Inflammation correction in micronutrient deficiency with censored inflammatory biomarkers

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ABSTRACT

Background: Biomarkers of micronutrient status vary with inflammation, and can be corrected by a regression-based approach [Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA)] using measured concentrations of inflammation biomarkers, e.g., C-reactive protein (CRP) and/or α1-acid-glycoprotein (AGP). However, this is confounded when inflammation is measured with multiple assays with variable limits of detection (LOD) and lower limits of quantification (LLOQ).

Objectives: We aimed to develop a probability approach for the estimation of prevalence of micronutrient deficiency using the distribution of true serum/plasma micronutrient concentrations in the population.

Methods: Left-censoring of an inflammation biomarker due to varying values of LOD or LLOQ was addressed by estimating the distribution of the inflammation biomarker at concentrations lower than the LOD and using this for the probability estimation of prevalence of nutrient deficiency. This method was evaluated using 2 publicly available data sets for children <5 y old: BRINDA and the Indian Comprehensive National Nutrition Survey. Each data set included measures of serum ferritin (SF), vitamin A, zinc, and CRP measured using different assays with variable LLOQs.

Results: The empirical distribution of SF after correction for CRP and AGP by the BRINDA method was comparable with the estimated probability distribution of SF, yielding similar estimates of iron deficiency prevalence when evaluated in the BRINDA data (17.4%; 95% CI: 15.2%, 19.7% compared with 16.8%; 95% CI: 13.9%, 20.0%; BRINDA compared with the probability method). The BRINDA method–adjusted iron deficiency prevalence was linearly associated with the proportion of left-censored CRP data, whereas these were not associated in the probability method. In the Indian survey data, estimates of prevalence of iron and zinc deficiency were comparable but vitamin A deficiency was lower by the probability method (17.6%; 95% CI: 16.7%, 20.2% compared with 15.7%; 95% CI: 15.2%, 16.3%; BRINDA compared with the probability method).

Conclusions: The proposed probability method is a robust alternate approach to the estimation of the prevalence of nutrient deficiency with left-censored inflammation biomarker data. *Am J Clin Nutr* 2021;113:47–54.

Keywords: inflammation biomarker, nutrient deficiency, BRINDA, probability method, left-censoring

Introduction

Serum ferritin, serum retinol, and serum or plasma zinc have been identified as biomarkers of nutritional deficiency (1). However, the plasma or serum concentration of biomarkers can get altered by the acute-phase response in response to microbial invasion, tissue injury, immunologic reactions, and inflammatory processes (2–4). Whereas the concentration of serum ferritin (SF) is elevated in the presence of inflammation, those of serum retinol and zinc are depressed. This can result in an underestimate of the prevalence of iron deficiency and an overestimate of the prevalence of vitamin A and zinc deficiencies (2–4).

To identify and quantify the extent and different stages of the acute-phase response, the concurrent measurement of acute-phase proteins like C-reactive protein (CRP) and α1-acid-glycoprotein (AGP), along with the nutrition biomarker, has been recommended by the WHO (5). Different methods have been proposed for the use of these acute-phase protein concentrations to adjust the estimation of the prevalence of iron, vitamin A, and zinc deficiencies, ranging from exclusion of nutrition biomarker

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Abbreviations used: AGP, α1-acid-glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CNNS, Comprehensive National Nutrition Survey; CRP, C-reactive protein; LLOQ, lower limit of quantification; LOD, limit of detection; SF, serum ferritin.

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values when these are elevated, to the more recent Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) method of correction (2–4), which does not require the exclusion or discarding of data, but salvages and uses all the valuable survey measurements collected.

However, a recent editorial by O'Callaghan and Roth (6) brings attention to a specific problem that could confound the use of the BRINDA method of adjusting for inflammation. They point out that the limits of detection (LODs) and the lower limits of quantification (LLOQs) for these assays may be unknown or even variable. This issue is particularly important in large surveys, where multiple laboratories may be used for sample analyses, with different standard operating procedures and different precision levels across different analytical methods, which are often poorly described. Excluding samples with values below the LLOQ, or imputing these values, or implementing single value substitution, can generate biased interpretations of micronutrient deficiencies when using the BRINDA approach. We offer a solution to the problem of multiple laboratories and assays/test kits in large-scale population surveys, through a probability approach for the estimation of micronutrient deficiency, where the parameters of distribution of the true serum/plasma micronutrient concentrations in the population are used in a direct estimation of the prevalence of deficiency.

Methods

The BRINDA method corrects the elevation or depression of the micronutrient biomarker that can be attributed to measured variations in the concentrations of either CRP or AGP, or both. The linear regression technique used by the approach either predicts an average outcome at any concentration of the predictors or estimates the rates of change in outcome for a change in the predictors. We also consider an intercept, in general, in a regression model that represents the population mean when influence of predictors on the outcome is absent. Similarly, the error variance is the population variance of the outcome which is assumed to be invariant over the concentration of predictors. Hence, per the aforementioned regression technique, the outcome, which is free from the influence of predictors, is assumed to be normally distributed with mean and variance as the intercept and error variance, respectively. The target for the BRINDA approach is this same distribution, which can be described through the regression technique. However, the remaining part of the BRINDA algorithm is not required, but can be termed as an alternative (nonparametric approach) to avoid the complicated probabilistic concept in a regression technique, although the desirable outcome of interest is stochastic in nature. We therefore propose a concept of inflammation correction that is aligned with the theoretical concept of regression in a stochastic phenomenon which may have a broader applicability in addressing some practical challenges, such as left-censoring measurements due to variation in the LODs and LLOQs.

Probability method of inflammation correction

We disaggregate the response biomarker or the micronutrient biomarker into 2 components: the first is the systematic component that is accounted for by inflammation; the second is a stochastic component which is accounted for by natural interindividual variability of the marker in a given population.

If "Y" is the measured biomarker of a nutrient intake and "X" is the measured biomarker of inflammation in a population, "Y" can be expressed as:

$$Y = \alpha + g(x) + \epsilon$$

where $g(x)$, the function of x, explains the deterministic part which is explained by x, and ϵ is a random component that explains the natural interindividual variability of the nutrient biomarker over population mean α. In a linear regression technique, ϵ is assumed to follow a normal distribution with mean 0 and variance σ^2.

A regression technique with valid parametric assumptions can directly estimate the true interindividual variability in a population after eliminating the systematic component. Thus, the inflammation-corrected prevalence of micronutrient deficiency can directly be estimated from this true probability distribution of the nutrient biomarker, that is, from the estimated distribution of the biomarker in a healthy population. Here, the manual elimination of the systematic part, as suggested by the BRINDA method, is not required, because the same correction automatically occurs in the intercept estimate, but at the population level.

In a simple linear regression with parametric assumption of normality we consider "Y" to be normally distributed with:

$$\text{mean}\ (Y) = \alpha + \beta x \ \text{and variance}\ (Y) = \sigma^2$$

By estimating the regression slope ($\hat{\beta}$), we can easily segregate the probability distribution of Y that accounts for natural interindividual variability as $N(\hat{\alpha},\ \hat{\sigma}^2)$:

$$\hat{\alpha} = \bar{y} - \hat{\beta}\bar{x}\ \&\ \hat{\sigma}^2 = \frac{1}{n-2}\sum_{i=1}^{n}\left(y_i - \hat{\alpha} - \hat{\beta}x_i\right)^2$$

Assuming "k" to be the cutoff for nutrient deficiency, the proposed probability method estimates the prevalence as follows:

$$\text{prevalence} = \text{probability}\ \{\epsilon < k\} = \Phi\left(\frac{k-\hat{\alpha}}{\hat{\sigma}}\right) \qquad (1)$$

where $\Phi(.)$ is the cumulative distribution function of a standard normal distribution in linear regression.

Probability method of inflammation correction with censored inflammation markers

For simplicity, let us assume that the inflammation marker measurements are left-censored owing to LODs or LLOQs at a fixed point. Let Y be the measured biomarker for any micronutrient intake and X be the measured inflammation marker such as CRP and/or AGP. We also assume that there are n_0 true X measurements and n_1 measurements that are left-censored at $\xi(n = n_0 + n_1)$. A dummy variable is defined for the i^{th} measurement as:

$$d_i = \begin{cases} 0, & if\ measured \\ 1, & if\ censored \end{cases}$$

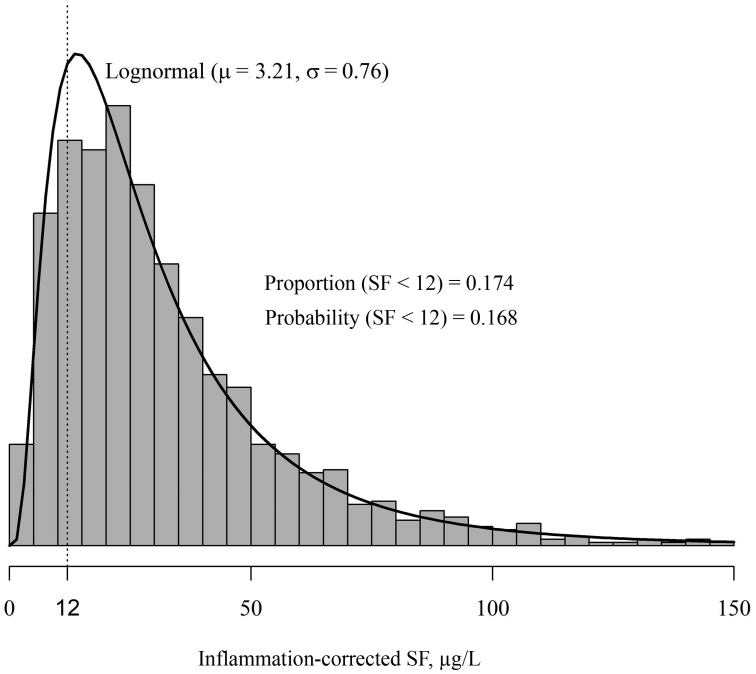

FIGURE 1 Comparison of inflammation-corrected SF by the BRINDA method and estimated probability distribution by the present probability method. Histogram: frequency density of SF (μg/L) with BRINDA method of inflammation correction using C-reactive protein and α1-acid-glycoprotein; smooth line: probability distribution of SF (μg/L) with the present probability method of inflammation correction; dotted line: cutoff value of iron deficiency (SF < 12 μg/L); proportion iron deficient after BRINDA correction is 0.174, proportion iron deficient after the probability method of correction is 0.168; *y* axis represents frequency for the histogram and probability density for the probability plot; BRINDA data were accessed and downloaded on March 1, 2020 from https://brinda-nutrition.org/the-brinda-approach/. BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; SF, serum ferritin.

We also define a new variable for "*X*" at the i^{th} measurement as follows:

$$Z_i = (1 - d_i)\ X_i + d_i U_i \quad (2)$$

where U_i is an unobserved random component with a suitable probability distribution. Then the regression model can be written as:

$$Y_i = \alpha + \beta Z_i + \epsilon_i = \alpha + \beta\{(1 - d_i)X_i + d_i U_i\} + \epsilon_i \quad (3)$$

where $\epsilon_i \sim N(0,\ \sigma_\epsilon^2)$ and $\log(U_i) \sim N(\delta_i,\ \sigma_u^2)$, because CRP or AGP have a positively skewed distribution.

Further, the parameters of the probability distribution of "*U*" can be estimated from the set of measured *X* below ξ, the censored point by maximum likelihood estimation considering $\delta_i = \delta$. However, for a better guess of those parameters, one can evaluate additional physical characteristics such as height, weight, or age that can further explain the variability of *X*, provided those are measured in both sets with *X*below ξ (true measurements and censored). If so, one can modify the estimate by regressing *X* on those physical characteristics as follows:

$$\hat{\delta}_i = \log\{E(U_i)\} = \hat{\gamma}_0 + \hat{\gamma}_1 h_i + \hat{\gamma}_2 w_i + \hat{\gamma}_3 a_i \quad (4)$$

and σ_u^2 can be estimated:

$$\hat{\sigma}_u^2 = \frac{1}{m-4}\sum_{j=1}^{m}\{\log(X_j) - \hat{\gamma}_0 - \hat{\gamma}_1 h_j - \hat{\gamma}_2 w_j - \hat{\gamma}_3 a_j\}^2 \quad (5)$$

where $\{X_j\}_1^m$ are the uncensored X_i's below ξ and $\{h_j,\ w_j,\ a_j\}$ are height, weight, and age, respectively.

The regression parameters of Equation 3 $\{\alpha,\ \beta,\ \sigma_\epsilon\}$ can be estimated by the Monte-Carlo simulation method.

The steps to be followed for the probability approach for a censored inflammation marker are as follows:

1) Generate a random number for each of $\{U_i^{(k)}\}_{n_0+1}^{n}$ from a lognormal distribution with mean and variance at log scale as in Equations 4 and 5, respectively. The inflammation biomarkers are assumed to have a lognormal distribution based on earlier reports (2–4) as well as observed data in the data set considered.
2) Derive Z_i's per Equation 2 as $Z_i^{(k)}$.
3) Regress Y_i on $Z_i^{(k)}$and estimate the regression parameters $\{\alpha^{(k)},\ \beta^{(k)},\ \sigma_\epsilon^{(k)}\}$.
4) Repeat all the above steps a sufficiently large number of times (say $r = 10{,}000$) and estimate the parameters as follows:

$$\hat{\alpha} = \frac{1}{r}\sum_{k=1}^{r}\alpha^{(k)};\ \hat{\beta} = \frac{1}{r}\sum_{k=1}^{r}\beta^{(k)};\ \hat{\sigma}_\epsilon = \frac{1}{r}\sum_{k=1}^{r}\sigma_\epsilon^{(k)} \quad (6)$$

The SE for the parameters can be estimated by:

$$\text{SE}\ (\hat{\alpha}) = \sqrt{\frac{1}{r}\sum_{k=1}^{r}(\alpha^{(k)} - \hat{\alpha})^2};\ SE\ \left(\hat{\beta}\right) = \sqrt{\frac{1}{r}\sum_{k=1}^{r}(\beta^{(k)} - \hat{\beta})^2}$$

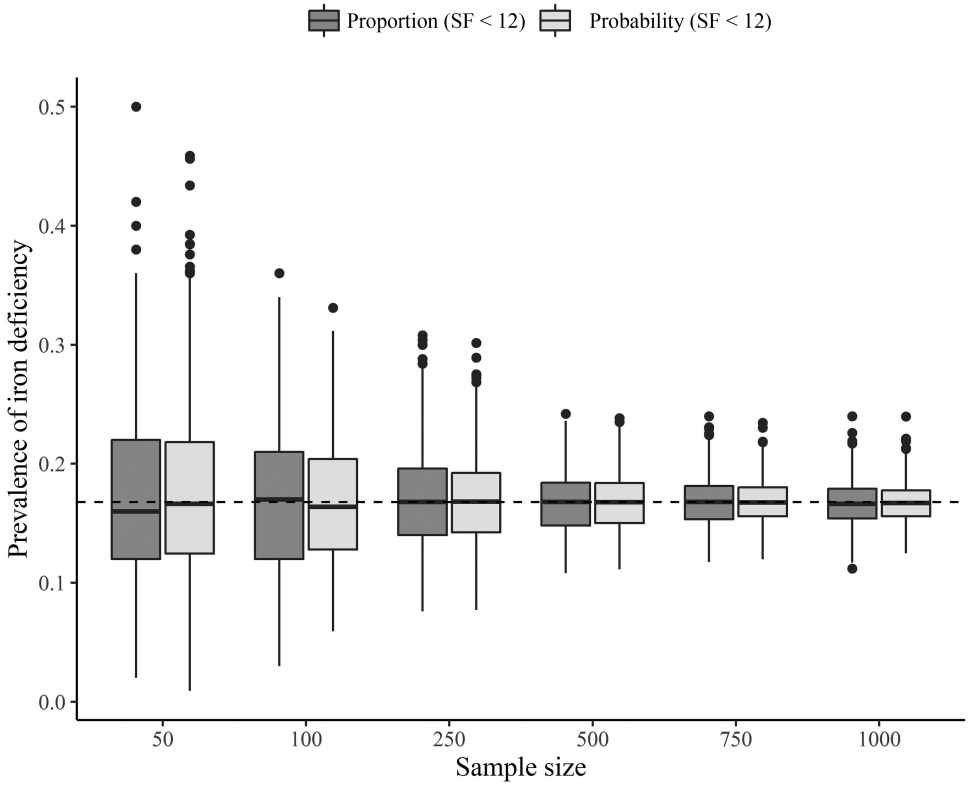

FIGURE 2 Comparison of estimates of the prevalence (proportion) of iron deficiency by the BRINDA method and the present probability method after inflammation correction with varying sample sizes. Dashed horizontal line indicates the true prevalence of iron deficiency (SF < 12 μg/L); BRINDA data were accessed and downloaded on March 1, 2020 from https://brinda-nutrition.org/the-brinda-approach/. BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; SF, serum ferritin.

$$\text{SE}\ (\hat{\sigma}) = \sqrt{\frac{1}{r}\sum_{k=1}^{r}(\sigma^{(k)} - \hat{\sigma})^2}$$

Alternatively, one can estimate the SE of the parameters at each of the k^{th} steps and then obtain the estimate with a similar approach as in Equation 6.

In the case of a symmetric biomarker for micronutrient intake, the prevalence of nutrient deficiency can be derived from Equation 1. However, for an asymmetric distribution of the biomarker, a lognormal distribution can be assumed for positively skewed distributions, followed by a modification of Equation 1 according to the cumulative distribution function of a lognormal distribution. Alternatively, this method can also be extended for a generalized linear model for a nonnormal distribution of the biomarker.

Validation based on a BRINDA SF data set

A sample data set that included SF, CRP, and AGP of 1102 school-going children was downloaded from the BRINDA website (https://brinda-nutrition.org/the-brinda-approach/). As the first step for the validation, we compared the probability method of inflammation correction with the defined BRINDA method. In this process, we validated the inflammation-corrected probability distribution of SF against the empirical distribution of SF after BRINDA correction. The prevalence of iron deficiency, estimated by both the methods with an arbitrary SF deficiency cutoff of 12 μg/L, was compared. Further, simulated data were generated through resampling of the sample data set to arrive at varying sample sizes. This was done to compare prevalence estimates by both the methods and their relative association with sample size. A set of censored data was generated by deliberately censoring CRP data at a value of 3 mg/L, with a varying censored percentage of data with CRP ≤3 mg/L. The association of the prevalence of iron deficiency with different proportions of censoring was explored by scatterplot and linear regression. Further, probability distributions of inflammation-corrected SF after substantial left-censoring such as 30%, 50%, and 80% of CRP were compared against the whole data set, without any censoring.

Application of the method to a recent Indian survey of serum retinol in children

The Comprehensive National Nutrition Survey (CNNS), 2016–2018 was conducted by the Ministry of Health and Family Welfare, Government of India, funded by UNICEF, across 30 states of India for nutritional deficiency assessment among preschool, school-going, and adolescent children. A detailed description of the survey has been published in the CNNS report (7). In this report, the CRP measurement was censored at 3, 3.1, and 3.2 mg/L, presumably owing to analysis by different

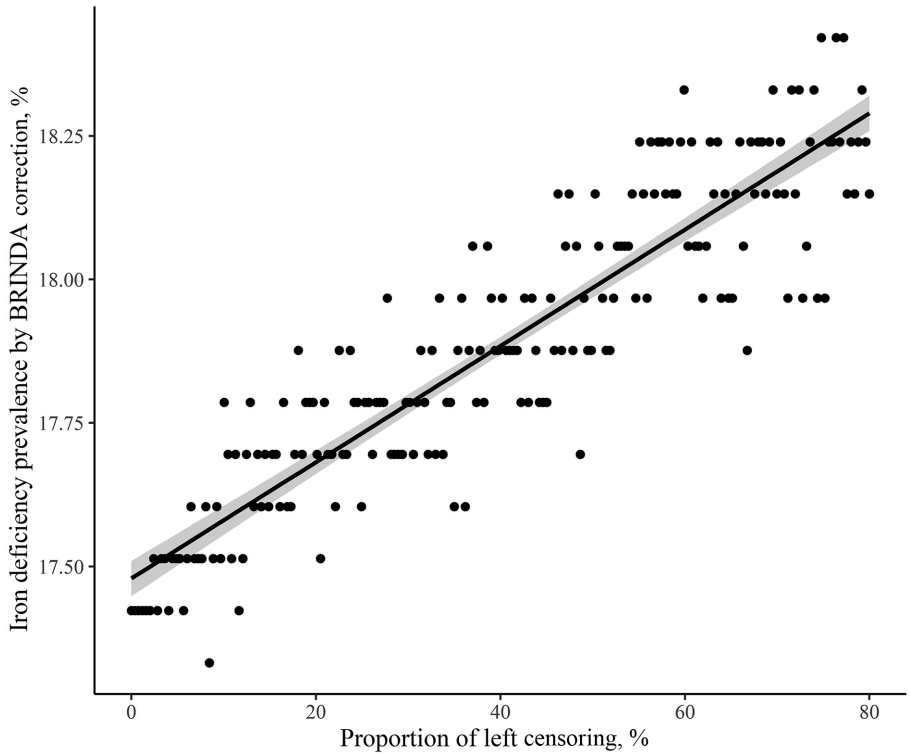

FIGURE 3 Scatter plot of iron deficiency prevalence after BRINDA correction at varying proportions of left-censored C-reactive protein measurements by a simulation study. The straight line is the regression line (95% CI) of prevalence on left-censoring proportion; simulated from BRINDA data accessed and downloaded on March 1, 2020 from https://brinda-nutrition.org/the-brinda-approach/. BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia.

assays, with varying LLOQs in ~80% of the sample. However, we assumed a single value of 3 mg/L as the censoring point. We applied the probability method of inflammation correction (for the participant flowchart see **Supplemental Figure 1**) and estimated the prevalence of iron deficiency and vitamin A deficiency among preschool children (1–4 y old). We then compared the prevalence estimates against the BRINDA method and the WHO-supported CRP >5 mg/L exclusion method (8). All statistical analyses and simulations were performed using R version 4.0.2 (R Foundation for Statistical Computing) (9).

Results

The empirical distribution of SF after inflammation correction for CRP and AGP by the BRINDA method compared well with the estimated probability distribution of SF by our probability method. Using an arbitrary SF cutoff of 12 μg/L, the prevalence of iron deficiency by the BRINDA method was 17.4% (95% CI: 15.2%, 19.7%) compared with 16.8% (95% CI: 13.9%, 20.0%) estimated by the probability method (**Figure 1**). In the simulation (**Figure 2**), we observed that both the bias and precision in the prevalence estimation were less than via the BRINDA method at smaller sample sizes. However, both estimates converged toward each other as the sample size increased. The simulated sample data set with varying levels of left-censoring for the CRP measurement showed that the over-estimation of the prevalence of iron deficiency by the BRINDA method was directly proportional to the percentage of data censored (**Figure 3**). However, almost no impact on the percentage of data censored was observed with the probability distribution method (**Figure 4**).

CRP was measured in the CNNS survey using 2 different analytical kits with distinctly different sensitivities. One kit could measure CRP at an LLOQ close to 0 (~0.2 mg/L), whereas for the other the LLOQ varied from 3 to 3.2 mg/L. Most CRP measurements were performed by the lower-resolution kit and therefore a large number (~80% <3.3 mg/L) of measurements were left-censored at 3, 3.1, or 3.2 mg/L (**Figure 5**). The estimates of the prevalence of iron, vitamin A, and zinc deficiencies were consistently more precise (based on the width of the 95% CIs) than via the BRINDA method, as well as the CRP-based exclusion method (**Figure 6**); prevalence of iron deficiency was 33.4% (95% CI: 31.0%, 35.9%) compared with 32.3% (95% CI: 31.5%, 33.1%), vitamin A deficiency was 17.6% (95% CI: 16.7%, 20.2%) compared with 15.7% (95% CI: 15.2%, 16.3%), and zinc deficiency was 19.0% (95% CI: 17.0%, 21.2%) compared with 17.4% (95% CI: 16.7%, 18.0%) by the BRINDA method compared with the probability method.

Discussion

The BRINDA consortium identified the weakness in excluding nutritional biomarker values from a survey that could potentially be elevated or depressed owing to inflammation, and developed a statistical method to utilize all available data. The BRINDA method is robust when nutritional and inflammation biomarkers

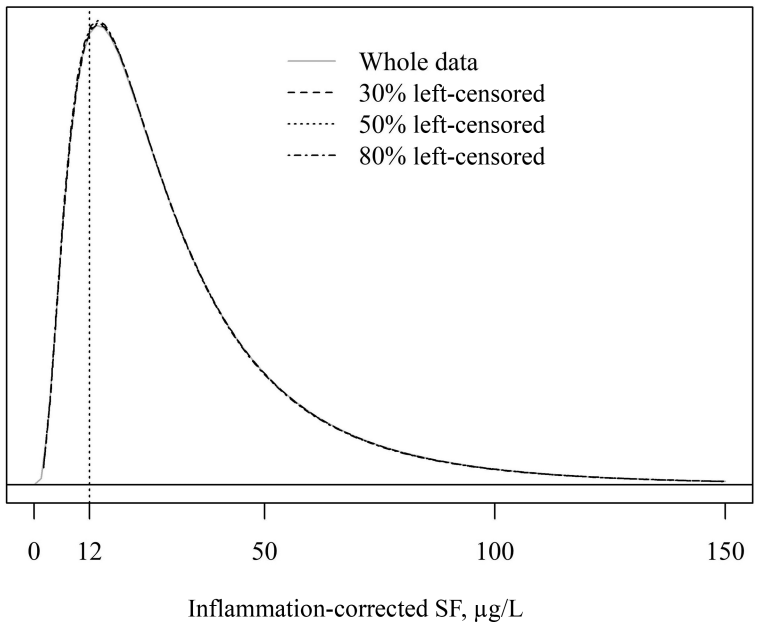

FIGURE 4 Estimated probability distribution of inflammation-corrected SF by the BRINDA method with 0% (whole data set), 30%, 50%, and 80% left-censored C-reactive protein measurements by simulation. Vertical dotted line: cutoff value of iron deficiency (SF = 12 µg/L); *y* axis represents probability density; overlapping curves: estimated distributions of SF over varying levels of censoring; simulated from BRINDA data accessed and downloaded on March 1, 2020 from https://brinda-nutrition.org/the-brinda-approach/. BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; SF, serum ferritin.

are measured using the same techniques and assays which have the same and low LODs and LLOQs within a single study (6). However, this is often not the case, and multiple assays with varying limits are used for operational reasons in many surveys. Then, the BRINDA approach is not sufficient to accurately estimate the inflammation-adjusted population prevalence of nutritional deficiencies, such as those of iron, vitamin A, and zinc. Thus, there is a requirement for greater rigor in reporting the details of assay precision, LLOQs, and protocols for specimen collection, handling, and laboratory procedures in surveys, but in the event of multiple assays of the inflammation biomarkers, with higher or varying LODs or LLOQs, there has been no alternative method available for estimating the prevalence of micronutrient deficiency. In this report, we have described

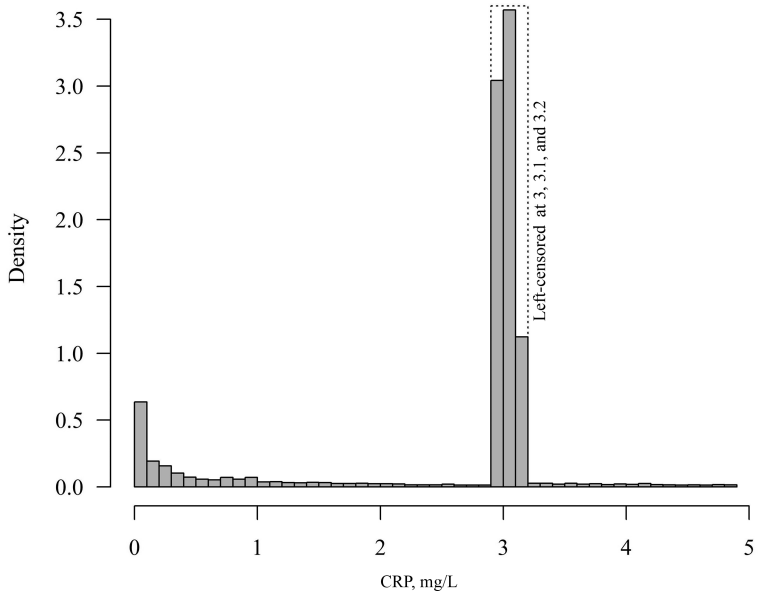

FIGURE 5 Distribution of CRP with left-censored values. Data used are Comprehensive National Nutrition Survey India data ($n = 35{,}997$). CRP, C-reactive protein.

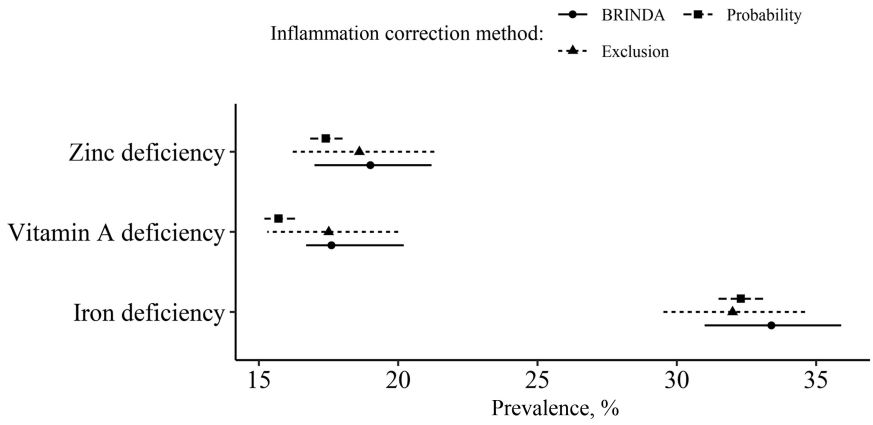

FIGURE 6 Prevalence estimates (95% CIs) after inflammation correction by the BRINDA method, exclusion method, and probability method. BRINDA method adapted from references 2–4. Exclusion method: nutrient biomarkers corresponding to C-reactive protein >5 mg/L excluded. Data used are Comprehensive National Nutrition Survey India data (n = 35,997). Iron deficiency: serum ferritin ≤12 μg/L; vitamin A deficiency: serum retinol <20 μg/g; zinc deficiency: serum zinc <65 μg/dL. BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia.

an extension of the method for estimation of micronutrient deficiency after inflammation correction, when left-censored inflammatory markers due to varying LLOQs are present, through a probability method by directly estimating the population distribution of inflammation-free nutritional biomarkers, which is unlike the nonprobabilistic and indirect approach adopted by the BRINDA method (2–4).

The BRINDA method of regression correction for all CRP values did give more precise estimates of micronutrient deficiency prevalence than the prior data exclusion method, and even demonstrated the correlation of CRP and AGP with the micronutrient biomarkers at concentrations that were below the inflammation cutoff of CRP and AGP (2–4). However, there is a possibility of overestimation of prevalence of deficiency by the BRINDA method with increasing levels of left-censoring in the inflammation biomarker (Figure 3). This is addressed by the probability approach, and the distribution of the inflammation-adjusted biomarker remains the same at multiple levels of censoring. The probability approach has been previously used in nutrition science for the estimation of dietary nutrient inadequacy where distributions of the requirement are skewed (10).

The method proposed in this article uses Monte-Carlo simulations to estimate the regression coefficient of the inflammation biomarker with greater precision when left-censoring renders varying amounts of data unusable. This is similar to the simulation techniques used in the estimation of iron deficiency anemia where Monte-Carlo simulations were used to separate uncertainty due to lack of data and variability due to biological variability in iron intake distributions (11). The recent Indian CNNS survey (7) posed a similar challenge, because multiple assays were used for the measurement of CRP. Here, the deficiency prevalence estimates of iron, vitamin A, and zinc were consistently more precise by the present method and relatively lower than those coming from the BRINDA approach. This difference was a little higher for vitamin A deficiency than for the other nutrients. The relative impact of the correction in the present method depends on the magnitude of the estimated regression slope of the inflammatory marker, the scale, and the shape of the distribution of the nutrient biomarker. The probability approach using censored inflammatory biomarker data will always improve the correlation between the nutrient and inflammatory biomarkers. Hence, a low scale and a regression slope relatively larger than the scale could be one reason for the lower prevalence estimate of vitamin A deficiency with the present method. In addition, the proportion of children within the neighborhood of the cutoff of the nutrient biomarker could also determine the magnitude of the impact of the correction. When data are clustered around the cutoff, as with serum vitamin A concentrations, even a small change in the slope of CRP may bring a substantial proportion of children on one side of the cutoff to the other, thus changing the prevalence estimate nontrivially. The present method could also be extended to the adjustment for AGP and malaria infection.

One of the limitations of our approach is that it is limited to population-level estimates of prevalence and is not applicable for the classification of individuals as nutritionally deficient or nondeficient. Therefore, the present probability method cannot be utilized directly for extended computations, for example, the calculation of body iron stores of an individual, with adjusted concentrations of SF and serum transferrin receptor. Our method of estimation of the prevalence of nutrient deficiency can be imprecise when the nutritional biomarker is itself censored; in this situation, further modifications in the proposed method will be required.

In summary, the proposed probability method of inflammation correction provides an alternative to the BRINDA method of inflammation correction, which is challenged in surveys with varying LLOQs for measurements of inflammation biomarkers and by left-censored data due to high LOD/LLOQs.

The CNNS study was conducted by the Ministry of Health and Family Welfare (MoHFW), Government of India, and UNICEF, with support from the Mittal Foundation. HSS designed the draft protocol of the CNNS with consultancy support from UNICEF, India. The data were provided to Indian researchers after a data user workshop conducted by UNICEF, India. AVK and HSS were members of the Technical Advisory Committee constituted by the MoHFW of the Government of India to oversee the conduct and analysis of the CNNS. We acknowledge the critical statistical review of the manuscript by T Bandyopadhyay of the Indian Institute of Management, Ahmedabad.

The authors' responsibilities were as follows——SG and TT: performed the modeling and statistical analyses; AVK and HSS: provided critical review of analyses and drafting of the manuscript; and all authors: read and approved the final manuscript. HSS is a member of the WHO Nutrition Guidance Expert Advisory Group (NUGAG) Subgroup on Diet and Health and a member of Expert Groups of the MoHFW on Nutrition and Child Health. AVK is a Nutrition Advisor to the Tata Trusts. SG and TT are UNICEF consultants for CNNS analyses.

Data availability

BRINDA data described in the article, code book, and analytic code are publicly and freely available without restriction at https://brinda-nutrition.org/the-brinda-approach/. The CNNS data described in the article will be made available upon request pending application and approval.

References

1. Raiten DJ, Namasté 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;94:633S–50S.
2. 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):359S–71S.
3. 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.
4. McDonald CM, Suchdev PS, Krebs NF, Hess SY, Ryan Wessells K, Ismaily S, Rahman S, Wieringa FT, Williams AM, Brown KH, et al. Adjusting plasma or serum zinc concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2020;111(4):927–37.
5. WHO. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations [Internet]. Vitamin and Mineral Nutrition Information System (WHO/NMH/NHD/MNM/112). Geneva, Switzerland: WHO; 2011. Available from: https://www.who.int/vmnis/indicators/serum_ferritin.pdf. [Accessed on 10th June, 2020].
6. O'Callaghan KM, Roth DE. Standardization of laboratory practices and reporting of biomarker data in clinical nutrition research. Am J Clin Nutr 2020;112(Suppl 1):453S–7S.
7. Comprehensive National Nutrition Survey (CNNS), Ministry of Health and Family Welfare (MoHFW), Government of India, UNICEF, and Population Council. Comprehensive National Nutrition Survey (CNNS) National Report. New Delhi. 2019.
8. WHO/CDC. Assessing the iron status of populations: report of a joint World Health Organization/ Centers of Disease Control and Prevention technical consultation on the assessment of iron status at the population level. Geneva: World Health Organization; 2005.
9. R Core Team. R: a language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.R-project.org/. [Accessed on 1st January, 2020].
10. Institute of Medicine. Dietary Reference Intakes: a risk assessment model for establishing upper intake levels for nutrients. Washington (DC): National Academy Press; 1998.
11. De Oliveira Mota J, Tounian P, Guillou S, Pierre F, Membré J-M. Estimation of the burden of iron deficiency anemia in France from iron intake: methodological approach. Nutrients 2019;11(9):2045.