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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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1 **Abstract**

2 *Background*

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

5

6 *Objective*

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

9

10 *Methods*

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

21

22 *Results*

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

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

28

29 *Conclusion*

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

35

36

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

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49 **Background**

50 Anemia is a major public health issue and affects an estimated 71% of young children (< 5
51 years) in west and central Africa (1). It can cause fatigue and has been associated with poor
52 cognitive and motor development (2). Iron deficiency (ID) is believed to be responsible for
53 50% of anaemia cases (3). Other causes of anemia include infectious diseases,
54 hemoglobinopathies and deficiencies of folate, vitamin B12 or vitamin A (2,4).

55

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

68 In order to interpret biomarkers of iron status in the presence of inflammation, Thurnham et al
69 (15,16) have suggested applying correction factors (CF) to measured concentrations of SF in
70 individuals with inflammation defined as elevated serum levels of the acute phase proteins
71 (APPs) serum c-reactive protein (CRP) and/or serum α_1 -acid glycoprotein (AGP). While SF
72 has been adjusted for inflammation in several studies (10,14,15,17–21), there is still some
73 debate as to whether it is useful to adjust sTfR concentrations (10,21–23).

74

75 The CF approach is easy to apply and has been used in a number of studies (10,14,17–20).
76 However, it relies on single cut-offs and therefore ignores that the impact of inflammation on
77 biomarkers of iron status depends on the severity of the inflammation (11,23) and may also
78 depend on the cause of inflammation. In contrast, regression modelling, which has been
79 proposed as an alternative to the CF approach (24), is not dependent on cut-offs and has the
80 advantage that it can take morbidity covariates into account. It may therefore be a better option
81 in populations with a high prevalence of infections. One concern about the use of linear
82 regression models is that the relationships between APPs, namely CRP and AGP, and
83 biomarkers of iron status are not linear (24) and it may thus be necessary to use more flexible
84 regression models. Regression models have previously been used to adjust for inflammation
85 (22,23) but more studies are needed, in particular in contexts where infections as well as
86 malnutrition are common.

87

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

92

93 **Materials and methods**

94 *Study area and population*

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

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

102

103 Children aged 6–23 months with moderate acute malnutrition, resident in the catchment area,
104 and whose parents/guardians provided consent for their children to participate were included.

105 Children who were hospitalised or treated for severe acute malnutrition in the previous two
106 months, children with a haemoglobin < 5 g/dL, children who were already enrolled in a
107 nutritional programme, and those who had medical complications requiring hospitalisation
108 were not included. Screening for participants was carried out by community health workers
109 using MUAC tapes and designated screening teams using both MUAC and weight-for-height
110 z-score (WHZ). In addition, children could be referred from a health centre or could present at
111 site on carer's initiative. Recruitment took place from September 2013 until August 2014.

112

113 *Data collection*

114 Socio-demographic data were collected by trained interviewers. Body weight was measured
115 to the nearest 0.1 kg using an electronic scale with double weighing function (Seca model
116 881 1021659). Length was measured to the nearest 0.1 cm using a standard UNICEF wooden
117 measuring board. All children were measured lying down. MUAC was measured on the left
118 arm to the nearest 1 mm. During clinical examinations and 14-day retrospective morbidity
119 interviews research nurses collected the following morbidity data: rash, skin infection, runny
120 nose, cough, ear discharge, upper respiratory infection, lower respiratory infection, diarrhea,
121 fever and malaria as well as history of fever, cough, diarrhea, vomiting, rash and swelling.
122 Venous blood (2.5 ml) was collected from the arm. One drop was used for diagnosis of
123 malaria using a rapid diagnostic test that detects histidine rich protein 2 synthesized by the

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

134

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

141

142 ***Data handling and statistical analysis***

143 Data were double entered into Epidata 3.1. software (Epidata Association, Odense, Denmark)
144 and double entry checks were carried out on a daily basis. All statistical analyses were carried
145 out using the statistical software R (31). P-values <0.05 were considered to be significant.
146 Characteristics of the study population were summarized as percentage, **mean \pm SD** or, if not
147 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

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

177

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

188

189 *Ethical considerations*

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

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

200

201 **Results**

202 *Sample population characteristics*

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

211

212 *Model selection: Serum ferritin*

213 Although the relationship between SF and APPs was not completely linear as shown in **Figure**
214 **1 A,B** and also confirmed by the generalized additive model (p-value of smooth terms <0.05),
215 SF appears to steadily increase for APP values above the cut-off indicating inflammation and
216 levels off at high concentrations of the acute phase proteins (**Figure 1 A,B**). In line with the
217 latter observation, cross-validation revealed no advantage of using more complex GAM and
218 quadratic models over linear models in terms of the RMSEs, which were 0.953, 0.952 and
219 0.957 for the GAM, quadratic and linear models with APPs in continuous form as predictors.
220 Since there appears to be no gain from using more complex models the remainder of the
221 analysis is based on linear models. While model type did not greatly affect the predictive

222 performance of the models, the choice of covariates had more of an impact. The RMSEs were
223 reduced if both APPs were included as continuous rather than a categorical variables and they
224 were further reduced if morbidity data were included in addition to APPs (**Table 2**). APPs,
225 malaria, lower respiratory tract infection as well as history of fever were significantly
226 associated with increased log SF levels (**Table 2**). RMSEs for models not presented in **Table**
227 **2** can be found in **Supplemental Table 1**.

228

229 *Model selection: Serum soluble transferrin receptor*

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

246

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

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

254

255 *Impact of adjustment on estimated prevalence of ID*

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

269

270 **Discussion**

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

275

276 To adjust SF for inflammation, the use of regression models is an alternative and may be
277 preferable to the CF approach for several reasons. First, the relationship between the APPs and
278 SF is fairly linear for concentrations above thresholds used to indicate inflammation, and in
279 terms of predictive performance, there does not appear to be any advantage of using more
280 flexible models. Second, we observed higher SF with increasing severity of inflammation and
281 as a result models performed better if CRP and AGP were treated as continuous rather than
282 categorical variables. Third, while the difference in RMSE between model 2 and 3 and
283 resulting prevalence of ID was small, the results indicate that including morbidity leads to a
284 more precise estimate and including morbidity is not possible in the CF approach. Lastly, the
285 estimated adjusted prevalence of ID based on the linear models 2 and 3 was similar to the
286 prevalence of ID in the subset of children without inflammation or without inflammation and
287 infection, respectively. Overall, as expected, adjustment of SF using the 3 models led to an
288 increase in estimated prevalence of ID, which is consistent with findings of previous studies
289 (10,18,20,22).

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

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

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

304

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

321 (37,38) so it is not possible to know whether a positive test reflects current or recent malaria.
322 However, as previously mentioned in contrast to our results others have observed positive
323 relationships between inflammation markers and sTfR (10–14) or shown that that sTfR
324 decreased in malaria (8). In addition to increased erythropoiesis, higher sTfR concentrations
325 in children with infections may also be due to poorer iron status. Adjusting for morbidity may
326 therefore lead to over-adjustment. Adjustment for CRP may however be justified since elevated
327 levels of CRP in our study were associated with lower levels of sTfR and inflammation may
328 therefore lead to underestimation of ID, but the impact in our study was small. Overall,
329 considering the inconsistencies in the literature regarding association between sTfR and
330 inflammation, the possible risk for over adjustment (if adjusting for morbidity as well as CRP),
331 and that the impact of adjustment was overall small, we believe there is no benefit in adjusting
332 sTfR, which is in agreement with findings from other studies (21,22).

333

334 We found a large difference in estimated prevalence of ID based on sTfR and SF, even after
335 adjustment, which is consistent with findings of other studies (10,14,22,39,40). Since SF and
336 sTfR measure different aspects of iron status, differences in prevalence may not be surprising.
337 However, the large difference in prevalences may also have other causes. First, it may be
338 related to the cut-offs used. There are no internationally agreed cut-offs for sTfR (4) and the
339 appropriateness of the 12 $\mu\text{g/L}$ cut-off for SF has also been questioned (41). However, although
340 both lower (41) and higher (42) cut-offs for SF in under 12 months old infants have been
341 suggested, the ESPGHAN committee on nutrition concluded in a position paper that the 12
342 $\mu\text{g/L}$ cut-off leads to over- rather than underestimation of ID (43), which would not explain the
343 differences we found. Secondly, it has also been suggested that SF and sTfR may not be useful
344 for diagnosis of ID until 9 months of age ID (41) but excluding children under 9 months did
345 not really impact prevalence of ID based on sTfR as well as adjusted or unadjusted SF (data

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

355

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

365

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367 BC, PK, HF, CR conceptualized the study. BC and CF conducted the research; BC and CR
368 analysed the data and BC wrote the first draft of the manuscript; BC had primary responsibility
369 for final content. BC, CR, CF, VBC, SF, HF & PK revised the manuscript. All authors read
370 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 Faso¹

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)	
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	
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	
< 11 g/L	10.0 ± 1.6 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.

²MUAC \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 Faso¹

	Log serum ferritin ($\mu\text{g/L}$) ²			Log serum soluble transferrin receptor (mg/L) ³		
	Coefficient (95% CI)	p-value	RMSE	Coefficient (95% CI)	p-value	RMSE
Model 1. Inflammation Categories³						
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 $R^2 = 0.113$.

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Table 3. Estimated prevalence of iron deficiency (ID) with and without adjustment in 1564 6-23 month old children with moderate acute malnutrition¹

	Serum ferritin ($\mu\text{g/L}$)		Serum soluble transferrin receptor (mg/L)		
	<i>n</i>	Median (IQR)	ID ⁵ %, (<i>n</i>)	Median (IQR)	ID ⁵ %, (<i>n</i>)
Without adjustment					
All participants	1564	33.4 (13.5-74.0)	21.0 (329)	12.6 (9.1-17.3)	82.9 (1296) ³⁷⁸
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) ³⁷⁹
Participants without inflammation	494	18.9 (9.5-40.4)	34.8 (172)	11.2 (8.4-15.3)	76.7 (379) ³⁸⁰
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) ³⁸¹
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 (1183) ³⁸²
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) ³⁸³
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 (1303) ³⁸⁴

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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). 386³ 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 $\mu\text{g/L}$ 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 group¹

	Study generated (<i>n</i> =1564)	Ratio (95% CI)			
		Metaanalysis ²			
		Infants (<i>n</i> =1278)	p-value ³	Children (<i>n</i> =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 ($\mu\text{g/L}$) and soluble transferrin receptor (mg/L) from 10-fold cross validation in 1564 6-23 months old children with moderate acute malnutrition.

	Serum ferritin ($\mu\text{g/L}$)	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