Baseline Iron Indices as Predictors of Hemoglobin Improvement in Anemic Vietnamese Women Receiving Weekly Iron-Folic Acid Supplementation and Deworming

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Abstract. Iron deficiency anemia is highly prevalent among women living in rural Vietnam. However, the utility and cut-offs of indices for diagnosing iron deficiency anemia in the public health context is ill defined. We assessed the ability of iron indices to predict the hemoglobin response (HBR) to weekly iron-folic acid supplementation (WIFS) in anemic rural Vietnamese women. We compared hemoglobin, serum ferritin, and soluble transferrin receptor in a cohort of 221 non-pregnant women of reproductive age before and after 3 months of WIFS and deworming. At baseline, anemia (Hb < 120 g/L) was present in 81/221 (36.7%) of subjects. After 3 months, anemia prevalence fell to 58/221 (26.2%), and the mean hemoglobin change was +3.5 g/L (95% confidence interval, 0.9, 6.6). A hemoglobin response was observed in 50/75 (66.6%) of anemic women. A ferritin cut-off < 30 ng/mL was a more sensitive predictor of response than ferritin < 15 ng/mL.

INTRODUCTION

Globally, it is estimated that 41.8% of pregnant and 30.2% of non-pregnant women are anemic, with the main burden occurring in Africa and Asia.1 The World Health Organization (WHO) and the United Nations Children’s Fund (UNICEF) have reiterated the importance of anemia control at the public health level.2 Iron deficiency is believed to be the main cause of anemia, and iron deficiency and anemia are linked to increased maternal morbidity and mortality and impaired functional capacity in women.3

Weekly iron-folic acid supplementation (WIFS) is currently proposed for non-pregnant women as an effective, safe, and practical anemia prevention strategy.4 Although the proportion of anemia attributable to iron deficiency varies in different settings,5,6 WHO currently recommends universal iron-folic acid supplementation for all women living in populations where the prevalence of anemia is >20%.7,8 Given that testing for iron indices is relatively expensive and requires specialized laboratory resources, WHO guidelines do not insist on specifically establishing the prevalence of iron deficiency.9 However, other causes of anemia, including vitamin A deficiency and genetic hemoglobinopathies, have been identified as common in Southeast Asian populations,9 and it is possible that population estimates of anemia in this region may not accurately reflect the burden of iron deficiency. This may influence the proportion of recipients of universal iron supplementation who could be expected to respond to such an intervention.

A firm diagnosis of iron deficiency anemia can be established if an improvement in hemoglobin to a trial of iron occurs.10 Iron indices may also be used to identify iron status: serum ferritin is an accurate indicator of total body iron stores, especially in the absence of inflammation,11 and is often used as an indicator of iron status in populations. For adults, a cut-off of < 15 ng/mL is recommended by WHO as an indicator of iron deficiency.12 However, as with all diagnostic tests, sensitivity and specificity depend on the cut-off used. Clinical studies have indicated that higher cut-offs for ferritin may be more sensitive for detection of iron deficiency and be more appropriate.13 Soluble transferrin receptor (TfR) reflects tissue iron status and is an alternate index, but its role in population evaluations of iron status remains unclear,13 and reference ranges differ between manufacturer’s assays.14 TfR is also elevated in patients with increased hematopoesis as is found in the hemoglobinopathies.15 The TfR/log (ferritin) ratio (TfR-F index) seems to closely reflect marrow iron.16 The selection of indices for use for monitoring field iron supplementation programs and the thresholds to define iron depletion or repletion require further evaluation.15

Estimates of prevalence of iron deficiency among women in Vietnam range from 22% to 86.3%,17,18 and up to 42.8% of non-pregnant women are anemic.19 We implemented a universal WIFS and deworming program for women of reproductive age in two districts of the northern mountainous province of Yen Bai, Vietnam starting in May 2006. The broad aims of this study were to determine the burden of anemia, iron deficiency, and soil-transmitted helminth infection in non-pregnant women of reproductive age in this region of Vietnam20 and to assess the change in these after a population-based intervention.

In this study, we aim to (1) describe the effect of WIFS and deworming in WRA by comparing the prevalence of anemia and iron deficiency in the baseline and 3-month surveys; (2) examine the predictors of a hemoglobin response in anemic women; and (3) examine the sensitivity and specificity of different cut-offs for ferritin, TfR, and TfR-F index to predict a hemoglobin response amongst anemic subjects.

MATERIALS AND METHODS

Study site and subjects. Yen Bai province, a mountainous, rural region of northwest Vietnam, was selected for implementation of the WIFS/de-worming program. Almost one third of communes in the area are considered to have “very poor” socioeconomic status.21 Malaria has been eradicated from this region, and there were only two imported cases reported in Yen Bai in 2005 (Phu L, Director Malaria Control Program, Yen Bai Province, personal communication).

Intervention. From May 2006, a program of WIFS and deworming every 4 months for all women of reproductive age living in Yen Bai province was implemented by active
distribution through the existing primary health structure. Women received ferrous sulphate/folic acid tablets (60 mg/0.4 mg; UNICEF, Copenhagen, Denmark) from village health workers. Albendazole (400 mg; UNICEF) was administered as observed treatment. Before implementation, the staff of each district Department of Preventive Medicine, two nurses from each commune health station, and all village health workers in the two districts had been trained about the causes, treatment, prevention, and health risks of anemia and hookworm infection and received promotional and educational materials for distribution to the population. More than 52,000 women living in the district had free access to the supplementation and deworming.

**Sampling.** Two districts in the province (of a possible seven districts) were chosen, based on their proximity to the main city and access to health services, for evaluating the program. The total population of each district was similar. A multi-stage cluster sampling design was used to select villages and individual women within villages. Primary sampling units (villages) were chosen using a “probability proportional to size” random sampling method, with one half the target sample of villages taken from each district. Secondary sampling units (women) were randomly selected from each village from provincial lists. Approximately 13 women were selected from each village, and a questionnaire covering basic demographics such as age, number of children, and poverty status was administered.

**Laboratory methods.** Blood samples were collected from each participant. Hemoglobin was evaluated by hemoglobinometer (HemoCue, Angelholm, Sweden) using capillary blood, and 3 mL of venous blood was collected into tubes containing a fast clotting agent. The tubes were spun at 4,000 rpm for 20 minutes at room temperature, after which serum was collected and stored at −4°C and transported on dry ice to Sydney, Australia, for analysis. Serum ferritin was evaluated using a sandwich immunoenzymatic assay (IEA; Beckman Coulter Access Reagents, Fullerton, CA), and TfR was estimated using enzyme-linked immunoassay (ELISA; IT: Orion Diagnostica, Espoo, Finland). The ratio of transferrin receptor to log (base 10) serum ferritin (TfR-F index) was calculated. Serum C-reactive protein (CRP) levels were assessed by an ELISA method (Beckman Coulter, Fullerton, CA). We chose a level of > 10 mg/L to represent a state of inflammation.

Anemia was defined as a hemoglobin concentration < 120 g/L and iron deficiency as a serum ferritin < 15 ng/mL, in accordance with WHO recommendations for non-pregnant women. TfR levels of 2.3 mg/L or above were considered to represent iron deficiency based on the manufacturer’s reference interval (0.8–2.3 mg/L). The TfR-F index has been previously found to provide an excellent indicator of iron stores, with depletion when the ratio exceeds 1.8, using the same kit as was used in this study.

**Ethics.** Consultation was undertaken between the project team and village, district, and provincial health staff and members of the community. The subjects were educated about the project by local village health workers, after which informed consent was obtained. The project was approved by the Human Research Ethics Committees of the National Institute of Malariaology, Parasitology, and Entomology (Hanoi, Vietnam), The Walter and Eliza Hall Institute of Medical research (Melbourne, Australia), and Melbourne Health Research Directorate (Melbourne, Australia).

**Statistical analysis.** A sample size of 220 at baseline and follow-up would ensure a power of 0.90 for detection of a reduction in the prevalence of anemia from 40% to 25% and a change in mean hemoglobin levels by 5 g/L. Hemoglobin data were normally distributed. The distribution of ferritin, TfR, and TfR-F index were approximately log-normal, and these data were log-transformed for subsequent analysis.

The prevalence of anemia, iron deficiency (using different indices and cut-offs), and iron deficiency anemia at baseline and follow-up were compared using a two-sample proportion test. We estimated the within-subject change in hemoglobin and iron status by calculating the hemoglobin change (hemoglobin_{3 months} − hemoglobin_{baseline}), geometric mean within-subject ratio of ferritin and TfR ([ferritin_{3 months}/ferritin_{baseline}] and [TfR_{3 months}/TfR_{baseline}], respectively), and calculating the mean for these for anemic, non-anemic, and overall groups.

We estimated that a subject responding to iron should therefore experience at least a rise of 10 g/L if there had been 12–14 doses (weekly dose for 3 months). However, a subject with mild anemia may undergo a smaller rise in hemoglobin to achieve resolution of the anemia. We therefore defined a hemoglobin response (HBR) in anemic women as a rise in hemoglobin by at least 10 g/L or resolution of anemia. Hematologic data were compared in subjects with and without an HBR using t tests. All estimates were obtained with robust confidence intervals (CIs) to allow for the sampling design. Receiver operator characteristic curves (ROCs) were used to show the sensitivity and specificity of ferritin at various cut-point values to predict an HBR. The area under the curve of the ROC (AUC^90%) was calculated to estimate the utility of each test of iron stores to distinguish between responders and non-responders. Statistical analysis was performed using Stata (Stata 9.2 for Windows; StataCorp, College Station, TX).

**RESULTS**

Of 349 subjects who had baseline hemoglobin assessments, 221 (63%) underwent repeat survey after 3 months of WIFS and one dose of albendazole. Their mean age was 31.4 years (95% CI, 30.4, 32.5), the mean number of children was 2.1 (95% CI, 2.0, 2.2), and 19/218 (8.7%; 95% CI, 4.9, 12.5) had a poor card (signifying the holder is a recipient of fully subsidized comprehensive health insurance provided to the poor through the government).

At baseline, the mean hemoglobin among these subjects was 122.5 g/L (95% CI, 120.1, 124.8), the geometric mean ferritin was 28.6 ng/mL (N = 210; 95% CI, 24.9, 32.8), geometric mean TfR was 1.8 mg/L (N = 214; 95% CI, 1.7, 1.9), and geometric mean TfR-F index was 1.3 (N = 208; 95% CI, 1.2, 1.4). CRP was < 10 mg/L in all but two subjects. Among the 128 women whom did not return for follow-up, the mean baseline hemoglobin was 121.9 g/L (95% CI, 119.2, 124.7), the geometric mean ferritin was 27.5 ng/mL (95% CI, 22.9, 33.1), the geometric mean TfR was 1.6 mg/L (95% CI, 1.5, 1.7), and geometric mean TfR-F index 1.2 (95% CI, 1.0, 1.4).

Table 1 depicts the baseline prevalence of anemia and indices indicating iron deficiency among the overall group, the prevalence of indices indicating iron deficiency within the anemic and non-anemic groups, and the improvements in these indices after 3 months of intervention. There was a reduction in anemia, iron deficiency, and iron deficiency anemia. The
prevalence of iron deficiency was higher when using a ferritin cut-off of <30 ng/mL compared with <15 ng/mL.

The mean within-subject changes in hemoglobin, ferritin, TIR, and TIR ratio after 3 months of the WIFS/deworming intervention are shown in Table 2 for the overall and anemic groups, indicating an improvement in iron status in the anemic and overall groups. The group that was anemic at baseline, highlighted in Tables 1 and 2, was evaluated for HBR. An HBR was achieved in 50/75 (66.6%) subjects with baseline anemia for whom hemoglobin, ferritin, and TIR data were available. Of the women with ferritin <15 ng/mL and anemia, 21/26 (80.8%) attained an HBR. Table 3 shows a comparison of hemoglobin and iron indices for clinical responders and non-responders at baseline and after 3 months. Among those individuals who were clinical responders, iron indices improved over 3 months, but there was no apparent change in non-responders. Responding women had more children compared with non-responding women (mean number of children, 2.3; 95% CI, 2.1, 2.6 versus 1.8; 95% CI, 1.4, 2.2; \( P < 0.05 \)) and were older (mean age responders, 32.9 years; 95% CI, 30.9, 34.9 versus 28.3 years; 95% CI, 24.9, 31.6). There was no difference between responding and non-responding anemic women in terms of category of level of education achieved (\( P = 0.56 \)). The proportion of responders and non-responders who held a poor card was similar (9% versus 12.5%; \( P = 0.64 \)).

The AUCROC for ferritin as a test for predicting HBR was 0.65 (95% CI, 0.51, 0.80) and for the TIR-F index was 0.68 (95% CI, 0.55, 0.81).

**DISCUSSION**

We reported a significant reduction in prevalence of anemia and iron deficiency and rise in mean hemoglobin in non-pregnant women of reproductive age after 3 months of an iron-folic acid and deworming program. A significant response in hemoglobin occurred in two thirds of anemic women, even though only a third had iron deficiency at baseline according to conventional iron indices (ferritin and TIR), suggesting iron deficiency plays a larger role in the burden of anemia than estimated by conventional iron indices and cut-offs alone.

Given the low prevalence of iron deficiency as defined by ferritin <15 ng/mL, it is surprising that so many (66%) anemic women achieved a HBR. This suggests that the present definition of iron deficiency (ferritin of <15 ng/mL) may be too specific and that women with higher ferritin levels also benefited. Increasing the cut-off of ferritin to 30 ng/mL improved the sensitivity for detection of HBR from 44% to 72%.
Determined cut-offs for diagnostic test results that define disease is an inherent trade-off between improved sensitivity or specificity, with the specific cut-point ideally determined based on the clinical information required from the test. Given that there are relatively few side effects from treating with iron, especially in adults living in non–malaria-endemic regions, we consider the benefit of an improvement in sensitivity outweighs the loss in specificity. This is particularly so when identifying cut-off levels for population-based programs. In addition, others have found that up to one quarter of patients without stainable bone marrow iron have ferritin levels > 15 ng/mL.24 Our results and conclusions also reflect those of a previous study in which the sensitivity of ferritin as an indicator of response to iron supplementation was improved if the cut-off increased from 15 to 30 ng/mL.12

Ferritin rose significantly in the group who achieved a HBR, but remained static in the non-response group. This latter group were more iron replete at baseline, and it is likely that a cause other than iron deficiency was the explanation for the anemia in this group. It is also possible that iron-replete, anemic subjects (Green T, personal communication). Furthermore, the folate dose (0.4 mg weekly) was relatively low,27 and there is little evidence that folate acid improves hemoglobin in non-pregnant women without megaloblastic anemia.

A response to the folic acid component of the supplement may have partially contributed to the high HBR rate. We do not believe this is likely because preliminary biochemistry results suggested that folate deficiency was uncommon in our subjects (Green T, personal communication). Furthermore, the folate dose (0.4 mg weekly) was relatively low,27 and there is little evidence that folic acid improves hemoglobin in non-pregnant women without megaloblastic anemia.

The AUC ROC reflects the overall ability of a continuous measure to discriminate between individuals with and without a disease.20 In this study, we used AUC ROC to determine the ability of baseline iron indices to discriminate between women who would and would not have a HBR to the WIFS/deworming intervention. The AUC ROC values for ferritin, TIR, and TIR-F index were each < 0.7, suggesting iron indices had only a moderate diagnostic value in predicting HBR.29 The AUC ROC value for determination of HBR for anemic individuals using ferritin was superior to that for TIR and similar to the TIR-F index, suggesting little added diagnostic benefit from assaying the baseline TIR in this population, despite significant additional cost.

Our analysis was limited by incomplete data collection. The baseline survey was comprised of 349 non-pregnant women, of whom 221 returned for follow-up analysis at 3 months, and com-

### Table 3
Comparison of baseline hemoglobin and iron indices between baseline anemic women who achieved a hemoglobin response of > 10 g/L to WIFS/deworming and those who did not

<table>
<thead>
<tr>
<th>Hemoglobin response, mean [95% CI]</th>
<th>No response, mean [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>3 month</td>
</tr>
<tr>
<td>Hemoglobin (g/L)†</td>
<td>122.5 [120.1, 124.8]</td>
</tr>
<tr>
<td>Ferritin (ng/mL)†</td>
<td>26.8 [24.9, 32.8]</td>
</tr>
<tr>
<td>TIR (mg/L)†</td>
<td>1.8 [1.7, 1.9]</td>
</tr>
<tr>
<td>TIR-F index†</td>
<td>1.3 [1.2, 1.4]</td>
</tr>
</tbody>
</table>

*Arithmetic mean.
† Geometric mean.
‡ P < 0.05 for difference between baseline hemoglobin in HBR response and nonresponse groups, two-sample t test.
§ P < 0.05 for change in mean between baseline and 3-month evaluation, paired t test.

### Table 4
Sensitivity and specificity of different ferritin cut-offs for ferritin, TIR, and TIR-F index to predict hemoglobin response among anemic subjects

<table>
<thead>
<tr>
<th>Ferritin cut-off (ng/mL)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 15</td>
<td>44</td>
<td>80</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>72</td>
<td>52</td>
</tr>
<tr>
<td>&gt; 1.8</td>
<td>76</td>
<td>52</td>
</tr>
<tr>
<td>&gt; 2.3</td>
<td>46</td>
<td>68</td>
</tr>
<tr>
<td>TIR-F index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1.1</td>
<td>76</td>
<td>56</td>
</tr>
<tr>
<td>&gt; 1.83</td>
<td>44</td>
<td>72</td>
</tr>
</tbody>
</table>

Figure 1. Sensitivity and specificity of different ferritin cut-offs for detection of hemoglobin response in women with access to a WIFS/deworming program.
plete biochemical data were available for 208. It is possible that women more interested in their health, or attended by more effective health workers, may have had better adherence to the program and been more likely to return for follow-up. This may have selected for a group of women with a greater response in hemoglobin because of better compliance. However, the baseline prevalence of anemia and iron deficiency was similar in women who returned for follow-up evaluation and those who did not. Anecdotally, village health workers reported that loss to follow-up was because of reluctance of women to undergo a second venipuncture. The other limitation of our study is the absence of a control group. This would have allowed us to establish the prevalence of iron deficiency, based on the proportion of women who responded to iron supplementation compared with those receiving placebo, as previously described. However, we did not feel it ethical to include a control arm in this study given that WIFS is an established strategy for reducing morbidity among women of reproductive age in developing countries. Deworming was provided to all women. We have previously shown that hookworm burden was weakly associated with ferritin levels but was not associated with hemoglobin in this population. Thus, hookworm burden is likely to have had little influence on hemoglobin response.

Estimates of the prevalence of iron deficiency among non-pregnant women in Vietnam have ranged widely from 22% to 86%, and estimates of anemia range from 24.3% to 42.8%. Our baseline estimate falls within this range, although it is slightly higher than other estimates conducted in the northern mountainous regions. Although we sampled women living in districts of the province that were geographically nearer to the major town, the living conditions in these areas are not dissimilar to the broader conditions in this mountainous province; nevertheless, it is possible that the prevalence of anemia may be higher in more remote areas. There may also be some variation in the prevalence of non-iron deficiency causes for anemia, such as hemoglobinopathy, across the province.

Our results support current WHO recommendations suggesting screening for iron deficiency anemia at the population level using hemoglobin alone when iron data are not available and providing iron supplementation where the prevalence of anemia exceeds 20%. These recommendations are appropriate in this population, because the majority of anemic women responded to 3 months of weekly iron and folic acid supplementation. Our results indicate that iron indices provided only moderate predictive value for hemoglobin response, and there was little additional gain from assaying TfR if ferritin was performed. When iron studies are performed, adjusting the upper limit of ferritin for detection of iron deficiency to 30 ng/mL may improve sensitivity for identifying anemic subjects (and thus populations) likely to respond to iron supplementation programs.

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