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Invited Commentary

Correcting nutritional biomarkers for the influence of inflammation

In this issue of the journal, Diana *et al.*[1] describe the use of two methods of correcting four serum micronutrient biomarkers for the effects of inflammation. The acute-phase proteins (APP) serum C-reactive protein (CRP) and α-1-acid glycoprotein (AGP) were used to assess inflammation. Concentrations of the APP were either used to detect inflammation and categorise the biomarker results into four groups (a reference and three groups with inflammation)[2,3] or the second method used regression analysis of each APP as independent variables against the biomarkers as dependant variables; the latter method was recently introduced by the Biomarkers Reflecting Inflammation And Nutritional Determinants Of Anemia (BRINDA) group[4,5]. The first method produced correction factors (CF), whereas the second used the slopes (regression coefficients) of the two APP to adjust the biomarkers. In both cases arbitrary factors influenced the outcome as inflammation is defined in the CF method by CRP > 5 mg/l and AGP > 1·0 g/l while the regression method uses the maximum of the lowest decile of each APP concentration to define the reference, that is the group with no inflammation. The CF method is criticised[1] as possibly missing some of the subjects with inflammation as for all the biomarker comparisons made by the authors, the regression method gave a greater correction of the respective biomarkers than did the CF method. That is the regression approach generated a greater prevalence of Fe and lower prevalence of vitamin A and Zn deficiencies than the CF approach compared with unadjusted prevalence estimates. The authors expressed no opinion on which method was preferable to use.

It is now well recognised that infection and inflammation alter the concentrations of some nutrient biomarkers in the blood. As nutrition workers use these concentrations to indicate nutritional status and evaluate the success or otherwise of nutritional interventions, it is important to know the extent to which biomarker concentrations may have been altered by disease. The problems are particularly important where people are apparently healthy but live in areas where there is a high prevalence of endemic disease. In such areas, apparently healthy subjects may have been recently infected and are incubating a disease or recently recovered and in convalescence. In both scenarios, sub-clinical infection or inflammation will have increased inflammatory proteins and altered nutrient biomarker concentrations.

We introduced methods to correct plasma retinol[2] and ferritin[3] concentrations using CF derived from the ratios obtained from people with and without sub-clinical inflammation drawn from a number of studies using a meta-analytical approach. Inflammation was categorised using the two APP, CRP and AGP to provide three CF for those incubating disease and those in acute or chronic convalescence. Diana *et al.* refer to this method as giving 'external CF' and they used the same method on their own data to calculate internal CF and compared both with the regression approach outlined by the BRINDA group. The first observation of note is that prevalence estimates for deficiencies of Fe and vitamin A using ferritin and retinol binding protein biomarkers, were similar irrespective of whether internal or external CF were applied. Similar findings were reported for vitamin A and/or Fe in Liberian children[6], Kenyan pre-school children[7] and in children with moderate acute malnutrition in Burkina Faso[8]. The finding of very similar outcomes using external and internal CF in children in Asia and East and West Africa suggest that the CF generated by the meta-analyses may be widely applicable in children in tropical environments.

The important question arising from this paper[1] however is which approach provides the better estimate to correct nutritional biomarkers for the effects of inflammation; CF or regression? When we originally proposed the CF method we believed its usefulness depended on subjects being apparently healthy. That is we excluded subjects with sicknesses such as, for example, diarrhoea, fever, respiratory tract infection, etc., in order that the subjects would not have very high APP and in general the inflammation would be relatively uniform and mild. We included groups in the meta-analyses where there may have been sub-clinical malaria as our CF results did not vary if the groups were included on not. Others however have shown that if sub-clinical malaria is identified, CF needed to be greater to remove the effects of inflammation[9].

In the paper by Diana *et al.*[1], the infants are described as apparently healthy with no evidence of chronic disease or acute malnutrition but, according to maternal reports at the study visits, 43–51 % had fever and/or cough and 11–18 % had vomiting or diarrhoea. It seems possible therefore that inflammation in the infants was not mild and CRP and AGP concentrations were reported to be as high as 100 and 3·6 g/l, respectively, at some visits. There are parallels in this report with the results of Cichon *et al.*[10] who did similar method comparisons on ferritin analyses in 1609 children with a mean age of 12·3 months in Burkina Faso. Physical examinations found 72 % had clinical symptoms (fever, malaria, upper and lower respiratory tract infections and diarrhoea) and more than 24 % had a CRP concentration >10 mg/l and 66 % an AGP concentration >1 g/l. In spite of the high proportion with clinical symptoms, internally calculated CF for ferritin did not

significantly differ from those calculated for all children in our meta-analysis[8]. However, the authors concluded that 'regression analysis is preferable to the CF approach when adjusting serum ferritin for inflammation as it accounts for severity of inflammation and morbidity, however, in clinical settings the use of the meta-analysis CF may be appropriate'. The latter conclusion was suggested in spite of the fact that 72% of their subjects had clinical symptoms.

As indicated above, a number of workers have shown internally calculated CF are statistically similar to those produced by the meta-analysis studies of retinol and ferritin. The meta-analysis studies were originally designed to be done on apparently healthy subjects with mild inflammation and correlations between the biomarkers and the APP would therefore be poor. The observations of Diana *et al.*[1] and Cichon *et al.*[8] suggest that some subjects with more severe inflammation can be included without seriously altering the CF obtained by meta-analysis. In the studies of Diana, Cichon and the BRINDA reports regression analysis produced greater corrections than the CF for all biomarkers studied. These data suggest that the biomarkers are significantly correlated with the APP and in the BRINDA reports there were strong correlations between biomarkers and APP in all countries[4,5]. The BRINDA data was from national surveys and although morbidity data were collected it would not have determined subject selection[11], therefore within the populations a number of subjects would not have been apparently healthy. If subjects with identifiable morbidity had been excluded, the regression slopes would have been shallower and the difference between the regression and CF approaches would probably have been smaller.

In conclusion, the CF method may wrongly classify some subjects with mild inflammation in the reference group because of the arbitrary cut-offs for CRP and AGP but, the CF approach would appear to be a method that enables workers to correct for inflammation and compare the nutritional status of different populations, at least for vitamin A and Fe. This is not currently possible with the regression method as the cut-off for no inflammation is arbitrarily defined as the upper value of the first decile of the CRP and AGP concentrations. This may differ between populations depending on endemic disease and make comparison between populations more difficult. In addition there was heterogeneity in the linear relations (slopes) between biomarkers and APP for different countries, so currently a unified formula for the regression approach is not available[4,5]. Thus the lower cut-points to define no inflammation and inclusion of a variable number of persons with disease probably explain the greater adjustment to biomarkers produced by the regression method in the studies of Diana, Cichon and BRINDA. Further comparative work is needed to determine if the greater sensitivity of the regression method to correct for inflammation outweighs the greater simplicity of the categorical CF approach in making inter-country comparisons of nutritional data.

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