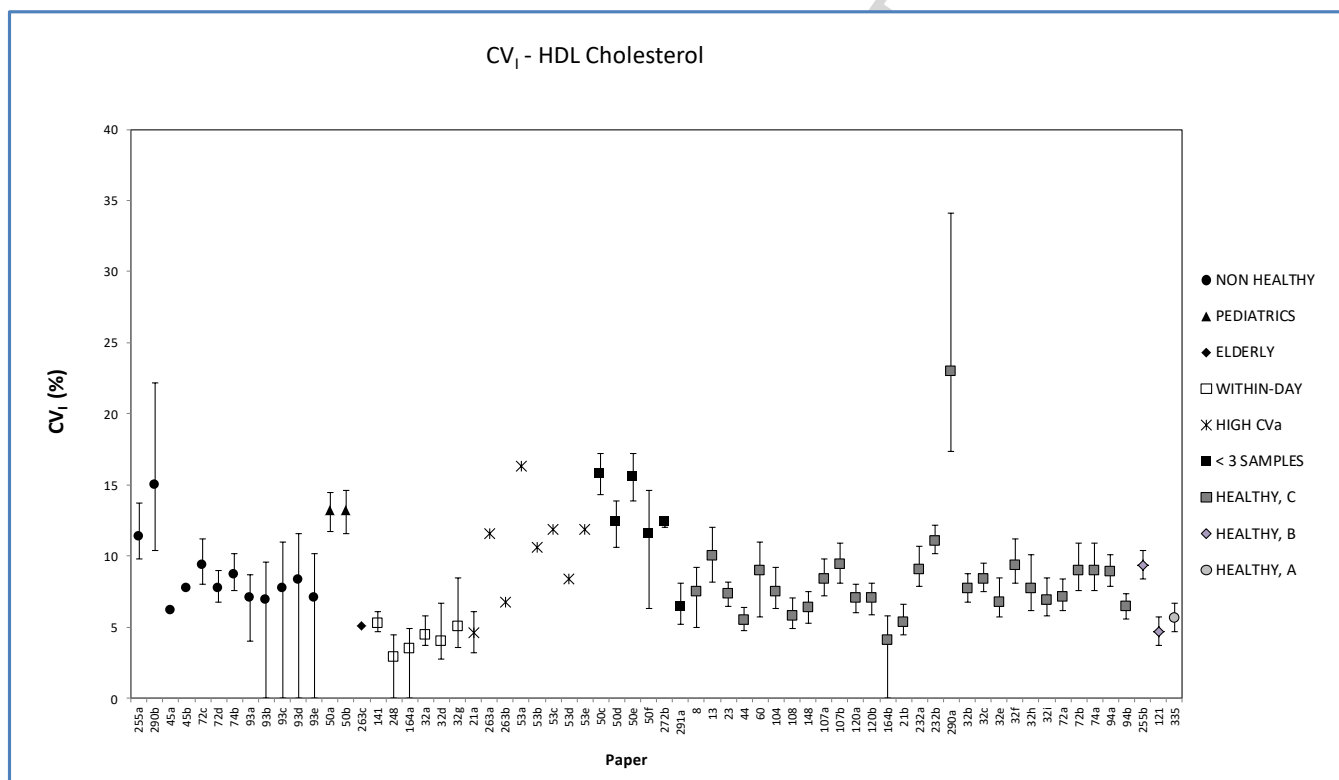
Figure 5. CV$_I$ and 95% confidence interval estimates for LDL-cholesterol. The different symbols indicate that estimates are derived from studies identified as: 1) BIVAC A, B and C graded studies performed in healthy adults, 2) the same in elderly and pediatric subjects, 3) those in non-healthy subjects and 4) estimates derived from within-day studies, 5) studies with less than 3 samples per subject. Publications (X axis) are identified by the articles number given in the supplemental Table 1, with letters indicating subset of data (subgroups) derived from the same publication.
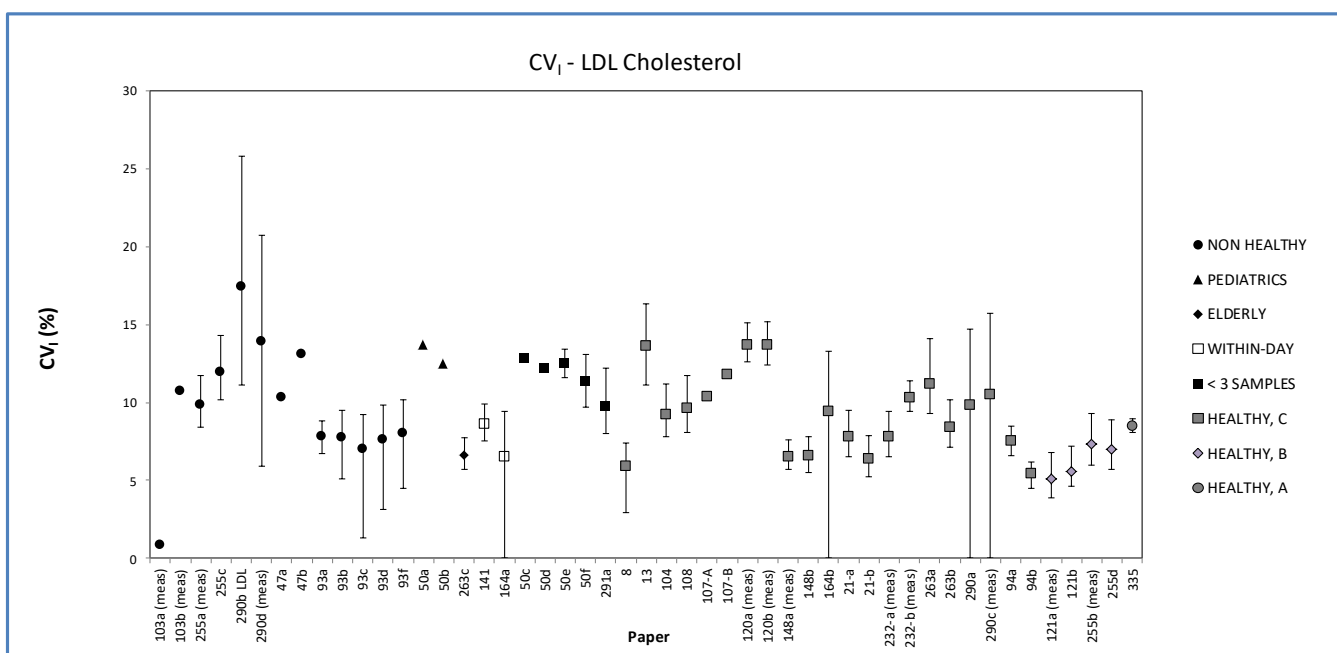
Highlights:

- Reliable biological variation (BV) estimates are necessary for optimal diagnosis and monitoring of cardiovascular risk.
- The Biological Variation Data Critical Appraisal Checklist has been applied to systematically evaluate BV studies for lipids
- This study provides updated and evidence-based estimates of within-subject (CV_I) and between-subject (CV_G) values for lipids based on BIVAC-compliant studies, delivered by meta-analysis
- Quality assessed BV data will in the future be made available in the EFLM Biological Variation Database.