Assessment of regression models for adjustment of iron status biomarkers for inflammation in children with moderate acute malnutrition in Burkina Faso [i-iv].

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[i] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the manuscript and from the same link in the inline table of contents at jn.nutrition.org.

[ii] List of abbreviations: Serum α_1-acid glycoprotein (AGP); acute phase proteins (APPs); correction factors (CF); serum c-reactive protein (CRP); generalized additive model (GAM); Iron deficiency (ID); mid-upper-arm-circumference (MUAC); root mean squared error (RMSE); Serum ferritin (SF); serum soluble transferrin receptor (sTfR); weight-for-height z-score (WHZ).

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Abstract

Background

Biomarkers of iron status are affected by inflammation. In order to interpret them in individuals with inflammation the use of correction factors (CF) has been proposed.

Objective

The objective was to investigate the use of regression models as an alternative to the CF approach.

Methods

Morbidity data were collected during clinical examinations and morbidity recalls in a cross-sectional study among 6-23 month old children with moderate acute malnutrition. C-reactive protein (CRP), α_1-acid glycoprotein (AGP), ferritin (SF) and soluble transferrin receptor (sTfR) were measured in serum. Generalized additive, quadratic and linear models were used to model the relationship between SF and sTfR as outcomes and CRP and AGP either as categorical variables (model 1; equivalent to the CF approach), continuous variables (model 2) or CRP and AGP as continuous variables and morbidity covariates (model 3) as predictors. The predictive performance of the models was compared using ten-fold cross-validation and quantified using root mean squared errors (RMSE). SF and sTfR were adjusted using regression coefficients from linear models.

Results

Cross-validation revealed no advantage of using generalized additive or quadratic models over linear models in terms of the RMSE. Linear model 3 performed better than models 2 and 1. Furthermore, we found no difference in CFs for adjusting SF and those from a previous meta-

analysis. Adjustment of SF and sTFR using the best performing model led to a 17% points increase and <1% point decrease in estimated prevalence of iron deficiency, respectively.

Conclusion

Regression analysis is an alternative to adjust SF and may be preferrable in research settings as it can take morbidity and severity of inflammation into account. In clinical settings the CF approach may be more practical.There is no benefit of adjusting sTfR. The trial was registered at the International Standard Randomised Controlled Trial Number Register (ISRCTN42569496).

Keywords: Inflammation, α_1-acid glycoprotein, correction factors, c-reactive protein, iron deficiency, regression analysis, serum ferritin, soluble transferrin receptor, young children.

Background

Anemia is a major public health issue and affects an estimated 71% of young children (< 5 years) in west and central Africa (1). It can cause fatigue and has been associated with poor cognitive and motor development (2). Iron deficiency (ID) is believed to be responsible for 50% of anaemia cases (3). Other causes of anemia include infectious diseases, hemoglobinopathies and deficiencies of folate, vitamin B12 or vitamin A (2,4).

Diagnosis of ID is necessary for a better understanding of the causes of anemia, identifying individuals who are most likely to benefit from iron supplements and evaluating effectiveness of interventions to combat anemia. It is, however, a challenge because biomarkers of iron status, namely serum ferritin (SF) and serum soluble transferrin receptor (sTfR), are affected by inflammation (4,5). More specifically, SF acts as a positive acute phase reactant (6). sTfR is believed to be less affected by inflammation (4), although there are discrepancies in the literature regarding the relationship between inflammation, infection and sTfR. Some studies have shown that sTfR decreased in presence of inflammation (6,7) and malaria (8) while others found higher levels of sTfR in individuals with malaria (9,10) or observed positive relationships between inflammation markers and sTfR (10–14). It is unclear what causes these discrepancies but they may be in part due to different levels of immunity, time course and severity of infection, as well as the infection causing the inflammation and anemia.

In order to interpret biomarkers of iron status in the presence of inflammation, Thurnham et al (15,16) have suggested applying correction factors (CF) to measured concentrations of SF in individuals with inflammation defined as elevated serum levels of the acute phase proteins (APPs) serum c-reactive protein (CRP) and/or serum α_1-acid glycoprotein (AGP). While SF has been adjusted for inflammation in several studies (10,14,15,17–21), there is still some debate as to whether it is useful to adjust sTfR concentrations (10,21–23).

The CF approach is easy to apply and has been used in a number of studies (10,14,17–20). However, it relies on single cut-offs and therefore ignores that the impact of inflammation on biomarkers of iron status depends on the severity of the inflammation (11,23) and may also depend on the cause of inflammation. In contrast, regression modelling, which has been proposed as an alternative to the CF approach (24), is not dependent on cut-offs and has the advantage that it can take morbidity covariates into account. It may therefore be a better option in populations with a high prevalence of infections. One concern about the use of linear regression models is that the relationships between APPs, namely CRP and AGP, and biomarkers of iron status are not linear (24) and it may thus be necessary to use more flexible regression models. Regression models have previously been used to adjust for inflammation (22,23) but more studies are needed, in particular in contexts where infections as well as malnutrition are common.

The objective of this study was to investigate the use of regression models in adjusting biomarkers of iron status for the effect of inflammation in young children with moderate acute malnutrition in Burkina Faso where, as previously shown, inflammation and morbidity are common (25).

Materials and methods

Study area and population

The data for this paper were baseline data collected as part of the TreatFOOD trial, a randomized trial with the objective to assess effectiveness of 12 supplementary foods for treatment of moderate acute malnutrition, defined as a weight-for-height between –3 and –2 z-scores and/or a mid-upper arm circumference (MUAC) between 115 and 125 mm. As

previously described (26), the trial was carried out in 5 health centers in the Province du Passoré, Burkina Faso. The study catchment area covered a total of 143 villages and a total population of ~ 258,000.

Children aged 6–23 months with moderate acute malnutrition, resident in the catchment area, and whose parents/guardians provided consent for their children to participate were included. Children who were hospitalised or treated for severe acute malnutrition in the previous two months, children with a haemoglobin<5 g/dL, children who were already enrolled in a nutritional programme, and those who had medical complications requiring hospitalisation were not included. Screening for participants was carried out by community health workers using MUAC tapes and designated screening teams using both MUAC and weight-for-height z-score (WHZ). In addition, children could be referred from a health centre or could present at site on carer´s initiative. Recruitment took place from September 2013 until August 2014.

Data collection

Socio-demographic data were collected by trained interviewers. Body weight was measured to the nearest 0.1 kg using an electronic scale with double weighing function (Seca model 881 1021659). Length was measured to the nearest 0.1 cm using a standard UNICEF wooden measuring board. All children were measured lying down. MUAC was measured on the left arm to the nearest 1 mm. During clinical examinations and 14-day retrospective morbidity interviews research nurses collected the following morbidity data: rash, skin infection, runny nose, cough, ear discharge, upper respiratory infection, lower respiratory infection, diarrhea, fever and malaria as well as history of fever, cough, diarrhea, vomiting, rash and swelling. Venous blood (2.5 ml) was collected from the arm. One drop was used for diagnosis of malaria using a rapid diagnostic test that detects histidine rich protein 2 synthesized by the

Plasmodium falciparum malaria parasite (Bioline, Malaria Ag P.f, Standard diagnostics Inc.) and one drop of blood was used to estimate haemoglobin concentration using a HemoCue device (HB 301, Ängelholm, Sweden). The HemoCue was calibrated at the end of every month with a control solution. The remaining blood was added to a sample tube with clot activator (BD reference #368492) and transported to the trial lab in a cold box at 2-8°C. Serum was isolated following centrifugation at 700 x g for 5 minutes (EBA 20 S Hettich) and stored at -20^{0}C until shipment to VitMin Lab in Willstaedt, Germany for analysis of CRP, AGP, SF and sTfR using a combined sandwich enzyme-linked immunosorbent assay (27). All samples were measured in duplicate and both intra- and interassay coefficient of variation were <10%. Samples were frozen and thawed only once prior to analysis.

The thresholds used for defining abnormal values were as follows: Hemoglobin <11 g/L (28), SF <12 µg/L (28), sTfR >8.3 mg/L (27), CRP >5 mg/L (24), AGP >1 g/L (24). Fever was defined as an axillary temperature ≥ 37.5 °C. Upper and lower respiratory tract infections were diagnosed by experienced paediatric nurses based on an adapted version of the Integrated Management of Childhood Illnesses guidelines (29,30). Diarrhoea was defined as three or more loose watery stools per day.

Data handling and statistical analysis

Data were double entered into Epidata 3.1. software (Epidata Association, Odense, Denmark) and double entry checks were carried out on a daily basis. All statistical analyses were carried out using the statistical software R (31). P-values <0.05 were considered to be significant. Characteristics of the study population were summarized as percentage, mean ± SD or, if not normally distributed, as median (interquartile range). Scatter plots with a best-fitting local

148 regression curve were used to display the possibly nonlinear relationships between biomarkers
149 of iron status and acute phase proteins.
150
151 Three types of models were used to predict logarithm-transformed SF and sTfR, namely
152 generalized additive models (GAM), which flexibly allow modelling of nonlinear
153 relationships, quadratic models, and linear models. For each of these three model types, five
154 models per iron status biomarker as outcome and with either i) CRP as continuous variable, ii)
155 AGP as continuous variable, iii) CRP and AGP as continuous variables, iv) both acute phase
156 proteins and morbidity covariates, or v) inflammation groups as independent variables were
157 built. The inflammation groups used were: no inflammation, incubation (CRP >5mg/L only),
158 early convalescence (CRP >5mg/L and AGP >1g/L) and late convalescence (AGP >1 g/L only)
159 as previously described by Thurnham et al (15). Stepwise backwards elimination was used for
160 variable selection. The first four models were fitted to the subset of the data consisting of
161 individuals who had a CRP >5mg/L and/or AGP >1g/L and the last model was built in the full
162 dataset, since the base category were children without inflammation. Model checking was
163 based on residual and normal probability plots.
164
165 The predictive performance of the models was compared using ten-fold cross-validation. More
166 specifically, the data set was randomly split into ten subsets of equal size. In turn each of these
167 one-tenth of the data set (test set) was left out and models fitted to the remainder part of the
168 data (training set). For both SF and sTfR predictive performance was evaluated using root mean
169 squared errors (RMSEs) between observed and predicted values, where a lower RMSE
170 indicates better performance.
171

Following the cross-validation, adjusted SF and sTfR concentrations were calculated using regression coefficients from the models. As an example, the formula for calculation of adjusted SF concentrations using the model with both CRP and AGP as independent variables would be: Adjusted SF= exp(log SF - β_{CRP}*CRP- β_{AGP} *AGP), where β_{CRP} is the regression coefficient from the model and logSF is logarithm transformed SF.

Only concentrations in individuals with CRP >5 mg/L and/or AGP >1 g/L were adjusted. Since back-transformed regression coefficients from logarithm-transformed model are equal to the ratio of geometric means, the model with inflammation groups as independent variable corresponds to the correction factor approach previously described by Thurnham et al (15,16) where ratios of geometric means are converted to correction multipliers by dividing 1 by the ratio. We compared our results to the ratios calculated in a recent meta-analysis (15) for both infants (<12 month) and children (up to 18 years) using approximate t-tests. Prevalence of iron deficiency was calculated for unadjusted and adjusted values as well as separately for individuals with and without inflammation based on the cut-offs for SF and sTfR mentioned above.

Ethical considerations

The study was approved by the Ethics Committee for Health Research of the government of Burkina Faso (2012-8-059) and consultative approval was obtained from the Danish National Committee on Biomedical Research Ethics (1208204). The study was carried out in accordance with the declaration of Helsinki. All children recruited in need of medical treatment received treatment free of charge according to an adapted version of the Integrated Management of Childhood Illnesses guidelines (29,30) and national protocol. Consent was obtained from carers, prior to inclusion, verbally and in writing (signature or fingerprints).

Data were kept confidential and in a locked facility. The trial was registered in the International Standard Randomised Controlled Trial Number registry under the number ISRCTN42569496.

Results

Sample population characteristics

As previously reported 1609 children were enrolled in the TreatFOOD study (25). Among these, 1564 children (82.1%) had baseline SF and sTfR data and were included in the analysis presented here. Background characteristics are presented in **Table 1** and have been described in more detail elsewhere (25). As previously reported, infections and inflammation were common (25). More than two thirds of children had a symptom or infection diagnosed during the physical examination, 35.8% (*n*=561) had elevated CRP and 66.4% (*n*=1039) had elevated AGP (**Table 1**). Only 11.1% (*n*=174) of children did not have any inflammation, history of illness or infections. Anaemia was also common (**Table 1**).

Model selection: Serum ferritin

Although the relationship between SF and APPs was not completely linear as shown in **Figure 1 A,B** and also confirmed by the generalized additive model (p-value of smooth terms <0.05), SF appears to steadily increase for APP values above the cut-off indicating inflammation and levels off at high concentrations of the acute phase proteins (**Figure 1 A,B**). In line with the latter observation, cross-validation revealed no advantage of using more complex GAM and quadratic models over linear models in terms of the RMSEs, which were 0.953, 0.952 and 0.957 for the GAM, quadratic and linear models with APPs in continuous form as predictors. Since there appears to be no gain from using more complex models the remainder of the analysis is based on linear models. While model type did not greatly affect the predictive

performance of the models, the choice of covariates had more of an impact. The RMSEs were reduced if both APPs were included as continuous rather than a categorical variables and they were further reduced if morbidity data were included in addition to APPs (**Table 2**). APPs, malaria, lower respiratory tract infection as well as history of fever were significantly associated with increased log SF levels (**Table 2**). RMSEs for models not presented in **Table 2** can be found in **Supplemental Table 1**.

Model selection: Serum soluble transferrin receptor

Similarly to SF the relationship between sTfR and APPs was not completely linear as demonstrated by **Figure 1 C, D** and confirmed by the generalized additive model (p-value of smooth terms <0.05). Soluble transferrin receptor concentrations appeared to steadily decrease as CRP increases for CRP concentrations >5mg/L. There appeared to be an inverted U-shaped relationship between sTfR and AGP with the apex around an AGP concentration of approximately 1.5g/L (**Figure 1 D**). In line with the observation that sTfR concentration appeared to decrease in individuals with inflammation, cross-validation revealed no advantage of using more complex GAM and quadratic models over linear models in terms of the RMSEs, which were 0.426, 0.427 and 0.429 for the GAM, quadratic and linear models including APPs in continuous form as predictors. The remainder of the analysis was therefore based on the linear models. RMSEs for models not presented in **Table 2** can be found in **Supplemental Table 1**. Similarly to the SF models, the sTfR models performed better if APPs were included in continuous as opposed to categorical form and performance was further improved if morbidity covariates were added (**Table 2**). If both CRP and AGP were included in the models, only CRP remained significant. CRP was associated with a decrease in sTfR, while malaria, fever and acute diarrhea were associated with higher concentrations of sTfR (**Table 2**).

Comparison of study generated to meta-analysis CFs for adjusting SF

The ratio of geometric means between reference and the inflammation groups did not differ from the ones calculated in children in the meta-analysis of Thurnham et al (15). The ratio for the early convalescence vs reference group generated based on our data was different from the one calculated by Thurnham et al. (15) for the subgroup of infants (<12 months) but did not differ for the other two groups **(Table 4).** However, if comparison was made only based on infants under 12 months old in our data as well, this difference disappeared (data not shown).

Impact of adjustment on estimated prevalence of ID

Adjusting SF concentrations for the impact of inflammation and infection led to a lower mean SF and a higher estimated prevalence of ID in the sample by 12, 14 and 17 percentage points for model 1 (linear model with inflammation categories as predictor), model 2 (linear model with APPs as continuous variables) and model 3 (linear model with APPs as continuous variables and morbidity covariates), respectively (**Table 3**). Impact of adjustment of SF is also shown in **Figure 2 A, B**. The estimated prevalence based on adjustment using models 2 or 3 were very close to the prevalence of ID in the subset of children without inflammation and without inflammation and/or infection, respectively (**Table 3**). Estimated prevalence calculated using model 1, which corresponds to the CF approach, was slightly lower than based on the other 2 models. Adjusting sTfR concentrations reduced the prevalence of ID by 7 percentage points based on model 1 and increased the prevalence of ID by 3 and < 1 percentage points if based on model 2 and model 3, respectively (**Table 3**). As also demonstrated in **Figure 2 C, D**, the impact of adjustment on sTfR was therefore small.

Discussion

Our results confirm that the relationship between the two APPs, CRP and AGP, and biomarkers of iron status is not completely linear. Nevertheless, linear models perform well and there was no advantage in using the more complex quadratic or GAM models to predict SF and sTfR concentrations.

To adjust SF for inflammation, the use of regression models is an alternative and may be preferable to the CF approach for several reasons. First, the relationship between the APPs and SF is fairly linear for concentrations above thresholds used to indicate inflammation, and in terms of predictive performance, there does not appear to be any advantage of using more flexible models. Second, we observed higher SF with increasing severity of inflammation and as a result models performed better if CRP and AGP were treated as continuous rather than categorical variables. Third, while the difference in RMSE between model 2 and 3 and resulting prevalence of ID was small, the results indicate that including morbidity leads to a more precise estimate and including morbidity is not possible in the CF approach. Lastly, the estimated adjusted prevalence of ID based on the linear models 2 and 3 was similar to the prevalence of ID in the subset of children without inflammation or without inflammation and infection, respectively. Overall, as expected, adjustment of SF using the 3 models led to an increase in estimated prevalence of ID, which is consistent with findings of previous studies (10,18,20,22).

A disadvantage of regression analysis is that it is more complex than the CF approach and requires available population data. It is unclear exactly how large a sample would be required to allow prediction models to be obtained from regression techniques but we estimate that for sample sizes below 50 the data would not be sufficiently informative.

While we believe that regression analysis using both APP and morbidity data would give a more reliable estimate of iron status and is preferable at population level for example when

evaluating effectiveness of interventions, it is not practical in a clinical setting for identification of ID in an individual unless the regression coefficients and devices are available to carry out the calculations. In this case the use of a CF would be better. Interestingly, even though morbidity appears to play an important role, we found no differences in CFs calculated in apparently healthy children as part of a meta-analysis (15) compared to the ones we calculated as part of our study. In clinical settings where regression approach would be impractical and where population data are not available, the use of meta-analysis correction factors may therefore be appropriate to adjust SF even in children with moderate acute malnutrition.

In the case of sTfR, the models also performed better if both APPs and morbidity covariates were included. However, while in the case of SF it makes sense to pick the best performing model for adjustment, this may not be the case for sTfR. As previously mentioned there is still some debate as to whether sTFR should be adjusted for inflammation (10,21–23) and there are discrepancies in the literature regarding the relationship between sTFR and inflammation or infection. We found a negative relationship between CRP and sTfR, as others have previously reported (6,7). Therefore, since lower sTfR is associated with better iron status, this would suggest better iron status in individuals with elevated CRP. sTfR is a marker of erythropoiesis as well as tissue iron deficiency (5) and lower levels of sTfR in children with inflammation may be a result of suppression of erythropoiesis, which occurs possibly through the actions of inflammatory cytokines (32). In contrast, we and others (9,10,33,34) found higher levels of sTfR in individuals with malaria. It is possible that erythropoiesis is depressed during and increases shortly after the acute malaria infection stage. In line with this it has been shown that while erythropoietin is increased in malaria (35,36), the bone marrow response to erythropoietin may be suppressed until parasites have been cleared (36). We measured malaria using an rapid diagnostic test, which can stay positive for over a month following treatment

(37,38) so it is not possible to know whether a positive test reflects current or recent malaria. However, as previously mentioned in contrast to our results others have observed positive relationships between inflammation markers and sTfR (10–14) or shown that that sTfR decreased in malaria (8). In addition to increased erythropoiesis, higher sTFR concentrations in children with infections may also be due to poorer iron status. Adjusting for morbidity may therefore lead to over-adjustment. Adjustment for CRP may however be justified since elevated levels of CRP in our study were associated with lower levels of sTfR and inflammation may therefore lead to underestimation of ID, but the impact in our study was small. Overall, considering the inconsistencies in the literature regarding association between sTfR and inflammation, the possible risk for over adjustment (if adjusting for morbidity as well as CRP), and that the impact of adjustment was overall small, we believe there is no benefit in adjusting sTfR, which is in agreement with findings from other studies (21,22).

We found a large difference in estimated prevalence of ID based on sTfR and SF, even after adjustment, which is consistent with findings of other studies (10,14,22,39,40). Since SF and sTfR measure different aspects of iron status, differences in prevalence may not be surprising. However, the large difference in prevalences may also have other causes. First, it may be related to the cut-offs used. There are no internationally agreed cut-offs for sTfR (4) and the appropriateness of the 12 µg/L cut-off for SF has also been questioned (41). However, although both lower (41) and higher (42) cut-offs for SF in under 12 months old infants have been suggested, the ESPGHAN committee on nutrition concluded in a position paper that the 12 µg/L cut-off leads to over- rather than underestimation of ID (43), which would not explain the differences we found. Secondly, it has also been suggested that SF and sTfR may not be useful for diagnosis of ID until 9 months of age ID (41) but excluding children under 9 months did not really impact prevalence of ID based on sTfR as well as adjusted or unadjusted SF (data

not shown). Furthermore, while we adjusted SF for inflammation we did not account for the fact that children with inflammation and/or infection may also be more iron deficient than children without and the estimated prevalence of ID after adjustment may be underestimated. Lastly, SF may also be affected by other factors such as liver disease (44) and there may be other unknown causes of elevated sTfR in this population, such as thalassemia (45), sickle cell anemia (5); a limitation of our study is that we did not collect data on hemoglobinopathies. A further limitation is that we were not able to compare adjustments to a gold standard for ID, namely bone marrow iron and it is therefore difficult to say which biomarker with which adjustment iron best reflects iron status in this population.

In conclusion, regression analysis is an alternative and may be preferable to the CF approach when adjusting SF for inflammation since it allows accounting for severity of inflammation and morbidity and we recommend investigating whether this approach would prove to be useful in other populations as well. However, in clinical settings where the regression approach would be impractical the use of meta-analysis CFs may be appropriate. We furthermore believe that there is no benefit of adjusting sTfR. Moreover, considering the large difference in estimated prevalence of ID based on SF and sTfR more research is needed as to which biomarker, using which cut-offs for the markers, and with which adjustment can best define iron status of children from low income areas with high infectious disease burden.

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BC, PK, HF, CR conceptualized the study. BC and CF conducted the research; BC and CR analysed the data and BC wrote the first draft of the manuscript; BC had primary responsibility for final content. BC, CR, CF, VBC, SF, HF & PK revised the manuscript. All authors read and approved the final manuscript.

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Tables and Figures

Table 1. Characteristics of 1564 6-23 month old children with moderate acute malnutrition in Burkina Faso[1]

Sex, male	45.1 (706)
Age, months	11.4 [8.2-16.2]
Anthropometry	
Inclusion category	
Low MUAC only[2]	29.0 (454)
Low WHZ[3] and low MUAC	50.1 (784)
Low WHZ only	21.0 (326)
Height-for-age z-score <-2	37.7 (590)
Morbidity	
Illness according to maternal recall[4]	37.5 (587)
Illness according to physical examination	71.6 (1121)
Malaria[4]	40.2 (626)
Laboratory tests	
Serum CRP, mg/L (IQR)	2.3 [0.8-9.4]
0-5 mg/L	64.1 (1002)
>5- 10mg/L	11.7 (183)
>10-20mg/L	9.2 (144)
>20-40 mg/L	7.0 (110)
>40 mg/L	8.0 (125)
Serum AGP, g/L	1.2 [1.19-1.24]
0-1 g/L	33.0 (517)
>1-2 g/L	52.4 (819)
>2-3 g/L	10.8 (169)
>3 g/L	3.8 (59)
Hemoglobin, g/L	10.0 ± 1.6
< 11 g/L	70 (1095)

[1] Values are % (*n)* for categorical variables, mean ± SD for continuous variables with a normal distribution, or median [IQR] for continuous variables with a skewed distribution**.** IQR, interquartile range; MUAC, mid upper arm circumference; WHZ, weight-for-height z-score; CRP, C-reactive protein; AGP, α_1-acid glycoprotein.
[2]MUAC $\geq$ 115mm and <125mm
[3] WHZ $\geq$ -3 & < -2 z-scores
[4] Data missing: Ill according to maternal recall (9), malaria (6)

Table 2. Prediction models for log-transformed serum ferritin and soluble transferrin receptor in 1564 young children from Burkina Faso[1]

	Log serum ferritin (µg/L)[2]			Log serum soluble transferrin receptor (mg/L)[3]		
	Coefficient (95% CI)	p-value	RMSE	Coefficient (95% CI)	p-value	RMSE
Model 1. Inflammation Categories[3]						
CRP >5mg/L	0.253 (-0.11, 0.625)	0.2		0.142 (-0.014, 0.299)	0.07	
CRP >5mg/L and AGP >1g/L	1.094 (0.969, 1.220)	<0.001		0.149 (0.096, 0.202)	<0.001	
AGP >1g/L	0.432 (0.305, 0.559)	<0.001	1.027	0.147 (0.094, 0.201)	<0.001	0.432
Model 2. Acute phase proteins in continuous form						
CRP	0.015 (0.012, 0.018)	<0.001		-0.003 (-0.004, -0.002)	<0.001	
AGP	0.454 (0.338, 0.571)	<0.001	0.957	-		0.429
Model 3. Acute phase proteins in continuous form and morbidity						
CRP	0.014 (0.010, 0.017)	<0.001		-0.004 (-0.006, -0.003)	<0.001	
AGP	0.348 (0.232, 0.463)	<0.001		-		
Malaria	0.426 (0.310, 0.541)	<0.001		0.259 (0.209, 0.309)	<0.001	
Lower respiratory tract infection	0.139 (0.008, 0.269)	0.04		-		
History of fever	0.316 (0.177, 0.455)	<0.001		-		
Fever	-			0.072 (0.009, 0.136)	0.03	
Acute diarrhoea	-		0.927	0.132 (0.029, 0.234)	0.01	0.410

[1] CRP, C-reactive protein; AGP, α_1-acid glycoprotein; RMSE, root mean squared error from 10-fold cross-validation.
[2] Model 1: Adjusted R^2 =0.159 ; Model 2: Adjusted R^2 = 0.238; Model 3: Adjusted R^2 =0.293
[3] Model 1: Adjusted R^2 =0.023; Model 2: Adjusted R^2 =0.023; Model 3: Adjusted R^2 = 0.113.

Table 3. Estimated prevalence of iron deficiency (ID) with and without adjustment in 1564 6-23 month old children with moderate acute malnutrition[1]

		Serum ferritin (µg/L)		Serum soluble transferrin receptor (mg/L)	
	n	Median (IQR)	ID[5] %, (*n*)	Median (IQR)	ID[5] %, (*n*)
Without adjustment					
All participants	1564	33.4 (13.5-74.0)	21.0 (329)	12.6 (9.1-17.3)	82.9 (1296)
Participants with inflammation (CRP>5 and/or AGP >1)	1070	44.4 (18.9-91.6)	14.7 (157)	13.3 (9.7-18.2)	85.7 (917)
Participants without inflammation	494	18.9 (9.5-40.4)	34.8 (172)	11.2 (8.4-15.3)	76.7 (379)
Participants without inflammation and/or illness	174	15.4 (9.3-29.2)	38.6 (66)	8.14 (8.05-8.23)	72.4 (126)
With adjustment					
Model 1. Linear model with inflammation categories [2, 3]	1564	19.6 (9.2-31.3)	32.9 (516)	11.4 (8.3-15.6)	75.6 (1182)
Model 2. Linear model with CRP and AGP as continuous variables[3,4]	1564	17.5 (8.7-33.5)	35.4 (553)	13.1 (9.6- 18.1)	86.1 (1347)
Model 3. Linear models with CRP, AGP and morbidity [3,5]	1564	16.0 (8.0-30.0)	38.3 (587)	12.4 (9.2-16.9)	83.6 (1305)

[1] ID, iron deficiency; IQR, interquartile range; CRP, C-reactive protein; AGP, α_1-acid glycoprotein.
[2] Inflammation categories were: i.no inflammation, ii.CRP >5mg/L, iii. CRP >5mg/L and AGP >1g/L and iv. AGP >1mg/L. Model 1 is equivalent to the CF approach described by Thurnham et al (15).
[3] Only biomarker concentrations in individuals with inflammation (CRP>5mg/L and AGP>1g/L) were adjusted (*n*=1070) but median and % ID refer to the full sample.
[4] In the sTfR model only CRP was significant.
[5] Morbidity variables included in the serum ferritin model were malaria, lower respiratory tract infection and history of fever and in the sTfR model malaria, fever and acute diarrhea.
[5] Cut-offs used to define ID were serum ferritin<12 µg/L and serum soluble transferrin receptor >8.3 mg/L.

Table 4. Comparison of study-generated and meta-analysis geometric mean ferritin ratios for inflammation groups versus no inflammation group[1]

	Ratio (95% CI)				
	Study generated	Metaanalysis[2]			
	(*n*=1564)	Infants (*n*=1278)	p-value[3]	Children (*n*=3695)	p-value[3]
CRP>5mg/L vs no inflammation	1.29 (0.89-1.87)	1.13 (0.9, 1.41)	0.54	1.56 (1.22-1.99)	0.36
CRP>5mg/L and AGP>1mg/L vs no inflammation	2.99 (2.63-3.39)	2.09 (1.66-2.63)	0.006	2.55 (1.37-4.72)	0.61
AGP>1mg/L vs no inflammation	1.54 (1.36-1.75)	1.42 (1.14-1.76)	0.52	1.53 (1.15-2.04)	0.97

[1] CI, confidence interval**;** CRP, c-reactive protein; AGP**,** α_1-acid glycoprotein.
[2] Geometric mean ferritin ratios for infants (aged <12 months) and children (aged up to 18 years) from a meta-analysis carried out by Thurnham et al **(15)**; [3] p values based on approximate t-tests

Figure 1. Relationship between acute phase proteins and biomarkers of iron status in 1564 6-23 month old children.
(A) Relationship between C-reactive protein (CRP) and serum ferritin (SF); (B) Relationship between CRP and soluble transferrin receptor (sTfR); (C) Relationship between α_1-acid glycoprotein (AGP) and SF; (D) Relationship between AGP and sTfR. Grey dots represent serum concentrations of iron status biomarkers (SF or sTfR). Solid black line is the best fitting local regression curve with 95% confidence interval (CI). Dotted line indicates the cut-off used to define inflammation, i.e. 5mg/L for CRP and 1 g/L for AGP.

Figure 2. Impact of adjusting biomarker concentrations on relationship with acute phase proteins in 1564 6-23 month old children in Burkina Faso.
(A) Impact of adjusting serum ferritin (SF) on relationship with C-reactive protein (CRP); (B) Impact of adjusting soluble transferrin receptor (STfR) on relationship with CRP; (C) Impact of adjusting serum ferritin (SF) on relationship with α_1-acid glycoprotein (AGP); (D) Impact of adjusting sTfR on relationship with AGP. Grey dots indicate unadjusted SF or sTFR concentrations and black dots indicate values adjusted for inflammation. Adjusted SF and sTfR concentrations were calculated using regression coefficients for CRP, AGP and morbidity covariates from linear models predicting log-transformed SF and sTfR concentrations.

Supplemental Table 1. Root mean squared error (RMSE) for predictive models for log-transformed serum ferritin (µg/L) and soluble transferrin receptor (mg/L) from 10-fold cross validation in 1564 6-23 months old children with moderate acute malnutrition.

	Serum ferritin (µg/L)	Serum soluble transferrin receptor (mg/L)
1.Generalized additive models		
1.1. CRP only	0.978	0.428
1.2. AGP only	0.992	0.43
1.3. CRP and AGP	0.953	0.426
1.4. CRP, AGP and morbidity covariates	0.925	0.408
2. Quadratic models		
2.1. CRP only	0.976	0.429
2.2. AGP only	0.992	0.430
2.3. CRP and AGP	0.952	0.427
2.4. CRP, AGP and morbidity covariates	0.924	0.409
3. Linear models		
3.1. Inflammation Categories	1.027	0.432
3.2. CRP	0.982	0.429
3.3. AGP	0.992	0.433
3.4. CRP and AGP	0.957	0.429
3.5. CRP, AGP and morbidity	0.927	0.410