Interpretation of serum ferritin concentrations as indicators of total-body iron stores in survey populations: the role of biomarkers for the acute phase response\textsuperscript{1–4}

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ABSTRACT

Background: Nutritional surveys use acute phase protein (APP) biomarkers such as C-reactive protein (CRP) and \( \alpha_1 \)-acid glycoprotein (AGP) to identify the influence of inflammation on the distribution of iron status biomarkers. Few, however, have examined which biomarker better identifies persons with spurious elevations in iron status markers.

Objective: We explored the relations of APP biomarkers to iron-status biomarkers in infants and school-age children.

Design: In screening surveys, we identified a sample of African American infants (\( n = 351 \)) and Guatemalan school-age children (\( n = 375 \)). We used a common set of APP and iron-status biomarkers to examine the association between the 2 sets of markers (laboratory variables).

Results: The overall prevalence of either inflammation or iron deficiency was <10% in both samples. The log AGP and CRP values were significantly correlated (\( r = 0.70 \)), but the unexplained variance still was >50%. Serum ferritin—but not transferrin receptor, transferrin receptor index, or serum iron—was related to APP concentrations, but poor positive predictive value (<72%) and low kappa scores were found. Ferritin concentrations >1 geometric SD above the geometric mean were poorly predicted by either elevated AGP or CRP. Qualitative CRP analysis was not effective in identifying persons who had other indications of mild inflammation.

Conclusions: These analyses show that a low prevalence of inflammation has little influence on the distribution of ferritin, and 2 common indicators of inflammation do not perform equally well in identifying persons who may have elevations in ferritin due to inflammation. 

KEY WORDS

Iron deficiency, ferritin, transferrin receptor, acute phase proteins, C-reactive protein, \( \alpha_1 \)-acid glycoprotein, children, Guatemala

INTRODUCTION

Current studies of the prevalence of iron deficiency often incorporate biomarkers of infection and inflammation to aid in interpreting some markers of iron status (eg, ferritin, serum iron, and transferrin saturation), which themselves are altered during acute phase reactions (1). Relatively few surveys have used multiple biomarkers of inflammation, and even fewer have attempted to determine which inflammation biomarker may be most useful in the identification of persons with alterations or distortions in iron-status variables due to inflammation (2–5).

The current study examined the performance of 2 of the most common biomarkers, C-reactive protein (CRP) and \( \alpha_1 \)-acid glycoprotein (AGP), in infants and young children.

Persons with acute and chronic infections have lower serum iron, transferrin saturation (TSAT), zinc, and serum retinol concentrations and higher ferritin concentrations than do persons without inflammation (6). A difficulty in nutritional surveys then arises in identifying persons with inflammations and in estimating how much influence the inflammatory response may have on the nutritional status variables being measured. At least 3 approaches have been explored. The first is to adjust the cutoff for the definition of a low ferritin concentration upward to account for the influence of inflammation on ferritin. This has been suggested in studies of children aged <5 y (7) and in studies of chronic inflammation in the elderly (8). In both situations, the suggestions of cutoffs extend the normal 12–15 \( \mu \)g ferritin/L cutoff to 30–50 \( \mu \)g/L. A second option is the use of acute phase protein (APP) information to exclude persons with inflammation from the analysis of the data set in an effort to ensure that the iron status of the persons analyzed is more accurately known (9). This approach is likely to be unsatisfactory in parts of the developing world where endemic parasitic infections may cause >50% of a particular sample to manifest inflammation (3, 10). A third option, not yet truly applied to the iron nutrition agenda, is to generate a correction factor on the basis of commonly used markers of inflammation to adjust for the elevation in ferritin based on the elevations in the sentinel APP (11). All these approaches have limitations that result from constraints of either biology or the nature of subject populations.

The temporal response to the induction of inflammation is different for each of the APPs and nutritional variables (12). Very...
few time course experiments are available from which to glean within-person information on the time of peak elevation in the acute phase response (13). However, it appears that serum iron and retinol concentrations drop within several hours of the onset of infection, and that those decreases are quickly followed by an elevation in CRP. AGP does not reach a maximum concentration until 24 h after a single stimulation with interleukin 6 (IL-6); ferritin does not reach its maximum until 48 h after such stimulation. The CRP concentrations begin to decline 24–48 h after the initiation of inflammation, whereas elevations in AGP persist for 5–6 d and those in ferritin for up to 10 d (13).

To address these issues, we measured CRP (both quantitatively (qn-CRP) and qualitatively (ql-CRP)) and AGP along with serum ferritin, serum transferrin receptor (TfR), and other measures of iron status in several studies by using identical methods in all cases. One study involved 351 samples from 9–12-mo-old African American infants from inner-city Detroit (14), and the other involved samples from 242 children aged 7–11 y in Guatemalan cities (NW Solomons, unpublished observations, 2005). Our objective in this analysis is to determine whether one APP has an advantage over another in the identification of persons who have elevated ferritin as a result of a current infection. In addition, we wanted to evaluate whether the ql-CRP assay is diagnostically useful in identifying the subgroup of persons who are at risk of inflammation.

SUBJECTS AND METHODS

Subjects

All procedures were approved by the Institutional Review Board of The Pennsylvania State University; the Human Subjects Committee of the Institutional Review Board of the Center for Studies of Sensory Impairment, Aging, and Metabolism (CeSSIAM) and the Zugho Mei (ZUGEME) Ethics Committee (for the Guatemala study); and the Wayne State University and University of Michigan institutional review boards (for the Detroit study). The methods used in this study were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Detroit sample

Caregivers of 9-mo-old infants presenting at the General Pediatric Clinic of the Children’s Hospital of Michigan, Wayne State University (Detroit, MI), were contacted as part of a study of the effects of early iron deficiency on brain development and behavior. Iron status was evaluated in all infants whose parent or guardian consented and who had a health maintenance visit (at age 9 mo) during the 1st year of subject enrollment (2002–2003). In the 2nd year, iron status (the initial criterion for inclusion in the brain development and behavior study) was determined only for full-term healthy African American infants. Infants in the brain and behavior study had another blood test at 12 mo, after 3 mo of oral iron or ferrous sulfate (22.5 mg elemental iron in a single daily dose). A total of 351 blood specimens were used for the current analysis: 264 samples at 9 mo only, 5 at 12 mo only, and 82 at both 9 and 12 mo. Infants with HIV/AIDS, sickle cell disease, or other major hemoglobinopathies received care in specialty clinics and were not part of the study.

The infants were predominantly African American (92%) and divided approximately equally by sex (51% male). Eight percent of the infants weighed ≤2500 g at birth. The average birth weight of the remaining children was 3.21 kg, and their gestational age was 39.9 wk. All but 9 infants (3%) were singletons. Virtually all of the mothers and infants participated in the Special Supplemental Food Program for Women, Infants, and Children, and almost all of the infants were fed formula with iron. Almost one-third of the infants (31%) had also been breastfed (average duration: 18 wk). None of the mothers reported having started giving their infants unmodified cow’s milk. Details of the data collection protocol and characteristics of the sample are provided elsewhere (14). The specimens used for the current analysis and the measured markers are shown in Figure 1.

Guatemala sample

The substudy in Guatemala was part of a prospective, randomized, controlled, supervised field-efficacy trial of an intervention in which a multimicronutrient-fortified beverage was consumed on 120 days of school attendance by healthy, nonanemic schoolchildren (aged 7–11 y; n = 158 at baseline and 133 at follow-up) of both sexes. As preparation for our main study, a cross-sectional pilot study was conducted in children (aged 7–11 y; n = 84) to train and standardize the study personnel. Because the longitudinal data were collected 4 mo apart, we treat them as independent samples, which resulted in a total sample population of 375 (84 from the pilot study and 291 from the longitudinal study).

Subjects were recruited from a public elementary school as well as a church-affiliated elementary school in Guatemala City. The parent or legal guardian of each child gave written informed consent to the child’s participation after the nature, purpose, benefits, risks and inconveniences, of study participation were explained in Spanish. The children also gave assent to their participation in the study and each of its component evaluations. Children of both sexes between the ages of 7 and 11 y were eligible for the study. Age was established from the school-enrollment records and calculated (in mo) at the time of screening for the study. Screening involved the assessment of iron status and inflammatory markers by studies of blood obtained from an antecubital vein and a brief physical examination. Subjects were excluded from the analysis if they were anemic (hemoglobin <123 g/L for children aged <96 mo and <117 g/L for children aged 8–11 y). These values were adapted from the international World Health Organization standards and adjusted for the altitude of Guatemala City. The parents of those children found to be anemic were advised of the diagnostic finding, and the study physician offered a 30-d supply of therapeutic iron elixir free-of-charge. Similarly, physical limitations (eg, low vision, impaired hearing, amputations, or deformities) interfering with participation in the performance evaluations were grounds for exclusion. Girls had to be premenarcheal. Children were also excluded if they consumed nutrient supplements.

Measurements

Detroit sample

An experienced clinic phlebotomist obtained up to 5 mL venous blood from each infant. Routine blood tests included a complete blood count with red blood cell indexes (Beckman Coulter Max-M; Beckman Coulter, Fullerton, CA, or SE-900 automated counter; Sysmax, San Jose, CA), lead level (atomic
absorption using a graphite furnace), and free erythrocyte protoporphyrin concentration [assayed by extraction followed by fluorometric measurement (15)]. The Detroit Medical Center University Laboratories performed the hematologic, zinc protoporphyrin, and lead assays. ARUP Laboratories (Salt Lake City, UT) performed the free erythrocyte protoporphyrin measurements. The mean lead concentration was $2.7 \pm 2.3 \mu g/dL$, which indicated little likelihood of lead toxicity in these infants; no infants had a blood lead concentration $>10 \mu g/dL$. When possible, an extra tube of blood was obtained; the blood was promptly centrifuged at $3000 \times g$ for 15 min at $4^\circ C$ and frozen at $-20^\circ C$ for subsequent assays: TSAT and serum ferritin by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA), serum TfR by enzyme-linked immunosorbent assay (16), and APPs by immunodiffusion (AGP and quantitative CRP; Kent Laboratories, Redmond, WA).

### Guatemala sample

Children were asked to come to school after an overnight fast on the day of their blood extraction. A venipuncture through a fixed hypodermic needle was used to collect whole venous blood. Blood was immediately aliquoted into 3 tubes (whole blood, plasma, and serum). The whole blood specimen was transferred on the same day as extraction to the clinical laboratory of Nuestra Señora de Pilar Hospital, where a full panel of hematologic variables was measured by using an automated method. Frozen serum was analyzed for iron and acute phase markers as noted previously. The ql-CRP assay was analyzed for iron and acute phase markers as noted previously. The ql-CRP assay was an agglutination assay (Wampole Laboratories, Princeton, NJ). All analyses were done at Pennsylvania State University (State College, PA).

### Blood sample analyses

All laboratories maintained strict quality control by using internal and external standards when available (not available for TfR). All samples were assayed in duplicate or triplicate. The within-assay variation for ferritin and TfR was 3.8% and 4.9%, respectively, and the between-assay CVs were 5.8% and 5.3%, respectively. The International Bureau of Standards ferritin was used as an external calibrator. The AGP measurement was validated against a similar technique in the laboratory of the senior author at the Department of Nutritional Sciences at Pennsylvania State University, and agreement of sample values was $>92\%$. The TfR-ferritin index was computed as the ratio of TfR to log ferritin (TfR/log ferritin) as previously reported (17, 18), and body iron was calculated by using the new approach of Cook et al (19).

### Statistical analysis

The approach to the analysis of the relation between iron-status biomarkers and 2 APPs occurred in 3 phases: 1) determination of the proportion of subjects in 2 samples that had elevated biomarkers of inflammation; 2) construction of contingency tables to determine the overlap of abnormal concentrations of AGP, CRP, and elevated ferritin; and 3) regression and correlation analysis between elevated ferritin and elevated biomarkers of inflammation. A detailed descriptive discussion of the hematologic data from both samples is provided elsewhere (14; NW Solomons, unpublished observations; CESSIAM@guate.net). The geometric mean $\pm SD$ were computed for each of the APP and iron-status variables. The geometric SD is an exponentiated
possible causes of anemia were not examined in either trial. [A
one prepared solely from serum ferritin concentrations. Other
body iron is positive, this distribution curve would be similar to
Because ferritin is the key component of this calculation, when
Fe/kg as noted in the cumulative distribution plot (Fig. 2).
The goal was to estimate, in the absence of a gold standard, the
validity (accuracy) of inflammation tests that are based on CRP
The second approach was to use inflammation tests to estimate
iron status, in the absence of a gold standard. The positive
predictive value (PPV) of elevated CRP concentrations
>3 mg/L, 97% also had low AGP concentrations, which left 3% of subjects
with indications of inflammation from AGP but not from CRP.
The positive predictive power (PPP) of elevated CRP concentrations
to predict an elevated AGP was between 23% and 45% in individual
data sets or in the combined data set. It is not possible to compute sensitivity and specificity with either of

TABLE 1
Tabulation of iron-status and acute phase protein biomarker concentrations in samples of infants from Detroit and school-age children from Guatemala City1

<table>
<thead>
<tr>
<th></th>
<th>Detroit (n = 351)</th>
<th>Guatemala (n = 375)</th>
<th>Combined (n = 726)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (µg/L)2</td>
<td>30 ± 12</td>
<td>43 ± 10</td>
<td>37 ± 12</td>
</tr>
<tr>
<td>TIR (mg/L)</td>
<td>5.0 ± 1.6</td>
<td>4.5 ± 1.6</td>
<td>4.9 ± 1.6</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>22.2 ± 4.1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Body iron (mg/kg)</td>
<td>3.57 ± 2.3</td>
<td>6.9 ± 2.2</td>
<td>5.8 ± 2.8</td>
</tr>
<tr>
<td>TIR/ferritin index</td>
<td>3.7 ± 2.3</td>
<td>2.8 ± 1.0</td>
<td>3.3 ± 1.9</td>
</tr>
<tr>
<td>AGP (mg/dL)</td>
<td>66.7 ± 6.8</td>
<td>75.9 ± 7.3</td>
<td>70.8 ± 7.1</td>
</tr>
<tr>
<td>qn-CRP (mg/dL)</td>
<td>0.22 ± 0.10</td>
<td>0.81 ± 0.79</td>
<td>0.41 ± 0.25</td>
</tr>
</tbody>
</table>

1 All values are ± SD. TSAT, transferrin saturation; TIR, soluble transferrin receptor; AGP, α1-acid glycoprotein; qn-CRP, quantitative C-reactive protein.
2 Geometric ± SD.

value of the SD of log-transformed value. In addition to descrip-
tive statistics for each sample, we used both regression and cor-
relation analysis to examine the association between iron-status
variables and CRP and AGP. Data (ie, ferritin, AGP, and CRP)
were log transformed to create normal distributions. The qn-CRP
is a categorical variable and was entered into the regression
models on that basis. Contingency tables were created by using
the published cutoffs for AGP (>133 mg/L) and CRP (>3 mg/L)
as recommended by the manufacturers and the concentrations of
ferritin that corresponded to 1 geometric SD above the geometric
mean.

The kappa statistic for extent of agreement was computed
by using PROC FREQ with the AGREE option in SAS for
WINDOWS software (version 9.1; SAS Institute, Cary, NC). The
goal was to estimate, in the absence of a gold standard, the
validity (accuracy) of inflammation tests that are based on CRP
or AGP. If the 2 tests disagree, then ≥1 test is probably incor-
crect. Thus, analysis of agreement data permitted inferences on
the likelihood that a test was correct. We also chose to use the
strength of linear association between the log-transformed val-
ues of CRP or AGP and iron-status variables. All statistical
analyses were performed by using SAS for WINDOWS soft-
ware (version 9.1; SAS Institute, Cary, NC).

RESULTS
Detailed hematologic data for the 351 samples from 9–12-
mo-old African American infants from inner-city Detroit and the
375 samples from Guatemala City are available elsewhere (NW
Solomons, unpublished observations; CESSIAM@guate.net).
The summary iron-status data, computed as geometric means,
are presented in Table 1. There are slight differences in the
battery of variables measured in the 2 studies: TSAT was mea-
sured in the Detroit but not the Guatemala sample, and qn-CRP
was measured in the Guatemala but not the Detroit sample. Very
few persons in the Detroit sample were anemic (3%), and none
were severely anemic. Anemic children were excluded from the
Guatemala sample. The mean body iron for the entire sample
was 5.8 mg Fe/kg body wt, and 6.2% of subjects had <0 mg body
Fe/kg as noted in the cumulative distribution plot (Fig. 2).
Because ferritin is the key component of this calculation, when
body iron is positive, this distribution curve would be similar to
one prepared solely from serum ferritin concentrations. Other
possible causes of anemia were not examined in either trial. [A

FIGURE 2. Cumulative distribution plot of body iron in the total sample (n = 626) compared with the total sample minus individuals without inflam-
mation [α1-acid glycoprotein (AGP) <133 and C-reactive protein (CRP) <3;
n = 548].

throughout discussion and presentation of anemia prevalence in
both studies is presented elsewhere (14).]
The first approach to the data analysis was to determine the
proportional distributions of infants and children defined as hav-
ing inflammation by virtue of having CRP or AGP values above
the threshold. Twenty-six infants from Detroit (7.8%) had CRP
>3 mg/L; 18 (5.4%) of 335 had an elevated AGP >133 mg/dL
(Table 2). Only 35% (9/26) of infants with an elevated CRP also
had an elevated AGP, which indicates that, most of the time, the
2 APPs did not agree with respect to the diagnosis of inflamma-
tion. The kappa statistic for agreement was 0.506.
Thirty-five of 280 children in Guatemala had CRP concentra-
tions >3 mg/L (12.5%), and 16 of 280 had elevated AGP concentra-
tions (5.7%); only 23% (8/35) of those with elevated CRP
centrations had elevated AGP concentrations. The kappa
score in this data set was 0.289. In the combined (Detroit and
Guatemala) analysis, when CRP concentrations were <3 mg/L,97%
also had low AGP concentrations, which left 3% of subjects
with indications of inflammation from AGP but not from CRP.
The kappa score was 0.361. Of infants and children who had AGP
concentrations ≤133 mg/dL, 5.4% of infants (17/317) and
10.2% of children (27/264) have elevated CRP concentrations
and would be identified by that marker as having inflammation.
The positive predictive power (PPP) of elevated CRP concentra-
tions to predict an elevated AGP was between 23% and 45%
in individual data sets or in the combined data set. It is not possible to compute sensitivity and specificity with either of

FIGURE 2. Cumulative distribution plot of body iron in the total sample (n = 626) compared with the total sample minus individuals without inflam-
mation [α1-acid glycoprotein (AGP) <133 and C-reactive protein (CRP) <3;
n = 548].
these APP biomarkers, because no gold standard that absolutely documents inflammation is available in either of these surveys.

In 280 samples from the Guatemala study, we utilized both qn-CRP and ql-CRP assays to test the usefulness of this newly popular approach to the evaluation of inflammation. The quantitative CRP was elevated in 4.1% of samples, whereas the ql-CRP analysis indicated inflammation in 10.3%. Ninety-two percent of children with elevated qn-CRP were also identified as having inflammation by the ql-CRP assay. The reverse, of course, was not true; most of the children identified by the ql-CRP assay were not identified by the qn-CRP assay. The kappa value here was 0.22.

**Contingency analysis**

The second step in the analysis was to determine whether infants and children with elevated inflammation biomarkers, AGP and qn-CRP, also have elevations in serum ferritin (Table 2). We first determined the distribution of ferritin in a subset of the population that had no anemia, no TSAT <15%, no TIR >8, and APP below cutoffs—that is, a nominally healthy, iron-sufficient population. We computed the geometric mean ferritin concentration (41.5 μg/L for Guatemala children, 31.7 μg/L for Detroit infants, and 35.5 μg/L for the combined data set) and then added 1 geometric SD to define “elevated ferritin” in each sample set. With this approach, we defined an elevated ferritin concentration as >40 μg/L (1 SD) for the Detroit infants, >52 μg/L (1 SD) for the Guatemala children, and >47 μg/L (1 SD) for the combined dataset. The contingency table of data from Detroit infants with AGP concentrations >133 mg/dL shows a 82% agreement between high AGP and a ferritin concentration >40 μg/L. The AGP cutoff was not nearly as useful in school-age children or in the combined dataset in the identification of persons with elevated ferritin. The corresponding analysis with CRP was also ineffective in identifying infants or school-age children with elevated ferritin (Table 2). The kappa values were low in all comparisons and thus were in general agreement with the contingency table analysis.

The effect of inflammation on the sample distribution of body iron was also examined (Figure 2). The removal of subjects with high AGP and CRP concentrations caused a uniform shift of the ferritin distribution curve to the left. To determine whether these biomarkers influenced another index of iron status, we computed the TIR-ferritin index. Only 13 subjects in the entire data set had an index above a theoretical threshold of 1.5, an index of iron deficiency (20, 21). The association of the TIR-ferritin index with either AGP or CRP was virtually nonexistent; in the 18 Detroit infants with elevated AGP concentrations, the TIR-ferritin index ranged from 2.3–4.19, whereas in the Guatemalan children, those with elevated AGP concentrations had a TIR-ferritin index ranging from 1.95 to 4.38. The usefulness of the ql-CRP assay was also examined; positive test results were unrelated to elevations in ferritin, TIR-ferritin index, TIR, or even serum iron concentrations (data not shown).

**Regression analysis**

The third approach to examining the relation of APP and iron-status indicators used correlation and regression analysis after log transformation. These associations were examined in both datasets independently and in a combined dataset of ~700 samples (Table 3). In infants and school-age children, positive correlations of r = 0.487 and 0.412, respectively, were found between log scores of AGP and CRP in individual data sets, and a correlation, r = 0.492, was found in the combined data set. Significant relations of CRP with TIR in the Guatemalan data set and with TSAT in the Detroit data set were found, but the relations were not consistently strong across data sets. Some modest but significant relations were found between log iron and log AGP (r = −0.198, P = 0.002) and between log iron and log CRP.

### Table 2

Contingency table of the distribution of infants in Detroit and young children in Guatemala City with biomarkers of inflammation and ferritin values in different categories of inflammation

<table>
<thead>
<tr>
<th></th>
<th>AGP ≤ 133 mg/dL</th>
<th>AGP &gt; 133 mg/dL</th>
<th>Kappa Statistic</th>
<th>Predictive power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detroit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP ≤3 mg/dL</td>
<td>300</td>
<td>9</td>
<td>0.506</td>
<td>NPP: 97</td>
</tr>
<tr>
<td>CRP &gt;3 mg/dL</td>
<td>17</td>
<td>9</td>
<td>0.506</td>
<td>PPP: 35</td>
</tr>
<tr>
<td>Guatemala</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP ≤3 mg/dL</td>
<td>237</td>
<td>8</td>
<td>0.289</td>
<td>NPP: 97</td>
</tr>
<tr>
<td>CRP &gt;3 mg/dL</td>
<td>27</td>
<td>8</td>
<td>0.289</td>
<td>PPP: 23</td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP ≤3 mg/dL</td>
<td>537</td>
<td>17</td>
<td>0.361</td>
<td>NPP: 97</td>
</tr>
<tr>
<td>CRP &gt;3 mg/dL</td>
<td>44</td>
<td>17</td>
<td>0.361</td>
<td>PPP: 28</td>
</tr>
</tbody>
</table>

1 n = 351 and 375 from Detroit and Guatemala City, respectively. AGP, α1-acid glycoprotein; CRP, C-reactive protein; NPP, negative predictive power; PPP, positive predictive power. Ferritin cutoffs are 1 geometric SD above the geometric mean of the corresponding sample.

2 Computed by using SAS software (PROC FREQ with the AGREE option; version 9.1; SAS Institute, Cary, NC).
A highly significant relation between serum ferritin and APP biomarkers was observed in both individual data sets and the combined data set. Detroit infants had a much stronger association of ferritin with AGP than with CRP, whereas Guatemalan children had nearly equally strong associations between ferritin and both APPs. Strong curvilinear relations were found between ferritin and CRP (Figure 3) and between ferritin and AGP (Figure 4). Both of these relations were highly significant \((P < 0.001)\). Correlations of TfR-ferritin index or body iron with APP were nonsignificant statistical relations.

Stepwise regression analysis failed to produce any significant effect beyond that suggested by the correlation analysis between ferritin and AGP or CRP. In the Detroit data set analysis, log AGP was the first significant predictor \((\beta = 0.693, R^2 = 0.1813, F_{1,331} = 73.07, P < 0.0001)\), and log CRP did not add significantly to the model. In the combined data set analysis, log CRP was the first variable to enter the model that predicted log ferritin \((\beta = 0.158, R^2 = 0.1145, F_{1, 614} = 79.23, P < 0.0001)\). Log AGP then was entered into the model and explained a significant additional 3% of the variance \((\beta = 0.1109, partial R^2 = 0.1145, F_{2, 612} = 20.77, P < 0.0001)\).

**DISCUSSION**

Numerous studies of iron status conducted in developing countries have used biomarkers of inflammation to determine the
influence of this inflammation on the distribution of iron-status markers (3, 7, 22–25). The current analysis of the strength of association between markers of iron status and markers of inflammation resulted in several very interesting findings: 1) biomarkers of inflammation are significantly related to one another, but the unexplained variance still is >50%; 2) serum ferritin is the only biomarker of iron status that is consistently related to either CRP or AGP; 3) the association between ferritin and AGP was stronger than that between ferritin and CRP in infants in Detroit, whereas the associations of ferritin with CRP and AGP were equivalent in school-age children in Guatemala; and 4) the qualitative assessment of CRP proves to be of no utility in identifying persons with altered iron-status markers due to inflammation.

In these 2 individual data sets, the overall influence of inflammation on the distribution of iron-status measures was small. That is, inclusion or exclusion of those subjects with elevated AGP or CRP did not significantly change either the body iron distribution or the geometric mean ferritin concentrations in either sample. This suggests that when the prevalence of clinically definable inflammation is low—in this case, <10%—little influence exists on the distribution of iron biomarkers in a large sample, and hence, little influence exists on estimates of prevalence of iron deficiency. We do not know at what point the prevalence of inflammation actually does cause a shift in the distribution of iron-status biomarkers. This context is the concern in other recent publications that focused on the utility of these biomarkers within the context of malaria and other situations of endemic infection. In fact, several research groups argued for the usefulness of an adjustment of ferritin cutoffs, TfR, and the TfR-ferritin index (2, 5, 7, 17, 26). The lack of association between the TfR-ferritin index or TfR and the APPs in our 2 data sets clearly precludes our advocating any such adjustment in a population with a low prevalence of inflammation (18, 27).

Ferritin, plasma iron, CRP, and AGP all follow different plasma kinetic patterns after the onset of infection (13, 27). Measurable changes in plasma iron and CRP occur quickly (within 12 h), and changes in AGP and ferritin occur more slowly (beyond 24–48 h). More important, CRP and plasma iron return to baseline values long before either plasma ferritin or AGP do so (12). Thus, it is not surprising that, in a cross-sectional sample with low prevalence of inflammation, only a modest correlation between these 2 APPs is seen. Perhaps when chronic malaria or parasitic infections are present, this correlation would increase and allow APP to be used in identifying those persons whose iron-status markers are distorted because of an acute phase response (28).

Implicit in that conclusion, however, is the assumption that most persons identified as having high CRP concentrations would also be identified as having high AGP concentrations and vice versa. Our data do not support that assumption. Our data indicate that in only ~40% of cases would this be true. The 3 choices in the analysis are 1) to ignore those who appear to have inflammation; 2) to ignore the effects of inflammation on iron-status markers; or 3) to try to identify the magnitude of the effect of inflammation on the iron-status indicators. None of these alternatives is attractive in most situations, especially those in which the vast majority of subjects appear to have infections (2). The actual prevalence of infection varied between 5% and 12% in the samples in the current study, which is well below the rates of >60% observed in some recent studies from Africa (3, 7, 29).

Our approach to a definition of a "high" ferritin concentration was that of >1 SD above the geometric mean ferritin concentration in the samples from subjects who had neither iron deficiency nor identifiable inflammation. But it is clear from our analysis that this sample was not a segment of either population of subjects that was strongly associated with an elevation in CRP or AGP. Whereas our regression analysis found powerful curvilinear relations between both CRP and AGP with ferritin, the contingency analysis with kappa values and modest positive predictive values for AGP and elevated ferritin were less convincing. In our data sets, the ability to identify hyperferritinemia that is likely due to inflammation was limited.

The q1-CRP assay has become quite popular in field studies to identify inflammation in subjects, but our results suggest that this is not a useful approach. The q1-CRP analysis did a very poor job of identifying even those with elevated qn-CRP, let alone those with elevated AGP. Most important, the predictive capacity for hyperferritinemia was very poor.

Receiver operator curve analyses have been used to define optimal cutoffs for the relation of APP with iron-status biomarkers (30). This approach is most useful when a bone marrow biopsy or another direct measure of iron delivery to tissue is available for identifying the optimal cutoffs. In our 2 data sets, no such gold standard existed, and thus no sensitivity analysis, per se, could be utilized. In these 2 study populations with low degrees of inflammation and only modest degrees of iron deficiency, no convincing evidence existed that either of the acute
phase biomarkers was related to the body iron distribution curve or serum ferritin measurements. This kind of analysis of the usefulness of 2 common APPs should be repeated. Our expectation was that urban Guatemalan children and inner-city infants would have appreciable amounts of infection, but that was not observed. Nonetheless, the 2 different data analysis approaches agree that there is little relation between APP concentrations and iron-status indicators. AGP appears to perform better overall, but the limited amount of inflammation in >700 persons in these studies clearly requires a follow-up analysis in populations with more inflammation.

In conclusion, our results indicate that future field studies of iron nutriture in populations should consider carefully which biomarkers are likely to be useful in their sample collection schemes. Not all biomarkers are created equal. We asked the important question, “Which APP biomarker is better at predicting elevated ferritin?” Neither inflammation biomarker adequately discriminated persons with low ferritin from those with high ferritin. In addition, the 2 markers were quite different in terms of performance. It is important to note that the entire distribution of ferritin or body iron was affected and not just the upper quartile or deciles of ferritin or body iron concentrations; this finding suggests that it is inappropriate for researchers to assume that a particular portion of the distribution is affected. With further effort, perhaps a refinement in cutoffs for APP concentrations can be generated and would be helpful in reflecting prima facie distortion of iron-status measures.

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JLB was responsible for designing the experiment; LEM-K participated in the laboratory analysis of the samples; LEM-K participated in the statistical analysis; NWS facilitated and directed the data collection in Guatemala; MLA facilitated and directed the data collection in Detroit; NWS, MLA, and JLB analyzed the data; FR participated in the analysis of the acute phase proteins and in the interpretation of the collected data; and JLB was responsible for writing the manuscript, with the assistance of LEM-K and FR. None of the authors had any personal or financial conflict of interest.

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