Standardization of laboratory practices and reporting of biomarker data in clinical nutrition research

Karen M O’Callaghan and Daniel E Roth

Laboratory-derived measures of nutritional status and related biochemical phenomena (e.g., inflammation and oxidative stress) are critical tools in the nutritional sciences but have well-known challenges and pitfalls (1, 2). Researchers routinely examine biological sources of between- and within-person variation in the analysis of biomarker concentrations (e.g., age, sex, pregnancy, inflammation, etc.). However, close attention to the standardization and validation of laboratory practices is required to reduce the error variation that arises from inconsistencies in specimen handling, assay selection, assay performance, and management and statistical analysis of biomarker data (3–5). Furthermore, complete disclosure of laboratory assay protocols, performance characteristics, and technical limitations is essential to ensure the interpretability of published findings and promote opportunities for coherent pooling of biomarker data in meta-analyses.

In this issue of the Journal, 2 contributions from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) consortium (6, 7) revisit the real-world challenges of inconsistent nutritional biomarker measurement and reporting methods (2). In both studies, investigators used data from multiple population-representative surveys to determine the extent to which biomarkers of micronutrient status (folate and vitamin B12 in 1 study, zinc in the other) are associated with 2 biomarkers of systemic inflammation—C-reactive protein (CRP) and α-1 acid glycoprotein (AGP). As in prior BRINDA studies, the fundamental idea is that if micronutrient and inflammatory markers are consistently correlated, then estimates of the population prevalence of deficiency of that particular micronutrient should include a correction for inflammation (8). However, in both studies, the authors considered the wide variability in laboratory methods used for the measurement of micronutrient biomarkers to be a barrier to pooling of data across surveys (6, 7). For example, Young et al. (6) attributed their decision not to conduct pooled analyses to unquantifiable differences in methods used to assess folate and vitamin B12 status. Similarly, in their application of BRINDA methods to correct zinc concentrations for systemic inflammation, McDonald et al. (7) raised concerns about variability in blood collection procedures and laboratory analyses of plasma zinc, CRP, and AGP concentrations. The decision to forego meta-analyses was reasonable, but the unfortunate consequence was a rather complicated multiplicity of survey-specific analyses. Therefore, while the BRINDA project has undoubtedly made important contributions to our understanding of the role of inflammation in the interpretation of micronutrient biomarker data, it also reminds us of other pervasive and potent sources of variability in micronutrient concentrations—sample collection and storage methods, assay selection and performance, and other laboratory procedures.

The BRINDA authors acknowledged the scant information available to them concerning the specific assays used for each survey included in their studies (6, 7). Yet, for several of their surveys, samples were analyzed at the VitMin lab (Juergen Erhardt; http://www.nutrisurvey.de/blood_samples/), which uses a sandwich ELISA method to measure ferritin, retinol-binding protein, soluble transferrin receptor, CRP, and AGP (9). The VitMin lab has been a valued resource in the global micronutrient research community for many years; an initial validation study of its ELISAs was promising (9), although recent comparisons of the VitMin method to a new commercial assay showed poor concordance (10). For the surveys for which samples were analyzed at the VitMin lab, detailed measures of assay technique, measures of precision, and limits of quantification could have been feasibly obtained and assessed as part of the BRINDA project. For example, for CRP—a biomarker of the acute-phase response that is central to many BRINDA analyses—the VitMin lab reports values down to and including zero. According to published reports, prior BRINDA analyses have not routinely taken into account varying precision of the assay at lower concentrations or the VitMin laboratory’s stated limit of detection (LOD) of 0.5 mg/L (11–15). The LOD was, however, considered in a limited set of post hoc sensitivity analyses in the 2 recent BRINDA studies in this supplemental issue of the Journal, and was not found to affect their conclusions (6, 7). The LOD—defined as the lowest concentration of an analyte that can be feasibly and consistently detected—refers to the concentration that is reliably distinguished from “analytical noise”; even highly sensitive assays will rarely have the ability to measure concentrations of a true null value (16, 17). The lower limit of quantification (LLOQ) may be higher than the LOD and is the lowest concentration that is acceptably quantified by a particular assay, taking into consideration a desired level of accuracy and precision, which typically vary across the assay’s reportable concentration or the VitMin laboratory’s stated limit of detection (LOD) of 0.5 mg/L (11–15). The LOD was, however, considered in a limited set of post hoc sensitivity analyses in the 2 recent BRINDA studies in this supplemental issue of the Journal, and was not found to affect their conclusions (6, 7). The LOD—defined as the lowest concentration of an analyte that can be feasibly and consistently detected—refers to the concentration that is reliably distinguished from “analytical noise”; even highly sensitive assays will rarely have the ability to measure concentrations of a true null value (16, 17). The lower limit of quantification (LLOQ) may be higher than the LOD and is the lowest concentration that is acceptably quantified by a particular assay, taking into consideration a desired level of accuracy and precision, which typically vary across the assay’s reportable
range (16, 17). For analytes such as CRP, LODs and LLOQs are critically important in epidemiological studies, as considerable proportions of healthy populations can have unquantifiable results even when relatively high-sensitivity assays are used (18).

The BRINDA investigators (6, 7) were likely faced with a wide range of LODs/LLOQs for CRP assays included in their studies, but for most surveys the LOD/LLOQ was unknown or could only be inferred empirically based on the lowest nonzero value in the dataset (assuming that in generating the dataset, the LLOQ was imputed for all unquantifiable samples). Yet, the implications of variable LLOQs may not be negligible; for example, in a survey from Ecuador, the lowest CRP value in the dataset was 1.9 mg/L, and a majority of preschool children had this value (suggesting that the value was imputed for any child with a CRP value at or below 1.9 mg/L) (7).

As with nearly all laboratory biomarkers, substantial between-assay variations in CRP measurements have prompted unheeded calls for assay standardization (19). To consider how nutritional researchers generally handle the analysis and reporting of CRP, we searched online publications in the American Journal of Clinical Nutrition from the latter 6 mo (June to December) of 2019 for articles that reported CRP. Not surprisingly, we found wide variability in CRP assay selection (i.e., manufacturers and platforms/kits) across the 20 studies identified (20–39). All of the named methods were antibody based assays, and most studies used commercially available kits; we found very few (>2) articles that clearly used the same assay, but details about the methods were usually sparse, and 5 of 20 articles (35–39) did not specify the laboratory instrument or assay used. The widespread reliance on antibody-based assays (i.e., immunoassay, ELISA) is common in nutritional research, yet many (if not most) commercial immunoassay/ELISA kits on the market lack adequate validation or standardization (40, 41).

TABLE 1 Reporting of laboratory characteristics for C-reactive protein in original research publications in the American Journal for Clinical Nutrition from June to December, 2019

| Laboratory assay characteristic | Publications reporting characteristic, n (%) (n = 20) |
|---------------------------------|----------------------------------------------------|
| LOD and/or LLOQ                 | 4 (20%)                                            |
| Data handling method below LOD/LLOQ | 0                                                  |
| ULOQ                            | 0                                                  |
| Data handling method above ULOQ | 0                                                  |
| Inter-assay and/or intra-assay CV | 7 (35%)                                           |
| Specific analyzer and/or assay manufacturer | 16 (80%)                                         |
| Duplicate measurements performed for each sample | 2 (10%)                                           |

Note: LLOQ, lower limit of quantification; LOD, limit of detection; ULOQ, upper limit of quantification.

In these articles, we found that summary tables in the supplementary material enabled relatively complete and transparent reporting of relevant characteristics of the assays and laboratory practices and were particularly useful where several assays were studied.

Very few of the articles reporting CRP that we reviewed provided information about assay limits of sensitivity or the handling of values below such limits (Table 1). Given the uncertainty surrounding values between the LOD and LLOQ (16), the LLOQ is often of more concern in clinical and nutritional research is fragile (46). In addition to efforts to formally standardize assay selection and laboratory practices (47, 48), open communication between laboratory personnel and the investigators who analyze the data is essential to ensure that data management and analysis appropriately accounts for assay characteristics, including LODs and LOQs. Peer-reviewed journals could encourage improved practices by instituting checklists and guidelines for describing specimen handling and laboratory assays, or even consider minimum reporting
TABLE 2  Assay quality and performance indicators that may be considered standard reporting requirements of laboratory practices and characteristics in nutritional research

| Category                                                                 | Definition                                                                                                                                                                                                 | Explanations and examples                                                                                                                                                                                                 |
|--------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Protocols for specimen collection and handling and laboratory procedures | Detailed outline of procedures and materials sufficient to enable another investigator to replicate the analysis.                                                                                      | • Specimen information should include special considerations where appropriate (e.g., trace mineral–free blood collection materials) and details of specimen storage relevant to analyte stability (e.g., number of freeze–thaw cycles). |
| LOQs and reportable range                                                | LLOQ and ULOQ—lowest and highest concentrations, respectively, of analyte that can be repeatedly measured with acceptable accuracy and precision (17). Reportable range is the range of values across which results may be quantified and reported for a specific assay in a particular laboratory, including values generated by any standardized pretreatment procedures (e.g., sample dilution) (16). | • LLOQ typically refers to the concentration of lowest standard on the calibration curve.                                                                                                                             |
| Precision                                                                | Closeness of individual repeated measurements of the same sample, usually described empirically as a measure of imprecision (45), and determined by both within- and between-assay comparisons of results of 2 or more replicates. | • SDs and CVs (inter- and intra-assay) of individual repeated measurements under controlled conditions may be used to express precision.                                                                                       |
| Accuracy                                                                 | Extent to which assay produces “true” results relative to the gold-standard. Bias is average systematic difference between the test result obtained and accepted reference value; also known as systematic measurement error, as distinguished from random error (49). | • CVs may be used to convey within-run as well as between-run variation across batches, personnel, etc.                                                                                                               |
| Participation and performance in external quality assessment program    | Where applicable, participation in accuracy-based performance testing and/or external quality assurance schemes is encouraged and should be reported.                                                        | • Single CV values for each analyte are less informative than multiple estimates spanning detectable or clinically relevant ranges (e.g., low-, medium- and high-concentration control materials). |

1DEQAS, Vitamin D External Quality Assessment Scheme; LLOQ, lower limit of quantification; LOD, limit of detection; LOQ, limit of quantification; QA, quality assurance; QC, quality control; ULOQ, upper limit of quantification; VITAL-EQA, Vitamin A Laboratory—External Quality Assurance.

requirements of laboratory-related parameters and performance (Table 2). Yet, reporting of standards can only go so far, and greater attention to the optimization and standardization of laboratory activities is essential to promote the validity and reproducibility of clinical and epidemiological research.

The authors’ responsibilities were as follows—DER: conceptualized and structured the design of the Editorial and had responsibility for the final content; KMOC: conducted literature screening and analyzed the data; and both authors: wrote, read and approved the final manuscript. The authors report no conflicts of interest.

References

1. Hoofnagle AN. Harmonization of blood-based indicators of iron status; making the hard work matter. Am J Clin Nutr 2017;106(Suppl 6):1615S–9S.
2. Raiten DJ, Namaste S, Brabin B, Combs G Jr, L’Abbe MR, Wasantwisut E, Darnton-Hill I. Executive summary—Biomarkers of Nutrition for Development: building a consensus. Am J Clin Nutr 2011;94(2):S33–50.

3. Pfehali M, Sciacovelli L, Bernardi D, Aita A, Antonelli G, Padoan A. What information on measurement uncertainty should be communicated to clinicians, and how? Clin Biochem 2018;57:18–22.

4. Mocking RJ, Assies J, Lok A, Ruhe HG, Koeter MW, Visser I, Bocking CL, Schene AH. Statistical methodological issues in handling of fatty acid data: percentage or concentration, imputation and indices. Lipids 2012;47(5):541–7.

5. Pico C, Serra F, Rodríguez AM, Gómez MA, Serra-Majem L. New tools for new approaches. Nutrients 2019;11(5):1092.

6. Young MF, Guo J, Williams A, Whitfield KC, Nasrin S, Kancherla S, Suchdev PS, Raiten DJ, Brown KH, Flores-Ayala R, Addo OY. Overview of the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2019, submitted to AJCN.

7. McNicol C, Suchdev P, Krebs N, Wessells KR, Najib S, Rahman S, Wierenga F, Williams A, Brown K, King J. Adjusting plasma or serum zinc concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2019, submitted to AJCN.

8. Suchdev PS, Namaste SM, Aaron GJ, Raiten DJ, Brown KH, Flores-Ayala R. Adjusting total body iron for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. Adv Nutr 2016;7(2):349–56.

9. Erhardt JG, Estes ME, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr 2004;134(11):3127–32.

10. Karakachuk CD, Henderson AM, Samson KLI, Aljaadi AM, Devlin AM, Becquey E, Wirth JP, Rohner F. Comparison of a new multiplex immunoassay for measurement of ferritin, soluble transferrin receptor, retinol-binding protein, and C-reactive protein against a widely-used sELISA method. Diagnostics (Basel) 2018;8(1):13.

11. Namaste SM, Rohner F, Huang J, Bhushan NL, Flores-Ayala R, Kupka R, Mei Z, Rawat R, Williams AM, Raiten DJ, et al. Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2017;106(Suppl 1):S559–71s.

12. Rohner F, Namaste SM, Larson LM, Addo OY, Mei Z, Suchdev PS, Williams AM, Sakr Ashour FA, Rawat R, Raiten DJ, et al. Adjusting soluble ferritin receptor concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2017;106(Suppl 1):372s–82s.

13. Mei Z, Namaste SM, Serdula M, Suchdev PS, Rohner F, Flores-Ayala R, Addo OY, Raiten DJ. Adjusting total body iron for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2017;106(Suppl 1):383s–98s.

14. Larson LM, Addo OY, Sandalinas F, Faigao K, Kupka R, Flores-Ayala R, Suchdev PS. Accounting for the influence of inflammation on retinol-binding protein in a population survey of Liberian preschool-age children. Matern Child Nutr 2017;13(2):e12298.

15. Larson LM, Namaste SM, Williams AM, Engle-Stone R, Addo OY, Suchdev PS, Wirth JP, Temple V, Serdula M, Northrop-Clewes CA. Adjusting retinol-binding protein concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2017;106(Suppl 1):390s–401s.

16. Vashist SK, Luong JH. Bioanalytical requirements and regulatory guidelines for immunoassays. Cambridge, MA: Handbook of immunoassay technologies; Elsevier; 2018. p. 81–95.

17. van der Meer RF, Ten Kate H, Hulscher M, Nauta SL, Barkan DA, van der Post RA. Assessment of dietary pattern on inflammation and energy metabolism. Am J Clin Nutr 2019;110(4):984–92.
34. Meng H, Matthan NR, Wu D, Li L, Rodriguez-Morato J, Cohen R, Galluccio JM, Dolnikowski GG, Lichtenstein AH. Comparison of diets enriched in stearic, oleic, and palmitic acids on inflammation, immune response, cardiometabolic risk factors, and fecal bile acid concentrations in mildly hypercholesterolemic postmenopausal women-randomized crossover trial. Am J Clin Nutr 2019;110(2):305–15.

35. Tuomainen M, Karkkainen O, Leppanen J, Auriola S, Lehtonen M, Savolainen MJ, Hermansen K, Riserus U, Akesson B, Thorsdottir I, et al. Quantitative assessment of betainized compounds and associations with dietary and metabolic biomarkers in the randomized study of the healthy Nordic diet (SYSDiet). Am J Clin Nutr 2019;110(5):1108–18.

36. Seo M, Yamada T, Tamaki S, Morita T, Furukawa Y, Iwasaki Y, Kawasaki M, Kikuchi A, Kawai T, Abe M, et al. Prognostic significance of serum cholinesterase in patients with acute decompensated heart failure: a prospective comparative study with other nutritional indices. Am J Clin Nutr 2019;110(2):330–9.

37. Sarin HV, Taba N, Fischer K, Esko T, Kanerva N, Moilanen L, Saltevo J, Joensuu A, Borodulin K, Mannisto S, et al. Food neophobia associates with poorer dietary quality, metabolic risk factors, and increased disease outcome risk in population-based cohorts in a metabolomics study. Am J Clin Nutr 2019;110(1):233–45.

38. Khalaf A, Hoad CL, Menys A, Nowak A, Radford S, Taylor SA, Latief K, Lingaya M, Falcone Y, Singh G, et al. Gastrointestinal peptides and small-bowel hypomotility are possible causes for fasting and postprandial symptoms in active Crohn’s disease. Am J Clin Nutr 2019;110(1):131–40.

39. Sundfor TM, Svendsen M, Heggen E, Dushanov S, Klemsdal TO, Tonstad S. BMI modifies the effect of dietary fat on atherogenic lipids: a randomized clinical trial. Am J Clin Nutr 2019;110(4):832–41.

40. Rifai N, Watson ID, Miller WG. Commercial immunoassays in biomarkers studies: researchers beware! Clin Chem 2012;58(10):1387–8.

41. Prassas I, Diamandis EP. Translational researchers beware! Unreliable commercial immunoassays (ELISAs) can jeopardize your research. Clin Chem Lab Med 2014;52(6):765–6.

42. Gustafsson MK, Romundstad PR, Stafne SN, Helvik AS, Stunes AK, Morkved S, Salvesen KA, Thorsby PM, Syversen U. Alterations in the vitamin D endocrine system during pregnancy: a longitudinal study of 855 healthy Norwegian women. PLoS One 2018;13(4):e0195041.

43. Hang D, Kvaerner AS, Mu W, Hu Y, Tabung FK, Nan H, Hu Z, Shen H, Mucci LA, Chan AT, et al. Coffee consumption and plasma biomarkers of metabolic and inflammatory pathways in US health professionals. Am J Clin Nutr 2019;109(3):635–47.

44. Jones KS, Meadows SR, Schoennakers I, Prentice A, Moore SE. Vitamin D status increases during pregnancy and in response to vitamin D supplementation in rural Gambian women. J Nutr 2019;00:1–13.

45. Andreaasson U, Perret-Liaudet A, van Waalwijk van Doorn LJ, Blennow K, Chiasserini D, Engelborghs S, Fladby T, Genc S, Kruse N, Kuiperij HB, et al. A practical guide to immunoassay method validation. Front Neurol 2015;6:179.

46. Garza C, Stover PJ, Ohlhorst SD, Field MS, Steinbrook R, Rowe S, Woteki C, Campbell E. Best practices in nutrition science to earn and keep the public’s trust. Am J Clin Nutr 2019;109(1):225–43.

47. Binkley N, Dawson-Hughes B, Durazo-Arvizu R, Thamm M, Tian L, Merkel JM, Jones JC, Carter GD, Sempos CT. Vitamin D measurement standardization: the way out of the chaos. J Steroid Biochem Mol Biol 2017;173:117–21.

48. Haynes BM, Schleicher RL, Jain RB, Pfeiffer CM. The CDC VITAL-EQA program, external quality assurance for serum retinol, 2003–2006. Clin Chim Acta 2008;390(1-2):90–6.

49. Frenkel R, Farrance I, Badrick T. Bias in analytical chemistry: a review of selected procedures for incorporating uncorrected bias into the expanded uncertainty of analytical measurements and a graphical method for evaluating the concordance of reference and test procedures. Clin Chim Acta 2019;495:129–38.

50. Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, Makin HLJ, Pattini P, Sempos CT, Twomey P, et al. Hydroxyvitamin D assays: an historical perspective from DEQAS. J Steroid Biochem Mol Biol 2018;177:30–5.