HHS Public Access

Author manuscript

J Nutr. Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:

J Nutr. 2012 January ; 142(1): 105–111. doi:10.3945/jn.111.146316.

Correcting for Inflammation Changes Estimates of Iron Deficiency among Rural Kenyan Preschool Children[1,2,3]

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Abstract

The assessment of iron status where infections are common is complicated by the effects of inflammation on iron indicators and in this study we compared approaches that adjust for this influence. Blood was collected in 680 children (aged 6–35 mo) and indicators of iron status [(hemoglobin (Hb), zinc protoporphyrin (ZP), ferritin, transferrin receptor (TfR), and TfR/ferritin index)] and subclinical inflammation [(the acute phase proteins (APP) C-reactive protein (CRP), and α-1-acid glycoprotein (AGP)] were determined. Malaria parasitemia was assessed. Subclinical inflammation was defined as CRP >5 mg/L and/or AGP>1 g/L). Four groups were defined based on APP levels: reference (normal CRP and AGP), incubation (raised CRP and normal AGP), early convalescence (raised CRP and AGP), and late convalescence (normal CRP and raised AGP). Correction factors (CF) were estimated as the ratios of geometric means of iron indicators to the reference group of those for each inflammation group. Corrected values of iron indicators within inflammation groups were obtained by multiplying values by their respective group CF. CRP correlated with AGP ($r = 0.65$; $P < 0.001$), ferritin ($r = 0.38$; $P < 0.001$), Hb ($r = -0.27$; $P < 0.001$), and ZP ($r = 0.16$; $P < 0.001$); AGP was correlated with ferritin ($r = 0.39$; $P < 0.001$), Hb ($r = -0.29$; $P < 0.001$), and ZP ($r = 0.24$; $P < 0.001$). Use of CF to adjust for inflammation increased the prevalence of ID based on ferritin < 12 μg/L by 34% (from 27 to 41%). Applying the CF

[1]Supported in part by an Ellison Medical Foundation/Nevin Scrimshaw International Nutrition Foundation fellowship (F.K.E.G.) and the CDC (P.S.S.). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC.

[2]Author disclosures: F. K. E. Grant, P. S. Suchdev, R. Flores-Ayala, C. R. Cole, U. Ramakrishnan, L. J. Ruth, and R. Martorell, no conflicts of interest.

[3]This trial was registered at clinicaltrials.gov as NCT101088958.

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strengthened the expected relationship between Hb and ferritin ($r = 0.10$; $P = 0.013$ vs. $r = 0.20$; $P < 0.001$, before and after adjustment, respectively). Although the use of CF to adjust for inflammation appears indicated, further work is needed to confirm that this approach improves the accuracy of assessment of ID.

Introduction

The WHO/CDC consultative group recommends that in addition to Hb[9], the concentrations of ferritin, and TfR should be measured in the assessment of ID (1). Additionally, ZP, a measure of bone marrow iron availability for erythropoiesis, is useful in identifying preanemic ID (2,3).

The assessment of the true burden of ID is complicated by the influence of infections, such as malaria and HIV, on iron indicators, especially in developing countries (4). This makes ID monitoring among children in these areas difficult, because inflammation influences Hb, ferritin, ZP, and to a lesser extent TfR (5,6). The serum ferritin concentration, an indicator of iron body stores, spikes during inflammations, even in the case of subclinical inflammation whose occurrence is reflected in elevated APP, namely CRP and AGP; this makes interpretation of iron status problematic (4). CRP levels increase within 10 h of the onset of acute inflammation and normalize rapidly, usually within 1 wk (6), whereas AGP levels begin to increase 24 h after the onset of inflammation but remain elevated well into convalescence (4). Levels of CRP and AGP may thus identify different but overlapping groups of people with respect to their inflammation status (6). Inflammation has a smaller influence on plasma TfR (an indicator of erythropoietic intensity and iron requirements) compared to its influence on ferritin (7,8). Therefore, TfR may be useful when estimating the prevalence of ID in the presence of inflammation. Thus, in areas of high inflammation burden such as in developing countries, the WHO/CDC recommends measurement of one or more of the APP (CRP and AGP) together with the above-mentioned iron indicators to assess iron status (1).

Three approaches have been proposed to adjust for inflammation when assessing iron status: *1*) upward adjustment of the commonly used cutoff for low ferritin values from 12–15 μg/L to 30–50 μg/L applied to the whole sample; *2*) exclusion of individuals with inflammation as indicated by elevated values of one or more of the APP; or *3*) the use of CF that use APP biomarker(s) to adjust iron status indicators for the effects of inflammation (6,9,10).

The first and third approaches do not exclude data. In areas where there are high rates of inflammation, exclusion of a large number of people may be impractical (6,10–13). The use of CF incorporates the effects of the APP on iron status indicators, information that is not fully captured by simply modifying the cutoff point for ferritin. The CF approach has been explored by only a few studies among preschool children in areas of high inflammatory stress and has not been applied to multiple iron indicators. Additionally, we are not aware of

[9]Abbreviations used: CF, correction factor; Hb, hemoglobin; ID, iron deficiency; TfR, soluble transferrin receptor; ZP, zinc protoporphyrin.

research exploring whether the use of CF modifies the relationship between malaria infection and iron indicators.

The objectives of this study were to: *1*) document the relationship between inflammation as indicated by the APP (CRP and AGP) and iron indicators (Hb, ZP, ferritin, TfR, and TfR/ferritin index); *2*) assess the effect of the use of the CF on the relationship between malaria infection and iron indicators; and *3*) explore various approaches for estimating ID in the presence of inflammation among preschool children in rural western Kenya.

Materials and Methods

Study area and study population

The data for this study are from the Nyando Integrated Child Health and Education project (14,15) and were obtained from a cross-sectional survey of children 6–35 mo of age in 60 randomly selected villages from the Nyando Division (population 80,000) in the Nyanza Province of western Kenya between March and May 2009. After developing community maps and completing a household census, households with children aged 6–35 mo were selected by population proportion to size cluster sampling using updated population registries of Nyando District. Children were selected if they were between the ages of 6 and 35 mo at the time of enrollment and lived within the catchment area of the study; children with Hb <70 g/L were referred to the nearest clinic for treatment of severe anemia.

Children were excluded if they were unavailable for enrollment on 3 separate household visits or parental refusal to give informed consent. Data were recorded in the field using Dell Axim personal digital assistant (DDH Software) and downloaded into an Access 2007 (Microsoft) database daily. All children participating in the survey, whether febrile (n = 190) or not, had thick and thin smears made from capillary blood samples along with the other biochemical laboratory testing. History of fever 24 h prior to the interview was obtained through caregiver recall.

Written informed consent was obtained from all participating households. The Ethics Committee of Kenyan Medical Research Institute in Nairobi, Kenya (protocol 1176) and the Institutional Review Board of the CDC in Atlanta, GA (protocol 5039) approved the study.

Laboratory analysis

Trained laboratory technicians obtained capillary blood from the children between 0800 and 1600 h by using single-use sterile micro-lancets (Becton Dickinson) in a purple top microtainer capillary blood collector with EDTA (Becton Dickinson). Hb was determined within 3 min of blood collection with the use of the HemoCue B-Hemoglobin machine. A single blood drop was placed on a microscope slide (Thermo Fisher Scientific) for thick malaria smear examination for detection of malaria parasitemia for malaria infection. Testing for presence of malaria parasites and the level of parasitemia was performed by the CDC laboratory in Kisian, Kenya. The number of malaria parasites per 300 white blood cells was counted on Giemsa-stained thick blood films. Malaria parasite counts were converted to parasite loads or densities on the basis of 8000 white blood cells in 1 μL of blood (16). Severity of malaria infection was defined by malaria parasite loads >5000

parasites in 1 μL of blood. Caretakers of all children who met the criteria for active malaria infection [i.e., reported fever + positive malaria smear (n = 42)] were notified and referred to the nearest government clinic for antimalarial treatment.

An additional 400–500 μL of capillary blood was collected into a microtainer with EDTA (Becton Dickinson). The blood was then transported on ice to the Kenyan Medical Research Institute/CDC laboratory in Kisian, Kenya, where, with the use of a pipette with a disposable tip, a drop of the whole blood was placed on a disposable glass cover slip (Aviv Biomedical) to assess ZP. A cutoff value of 80 μmol/mol heme was used to indicate elevated ZP level and hence ID (3). Testing was done in duplicates. A CF was applied to the results of the ZP on the advice of the manufacturers and the CDC quality assurance laboratory. The rest of the blood was then centrifuged at 1500 × g for 5 min at 27°C and plasma was removed and stored in cryovials (Thermo Fisher Scientific) at −20°C within 12 h after blood collection. Samples were transported to Germany for subsequent laboratory analysis of ferritin, TfR, AGP, and CRP at the laboratories of the DBS–Tech by a procedure that combines all four analytes using sandwich ELISA technique (17). All samples were measured in duplicate and the intra- and interassay CV were <10%. The thresholds for defining abnormal values for the above biochemical indicators were as follows: ferritin, <12 μg/L; TfR, >8.3 mg/L (TfR, Ramco Laboratories); CRP, >5 mg/L; and AGP, >1.0 g/L (10). The TfR/ferritin index was calculated by dividing TfR (μg/L) by ferritin (μg/L),with elevated TfR/ferritin index defined as >500 (18). Anemia was defined according to the WHO threshold as Hb <110 g/L for children 0.5–4.99 y (19). The HemoCue machines were calibrated and checked for accuracy twice daily (morning before fieldwork and evening after fieldwork) using Hemocontrols and control cuvettes, respectively.

Statistical analysis

All statistical analyses were done using SAS 9.2 (SAS Institute). Significance was defined as $P < 0.05$. The distributions of the various biomarker concentrations were assessed for normality by the use of normality plots and Kolmogorov-Smirnov tests. The distributions of ferritin, TfR, ZP, CRP, and AGP were non-Gaussian. Both parametric and nonparametric approaches were used for analyses and reported as medians and interquartile ranges, or geometric means and SD, or arithmetic means and SD.

Spearman rank correlation coefficients were determined to assess relationships among the iron status indicators (Hb, ferritin, TfR, ZP, and TfR /ferritin index) and also between the iron indicators and the APP, CRP and AGP. Where correlations between any iron indicator and an APP were significant, we adjusted values by multiplying them by group-specific CF. Four groups were defined: *1*) nonelevated state or reference (CRP≤5mg/L and AGP≤1 g/L); *2*) incubation (CRP>5 mg/L and AGP≤1 g/L); *3*) early convalescence (CRP >5 mg/L and AGP >1 g/L); and *4*) late convalescence (CRP #5 mg/L and AGP >1 g/L). The CF were defined as the ratios of the geometric mean values of the iron indicator for the reference group to those in groups 2, 3, and 4 (10,13). We calculated adjusted corrected concentrations of the iron indicators by multiplying the individual values by their group-specific CF (10,11,13,20). Spearman rank correlations were computed again between the corrected iron indicator concentrations and the APP. We then tested the equality of the two observed

correlations (between uncorrected indicators and APP and between corrected indicators and APP) with Fisher's z transformation using PROC CORR with the FISHER option in SAS (version 9.2; SAS Institute). The objectives were to check that the effect of inflammation on iron indicators was attenuated by use of the CF and to explore whether expected relationships among iron indicators were masked by inflammation. We hypothesized a priori that correcting for inflammation would uncover or strengthen the following relationships: *1*) a positive relationship between Hb and ferritin and between TfR and ZP; and *2*) an inverse relationship between Hb and TfR, Hb and ZP, ferritin and TfR, and ferritin and ZP. The mean concentrations of iron status indicators for children with malaria and those without malaria were contrasted using Student's *t* test. Paired Wilcoxon's test was applied for the comparison of median concentrations of corrected and uncorrected iron status indicators, and McNemar's chi-square of proportion was used to compare the prevalence of ID and anemia in the corrected and uncorrected iron indicators.

We evaluated the extent to which correcting for inflammation changed estimates of ID by comparing the median values and proportions of abnormal values (i.e., anemia or ID) of the uncorrected indicators with those of the corrected indicators. We also explored if correcting for inflammation modifies the association between malaria infection and iron status indicators. Finally, we compared the prevalence of ID generated by each of the three approaches generally used to adjust for inflammation effects on iron indicators.

Results

Fifty-one percent of the study participants were males and the mean age was 21 mo (Table 1). The prevalence of reported recent fever was high, whereas the prevalence of malaria parasitemia, all as a result of *P. falciparum*, was lower than expected. Twenty-two percent (n = 42) of children who presented with recent fever had malaria parasitemia. The prevalence of inflammation, indicated by CRP and AGP, was high.

There were positive correlations between malaria parasite density and CRP (r = 0.33; P < 0.001) and AGP (r = 0.26; P < 0.001). Children who tested positive for malaria had higher CRP [median (25th, 75th quartiles): 6.6 (1.7, 20.5) vs. 1.1 (0.3, 4.1) mg/L; P < 0.001] and AGP [median (25th, 75th quartiles): 1.5 (1.1, 1.8) vs. 1.0 (0.8, 1.3) g/L; P < 0.001] levels than noninfected children.

There were positive correlations between CRP and AGP and among CRP, AGP, and ferritin (see uncorrected values, Table 2). Hb (negatively), ZP (positively), and TfR (positively) correlated with the two acute phase proteins. The relationships between the iron indicators and Hb indicated strong to moderate inverse (Hb with ZP, TfR, and TfR/ferritin index) correlations but a weak positive correlation with ferritin (see uncorrected values, Table 2).

Geometric mean concentrations of iron indicators were assessed by inflammation status defined by four APP groups: reference, incubating, and early and late convalescence. There were differences (P < 0.05) among groups for all indicators (Table 3). As an example, we found higher geometric mean ferritin concentrations in the incubation (27.4 μg/L), early convalescence (42.1 μg/L), and late convalescence (23.9 μg/L) groups than in the reference

group (14.9 μg/L) (all $P < 0.001$). These values translated into CF of 0.54, 0.35, and 0.62, respectively, to correct ferritin values in the 3 inflammation stages. The use of the CF resulted in attenuation of the correlation coefficients between all iron indicators and the APP (Table 2). The correlation between CRP and ferritin was reduced from 0.38 to 0.08 after correction ($P < 0.001$) and that between AGP and ferritin from 0.39 to 0.04 ($P < 0.001$).

Malaria parasitemia was significantly associated with the iron indicators (Table 4). The presence of malaria parasitemia was generally associated with lower levels of Hb and TfR/ferritin index but higher levels of ferritin, ZP, and TfR regardless of whether the indicators had been corrected for inflammation.

There were differences in both the median concentrations and the estimated prevalence of anemia and ID in all indicators when the corrected and uncorrected indicators were compared (Table 5). After correcting for inflammation, the ferritin concentration decreased by 6.8 μg/L or 47% and the prevalence of ID (ferritin <12 μg/L) increased by 34% (from 27 to 41%). Applying the CF strengthened the expected relationship between Hb concentration and ferritin that was potentially obscured by inflammation ($r = 0.10$; $P = 0.013$ vs. $r = 0.20$; $P < 0.001$, before and after correction, respectively) (Table 2). In addition to correcting the data for inflammation, we also tested the effects of ignoring one or the other of the 2 acute phase proteins, particularly for ferritin (Table 6). The use of CF increased the prevalence of ID (ferritin <12 μg/L) from 27 to 41%. Exclusion of inflammation cases resulted in prevalence values that ranged from 32 to 40% and use of a higher ferritin cutoff point (ferritin <30 μg/L) resulted in a prevalence of 66%. Taking the ID prevalence of 41% obtained by using CF as the reference, the uncorrected prevalence was 33% lower and that obtained by using a higher cutoff point was 62% higher.

Discussion

Our data indicate that correcting for the influence of inflammation increases the estimated prevalence of ID in children, particularly when the iron indicator is ferritin. The prevalence of subclinical inflammation as indicated by elevated APP was high in this sample of preschool children in western Kenya. We applied cutoff values commonly used to facilitate comparison with other studies (10). The proportion of children with malaria parasitemia was 12%, similar to prior reports in this setting (21). The elevated levels of AGP and CRP in the current study may potentially be explained by the inflammatory response to malaria as well as other common childhood infections in the population, such as diarrhea, upper respiratory infections, and HIV (4,22). The iron indicators Hb, ferritin, and ZP significantly correlated with the APP (Table 2).

The concentration of ferritin increases rapidly in parallel to CRP in the first 20 h after the onset of an acute inflammation, during which AGP levels show only small elevations, and in chronic inflammation continues to a maximum level at ~48 h, during which time the AGP level peaks and CRP declines (4,9,10). CRP is therefore a better indicator of how early inflammation influences ferritin and other iron indicators such as ZP and Hb, whereas AGP may better predict this influence in the latter inflammation stages (5,11–13,20). Thus, both CRP and AGP levels should be measured in studies, because they identify different but

overlapping groups of people with respect to status of inflammation in a community (6,10). However, our data suggest that AGP is more important than CRP in its association with the various iron indicators we assessed.

Group-specific CF were used in this study to correct for the effect of inflammation on iron indicators and thus adjust the estimation of the prevalence of ID in preschoolers in an area of high inflammation without discarding data (i.e., cases with elevated APP), altering the cutoff levels for low ferritin values or masking potentially important associations (10). Various studies have proposed the usefulness of CF in adult populations with little or no evidence of its usefulness in preschool children (11,12,20). Some investigators have argued that in areas of high inflammation, there may not be enough children to form a reference group to enable the estimation of reliable CF (5). However, in our study this was not an issue, because about one-third of the sample did not show inflammation (i.e., APP were not elevated). At the incubating and convalescence stages, ZP and ferritin levels were high, a potential indication of their sensitivity to the influence of inflammation. During an acute inflammation, redistribution of iron into the liver and mononuclear phagocyte system mediated by cytokines may occur resulting in increased ferritin levels and low serum iron (4). This limited iron supply to developing RBC results in excess ZP formation as a by-product of the heme biosynthetic pathway (3). In our study, ferritin and ZP levels were higher in the early convalescence (both elevated CRP and AGP) compared to the reference group. Similar trends for ferritin levels were observed among apparently healthy HIV-positive adults in Kenya (12), young Zanzibari children (5), and Indonesian infants (6).

In our study, the proportions of children in the incubating, early convalescence, and late convalescence groups were 1.6% (n = 11), 22% (n = 147), and 33% (n = 223), respectively. Similar observations by group were made in Pakistani preschoolers (23): incubating (0.2%) and early (11.0%) and late convalescence (50.7%). This is because the incubation period is very short (~48 h) and a high intensity of inflammation is required for numbers in this group to be large, whereas the late convalescence period is usually longer, especially in situations of poor nutritional status and slow recovery from disease (10). The presence of chronic inflammation in the children in our study area may be explained in part due to endemic and recurrent episodes of malaria and other infections that resulted in longer periods of convalescence (11). However, adults have reduced convalescence periods, probably due to a more fully developed humoral immunity and hence quicker recovery from inflammation (10). Data from this study indicate that inflammation due to malaria is associated with lower Hb levels and higher ferritin, ZP, and TfR levels. The inflammatory response to malaria is demonstrated by higher serum levels of the APP and ferritin with malaria-associated hemolysis, resulting in lower Hb (21). This influence has implications for the interpretation of ID. Malaria may also induce ID through reduced iron absorption or iron loss in urine after hemolysis as well as sequestration of iron in macrophages of the mononuclear phagocyte system.

The relationships between Hb levels and ZP, TfR, and TfR/ferritin index were not significantly affected by the APP. After correcting for the influence of CRP and AGP on these iron indicators, the inverse correlations between them and Hb levels remained significant. However, this was not the case for ferritin; the weak direct association between

Hb and ferritin became stronger after correcting for the influence of the APP on ferritin, indicating a strong influence of inflammation on ferritin levels as explained. Similar results have been reported elsewhere after adjusting for recent fever, CRP, and AGP (5). Thus, in the absence of inflammation, ferritin levels reflect iron stores (5).

As expected, when we assessed the prevalence of ID in the reference group (by discarding data for children with inflammation based on both elevated CRP and AGP, as is the practice in most studies), we obtained a prevalence of ID similar to that in the corrected data. However, these reference children constitute only 44% of the sample. If this reference group differed from the group with inflammation on other independent risk factors for iron status, discarding or adjusting data from inflamed participants may bias the prevalence estimates. Because the objective of the use of the CF is to correct the data of the inflamed group and thus restore the data distribution to that of the community as represented by the reference group, our internally generated CF produces identical geometric means across the 3 groups of inflammation (incubation and early and late convalescence). However, as noted, the CF approach retains all cases. The fact that associations among iron indicators are unmasked by the use of CF suggests that the CF approach generates iron status variables closer to what would have been observed in the absence of inflammation; unfortunately, we do not have a means for testing this directly.

A limitation of the study was that we did not access body fat, a potential confounder of inflammation. The CF estimated in this study may not be generalizable or applicable to other populations with different severity and frequency of infections and malnutrition status. However, when we compared the prevalence of ID (ferritin <12 μg/L) using our CF and the meta-analysis CF for ferritin for children #5 y old (10), the results were similar (Table 6). The strengths of this study include the use of the ratios of the geometric means of iron indicators in estimating the CF as suggested by the literature and also because it allows for comparison with future studies in other settings (10). Also, we measured a wide range of iron indicators and subclinical inflammation biomarkers, which allowed for the assessment of the influence of inflammation on all available iron biomarkers.

This study showed that the use of a CF can modify the levels and prevalence of anemia and ID, using various iron indicators known to be affected by inflammation. The CF approach helps in retaining all biochemical data from the study population without discarding essential data that may unmask associations between iron indicators. Using the CF approach may help interpret the assessment of iron status in populations with high rates of subclinical inflammation.

The variability of ID prevalence as measured by the different approaches may be important in public health terms (Table 6), especially for ferritin. Our study goes beyond the specific results in proposing the CF approach for addressing the issue of ID measurement in areas of high inflammation.

In conclusion, subclinical inflammation was associated with all the iron biomarkers (and not only ferritin) and not correcting for such inflammation altered the measures of ID. However,

further work is needed to identify whether the CF approach proposed in this study improves the accuracy of assessment of ID.

Acknowledgments

The authors thank Dr. Juergen Erhardt for analyzing the samples in his laboratory. They also thank Alie Eleveld of the Safe Water and AIDS Project and Vincent Were of KEMRI/CDC Kenya and the Nyando Integrated Child Health and Education Project, Cliff Ochieng, and Jared Oremo of the Nyando Integrated Child Health and Education Project. This study is dedicated to the memory of Alfredo Obure, the Nyando Integrated Child Health and Education project study coordinator. P.S.S., L.J.R., F.K.E.G., R.M., and R.F-A. were responsible for the study design; F.K.E.G., L.J.R., and P.S.S. were responsible for conducting the study and the data collection; F.K.E.G., P.S.S., R.F-A., C.R.C., U.R., and R.M. were responsible for data analysis; and F.K.E.G. wrote the initial manuscript. All authors read and approved the final manuscript.

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TABLE 1

Demographic, infection, and biochemical characteristics in a sample of 6- to 35-mo-old Kenyan children[1]

Characteristic	
Sex, % *male*	51.4
Age, *mo*	21.1 ± 9.2
WAZ < −2 SD, %	12.4
HAZ < −2 SD, %	28.3
WHZ < −2 SD, %	5.2
Recent fever, %	28.0
Malaria parasitemia, %	12.4
Hb, *g/L*	
Median (IQR)	111 (100, 119)
Mean ± SD	109 ± 15.4
Ferritin, µ*g/L*	21.2 (11.3, 39.3)
ZP, µ*mol/mol* heme	124 (89.9, 187)
TfR, *mg/L*	9.0 (7.4, 11.3)
TfR/ferritin index	399 (211, 855)
CRP, *mg/L*	0.9 (0.2, 4.6)
AGP, *g/L*	1.0 (0.8, 1.4)

[1] Values are means ± SD or median (25th, 75th percentiles), n = 680 or percent. HAZ, height-for-age Z-score; Hb, hemoglobin; TfR, plasma soluble transferrin receptor; WAZ, weight-for-age Z-score; WHZ, weight-for-height or length Z-score; ZP, whole blood zinc protoporphyrin.

TABLE 2

Spearman correlation coefficients relating biomarkers of inflammation to iron status indicators in a sample of 6- to 35-mo-old Kenyan children before and after correcting for inflammation[1,2]

	Ferritin	ZP	TfR	TfR/ferritin index	CRP	AGP
Hb						
Uncorrected	0.10*	−0.64**	−0.56**	−0.24**	−0.27**	−0.29**
Corrected	0.20**	−0.58**	−0.53**	−0.33**	−0.07	−0.04
Ferritin						
Uncorrected	—	−0.30**	−0.28**	−0.95**	0.38**	0.39**
Corrected	—	−0.40**	−0.25**	−0.94**	0.08*	0.04
ZP						
Uncorrected	—	—	0.76**	0.48**	0.16**	0.24**
Corrected	—	—	0.75**	0.57**	0.11*	0.04
TfR						
Uncorrected	—	—	—	0.53**	0.15**	0.15**
Corrected	—	—	—	0.61**	0.04	0.03
TfR/ferritin index						
Uncorrected	—	—	—	—	−0.30**	−0.30**
Corrected	—	—	—	—	−0.06	−0.04
CRP	—	—	—	—	—	0.65**

[1] Values are Spearman correlation coefficient, $n = 680$. *$P < 0.05$, **$P < 0.001$. Hb, whole blood hemoglobin; TfR, plasma soluble transferrin receptor; ZP, whole blood zinc protoporphyrin.

[2] Correction of data achieved using group specific CF estimated from the ratios of geometric means.

TABLE 3

Estimation of CF using ratios of iron status indicator geometric means by inflammatory status in a sample of 6- to 35-mo-old Kenyan children[1]

Biomarker	Concentration[2]	Ratio (95% CI)[3]	CF[4]
Hb, *g/L*			
Reference	111 ± 11.5		
Incubation	111 ± 10.9	1.01 (0.87, 1.13)	0.99
Early convalescence	98.9 ± 12.2	0.89 (0.86, 0.93)	1.12
Late convalescence	108 ± 11.5	0.97 (0.94, 1.02)	1.03
Ferritin, μ*g/L*			
Reference	14.9 ± 2.2		
Incubation	27.4 ± 1.5	1.84 (0.88, 3.86)	0.54
Early convalescence	42.1 ± 2.7	2.83 (2.22, 3.61)	0.35
Late convalescence	23.9 ± 2.4	1.61 (1.30, 1.99)	0.62
ZP, μ*mol/mol heme*			
Reference	126 ± 1.7		
Incubation	113 ± 1.6	0.90 (0.56, 1.43)	1.11
Early convalescence	166 ± 1.7	1.31 (1.13, 1.53)	0.76
Late convalescence	130 ± 1.7	1.03 (0.90, 1.18)	0.97
TfR, *mg/L*			
Reference	9.2 ± 1.5		
Incubation	8.9 ± 1.3	0.97 (0.69, 1.35)	1.03
Early convalescence	10.6 ± 1.6	1.15 (1.03, 1.28)	0.87
Late convalescence	9.6 ± 1.4	1.04 (0.94, 1.14)	0.96
TfR/ferritin index			
Reference	620 ± 2.7		
Incubation	325 ± 1.6	0.53 (0.22, 1.28)	1.89
Early convalescence	251 ± 3.3	0.41 (0.30, 0.54)	2.44
Late convalescence	400 ± 2.7	0.65 (0.50, 0.84)	1.54

[1] CF, correction factor; Hb, hemoglobin; TfR, plasma soluble transferrin receptor; ZP, whole blood zinc protoporphyrin.

[2] Values are geometric mean ± SD, n = 299 (reference), 11 (incubation), 147 (early convalescence), or 223 (late convalescence).

[3] Geometric mean ratio that compares the iron biomarker concentration at each inflammatory stage with that of the reference group (10).

[4] Correction factors were estimated from the ratios of the geometric mean as explained in reference (10). Example: ferritin correction factor at incubation stage (i.e., incubation vs. reference) = 1.0/1.84 = 0.54; early convalescence stage = 1.00/2.83 = 0.35; and late convalescence stage = 1.00/1.61 = 0.62.

TABLE 4

Association of malaria infection with iron indicators in a sample of 6- to 35-mo-old Kenyan children (n = 680)[1]

	Uncorrected			Corrected		
Iron status indicators	**Present**	**Absent**	***p*[2]**	**Present**	**Absent**	***p*[2]**
Hb, *g/L*	96.7 ± 16.5	110 ± 14.5	<0.001	103 ± 16.4	114 ± 14.9	<0.001
Ferritin, μ*g/L*	28.5 ± 2.9	21.2 ± 2.5	0.019	25.2 ± 2.4	13.9 ± 2.3	<0.001
ZP, μ*mol/mol* heme	183 ± 1.6	130 ± 1.7	<0.001	147 ± 1.6	119 ± 1.7	0.001
TfR, *mg/L*	10.8 ± 1.5	9.4 ± 1.5	0.008	11.4 ± 1.6	8.9 ± 1.4	<0.001
TfR/ferritin index	379 ± 3.5	445 ± 2.9	0.27	451 ± 3.0	648 ± 2.8	0.005
CRP, *mg/L*	4.8 ± 5.3	1.02 ± 6.4	<0.001	–	–	–
AGP, *g/L*	1.4 ± 1.4	1.04 ± 1.4	<0.001	–	–	–

[1] Values are geometric mean ± SD except hemoglobin (arithmetic mean ± SD), n = 84 (present) or 596 (absent). Hb, hemoglobin; TfR, plasma soluble transferrin receptor; ZP, whole blood zinc protoporphyrin.

[2] Student's t test: contrasting mean concentrations of iron status indicators between children with malaria and those without malaria.

TABLE 5

Comparison of median concentrations and proportions with abnormal values of uncorrected and corrected iron-status indicators in a sample of 6- to 35-mo-old Kenyan children (n = 680)[1,2]

Biomarker			P[3]	Abnormal value threshold[4]	Abnormal, n (%)	P[5]
Hb, *g/L*	Uncorrected	111 (100, 119)[2]	<0.001	<110 g/L	46.0 (313)	<0.001
	Corrected	114 (104, 122)			37.4 (254)	
Ferritin, µ*g/L*	Uncorrected	21.2 (11.3, 39.3)	<0.001	<12 µg/L	26.9 (183)	<0.001
	Corrected	14.4 (8.4, 25.5)			40.7 (277)	
ZP, µ*mol/mol*	Uncorrected	124 (90, 187)	<0.001	>80 µmol/mol	82.8 (562)	<0.001
	Corrected	116 (85.7, 174)			79.9 (543)	
TfR, *mg/L*	Uncorrected	9.0 (7.4, 11.3)	<0.001	>8.3 mg/L	61.2 (416)	<0.001
	Corrected	8.7 (7.1, 10.7)			56.6 (385)	
TfR/ferritin index	Uncorrected	399 (211, 855)	<0.001	>500	43.1 (293)	<0.001
	Corrected	579 (307, 1118)			55.9 (380)	

[1] Correction of data achieved using group-specific CF estimated from the ratios of geometric means. CF, correction factor; Hb, hemoglobin; TfR, plasma soluble transferrin receptor; ZP, whole blood zinc protoporphyrin.

[2] Values are median (25th, 75th percentiles).

[3] Paired Wilcoxon's test of medians.

[4] The thresholds for defining abnormal values for the above biochemical indicators were as follows: Hb, <110 g/L for children 0.5–4.99 y (19); ferritin, <12 µg/L; ZP, >80 µmol/mol heme (3); TfR, >8.3 mg/L (TfR, Ramco Laboratories); CRP, >5 mg/L; AGP, >1.0 g/L (10); and TfR/ferritin index defined as >500 (18).

[5] McNemar's chi-square of proportion with abnormal iron status values and anemia.

TABLE 6

Comparison of estimated prevalence of ID in the presence of inflammation based on different methods in a sample of 6- to 35-mo-old Kenyan children[1]

	Full sample			Exclusion of inflammation cases		
Iron status indicator	**Uncorrected**	**CF, this study**	**CF, from meta-analysis[2]**	**Elevated CRP and AGP[3]**	**Elevated CRP only[4]**	**Elevated AGP only[5]**
n	680	680	680	299	522	310
			% (*n*)			
Ferritin <12 μg/L	26.9 (183)	40.7 (277)	39.1 (266)	39.8 (119)	32.0 (167)	38.4 (119)
ZP >80 μmol/mol	82.8 (562)	79.9 (543)	–	79.6 (238)	81.1 (424)	79.4 (247)
TfR >8.3 mg/L	61.2 (416)	56.6 (385)	–	55.5 (166)	60.0 (313)	55.2 (171)
TfR/ferritin index >500	43.1 (293)	55.9 (380)	–	56.2 (168)	49.0 (256)	54.8 (170)

[1] CF, correction factor; ID, iron deficiency; TfR, plasma soluble transferrin receptor; ZP, whole blood zinc protoporphyrin.

[2] Correction of data achieved using CF for children ≤5 y (10).

[3] CRP ≤5 mg/L; AGP ≤1 g/L.

[4] CRP ≤5 mg/L.

[5] AGP ≤1 g/L.