Schistosomiasis japonica, anemia, and iron status in children, adolescents, and young adults in Leyte, Philippines

Tjalling Leenstra, Luz P Acosta, Gretchen C Langdon, Daria L Manalo, Li Su, Remigio M Olveda, Stephen T McGarvey, Jonathan D Kurtis, and Jennifer F Friedman

ABSTRACT
Background: Observational and interventional evidence supports a relation between human schistosomiasis and anemia; however, the exact causal mechanisms remain unclear. Eggs translocating across the intestinal or bladder wall may result in extracorporeal blood loss with subsequent iron deficiency. Alternatively, anemia may result from cytokine-mediated dyserythropoiesis, as seen in anemia of inflammation.

Objectives: By evaluating the cross-sectional relation between the intensity of Schistosoma japonicum infection, hemoglobin concentration, and iron status in 7–30-y-old persons from S. japonicum–endemic rice-farming villages in the province of Leyte, Philippines, we assessed the relative contribution of iron deficiency and anemia of inflammation to schistosomiasis-associated anemia.

Design: We enrolled 627 S. japonicum–infected and 111 S. japonicum–uninfected persons. We obtained stool samples to quantify S. japonicum infection and venous blood samples for hemograms and measures of iron status and inflammation.

Results: Intensity of S. japonicum infection was independently associated with hemoglobin (β = −0.24; 95% CI: −0.31, −0.17). Persons with high-intensity infection had a greater risk of iron deficiency anemia (adjusted prevalence odds ratio: 6.6; 95% CI: 2.9, 14.7), but there was no evidence of this relation in low-intensity infections. In contrast, anemia without iron deficiency was prevalent across all intensities (adjusted prevalence odds ratio: 3.8; 95% CI: 1.5, 9.5).

Conclusions: Storage iron deficiency is a major contributor to anemia in high-intensity S. japonicum infection. A high prevalence of anemia without iron deficiency, exclusion of other mechanisms of anemia, and the evidence of low bioavailable iron suggest that anemia of inflammation contributes to S. japonicum–associated anemia at all infection intensities.

KEY WORDS Schistosoma japonicum, anemia, iron deficiency, anemia of inflammation, Philippines

INTRODUCTION
Schistosomiasis remains a global public health problem: an estimated 600 million persons reside in endemic regions, and ≈200 million are infected at any given time (1, 2). Schistosoma japonicum infects ≈2.4 million persons, and 70 million are at risk of infection, mainly in China and Southeast Asia (2). Evidence from cross-sectional studies and randomized controlled trials supports a relation between schistosomiasis and anemia, but little is known regarding the mechanisms of this relation (1, 3, 4). A recent meta-analysis reassessing the global burden of disease due to schistosomiasis posited anemia as a major contributor to the disease-specific disability of schistosomiasis (4).

S. japonicum may cause intestinal blood loss and subsequent iron deficiency (ID) as eggs pass through the intestinal wall into the lumen of the gut. However, there is little evidence that the quantity of blood lost is sufficient to produce ID and anemia in the context of S. japonicum, except possibly at higher infection intensities (5, 6). Few studies have reported an association between schistosome-egg counts and decreased iron stores (7, 8), but the traditional markers of iron status used in these studies (ie, ferritin and erythrocyte protoporphyrin) are influenced by inflammation, which complicates their interpretation. Moreover, because poverty increases the risk of both schistosomiasis and dietary ID, studies that do not adjust for socioeconomic status (SES) may be confounded by poor iron intakes.

Schistosomiasis also may produce anemia by inducing a proinflammatory cytokine-mediated dyserythropoiesis, as seen in anemia of inflammation (AI) (9, 10). Anemia in the setting of acute or chronic inflammation is mediated by decreased erythropoietin production or responsiveness of erythrocyte precursors in the bone marrow (or both), decreased erythrocyte life span, shunting of bioavailable iron to storage forms, and, possibly, reduced uptake of dietary iron in the gut. In addition to AI, schistosomiasis may produce anemia secondary to increased sequestration of erythrocytes or increased hemolysis in the spleen of persons with schistosomiasis-associated splenomegaly (or both) (11–13).

The objectives of this study were to assess the association between S. japonicum and hemoglobin after adjustment for potential confounders and to explore mechanisms mediating this


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relation, including ID and aberrations in iron metabolism mediated by inflammation, in a cross-sectional sample of 7–30-y-old inhabitants of the province of Leyte, Philippines. We hypothesized that mechanisms other than extracorporeal blood loss—in particular, AI—contribute to schistosomiasis-associated anemia.

SUBJECTS AND METHODS

Study area and population

This cross-sectional study was conducted in 3 *S. japonicum*–endemic rice-farming villages (Macaniip, Buri, and Pitogo) in Leyte. Malaria is not endemic in this study area. *S. japonicum*–infected persons were recruited as part of a longitudinal study investigating immune correlates of resistance to reinfection. In total, 74.3% (n = 1262) of the 1699 persons aged 7–30 y who resided in these 3 villages were screened for the presence of *S. japonicum* infection by duplicate Kato-Katz examination of 3 stool samples before enrollment. The prevalence of infection with *S. japonicum* in persons in this age range was 60.0%. Subjects were eligible if they were infected with *S. japonicum*, were living primarily in a study village, were aged 7–30 y, and were not pregnant or lactating; in addition, both the assent of the child and parental or adult consent had to be provided. Nonparticipation was due to refusal to participate, residence outside the study area, or pregnancy or lactation. In addition, 111 *S. japonicum*–uninfected persons aged 7–18 y from the same villages were recruited as control subjects. Control subjects were recruited until the target sample size of ≈100 control subjects was obtained. Participants were enrolled in 2 separate cohorts, in October 2002 and April 2003. They were scheduled to come to the field laboratory on a designated day, and they were transported by study staff. All participation rates and analyses include the entire sample of uninfected and infected persons unless stated otherwise.

Written informed consent was obtained from each adult participant or from the parents of assenting children. The study was approved by the institutional review boards of Brown University and The Philippines Research Institute of Tropical Medicine.

Stool examination

Parasite burden was determined by examination of 3 consecutive stool specimens obtained from each study participant. Each of the 3 stool specimens was examined in duplicate for *S. japonicum*, *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm by the Kato-Katz method within 24 h of collection. For each of the stool specimens, the average number of eggs per gram (epg) of the duplicate test was ascertained. The overall mean epg was derived by averaging the parasite burden of the 3 individual specimens. Intensity of infection for each helminth was determined by using the following World Health Organization (WHO) criteria: low-, medium-, and high-intensity *S. japonicum* infection was defined as 1–99, 100–399, and ≥400 epg, respectively; low-, medium-, and high-intensity *A. lumbricoides* infection was defined as 1–4999, 5000–49,999, and ≥50,000 epg, respectively; low-, medium-, and high-intensity *T. trichiura* infection was defined as 1–999, 1000–9999 and ≥10,000 epg, respectively; and low-, medium-, and high-intensity hookworm infection was defined as 1–1999, 2000–3999, and ≥4000 epg, respectively (14, 15). For 10 hookworm larvae, obtained by culturing stool samples (16) from 203 study participants, the species was identified by using polymerase chain reaction; only *Necator americanus* species were detected.

Blood collection and processing

Venipuncture was performed and blood was collected into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing EDTA as anticoagulant (for hemogram) or serum separator gel (for serum assays). A complete hemogram (white and red blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, and platelet and lymphocyte counts and percentage) was obtained by using a Serono Baker 9000 hematology analyzer (Serono Baker Diagnostics, Allentown, PA). The hematology analyzer was maintained in compliance with College of American Pathologists’ guidelines, which include daily controls and necessary calibration. Serum samples were aliquoted and stored at −80 °C. Serum ferritin (SF), serum transferrin receptor (sTfR), C-reactive protein (CRP), and interleukin 6 (IL-6) were analyzed on a multianalyte Bio-Plex analyzer (Bio-Rad, Hercules, CA) by using in-house–produced sandwich- or competitive-style bead kits and commercial controls as described previously (17). Total and direct bilirubin assays were conducted with the use of commercial kits (Thermo DMA, Louisville, CO), as a measure of hemolysis. The bead assay kits showed <2% interanalyte interference, and the median interassay CV was 15% as assessed with 48 replicate controls on consecutive plates.

Definitions

Anemia was defined on the basis of age- and sex-specific hemoglobin cutoffs recommended by the WHO (18): hemoglobin <11.5 g/dL for children aged <12 y, <12 g/dL for children aged 12–14 y and nonpregnant females ≥15 y, and <13 g/dL for males aged ≥15 y. Mild, moderate, and severe anemia were defined as hemoglobin concentrations below the WHO cutoff but ≥9 g/dL, ≥7 but <9 g/dL, and <7 g/dL, respectively. ID was defined as SF <12 ng/mL for persons aged <15 y and women of all ages and SF <18 ng/mL for men aged ≥15 y. Iron-deficient anemia (IDA) was defined as anemia with concurrent ID. Non-iron-deficient anemia (NIDA) was defined as anemia without concurrent iron deficiency. IL-6 responders were defined as persons with detectable concentrations of IL-6 (>1.45 pg/mL) in serum. Menstruation was defined as having reported a first day of last menstrual period at enrollment.

Socioeconomic status

Individual SES scores were based on questionnaire data on parental and child educational status, occupational status, ownership status of home or land, and other assets. The questionnaire had good internal consistency with a Cronbach’s alpha of 82.4% for all questions. A summary SES score composed of all questionnaire items was calculated by using principal components analysis to appropriately weight questionnaire items, as described by Filmer and Pritchett (19). Because of missing data, it was not possible to calculate a summary SES score for 84 persons. For those subjects, SES scores were imputed from scores of another person from the same household, if available, or of the overall mean score of persons of the same age and sex if a household value was not available.
Ultrasound

Study subjects were evaluated with the use of ultrasound on a Hitachi EUB-200 with a 3.5-MHz probe (Hitachi Medical Corp, Tokyo, Japan). Spleen size was measured (in cm) in the left intercostal oblique view. Reference measurements for spleen size among healthy Filipinos were not available, and hence normal values from a healthy Chinese population were used (20). Splenomegaly was defined as > 2 SDs above the mean.

Data management and statistical analyses

Data forms collected in the field were bar-coded and entered with the use of FILEMAKER software (version 5.5; Filemaker Inc, Santa Clara, CA). Normality diagnostics were performed, and nonnormally distributed variables, including egg counts, and measurements of SF, sTfR, CRP, and unconjugated bilirubin were loge transformed [Ln (value + 1)]. All final analyses were performed with the use of SAS software (version 8.02; SAS Institute, Cary, NC). P values < 0.05 were considered statistically significant.

A significant proportion of the variance in our outcome measures was attributable to clustering within households. Therefore, multilevel statistical analyses were used to adjust for clustering at the household level. Specifically, multivariate random-intercept models were implemented by using PROC MIXED (with household as random effect and with the use of a compound symmetry correlation matrix) for continuous outcomes, and generalized estimating equation models were implemented by using PROC GENMOD (with household as repeated effect and with the use of a compound symmetry correlation matrix) for dichotomous outcomes. Unconditional models were used to estimate the intraclass correlation and descriptive measures (means and proportions) adjusted for the nonindependence of observations within households. For all measures, the empirical (robust) SEs are reported to protect against misidentification of the correlation matrix. Least-squares mean values represent the mean adjusted for confounders in multivariable models.

RESULTS

In total, 1262 individuals aged 7–30 y were screened; 60% of these 1262 were infected with S. japonicum. Of that total, 683 S. japonicum–infected and 111 S. japonicum–uninfected persons were potentially eligible to participate. Of these, 38 did not meet other eligibility criteria (29 were pregnant or lactating, and 9 were potentially eligible to participate. Of these, 38 did not meet other eligibility criteria (29 were pregnant or lactating, and 9 lived outside the study area) and 18 refused to participate, which gave a participation rate of 97.6% and a study sample of 738 persons.

Characteristics of the study sample are shown in Table 1. The distribution of the intensity of S. japonicum infection in this sample is shown in Table 2. The geometric mean egg count in S. japonicum–infected persons was 43 epg (95% CI: 38, 48 epg).

Prevalence of anemia and demographic determinants of hemoglobin concentration

Overall, 31.7% of the subjects were anemic, and 14 (2.0%) were severely anemic. Age and hemoglobin concentration were significantly (P < 0.0001) correlated (linear regression: \( \beta = 0.18 \)), and large differences were found in the prevalence of anemia between young adults and children of all ages (P < 0.0001); 36.9% of children aged < 12 y and 38.0% of children aged 12–17 y but only 16.8% of young adults (aged 18–30 y) were anemic. Because all subjects aged > 18 y were infected with S. japonicum, this prevalence represents that in infected persons, not that in the whole population from which this age group is drawn. There were no significant sex differences in mean hemoglobin concentration (P = 0.17), but the prevalence of anemia was significantly higher in males than in females (35.6% and 25.3%, respectively; P = 0.002). Severely anemic subjects were younger (\( \bar{x} = 13.5 \) and 15.6 y, respectively; \( P = 0.072 \)) and more likely to be male (prevalence of severe anemia: 2.9% in males and 0.4% in females; \( P = 0.035 \)) than those without severe anemia; i.e., only one female had severe anemia. SES was a strong determinant of hemoglobin concentration, independent of age and sex (adjusted linear regression: \( \beta = 0.44; P < 0.0001 \)).

Hemoglobin, red cell indexes, and S. japonicum intensity

As previously described for this study population (21), the intensity of the S. japonicum infection had a significant negative association with hemoglobin concentration, independent of age, sex, menstrual status, SES, splenomegaly, and hookworm infection (adjusted linear regression: \( \beta = -0.24; 95\% \text{ CI: } -0.31, -0.17 \)). Furthermore, this negative association corresponded with an increase in the prevalence of anemia (Figure 2). It is notable that the increased prevalence of anemia in persons with high-intensity infection was largely due to an increased prevalence of severe anemia [adjusted prevalence odds ratio: 10.5; 95% CI: 3.2, 34.6 (comparing high-intensity infection to negative or low- or medium-intensity groups pooled)].

Intensity of schistosomiasis japonica had a negative association with the red cell indexes of mean corpuscular volume (adjusted linear regression: \( \beta = -0.81; 95\% \text{ CI: } -1.11, -0.51 \)) and mean corpuscular hemoglobin concentration (adjusted linear regression: \( \beta = -0.16; 95\% \text{ CI: } -0.23, -0.09 \)), independent of age, sex, menstruation, SES, and hookworm infection. Correspondingly, the prevalence of microcytosis and hypochromatosis increased with intensity of infection (data not shown). Only one subject (0.1% of the cohort) had a mean corpuscular volume > 100 μm³ and was classified as macrocytic. That subject was not anemic.

Iron-deficiency anemia and S. japonicum

Measures of iron status were available for 727 subjects (98.5%). As expected, SF was positively correlated with CRP, even after adjustment for intensity of S. japonicum infection (\( R^2 = 0.06, P < 0.0001 \)). The overall prevalence of ID was high (18.6%). Of the anemic subjects, 36.3% were iron deficient; of that subgroup, 61.7% were anemic. Of the 14 severely anemic subjects, 11 (84.6%) were classified as iron deficient. In- fluamation (CRP > 8.2 μg/mL) was prevalent (25.4%), and therefore the reported prevalences of ID likely were underestimated. Adjusted mean hemoglobin concentration in the IDA group was 9.1 g/dL (95% CI: 8.4, 9.7 g/dL) after adjustment for age, sex, menstruation, SES, splenomegaly, and concurrent hookworm infection.

The positive association between S. japonicum intensity as a continuous variable and IDA described a quadratic function (−2 log likelihood ratio test, \( P = 0.043 \)). IDA was ≈4 times more prevalent in the high-intensity S. japonicum group than in the
negative or low- or medium-intensity groups when pooled (adjusted prevalence ratio: 6.6; 95% CI: 2.9, 14.7; Figure 3). IDA was more prevalent in the medium-intensity <i>S. japonicum</i> group than in the low-intensity group (adjusted prevalence odds ratio: 1.9; 95% CI: 1.0, 3.6; Figure 3).

IDA was markedly more prevalent in subjects with medium- or high-intensity hookworm infection than in those with low-intensity or no hookworm infection, even after adjustment for age, sex, menstruation, SES, and concurrent <i>S. japonicum</i> infection (adjusted prevalence odds ratio: 8.4; 95% CI: 3.3, 21.4).

### TABLE 1
Characteristics of the study sample and comparison of <i>Schistosoma japonicum</i>–infected and –uninfected subgroups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Entire sample (n = 738)</th>
<th>Infected (n = 647)</th>
<th>Uninfected (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>15.5 (15.0, 16.0)</td>
<td>15.8 (15.3, 16.4)</td>
<td>13.0 (12.3, 13.7)</td>
</tr>
<tr>
<td>Males [n (%)]</td>
<td>458 (62.1)</td>
<td>417 (64.5)</td>
<td>41 (45.1)</td>
</tr>
<tr>
<td>Menstruation [n (%)]</td>
<td>86 (37.4)</td>
<td>222 (33.9)</td>
<td>14 (15.9)</td>
</tr>
<tr>
<td>Socioeconomic status score</td>
<td>2.21 (2.12, 2.30)</td>
<td>2.20 (2.11, 2.29)</td>
<td>2.27 (2.15, 2.39)</td>
</tr>
<tr>
<td>Hemoglobin (&lt; WHO cutoff [n (%)])</td>
<td>82.3 (81.6, 83.1)</td>
<td>82.2 (81.3, 83.0)</td>
<td>83.5 (82.3, 84.7)</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>32.9 (32.8, 33.1)</td>
<td>32.9 (32.8, 33.1)</td>
<td>33.0 (32.7, 33.2)</td>
</tr>
<tr>
<td>CRP (µg/mL)</td>
<td>5.2 (4.8, 5.7)</td>
<td>6.5 (5.1, 6.2)</td>
<td>3.0 (2.5, 3.6)</td>
</tr>
<tr>
<td>Serum ferritin (ng/mL)</td>
<td>34.3 (31.3, 37.5)</td>
<td>35.5 (32.3, 39.0)</td>
<td>26.5 (21.6, 32.5)</td>
</tr>
<tr>
<td>Iron deficiency [n (%)]</td>
<td>138 (18.6)</td>
<td>117 (23.8)</td>
<td>21 (17.9)</td>
</tr>
<tr>
<td>IDA [n (%)]</td>
<td>86 (11.8)</td>
<td>79 (12.3)</td>
<td>7 (7.9)</td>
</tr>
<tr>
<td>sTIR (ng/mL)</td>
<td>5464 (5195, 5747)</td>
<td>5552 (5256, 5865)</td>
<td>4861 (4393, 5379)</td>
</tr>
<tr>
<td>Hookworm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive [n (%)]</td>
<td>420 (57.7)</td>
<td>387 (60.6)</td>
<td>33 (38.0)</td>
</tr>
<tr>
<td>epg</td>
<td>212 (181, 247)</td>
<td>216 (184, 254)</td>
<td>171 (104, 280)</td>
</tr>
<tr>
<td>Ascaris lumbricoides [n (%)]</td>
<td>539 (74.0)</td>
<td>477 (74.7)</td>
<td>62 (69.3)</td>
</tr>
<tr>
<td>epg</td>
<td>7909 (6598, 9481)</td>
<td>7422 (6096, 9036)</td>
<td>12658 (8630, 18563)</td>
</tr>
<tr>
<td>Trichuris trichiura [n (%)]</td>
<td>668 (91.9)</td>
<td>586 (92.3)</td>
<td>82 (91.1)</td>
</tr>
<tr>
<td>epg</td>
<td>784 (684, 899)</td>
<td>768 (667, 884)</td>
<td>905 (666, 1230)</td>
</tr>
</tbody>
</table>

1 WHO, World Health Organization; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; CRP, C-reactive protein; sTIR, serum transferrin receptor; epg, egg count/g stool; IDA, anemia with concurrent iron deficiency; NIDA, non-iron-deficiency anemia.

2 Geometric mean.

3 Defined as serum ferritin < 12 ng/mL in persons < 15 y old and all women and < 18 ng/mL in men ≥ 15 y old.

4 n = 280.

5 n = 230.

6 n = 50.

7 Summary score of all questionnaire items, calculated by using principal components analysis to appropriately weight items (19).

8 n = 727.

9 n = 640.

10 Geometric mean.

11 Defined as serum ferritin < 12 ng/mL in persons < 15 y old and all women and < 18 ng/mL in men ≥ 15 y old.

12 n = 722.

13 n = 636.

14 n = 86.

15 n = 726.

16 n = 90.

17 Geometric mean.

18 Defined as serum ferritin < 12 ng/mL in persons < 15 y old and all women and < 18 ng/mL in men ≥ 15 y old.

19 n = 722.

20 Geometric mean.
Neither *T. trichiura* nor *A. lumbricoides* infection was associated with IDA, and neither was a confounder of any of the presented associations (data not shown).

Non-iron-deficient anemia and *S. japonicum*

The adjusted mean hemoglobin concentration in the NIDA group was 10.2 g/dL (95% CI: 9.7, 10.8 g/dL) after adjustment for age, sex, menstruation, SES, splenomegaly, and concurrent hookworm infection. NIDA was significantly more prevalent in *S. japonicum*–infected persons than in *S. japonicum*–uninfected persons (adjusted prevalence odds ratio: 3.8; 95% CI: 1.5, 9.5; Figure 3). Moreover, there was a significant linear relation between NIDA prevalence and *S. japonicum* intensity when assessed as a continuous variable, even after exclusion of uninfected subjects from the analysis (adjusted log odds increase per log epg change: 0.27; 95% CI: 0.12, 0.41). None of the geohelminth infections (hookworm, *T. trichiura*, or *A. lumbricoides*) was associated with differences in prevalence of NIDA, and none was a confounder of any of the above associations (data not shown).

**TABLE 2**

<table>
<thead>
<tr>
<th>WHO intensity group</th>
<th>Value</th>
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<tbody>
<tr>
<td>Negative</td>
<td>n (12.3)</td>
</tr>
<tr>
<td>1–99 epg</td>
<td>452 (61.4)</td>
</tr>
<tr>
<td>100–399 epg</td>
<td>152 (20.2)</td>
</tr>
<tr>
<td>≥400 epg</td>
<td>43 (5.8)</td>
</tr>
</tbody>
</table>

† WHO, World Health Organization; epg, egg count/g stool.

Serum transferrin receptor, inflammation, and *S. japonicum*

Mean sTfR concentrations were significantly higher in *S. japonicum*–infected subjects than in *S. japonicum*–uninfected subjects, but no significant differences between *S. japonicum* intensity groups were observed (Figure 4). However, there was a linear relation between sTfR and intensity of infection as a continuous measure, even after exclusion of the uninfected and the high-intensity groups from analysis and after adjustment for SF (adjusted linear regression: β = 0.05; 95% CI: 0.01, 0.08). On average, sTfR was higher in subjects with NIDA than in nonanemic subjects (adjusted mean difference: 993; 95% CI: 365, 1695) and in subjects with IDA than in subjects with NIDA (adjusted mean difference: 1887; 95% CI: 693, 3294).

Recent evidence has pointed to IL-6 as a mediator of functional ID (ie, iron trapping in the reticuloendothelial system), one of the processes central to the etiology of AI (22). In the current study, 161 (22.9%) of 727 subjects had detectable concentrations of IL-6 in serum, and the log odds for IL-6 expression increased with the intensity of the *S. japonicum* infection (adjusted log odds increase per log epg change: 0.19; 95% CI: 0.10, 0.28; Figure 4). It is interesting that the mean sTfR concentration was significantly higher in IL-6 responders than in nonresponders, even after adjustment for the intensity of the *S. japonicum* infection and SF (adjusted difference in mean sTfR: 1667; 95% CI: 1094, 2184). When analyzed by *S. japonicum* intensity group, both sTfR and the prevalence of IL-6 responders increased with increasing infection intensity (Figure 4).

**FIGURE 1.** Mean hemoglobin concentration by intensity of *Schistosoma japonicum* infection. Diamonds represent least-squares mean hemoglobin by intensity of *S. japonicum* infection after adjustment for age, socioeconomic status, concurrent hookworm infection, splenomegaly, and nonindependence of observations within households in multilevel linear regression. Error bars represent empirical SEs for the least-squares means. Different letters represent significant differences between intensity groups in means.
Splenomegaly and intensity of S. japonicum infection

Spleen size was determined by ultrasound in 731 subjects. Overall, 12 subjects (1.7%) had an enlarged spleen, and all were infected with S. japonicum. Splenomegaly was significantly more common in subjects with high-intensity infection than in the pooled group of those with negative or low- or medium-intensity infection (adjusted prevalence odds ratio: 4.1; 95% CI: 1.12, 14.70). Subjects with splenomegaly had significantly lower hemoglobin concentrations than did those with normal-size spleens, even after adjustment for intensity of S. japonicum infection, age, sex, menstruation, SES, and hookworm infection (adjusted mean difference: −2.5; 95% CI: −4.0, −1.0). Splenomegaly was not associated with ID or mean differences in sTfR (data not shown). It also was not associated with any difference in mean unconjugated bilirubin, and none of the participants had hyperbilirubinemia (unconjugated bilirubin > 1 mg/dL) (data not shown).

DISCUSSION

Observational studies and a randomized controlled trial of praziquantel identified a relation between schistosomiasis japonica and anemia (1, 3, 4), but the mechanisms through which S. japonicum causes anemia remain unknown. Our cross-sectional study provides additional evidence for the association between S. japonicum infection and anemia. We found a significant dose-response relation between the intensity of the infection and the prevalence of anemia and mean hemoglobin concentration in our study sample. This association was evident across all infection intensities and after adjustment for the confounding effects of SES and concomitant hookworm infection. In addition, we report for the first time evidence suggesting that AI contributes to S. japonicum–associated anemia.

To assess whether S. japonicum infection leads to decreased iron stores, a mechanism generally thought to be central in the etiology of schistosomiasis-related anemia, we evaluated differences in the prevalence of IDA across infection intensities. We detected a markedly greater prevalence of IDA in subjects with high-intensity S. japonicum infection and a slightly greater prevalence in subjects with medium-intensity S. japonicum infection than in those with low-intensity infection. There was no evidence for increased prevalence of IDA in most (61.2%) of the S. japonicum–infected subjects. In contrast, prevalence of anemia not explained by iron deficiency (ie, NIDA) was significantly higher in all S. japonicum–infected subjects than in S. japonicum–uninfected subjects. Taken together, these data suggest that decreased iron stores, due to extracorporeal blood loss or impaired absorption of iron in the intestine (or both), may contribute to anemia in the more intense infections but not in most (predominantly low-intensity) infections. ID did not explain most of the S. japonicum–associated anemia in this study population.

We found no evidence of increased hemolysis in persons with schistosomiasis. Splenomegaly occurred in only 1.7% of our study sample, and therefore sequestration could not account for most of the S. japonicum–associated anemia. Clinically relevant hereditary hemoglobinopathies are probably uncommon in our study area (23, 24). Vitamin A deficiency is a known contributor to anemia (25), but tissue retinol concentration is not influenced by S. japonicum infection (26), which excludes schistosomiasis-induced vitamin A deficiency as a contributing mechanism. Only one subject in this population had macrocytosis, which excludes vitamin B-12 and folate deficiency as a potential cause of anemia. The prevalence of anemia was higher in males than in females. Many studies have noted that men are more susceptible to schistosomiasis-related morbidity, including undernutrition and...
hepatic fibrosis, than are women (17, 21, 27, 28), possibly as a result of sex-dependent immune responses (29, 30). The lack of evidence for potential contributing factors of anemia other than ID suggests that processes involved in AI may be involved in the etiology of *S. japonicum*-associated anemia in this population.

We found a significant association between the intensity of *S. japonicum* infection and the mean sTfR concentration, independent of SF. sTfR correlates closely with its expression on the cell surface of red cell precursors. Elevated sTfR concentrations are found in 3 instances: ID, which is due to either depleted stores (ie, storage ID) or decreased availability of iron at the site of erythropoiesis independent of iron stores (ie, functional ID, such as that due to impaired iron mobilization in AI); increased numbers of erythrocyte precursors (hemolytic disorders including hereditary hemoglobinopathies); and megaloblastic macrocytic anemias (31, 32).

Megaloblastic macrocytic anemia and hemolysis were excluded in this population, which suggests that the dose-response relation between sTfR and *S. japonicum* intensity reflects ID. The significantly elevated mean sTfR in the subgroup with anemia when ID was excluded as a cause suggests that, rather than storage iron deficiency, elevated sTfR concentrations in this subgroup reflect functional ID. Impaired iron mobilization and utilization, even in the presence of adequate iron stores, is one of the cardinal features of AI (9, 10). Also supporting this conclusion is evidence such as the significantly higher mean sTfR concentration in IL-6 responders than in nonresponders and the parallel associations between *S. japonicum* infection and mean sTfR and between *S. japonicum* infection and the prevalence of IL-6 responders (Figure 4). Recently, IL-6 has been implicated as the key regulator of hepcidin, a hepatocyte-derived protein that mediates AI by regulating iron release from intestinal cells, which regulates iron absorption, and reticuloendothelial cells, which regulates iron availability in the bone marrow (22, 33, 34).

We acknowledge several limitations of the current study. First, the cross-sectional design of our study remains susceptible to residual confounding by measured factors such as SES and by unmeasured factors such as dietary iron intake. Second, the measurement of iron status in the setting of chronic inflammation remains suboptimal because of the artifactual elevation of SF during inflammation. Because the prevalence of ID, defined by using a simple SF cutoff, is invariably underestimated in the setting of prevalent inflammation, it is generally recommended that persons with an elevated CRP concentration be excluded when estimations of the prevalence of ID are being formed (18). Although of use for population estimates, this strategy may lead to additional (and difficult-to-interpret) misclassification, when groups with different magnitudes of inflammation are compared (ie, iron-replete persons may be excluded differentially). Moreover, the inflamed subgroup is of considerable interest to our analyses, and exclusion of this group would have led to a biased representation. Therefore, to aid interpretation, we present a unadjusted definition of ID together with the prevalence and magnitude of inflammation in each subgroup. An alternative

**FIGURE 3.** Prevalence of non-iron-deficient anemia (NIDA) and iron-deficient anemia (IDA) and mean C-reactive protein (CRP) by Schistosoma japonicum intensity. Iron deficiency is defined as serum ferritin < 12 ng/mL in children aged <15 y and women of all ages and as serum ferritin < 18 ng/mL in men aged ≥15 y. IDA is defined as anemia with concomitant iron deficiency. NIDA is defined as anemia without concomitant iron deficiency. Gray and white bars (left y-axis) represent prevalence estimates of NIDA and IDA, respectively, by *S. japonicum* intensity after adjustment for age, sex, menstruation, socioeconomic status, concurrent hookworm infection, and nonindependence of observations within household in multilevel logistic regression. Diamonds (right y-axis) represent back-transformed least-squares mean CRP by *S. japonicum* intensity. Error bars represent empirical SEs for least-squares mean CRP. Different letters represent significant differences between *S. japonicum* intensity groups (compare lower-case and upper-case letters separately).
definition of ID [ie, using a higher SF cutoff of 50 ng/mL for persons with inflammation (35)] led to the same conclusions (data not shown). Finally, the diagnosis of AI, for which no gold-standard diagnostic test exists, is made on the basis of exclusion of other causes, which makes it impossible to present direct evidence of the phenomenon. In addition, because it is plausible that AI and ID occur simultaneously—and, in our analyses, subjects with both were classified as having IDA—we were unable to evaluate the full contribution of AI to S. japonicum–associated anemia.

In conclusion, our findings underscore anemia as an important manifestation of chronic S. japonicum infection, even in low-intensity infection. On the basis of the lack of evidence for ID as the central cause of anemia associated with schistosomiasis of low or medium intensity, the exclusion of other potential causes of anemia, and the effects of S. japonicum infection on functional iron status, we conclude that AI is an important contributor to S. japonicum–associated anemia. Our findings have critical public health ramifications because iron therapy in the context of AI may have a significantly reduced clinical efficacy. We believe these findings support a clinical trial of combination therapy for schistosomiasis and anemia to identify optimal treatment strategies and regimens.

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