An alternative approach to adjust the iron status for inflammation in population: an exploratory study

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Abstract

Background: Iron deficiency (ID), one of the major micronutrient deficiencies globally, is challenging to measure. This is due to the dual character of ferritin which measures both the ID and concurrently affected by inflammation and infection. To address this, inflammation biomarkers—e.g., CRP (C-Reactive Protein) and AGP (Alpha-1- Acetylated Glycoprotein) are used through various standard methods to report inflammation-adjusted ID. However, there are problems with these methods and their underlying principles. An exploratory study was conducted to suggest an alternative way for inflammation adjustment with the focus on changing the inflammation-cut off point of the biomarker.

Methods: Data of the Bangladesh National Micronutrient Survey 2011-12 were used in three population groups—preschool age children (6-59 month), school age children (6-14 years) and non-pregnant non-lactating women of reproductive age. Spearman Rank correlation was used between the iron status markers (ferritin, hemoglobin) vs. the inflammation biomarkers (CRP; AGP) at various cut-off levels of the inflammation markers, e.g., all-values, ≤5mg/L, ≤1mg/L, and ≤0.35mg/L. ID was estimated over specified ranges of percentiles of the inflammation biomarker.

Results: Spearman Rank correlation between CRP and ferritin showed statistically significant coefficients at various cut-offs of CRP, except at CRP≤1mg/L in preschool children and at CRP≤0.35mg/L in school age children. The decrease in ferritin concentration was 6.7%-11.1% across the studied populations between the inflammation-unadjusted concentration and at CRP≤1 mg/L. Generally, the prevalence of ID was higher at CRP≤1mg/L than at CRP>1mg/L. These findings suggested that a single inflammation biomarker, CRP with a cut-off of >1mg/L excluded the corresponding cases of inflammation-elevated ferritin, allowing for a simpler estimation of the inflammation-adjusted ID in this setting.

Conclusion: Usage of CRP with the cut-off ≤1mg/L is a useful, simplified and economic way to report the inflammation-adjusted ferritin-based ID in Bangladesh and similar settings with a modest infection burden. The method needs assessment in areas where the infection-burden is higher or in settings where malaria is endemic.

Introduction

Iron is a crucial element with vital functions such as oxygen transport, synthesis of DNA and muscle metabolism (Ordway and Garry 2004, Netz et al. 2014). Iron deficiency is the central cause of anemia, which is one of the most prevalent nutritional deficiencies worldwide, estimated eight years ago to affect 43% of children, 29% of non-pregnant women, and 38% of pregnant women worldwide (Stevens et al. 2013). A common indicator of iron deficiency (ID) is a concentration of serum ferritin lower than specified cut-off points, depending on the age of the population group (WHO 2011). However, ferritin is an acute phase reactant; it responds positively in the presence of inflammation and/or infection, overestimating iron status (Tomkins et al. 2003). Therefore, assessment of iron status in populations is difficult. The standard methods to adjust for infection/inflammation in order to report unbiased estimates of iron status commonly employ two inflammation biomarkers – C-Reactive Protein (CRP) and Alpha-1-Acetylated Glycoprotein (AGP). The frequently used cut-offs are CRP≤5 mg/L and AGP≤1g/L to define the levels below which the infection-induced bias in the estimates of iron status is unimportant (WHO 2020). Based on these cut-offs, the standard methods for adjustment for infection/inflammation are established. In the exclusion method, when CRP values are >5mg/L, those cases are excluded from analysis. In the Correction Factor (CF) method, the studied subjects are grouped into four categories, such as (1) **reference** (both CRP concentration ≤5mg/L and AGP concentration ≤1g/L); (2) **incubation** (CRP concentration >5mg/L and AGP concentration ≤1g/L); (3) **early convalescence** (both CRP concentration >5mg/L and AGP concentration >1g/L); and (4) **late convalescence** (CRP concentration ≤5mg/L and AGP concentration >1g/L) in order to calculate correction factors (Thurnham et al. 2010). The most recent, the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) regression method (Namaste et al. 2017), uses the linear regression of CRP and AGP vs. ferritin, assuming that such association exists.

However, it is observed that in the hours after the onset of an infection/inflammatory event, the CRP rises sharply and reaches its peak within 1-2 days (Calvin et al. 1988, Feedlers et al. 1998). Complementing this, serum ferritin rises abruptly (Feedlers et al. 1998). CRP is a major acute phase protein (APP) which typically shows a 10-100 fold elevation in response to an inflammatory event. Gabay and Kushner (1999) noticed that CRP values exponentially rise within hours of the onset of inflammation and reach a peak at >30,000% of the base value within 1-2 days. It then plummeted and by the day 4 the concentration was still 150% of the base value (Gabay and Kushner 1999). Beard et al. (2006) showed that ferritin concentrations (~15ng/ml) started rising at a CRP concentration as low as 0.3mg/L; and its concentration nearly doubled at a CRP concentration of 1mg/L. Any further rise of CRP beyond 1mg/L was associated with the exponential rise in the ferritin; and at the CRP concentration 3mg/L, the ferritin concentration hit the 140ng/ml mark. Hence, the standard cut-off of CRP≤5mg/L to report the ID status appears to be admixed with a profound inflammatory impact.

In contrast to CRP, AGP is a moderate APP, demonstrating 2-10 fold increases (Ceron et al. 2005). This raises questions regarding the validity of the two methods for adjustment of ferritin concentration for infection/inflammation which use the CRP≤5 mg/L cut-off. Another method, the BRINDA regression correction (RC) approach, developed by the BRINDA project, does not rely on the CRP cut-off ≤5 mg/L (Namaste et al. 2017). Nonetheless, Castberg et al. (2018) have shown that in a malaria endemic setting the respective values of CRP and AGP came down to

below the ≤5mg/L and ≤1g/L (i.e., healthy concentrations) two weeks earlier than that of ferritin which remained elevated at a substantial level. This suggests that the RC method, based on an assumption of linear regression between CRP/AGP and ferritin, may not be sufficient to fully compensate for the effect of malaria/inflammation on ferritin. Additionally, the BRINDA regression correction method used the values of the BRINDA meta-analyzed coefficients for CRP and AGP. These values are calculated on the basis of a pooled analysis of data pertaining to multiple diverse settings. Hence, its application to calculate the adjusted ferritin status of a particular setting might be inappropriately influenced by issues present in other settings.

Therefore, the present study explored the implications of using a new cut-off of the inflammation biomarkers to adjust for inflammation/infection for assessment of ferritin status. The objective is to examine the feasibility of using a single inflammation biomarker; and using a new cut-off at ≤x units to derive the inflammation-adjusted status of ID. The motivation for using a single inflammation biomarker stems from the fact of the relative higher responsiveness of one of the inflammation biomarkers in response to inflammatory events. The other reason for exploring a single inflammation biomarker is the cost implication, as the use of multiple biomarkers is expensive, particularly in the context of the low-income countries.

Methods

Setting

The study was conducted using the primary data of the first Bangladesh National Micronutrient Status Survey 2011-12. Bangladesh is a Low-Mid-Income-Country in South Asia with a per capita Gross National Income of US$ 1940 (World Bank Factsheet 2021) and a medium rank in the Human Development Index (Gini Index 2021). The national prevalence of stunting in under-five year old children is 28% (Multiple Indicator Cluster Survey 2019). The prevalence of anemia in preschool children and non-pregnant women is 33.1% and 26% respectively (Rahman et al. 2016). Hemoglobin, ferritin, CRP and AGP data from three population groups –preschool children (PSC) aged 6-59 months, school age children (SAC) aged 6-14 years and non-pregnant non-lactating women of reproductive age (NPNLW, 15-49 years) were used.

Hemoglobin was measured on a venous blood sample by a portable photometer (Hemocue 301, Hemocue AB, Angleholm Sweden). Ferritin, CRP and AGP parameters were measured by Sandwich ELISA. All biochemical analyses were done in the nutritional biochemistry laboratory of the International Centre of Diarrhoeal Diseases Research, Bangladesh (ICDDR,B). To control the quality of laboratory analyses, precinorm protein and precipath protein (Roche Diagnostics GmbH, Mannheim, Germany) were used as quality controls to check the accuracy and precision of ferritin, CRP and AGP assays. Quality control was performed and the coefficient of variation (CV) was calculated from the cumulative mean and standard deviation. The CV for ferritin, CRP and AGP was 3.7%, 3.9% and 5.9%, respectively.
These data from the national micronutrient survey 2011-12 were obtained through permission of the National Nutrition Services, Directorate General of Health Services, Ministry of Health, Government of Bangladesh. Ethical clearance for the survey was provided by the Institutional Review Board of ICDDR,B.

General approach to the study

The general approach to the study was adopted from the methodologies followed in similar studies (Namaste et al. 2017, McDonald et al. 2020).

First, correlation of the inflammation biomarkers (CRP, AGP) and ferritin/hemoglobin concentrations were examined at the specified cut-offs of the inflammatory biomarkers. The intent was to assess the responsiveness of ferritin to the changes in the inflammation biomarker concentrations (CRP/AGP). The correlation study was supplemented with a graphical depiction of the association. This was the initial step in the selection of the inflammatory biomarker/s and the cut-off concentration.

Second, the percentile distribution of CRP (and AGP) was examined vis-à-vis the various cut-off points of these biomarkers. This aided in understanding the extent of sample loss of the CRPs/AGPs (and thus of the ferritin) concomitant to the cut-offs. The first and second steps aided in identifying the inflammatory biomarker to consider and the proposed new cut-off point for exclusion of the cases with higher levels of the biomarker alongside the exclusion of the corresponding ferritin values.

Third, graphical displays were made to observe the pattern of the ferritin and hemoglobin concentrations across the various ranges of the values of the selected inflammation biomarker. This aided in demonstrating the extent of adjustment of ferritin value at the proposed cut-off concentration of the inflammation marker.

Fourth, the prevalence of ID was compared across the various ranges of the percentiles for the selected inflammation biomarker. This supported in consolidation of the proposed cut-offs.

Fifth, the prevalence of ID was measured in the same populations using the proposed method and the standard methods –CF (Thurnham et al. 2010) and RC (Namaste et al. 2017); and the changes were appraised.

Statistical analysis

The normality distribution of the data was assessed by the Shapiro-Wilk normality test (Results not shown). As the data of CRP (and AGP) were non-normally distributed, Spearman Rank correlation was performed to assess the association of CRP and ferritin (1) In the total data, (2) considering the CRP≤5mg/L, (3) considering the CRP≤1mg/L, and (4) considering the CRP≤0.35mg/L. Similarly, the Spearman Rank correlation was performed to assess the association of CRP and hemoglobin in the total sample, with CRP≤5 mg/L, CRP≤1mg/L and CRP≤0.35mg/L. Scatter plots were generated to show the associations. Line graphs were generated to depict the patterns of the serum ferritin vis-à-vis hemoglobin concentration at the aforementioned concentrations of CRP. Similarly, association between AGP vs. ferritin and hemoglobin were assessed. The prevalence of ID was assessed over the defined ranges of percentile distribution of the inflammatory markers.

Prevalence of ID was estimated on the same dataset using the following three methods for adjustment for inflammation: the Correction Factor (CF), the BRINDA Regression Correction (RC) and the proposed method (inflammatory biomarker<x units); and the relative magnitude of ID by these methods was appraised. The methods of adjustment of ferritin for inflammation by

the CF and RC are described elsewhere (Thurnham et al. 2010, Namaste et al. 2017). For the proposed method, the values of the ferritin corresponding to the selected inflammatory biomarker concentration >x units were excluded from analysis.

Iron deficiency was considered present when the infection-adjusted ferritin (by any method of adjustment) was <12ng/ml in children 6-59 months; and <15ng/ml in children 6-14 years and in the non-pregnant non lactating women (WHO 2020). Statistical analyses were done by using STATA 13 (STATA Inc. College Station TX).

Results and Discussion

Correlation of CRP vs. ferritin and hemoglobin

Table 1 depicts the correlation of hemoglobin and ferritin vs. the inflammation biomarker CRP at the latter's various levels of values in the three population groups—non-pregnant non lactating

Table 1. Spearman correlation of CRP vs. hemoglobin and ferritin concentrations

	CRP vs. hemoglobin			CRP vs. ferritin		
NPNLW	n	Spearman rho	Statistical significance	n	Spearman rho	Statistical significance
All-CRP values	883	0.07	0.03*	880	0.21	0.0000*
CRP≤5mg/L	799	0.1	0.006*	796	0.16	0.0000*
CRP≤1mg/L	497	0.06	0.18†	496	0.17	0.0001*
CRP≤0.35mg/L	250	-0.07	0.27†	250	0.14	0.02*
PSC						
All-CRP values	425	-0.09	0.058†	441	0.21	0.0000*
CRP≤5mg/L	383	-0.06	0.23†	400	0.14	0.0066*
CRP≤1mg/L	297	-0.04	0.44†	306	0.06	0.27†
CRP≤0.35mg/L	171	0.03	0.64†	174	0.15	0.04*
SAC						
All-CRP values	1273	-0.03	0.31†	1295	0.21	0.0000*
CRP≤5mg/L	1210	-0.004	0.88†	1231	0.15	0.0000*
CRP≤1mg/L	1066	0.03	0.37†	1081	0.11	0.0001*
CRP≤0.35mg/L	773	0.07	0.04*	784	0.02	0.51†

*Statistically significant ($p<0.05$) † Statistically non-significant

women (NPNLW), preschool age children (PSC) and the school age children (SAC) from a nationally representative survey of Bangladesh. In general, it was evident that ferritin was positively responsive to almost any level of values of CRP. Complementing this, Fig. 1 demonstrates that in NPNLW, there was a statistically significant correlation of ferritin and the CRP at all the cut-off concentrations of the latter. However, the line graphs of correlation appear

to be progressively flatter from all-CRP values to the CRP≤0.35mg/L, indicating smaller degree of associations as the CRP is restricted to lower and lower values.

Figure 1. Correlation of CRP and ferritin in NPNLW

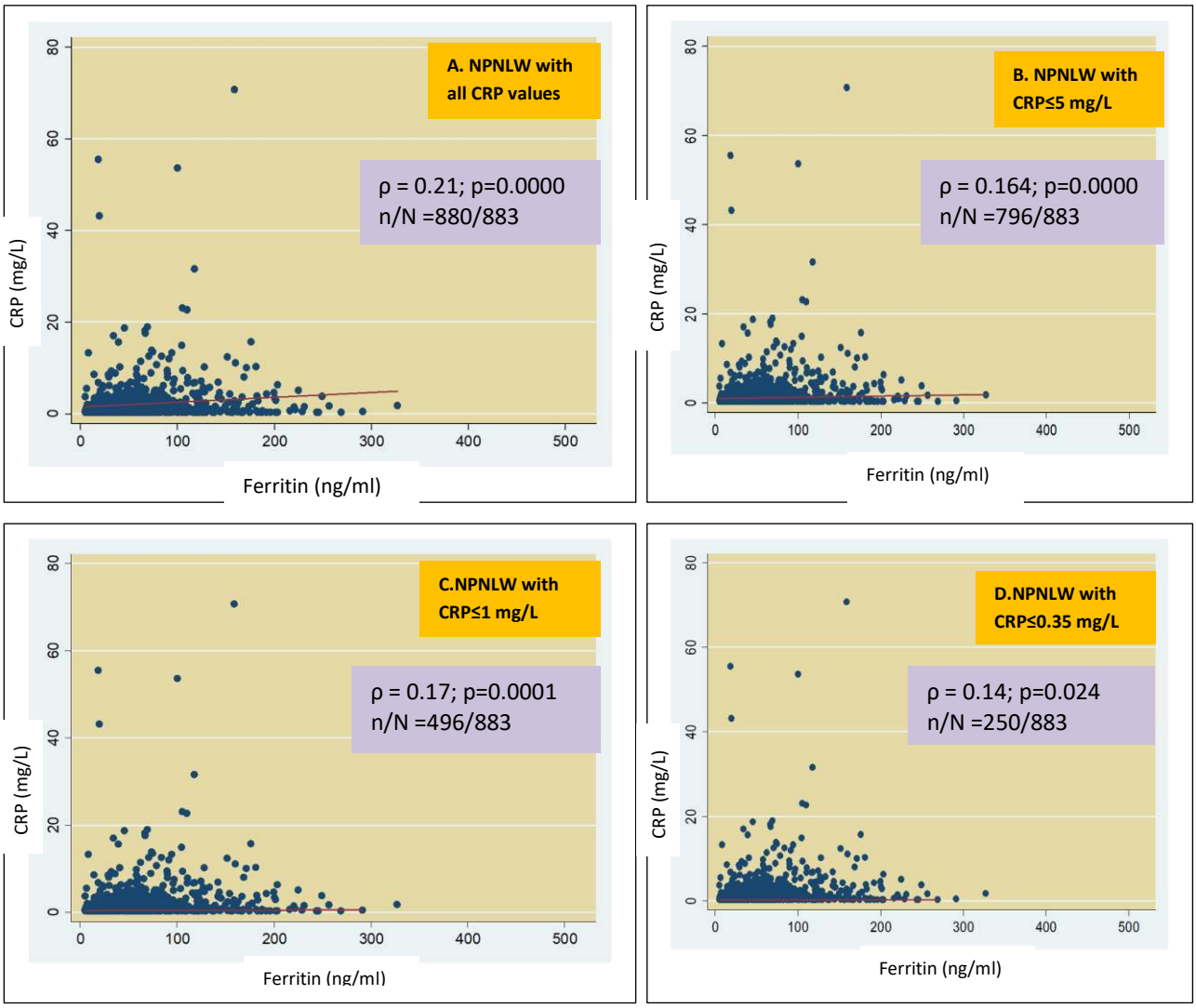


Figure 2. Correlation of CRP and ferritin in PSC

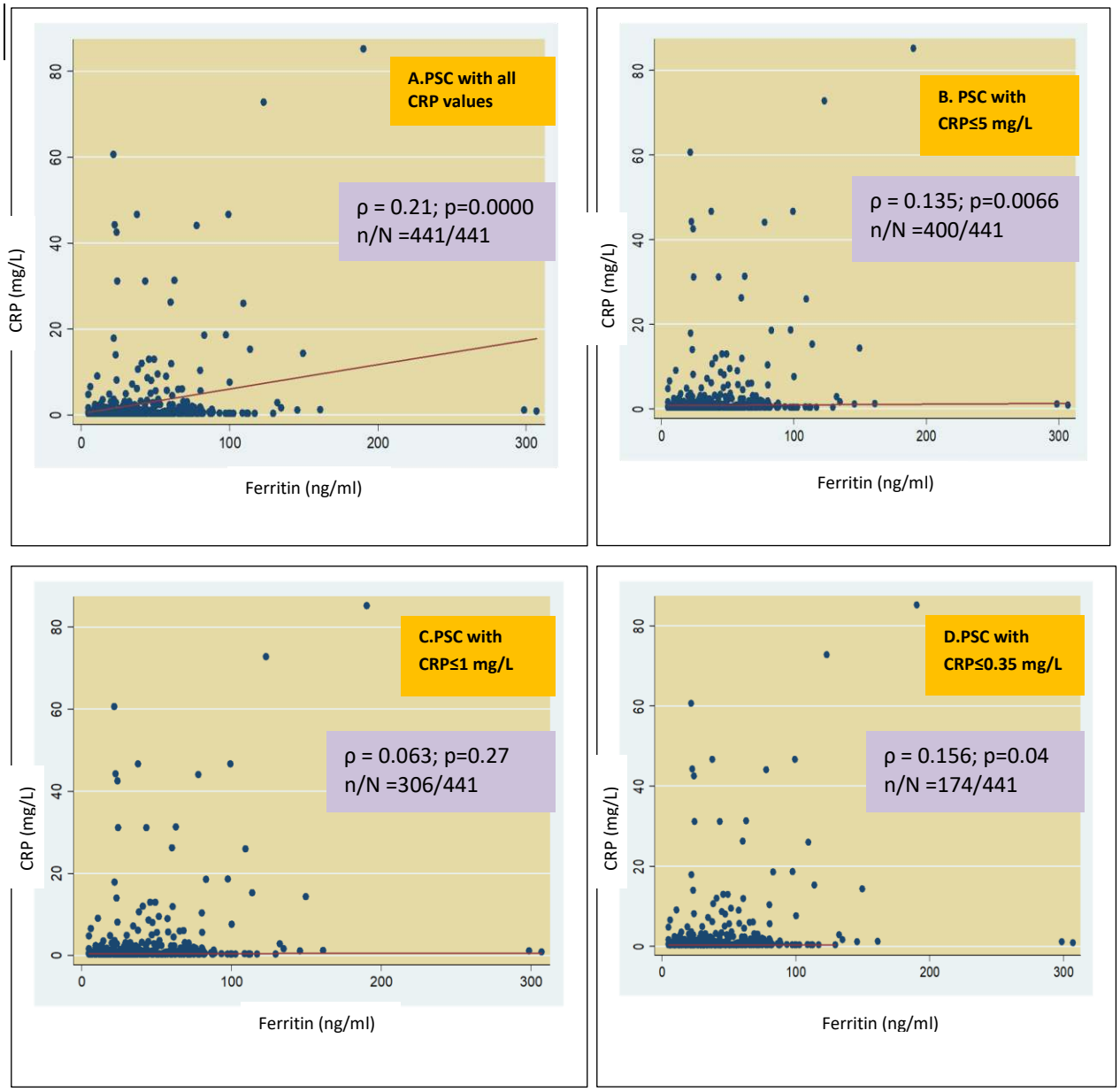


Fig. 2 shows the correlation of CRP and ferritin in PSC. The Spearman Rank coefficient rho for ferritin vs. all CRP values and CRP≤5mg/L were statistically significant. The Spearman rho for ferritin vs. CRP≤1mg/L was statistically non-significant and was smaller in size. The line graphs pertaining to the correlations flattened markedly from all-CRP values to the analyses restricted to lower concentrations of CRP. Though there was a non-significant coefficient at CRP≤1mg/L in case of the PSC, by and large a positive responsiveness of ferritin to CRP was observed in this study. This is consistent with other studies showing a rising trend of ferritin at CRP concentration 0.50mg/L (Namaste et al. 2017, Beard et al. 2006). The present study has additionally observed that the trend of the rise of ferritin occurs at the initial stages of the increment of CRP (0.3-0.35mg/L) [Fig. 2A]; and this is complemented by the significant correlation at CRP≤0.35mg/L in PSC and NPNLW (Table 1).

It is inappropriate to consider the CRP cut-off ≤0.35mg/L for minimization of the inflammatory effect on the ferritin status because of the large reduction of sample size which this would entail, potentially compromising the precision of estimates of the population iron status. On the other hand, the present cut-off of CRP≤5mg/L (WHO 2020, Thurnham et al. 2010) seems inappropriate in this setting. This is evident from statistically significant correlation of CRP and ferritin across all the study populations considered at the CRP≤5mg/L (Table 1). Moreover, the CRP cut-off ≤5mg/L corresponded to the 91stpercentiles of the distribution of CRP in NPNLW; 90.5th percentiles in the PSC and 95th percentiles in SAC populations (Fig. 4). Since this cut-off corresponds to a large majority of the CRP distribution across all populations, usage of this would result in high degree of admixing of ID with inflammatory effects, given the fact that ferritin rises exponentially at the CRP concentration as low as 1 mg/L (Beard et al. 2006).

Correlation of AGP and Ferritin

Table 2 shows the correlation of ferritin and AGP over all AGP values and at the different cut-offs in the studied population groups. All AGP values and AGP>1g/L (i.e., standard cut-off indicating an elevated AGP) were expectedly in positive correlation with ferritin in PSC and SAC but not in the NPNLW. As anticipated, the coefficient for AGP≤1g/L vs. ferritin is non-significant in NPNLW and PSC; but a similar finding was not observed in the SAC. Non-correlation of AGP vs. ferritin across all the study groups was observed when the AGP cut-off was lowered to ≤0.5g/L. However, such lowering incurred 87-94% loss of the AGP samples across the population groups, thus rendering the cut-off untenable to use to report population status of ID. Taking into consideration these observations, lowering of the AGP cut-off from the present standard (≤1g/L) cannot be suggested in this setting.

Consequently, through overall appraisal of the correlations of ferritin vs. CRP and AGP across various concentrations, we see little additional value obtained by including AGP. Thus, we propose the use of CRP as a single inflammation biomarker to adjust the ferritin status for inflammation and infection. Further research identifying a cut-off point below 5mg/L, but perhaps not so low as to reduce the sample size as much as would occur if CRP>1mg/L could be interesting. However, a CRP cut-off point between 1mg/L and 5mg/L seems far from an ideal solution, because of the considerable admixture of the inflammation-induced elevation of ferritin up to the CRP concentration 3mg/L (Beard et al. 2006); and this is additionally evident in the present study (Fig. 2A).

Table 2. Spearman correlation of AGP vs. ferritin concentration

NPNLW	N	Spearman rho	p-value
All-AGP values	881	0.06	0.056†
AGP>1g/L	125	0.14	0.11†
AGP≤1g/L	756	0.05	0.19†
AGP≤0.5g/L	87	0.09	0.40†
PSC			
All-AGP values	442	0.20	0.0000*
AGP>1g/L	132	0.008	0.92†
AGP≤1g/L	310	0.005	0.19†
AGP≤0.5g/L	23	-0.25	0.25†
SAC			
All-AGP values	1295	0.22	0.0000*
AGP>1g/L	191	0.19	0.007*
AGP≤1g/L	1104	0.11	0.003*
AGP≤0.5g/L	170	0.03	0.34†

*Statistically significant; †Statistically non-significant

Fig. 3 depicts ferritin and the corresponding hemoglobin concentrations sorted by various concentrations of CRP. Generally, a decreasing trend of ferritin is observed from the point when all-CRP values were considered to the point when CRP≤0.35mg/L was considered. The inflammation-adjusted decrease of ferritin by the proposed cut off of CRP (≤1mg/L) was 6.7%, 11.1% and 9.2% in the NPNLW, PSC and SAC respectively. Due to this adjustment, a slight upward trend of hemoglobin concentration was observed, especially in PSC and SAC.

Fig. 4 shows the prevalence of ID sorted by specified ranges of percentiles of the CRP distribution. Generally, over the lower percentiles of CRP, the ID was higher, e.g., in PSC the IDs were 14.4% and 21.6% over 20-40th percentile and 40-60th percentile ranges respectively. However, ID was lower at the upper CRP percentiles, e.g., 8.8% and 6.7% over 70-80th and 80-100th percentile ranges respectively. A similar pattern of high and low prevalence of ID over the low and high CRP percentile ranges respectively was observed in NPNLW and SAC. In PSC, the prevalence of ID at CRP≤1mg/L which was the 70th percentile of CRP was 15.4%. Above the CRP>1mg/L, the ID declined to 7.4%. A similar pattern of ID was observed over both the sides of the CRP cut off ≤1mg/L in the NPNLW and SAC. This observation is anticipated--the higher prevalence of ID below the 1mg/L concentration is suggestive of low degree of admixing of the inflammatory flare up of ferritin; and as such these prevalence's are more likely to accurately represent iron status.

Fig. 5 depicts the relative prevalence of ID in the studied populations when the ferritin is unadjusted and adjusted for inflammation by applying the proposed method (CRP≤1mg/L) and the two of the commonly used methods—Correction Factor (CF) and the BRINDA Regression Correction (RC). The pattern of ID by the various methods was consistent across the studied populations. The difference in ID between the unadjusted ferritin and the CF was small, albeit slightly higher by the latter. On the other hand, the ID prevalence was considerably increased by the RC method. The proposed method estimates the ID at a level between these two.

Figure 3. Ferritin and hemoglobin concentrations by CRP concentrations

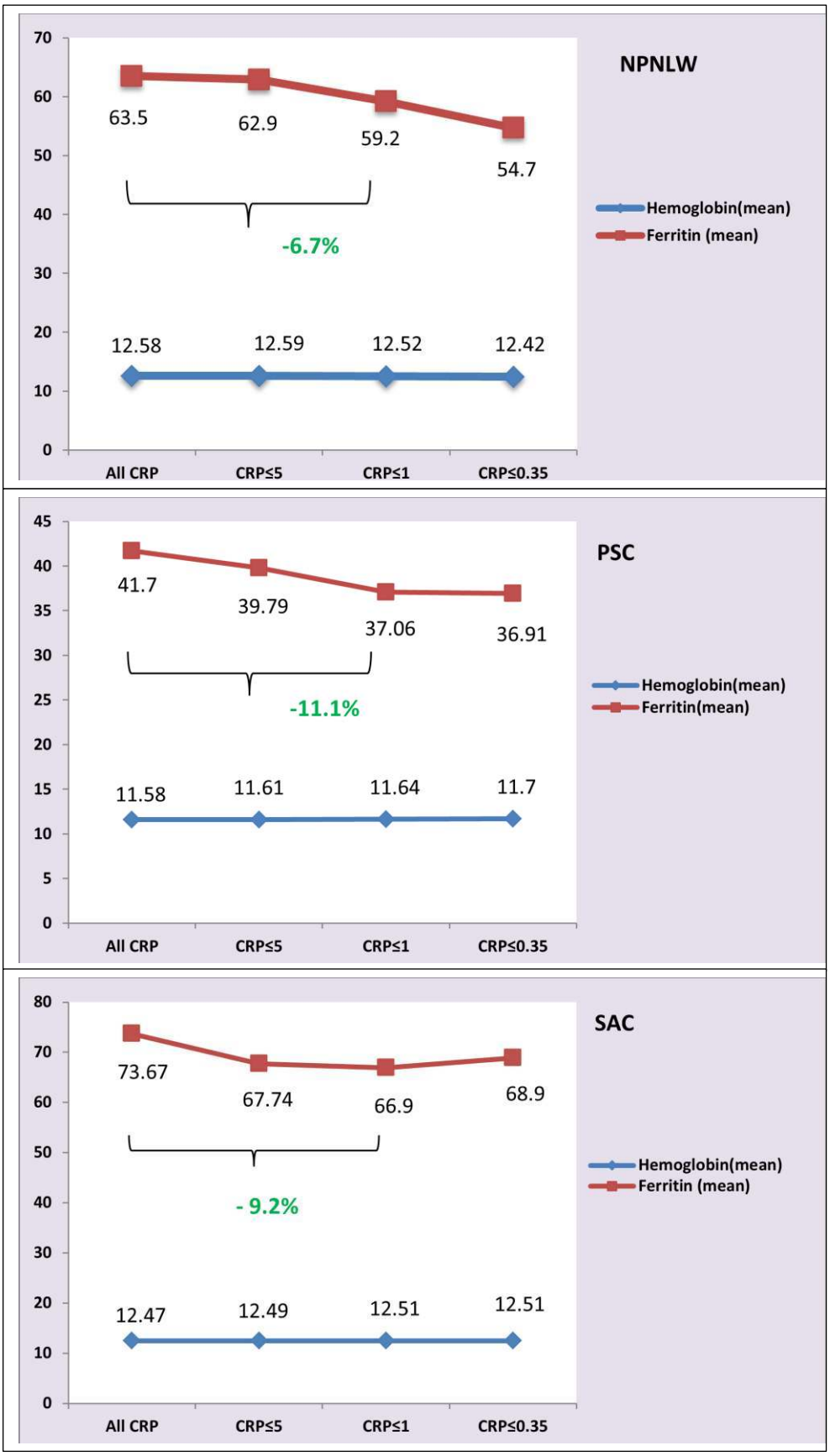
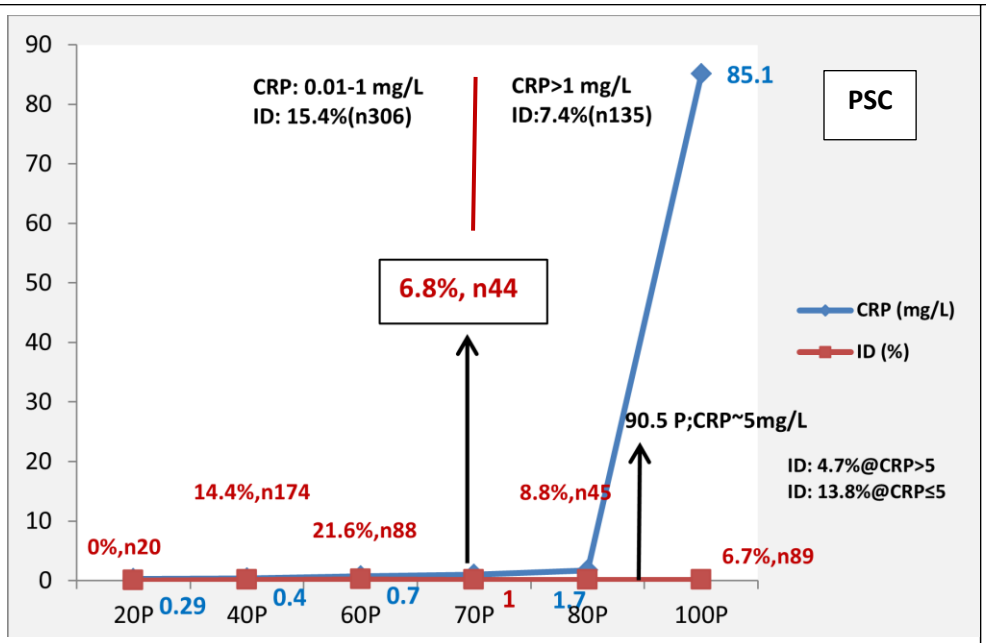
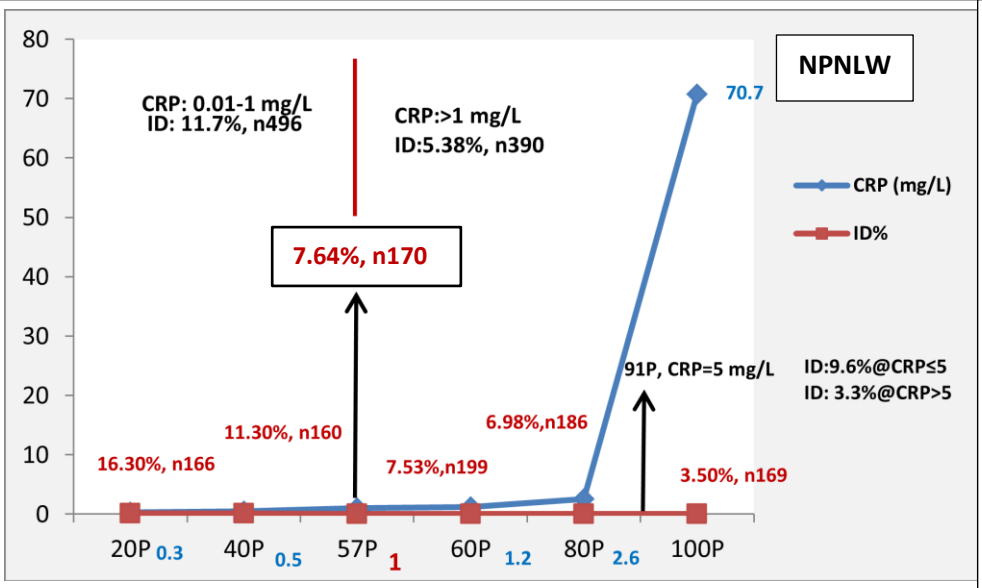


Figure 4. Prevalence of ID over the ranges of percentile distribution of CRP

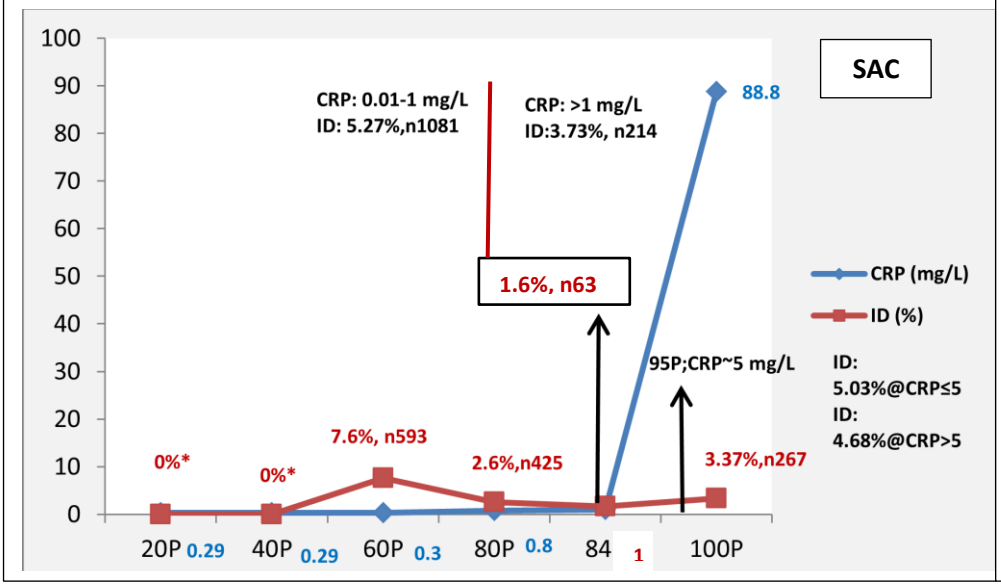


ID%: 0% @ CRP 1P-20P; ID%:14.4% @ CRP 20P-40P; ID%: 21.6% @CRP 40P-60P;

ID%: 6.8% @CRP 60P-70P; ID%: 8.8% @CRP 70P-80P; ID%: 6.7% @ CRP 80P-100P



ID: 16.3% @ CRP 1P-20P; ID: 11.3% @ CRP 20P-40P; ID: 7.64% @CRP 40P-57P ID: 7.53% @CRP 40P-60P; ID: 6.98% @CRP 60P-80P; ID: 3.5% @ CRP 80P-100P



ID: 0% @ CRP 1P-20P; ID: 0% @ CRP 20P-40P; ID: 7.6% @CRP 40P-60P ID: 2.6% @CRP 60P-80P; ID: 1.6% @CRP 80P-84P; ID: 3.37% @ CRP 80P-100P

*No samples were available over the percentile ranges

Figure 5. A comparison of the prevalence of iron deficiency with the inflammation unadjusted ferritin, standard methods for adjustment and the proposed method

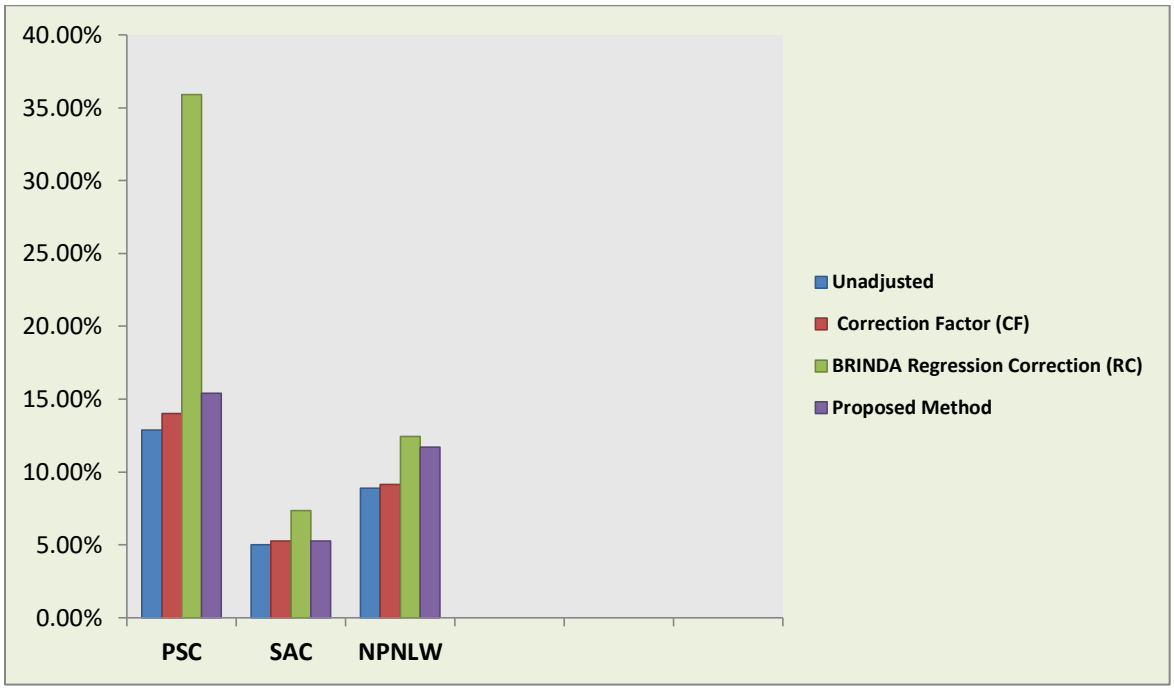
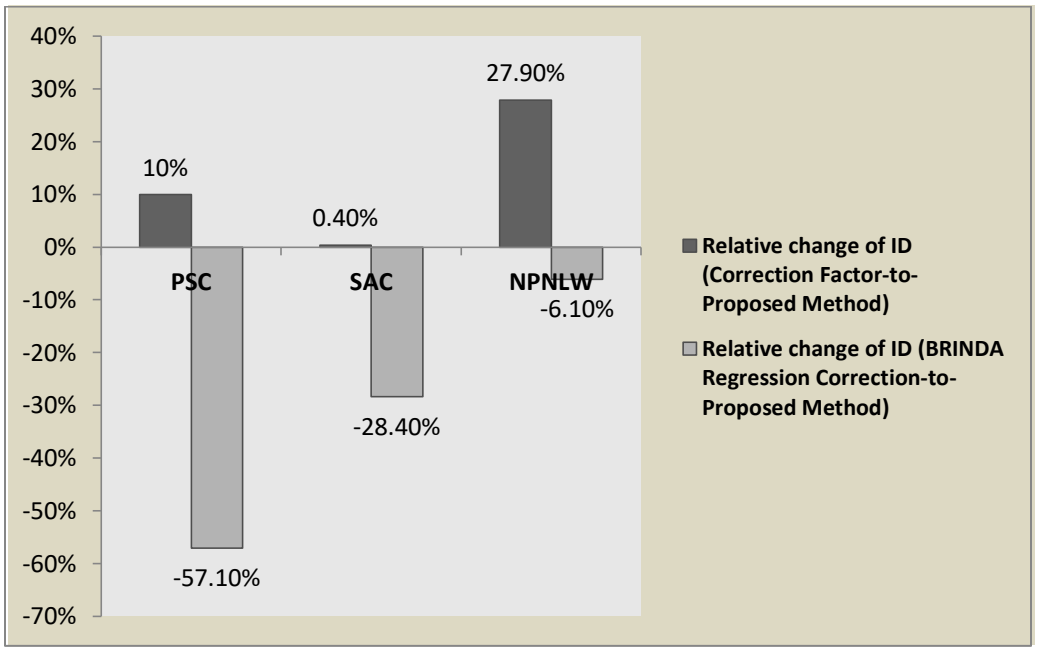


Figure 6 shows the relative change in the prevalence of ID by the proposed method compared to the CF and RC methods. The proposed method increased the ID relative to the CF by 10%, 0.4% and 27.9% in PSC, SAC and NPNLW respectively. The proposed method decreased ID estimates relative to the RC by 57.1%, 28.4% and 6.1% in PSC, SAC and NPNLW respectively. From Fig 5 and Fig 6, it is apparent that the RC method of correction for inflammation results in substantially higher estimate of ID compared to other correction methods and the unadjusted ID. This is difficult to explain. The RC method employs internal survey-specific coefficients as well as meta-analyzed BRINDA data to standardize the estimation of the inflammation adjustment for a particular setting. This meta-analyzed BRINDA data is a composite number derived from multiple settings with diverse background attributes in relation to infection burden, malaria prevalence and socio-economic status. Hence, application of the BRINDA RC approach to calculate the adjustment of ferritin for inflammation in a given context might have some extraneous unwanted influences.

As stated earlier, the proposed method put the inflammation-adjusted ID estimate between the estimates measured by the CF and RC methods and pulled it back from the high estimate measured by the latter (especially in PSC). This moderation of ID by the proposed method appears reasonable on the premise of relatively low burden of infection/inflammation as indicated by elevated CRP>5 mg/L in only 4.9-9.5% subjects among the studied population groups. Elevated AGP (>1 g/L) found a chronic inflammation burden of 14% (NPNLW), 14.7% (SAC) and 30% (PSC) (results not shown). Compared to other settings (Namaste et al. 2017), the burden of common infections is somewhat low in the country compared to settings where the malaria burden is high.

Figure 6. Relative change of the prevalence of ID with the proposed method to the Correction Factor and the Regression Correction methods



After applying the proposed method, the inflammation adjusted ferritin was decreased, but still remained at high values (Fig. 3), especially relative to the cut-off defining ID status (12-15ng/ml). This is not due to any failure of the proposed method to adjust for inflammation, but possibly explained by iron-rich groundwater, the principal source of drinking water in Bangladesh (97%, British Geological Survey 2001). Studies in this setting have reported a positive association of drinking iron rich groundwater and iron status in populations (Merrill 2011, Rahman 2016, Rahman 2019, Ahmed 2018) and a low burden of ID in some population groups (NMS 2011-12, Ahmed 2018, Rahman 2019). In high iron groundwater settings, the daily intake of iron from the drinking groundwater source closely approximates to the Upper Level (UL) of intake in non-pregnant women (Merrill et al. 2011) and in under-five year old children the intake of water iron was ~50% higher than the dietary intake of iron (4.8mg/d vs. 3.1mg/d) (Rahman et al. 2019). Consumption of iron-rich natural water has been reported to have a high level of bioavailability (Worwood et al. 1996). Hence, the high ferritin status in Bangladeshi population may predominantly be due to iron consumed from groundwater.

After correction of ferritin for inflammation using the proposed method, the role of any unaccounted inflammatory factor in increasing ferritin levels is likely to be insignificant. The high value of ferritin existing even after inflammation adjustment using the proposed cut-off CRP≤1mg/L (Fig. 3) may thus largely be attributed to drinking water iron. As the preschool children had a higher inflammation burden (9.5%), the degree of ferritin adjustment was highest in the group (11.1%).

The correlation coefficients between CRP vs. ferritin (Table 1) and AGP vs. ferritin (Table 2) were weak, probably due to the modest burden of infection. Additionally, as said earlier, in Bangladesh at the population level ferritin is largely reflecting drinking groundwater iron. The contribution of iron from the largely cereal-based diet is likely very low (National Micronutrient Status Survey 2011-12, Rahman et al. 2020). This is the reason for small correlational coefficient; despite being statistically significant (possibly induced by high sample size). Coupled with suboptimum dietary iron and low infection burden, the dominance of the drinking water acquired iron to the buildup and thus intractably maintaining the ferritin concentration imply that the overall ferritin responsiveness to the CRP elevation (i.e., correlational coefficient) was plausibly low in magnitude. Should there have been no or insignificant influence of water iron, perhaps the correlation coefficient would have been larger. Hence, some environmental and biological relevance is apparent on the findings of the correlation analyses.

The proposed method offers a reasonably inflammation-adjusted, simplified way of reporting ID status in populations. The existing methods for adjustment yield highly variable estimates of ID in the same population. The proposed method results in values located between those arrived at by the other two methods, making it an attractive alternative.

Value-for-money

Consideration of a single biomarker instead of two for adjustment of inflammation would be cost-effective in this setting which is constraint by resources. As shown in Table 1, the CRP>1mg/L was 44%, 16.5% and 30.4% in NPNLW, SAC and PSC respectively. AGP measurement added no additional useful data. Unit costs of the measurement of ferritin, CRP and AGP are similar. If only CRP is used to estimate inflammation, in this setting the supposed need would be to over count the sample size for ferritin (i.e. for CRP>1 mg/L) above the required sample size by +44% (NPNLW), +16.5% (SAC) and +30.4% (PSC). This entails some over-adjustment of the sample size of ferritin which will be additionally accompanied by the inflation of the sample size of the CRP by similar magnitude. Despite that, utilization of only one marker will incur a lower financial burden, especially in children (Supplementary Information 1).

Strengths/limitations

The strength of the approach lies in the lower cut-off of the CRP which offsets considerable distortion caused by inflammation in elevating ferritin values and thus overestimating ID. The proposed use of only a single biomarker incurs lower costs in measuring population ID status, which is especially important in resource-poor settings.

Since a substantial number of cases are excluded (CRP>1mg/L), the precision of the ID estimates might be compromised. However, the approach suggests the contingent up-adjustment of sample size of the ferritin and thus restoring the precision. Similar to the exclusion method, the exclusion of the cases with elevated marker (CRP>1mg/L) from analysis might induce some bias. Further research could identify a cut-off point between 1mg/L and 5mg/L that could achieve a more balanced trade-off.

Conclusions

Usage of the single inflammation biomarker—CRP with the lowering of cut-off to >1mg/L for defining elevated inflammation status is a useful, simplified way for reporting the inflammation-

adjusted iron status in settings with modest infection burdens. The method is less costly and thus might be useful in resource-poor settings. The validity of the method needs assessment in settings with high burden of infection and/or malaria.

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Annex: Cost implications

A hypothetical example in Bangladesh context

Suppose,

- 100 ferritin samples needed for a desired precision of ID prevalence estimate
- Hence, 100 CRP samples are required to analyze to assess for the inflammation-adjusted ferritin.
- It appears that in preschool age children for example ~30% have CRP values>1 mg/L [From the present study]
- So, 30% of ferritin has to be discarded, limiting the desired precision of ID
- To retain the desired precision of the ID estimate, 100+30= 130 ferritin samples are required
- To complement for the possible exclusion for elevated CRP (>1 mg/L) on the added ferritin samples (n=30), the increase in CRP needs to be by +30%

	PSC			
	Ferritin*	CRP*	AGP*	N (Total)
Existing method**	n=100	n=100	n=100	300
Proposed method***	n=100+30	n=100+30	n=00	260
				Cost saving:16%
	SAC			
	Ferritin	CRP	AGP	N (Total)
Existing method**	n=100	n=100	n=100	300
Proposed method***	n=100+16	n=100+16	n=00	232
				Cost saving:23%
	NPNLW			
	Ferritin	CRP	AGP	N (Total)
Existing method**	n=100	n=100	n=100	300
Proposed method***	n=100+44	n=100+44	n=00	288
				Cost saving:4%

*Ferritin, CRP and AGP are assumed to cost the same
**Combines ferritin, CRP and AGP for the correction factor (CF) and the regression correction (RC) methods for inflammation adjusted ID
***Combines ferritin and CRP for inflammation adjusted ID

Thus, on average, a standard ID status survey might save ~14.5% on the cost for the inflammation adjusted ID parameters.