Handling Data when Inflammation is Detected

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In a recent report on nutritional status among pregnant women in the Sidama Region of Southern Ethiopia, eight afebrile women (10%) were found to have elevated C-reactive protein (CRP) concentrations (>5 mg/L)[1] *(see article, Nutritional Status in the Sidama Region of Southern Ethiopia, p41).* Results from these women were removed from most of the analyses because of the known effects of inflammation on a large number nutritional biomarkers.[2] However, the authors showed that inflammation made an important contribution in explaining the variance in hemoglobin and that the apparently-healthy women with elevated CRP concentrations had lower hemoglobin, red cell counts and retinol concentrations – although none of the other biomarkers that were measured differed between those with and without inflammation.

The question has to be asked whether, by excluding the women with inflammation, the data were biased and if the data had been included, would that have altered (improved) the interpretation of some of the other analyses? For example, were the data from the women removed all from one or other of the two groups and, if so, did it say anything about the dietary habits of a specific group? In a recent paper, we suggested a general method that can be applied to most data sets that avoids the loss of data,[2] and we have shown that the method can work well for retinol[3] and ferritin.[4] However, the method does require the analysis of a second acute phase protein (APP), namely α_1-acid glycoprotein (AGP).

The need to measure chronic inflammation

Inflammation affects the concentration of many nutritional biomarkers[2] and studies have shown that the number with inflammation in a community can be large. A recent study found that 95% of Gambian infants had inflammation in the first 12 months of life.[5] Not all this inflammation will be revealed by CRP alone and we suggest that one chronic APP, i.e AGP, and one acute APP, i.e CRP, are measured.[3] AGP is a marker of chronic inflammation and, in the presence of infection or trauma, the concentration of AGP rises more slowly than CRP, taking 4–5 days to

Table 1: Change in biomarker concentrations in inflammation categories[1, 2]

Nutritional biomarker	Incubation (elevated CRP only)	Early convalescence (elevated CRP and AGP)	Late convalescence (elevated AGP only)
Retinol (both sexes)	-13	-24	-11
Ferritin (men)	+58	+505	+225
Ferritin (women)	+22	+419	+160

[1] Data are percentages
[2] Changes in retinol and ferritin concentrations from references 3 and 2, respectively

Table 2: Correction factors for ferritin based on data in Table 1[1]

Gender	Reference group: No inflammation Median (quartiles)	Correction factors[2] Incubation	Early convalescence	Late convalescence
Men	166 (94, 277)	0.630	0.165	0.307
Women	41 (13, 150)	0.820	0.190	0.380

[1] Data from reference.[2] Correction factors are the ratios of the medians of the reference group to the respective inflammation groups.
[2] Correction factors were the ratios of the medians of the groups with no inflammation (reference groups) to those of the respective inflammation groups.

reach plateau concentrations (>1 g/L). However, it remains elevated for longer than CRP after clinical signs disappear.[6] Use of the two APPs enables the nature of the inflammation to be described more fully and equated to the known effects of inflammation on nutritional biomarkers. **Figure 1** describes the phases of inflammation, by CRP and AGP.[2, 7]

In apparently-healthy people, these two APPs, when elevated, can reveal three scenarios. At the onset of infection, some APPs, like CRP, increase very rapidly before the onset of clinical signs. So, an elevated CRP concentration alone can indicate people in the incubation phase of disease. If, however, an apparently-healthy person has a raised CRP and a raised AGP, the person has recently recovered from disease, i.e. is in early convalescence. Normal CRP (< 5 mg/L) but elevated AGP indicates late convalescence. Thus, using two APPs, apparently-healthy people can be categorized into four groups: healthy (or reference group, with no raised APPs), incubation, early convalescence, and late convalescence.[3]

Inflammation also influences concentrations of nutritional biomarkers. Changes in retinol and ferritin are shown in **Table 1**. The results for retinol were obtained from a meta-analysis of 15 studies so they represented average results from studies in Africa, Asia and South America. In contrast, the results for ferritin were obtained in one study of apparently-healthy HIV-positive, adult Kenyan men and women, and may not be representative of other communities. The results do indicate that the change in the nutritional biomarker can be very different depending on the stage of inflammation. Early convalescence is usually the phase where most changes in biomarker concentration are seen, but even in the incubation phase, median ferritin concentrations increased by over 50% in men.

Effect of inflammation not predictable on nutritional status from CRP alone

Results in **Table 1** indicate the importance of measuring two APPs. If CRP only is elevated, you do not know whether your subject is incubating a disease or in early convalescence. Correcting retinol by a factor of 13% is very different from 24% and could make a big difference to the assessment of status in the community. Even if verbal confirmation of recent diseases is sought to distinguish whether a subject is in the incubation phase or early convalescence, if only one APP is measured, there is no way of knowing whether a person has, for example, been bitten by a malaria-positive mosquito in the last 24 hours and that the incubation phase is now superimposed on a late convalescence.

In the case of ferritin, the under-estimation of iron deficiency could be enormous, depending on what decision is made. In fact with one APP, the only decisions that can be made with the results from subjects with elevated APPs is to discard or ignore the results.

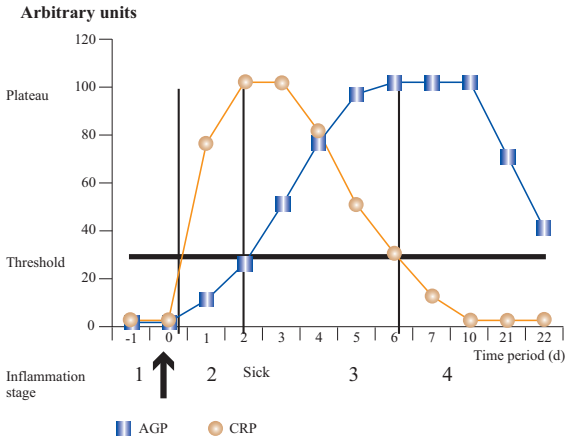

Figure 1: Phases of inflammation, characterized by CRP and AGP[2, 7]

In the case of the Ethiopian women, only 10% had elevated CRP concentrations and the results were discarded.[1] But if 95% of apparently-healthy subjects had had elevated APP as in the Gambian infants,[5] the results would have to be used and the inflammation ignored.

We proposed that instead of discarding data from subjects with inflammation, that the data are adjusted to remove the influence of inflammation.[2] In the case of retinol, the effects of inflammation were broadly similar across communities and genders. In the case of ferritin, from the limited amount of work we have done to date, the effect of inflammation on ferritin appears to be proportional to the pre-infection ferritin concentration,[2, 4] and the correction factors for men and women in the respective inflammation groups are broadly similar **(Table 2)**. Ferritin concentrations in subjects with no inflammation are shown in **Table 2** and they are assumed to represent pre-infection ferritin concentrations of the community. The effect of inflammation on ferritin is removed by multiplying the individual ferritin concentrations of subjects in the inflammation groups by the respective correction factors. In this way, individual values are adjusted to compensate for the effect of inflammation, the median values of all four groups become the same, and all data can be retained for further analysis. However, although the correction factors for ferritin within inflammation groups were of the same order, there were differences between men and women. In the Kenyan study, the number of men (n=56) was smaller than the number of women (n=107), and the numbers of subjects in the incubation (n=18) and in the late convalescent groups (n=16) were small. These factors probably accounted for the different correction factors seen between the sexes but use of these correction factors nevertheless appear to improve the consistency of the data and its ability to demonstrate the effect of iron intervention on ferritin and hemoglobin.[4]

In different communities, ferritin concentrations differ considerably. Among the pregnant Sidaman women, the mean ferritin concentration was 13.9 µg/L, whereas in the non-pregnant Kenyan women, it was 41 and, in men, 203 µg/L (the latter two groups without inflammation, **Table 2**). We do not yet know whether the correction factors shown in **Table 2** will apply universally. A meta-analysis is currently underway to examine this point. The big differences in ferritin concentrations between the sexes do indicate, however, that just elevating the acceptable threshold for ferritin (12 µg/L[1]) to 50 µg/L, as suggested by some authors,[8] is a very blunt instrument to prevent underestimation of iron deficiency and probably unsuitable in most situations since changes in ferritin depend on the stage of infection or recovery.

Can we avoid having to measure two APPs (see Figure 2)?

Measuring biochemical markers is expensive and there is resistance in the international community to measuring two APPs. CRP is widely measured and understood, and there are field techniques available but AGP is little known and measured in only a few laboratories. Can we make assumptions on the proportions of people in the different inflammation categories if we only have a measurement of CRP? For example, in Pakistani preschool children, where acute and chronic APPs were measured on 2,514 children, we found 38% with no inflammation and 1%, 10% and 51% in the incubation, early convalescent and late convalescent groups, respectively[3, 9]

An examination of the distribution of results used in the meta-analysis on vitamin A shows that, usually, the least number of subjects is

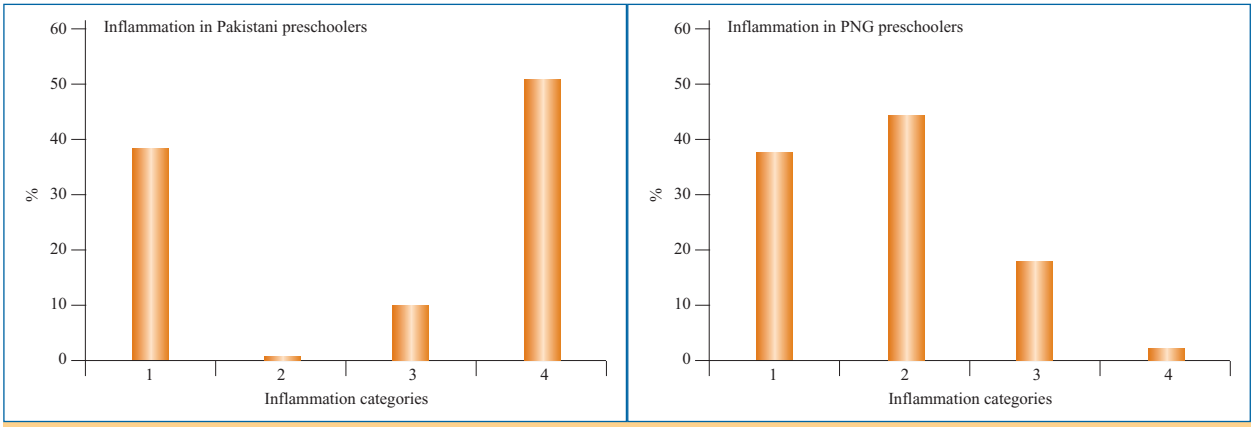

Figure 2: Proportions of apparently-healthy preschool children in different inflammation categories

found in the incubation group as in the Pakistani preschool children[3] – however, in a malaria-endemic area, this situation appears to be reversed. In preschool children in Papua New Guinea (PNG), there were 44%, 17% and 2% in the incubation, early convalescent and late convalescent groups, respectively. We have recently found a very similar distribution of subjects in two other malaria-endemic communities (unpublished) so the PNG situation does not appear to be unique. No doubt the distribution of subjects within the different inflammation groups tells us something about the type and prevalence of disease in a community but predictions on such distributions based on the result of one APP appear unlikely in the near future.

Conclusions

Measuring one APP alerts the investigator to the presence of inflammation in a community but does not enable any correction of data for the influence of inflammation. Results can only be discarded or the inflammation ignored. Measuring plasma CRP (acute) and AGP (chronic) concentrations enables subjects with inflammation to be categorized and some corrections to be made for the influence of inflammation on biochemical markers. Predictions of inflammation categories based on one APP measurement appear to be unlikely at this time. To reveal the inflammation in a community and to be able to correct important nutritional indices for the presence of inflammation requires the measurement of two APPs.

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