

# Anemia and Iron Deficiency in School Children, Adolescents, and Adults: A Community-Based Study in Rural Amazonia

Marcelo U. Ferreira, MD, PhD, Mônica da Silva-Nunes, MD, Carla N. Bertolino, RD, Rosely S. Malafronte, PhD, Pascoal T. Muniz, PhD, and Marly A. Cardoso, PhD

We investigated the prevalence and risk factors of anemia and iron deficiency in 398 rural Amazonians aged 5–90 years in Acre, Brazil. Anemia and iron deficiency were diagnosed in 16% and 19% of the population, respectively. Anemia was likely to have multiple causes; although nearly half of anemic school children and women had altered iron status indicators, only 19.7% of overall anemia was attributable to iron deficiency. Geohelminth infection and a recent malaria episode were additional factors affecting iron status indicators in this population. (*Am J Public Health*. 2007;97:237–239. doi:10.2105/AJPH.2005.078121)

Because global estimates for iron deficiency prevalence are not available, anemia, which affects 30% of the world population,<sup>1</sup> has been used as an indicator of iron deficiency and iron deficiency anemia. Hemoglobin determination, however, is neither sensitive nor specific as a screening test for iron deficiency. The former occurs because a large proportion of total body iron must be lost before hemoglobin levels fall below the laboratory definition of anemia.<sup>2</sup> The low specificity stems from other causes of anemia, such as other nutritional deficiencies, infections, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and hemoglobinopathies.<sup>3–6</sup>

## METHODS

We performed a cross-sectional survey in the agricultural settlement known as Ramal do Granada in Acre, Brazil (elevation, 100–208 m above sea level). All 473 inhabitants were invited to participate, and 467 (98.7%) respondents in 113 households were enrolled. Participants aged 5 years or older were invited to contribute a 5 mL venous blood sample and a stool sample. The 389 participants who provided blood samples (96.0% of those eligible) comprised the study population we analyzed.

Two experienced microscopists examined Giemsa-stained, thick blood smears from 386 (95.3%) participants. Hemoglobin concentration in 388 (95.8%) participants was measured using a HemoCue photometer (HemoCue, Angelholm, Sweden), and anemia was defined according to World Health Organization cut-off values.<sup>6</sup> Serum ferritin and soluble transferrin receptor concentrations in 379 (93.6%) participants were measured using an enzyme immunoassay (Ramco, Houston, TX). The normal range of soluble transferrin receptor levels, determined by the manufacturer, is 2.9–8.3 mg/L. A total of 356 (87.9%) participants were screened for G6PD deficiency using the colorimetric method of Tantular and Kawamoto (Dojindo, Kumamoto, Japan).<sup>7</sup> Stool specimens from 363 (89.6%) participants were examined for intestinal parasites.<sup>8</sup>

We used principal component analysis to derive a wealth index from information on ownership of 13 household assets.<sup>9</sup> We used multiple linear regression analysis to describe independent associations between concentrations of hemoglobin, serum ferritin, and soluble transferrin receptor (dependent variables) and demographic, socioeconomic, and morbidity covariates. We used natural log transformation of serum ferritin to improve the fit of linear regression models. We conducted multiple unconditional logistic regression analysis using SPSS, version 13.0 (SPSS Inc., Chicago, IL), to estimate adjusted odds ratios (AORs) for associations between anemia and the covariates. Attributable fractions<sup>3</sup> were estimated for risk factors for anemia associated with AORs significantly greater than 1 ( $P < .05$ ); AORs were converted to adjusted prevalence ratios, as previously described.<sup>10</sup>

## RESULTS

Anemia (overall prevalence, 16%) was most common in school children and women (Table 1); no cases of severe anemia (hemoglobin < 70 g/L) were diagnosed. Anemia was uniformly prevalent across all socioeconomic strata (16.1% among the poorest and 18.2% among the least poor). Iron deficiency was found in 19% of subjects, with the highest prevalence among school children and women, but only 30% of iron-deficient subjects were anemic. The overall prevalence of iron deficiency anemia was 5.6%.

In addition to age and gender, pregnancy was the only significant predictor of hemoglobin levels in multiple linear regression models (Table 2). Because we did not impose prior expectations on the relation of hemoglobin to iron status indicators, the hemoglobin model did not include serum ferritin or soluble transferrin receptor as covariates (Table 2). Separate analyses identified both serum ferritin and soluble transferrin receptors as strong independent predictors of hemoglobin levels. A decrease of 2.72  $\mu\text{g/L}$  (1 log unit) of serum ferritin was associated with a 4 g/L decrease in hemoglobin, and an increase of 1 mg/L of soluble transferrin receptor was associated with a 1.4 g/L decrease in hemoglobin ( $P < .001$ , for both). Geohelminth infection, i.e., infection with geohelminths (soil-transmitted helminths), and recent malaria were significant predictors of serum ferritin and soluble transferrin receptor levels, respectively (Table 2). Iron deficiency was the only significant predictor of anemia identified by logistic regression models in the overall population (OR = 3.03; 95% confidence interval = 1.40, 6.10), with an attributable fraction of 19.7%. Among females aged 12–45 years ( $n = 100$ ), 11.9% of all cases of anemia were attributable to a current pregnancy.

## DISCUSSION

As estimated by DeMayer and Adiels-Tegman in 1985,<sup>1</sup> half of anemic school children and women in rural Amazonia had iron deficiency. However, because more than 20% of anemia in the population was attributable to iron deficiency, widespread iron supplementation alone is likely to have only a limited

**TABLE 1—Iron Status Indicators and Diagnostic Categories of Anemia and Iron Deficiency Among Rural Amazonians, by Age: Brazil, 2004**

	School Children, 5–11 y	Adolescents, 12–14 y	Men, ≥15 y	Women, ≥15 y	All Age Groups
Hemoglobin (g/L) <sup>a</sup>					
Median (IQR)	126 (116–135)	131 (125–144)	148 (138–158)	133 (121–145)	137 (125–150)
Proportion below cut-off, % (95% CI)	20.5 (13.2, 30.4)	15.8 (7.5, 30.5)	9.0 (5.4, 14.8)	21.1 (14.9, 29.2)	16.0 (12.7, 20.0)
SF (μg/L) <sup>b</sup>					
Median (IQR)	43.0 (30.0–75.5)	55.0 (38.5–73.2)	111.0 (56.5–179.5)	54.0 (33.5–105.5)	63.0 (39.0–122.0)
Proportion <15 μg/L, % (95% CI)	2.5 (0.8, 8.5)	2.8 (0.7, 14.2)	0	7.4 (3.6, 13.0)	3.2 (1.8, 5.4)
Proportion <30 μg/L, % (95% CI)	25.9 (17.6, 36.4)	8.3 (3.0, 21.9)	5.7 (2.9, 10.8)	19.0 (13.0, 26.9)	14.5 (11.3, 18.4)
sTfR (mg/L) <sup>b</sup>					
Median (IQR)	5.7 (4.5–7.2)	4.9 (3.8–5.9)	4.9 (4.0–5.8)	4.6 (3.9–6.2)	5.0 (4.0–6.2)
Proportion >8.3 mg/L, % (95% CI)	13.6 (7.8, 22.7)	2.8 (0.6, 14.2)	4.2 (2.0, 9.0)	7.4 (4.0, 13.5)	7.1 (4.9, 10.2)
Proportion in each diagnostic category, % (95% CI)					
Iron sufficiency <sup>b,c</sup>	64.2 (53.3, 73.8)	88.9 (74.6, 95.5)	91.5 (85.7, 95.0)	77.7 (69.5, 84.2)	81.0 (76.7, 84.6)
Possible ID <sup>b,d</sup>	29.6 (20.8, 40.4)	8.3 (3.0, 21.9)	7.1 (3.9, 12.6)	14.0 (9.0, 21.4)	14.2 (11.1, 18.1)
Probable ID <sup>b,e</sup>	6.2 (2.7, 13.7)	2.8 (0.7, 14.2)	1.4 (0.4, 5.0)	5.0 (2.3, 10.4)	3.7 (2.2, 6.1)
Definite ID <sup>b,f</sup>	0	0	0	3.3 (1.3, 8.2)	1.1 (0.4, 2.7)
ID anemia <sup>g</sup>	10.0 (5.2, 18.5)	2.8 (0.7, 14.2)	0.7 (0.2, 3.9)	9.1 (5.2, 15.6)	5.6 (3.7, 8.4)
No. of participants with ID anemia/total no. of anemic participants (%)	8/16 (50.0%)	1/5 (20.0%)	1/12 (8.3%)	11/23 (47.8%)	21/56 (37.5%)

Note. CI = confidence interval; ID = iron deficiency; IQR = interquartile range; SF = serum ferritin; sTfR = soluble transferrin receptor.

<sup>a</sup>Results available for 83 children, 38 adolescents, 144 men, and 123 women (total, n = 388).

<sup>b</sup>Results available for 81 children, 36 adolescents, 141 men, and 121 women (total, n = 379).

<sup>c</sup>Iron sufficiency: SF ≥ 30 μg/L and sTfR ≤ 8.3 mg/L.

<sup>d</sup>Possible ID: SF 15–30 μg/L and sTfR ≤ 8.3 mg/L, or SF ≥ 30 μg/L and sTfR > 8.3 mg/L.

<sup>e</sup>Probable ID: SF = 15–30 μg/L and sTfR > 8.3 mg/L, or SF < 15 μg/L and sTfR ≤ 8.3 mg/L.

<sup>f</sup>Definite ID: SF < 15 μg/L and sTfR > 8.3 mg/L.

<sup>g</sup>ID anemia = hemoglobin below WHO cut-off value for age and gender plus any evidence of ID (SF < 30 μg/L or sTfR > 8.3 mg/L). Results available for 80 children, 36 adolescents, 140 men, and 121 women (total, n = 377).

impact on the overall prevalence of anemia among subjects aged 5 years or older. The multifactorial etiology of anemia putatively includes other nutritional deficiencies (folate, vitamin A), as well as genetic and infectious conditions. G6PD deficiency, which is infrequent in the Ramal do Granada population (3.9%) and other Amazonian populations,<sup>11</sup> had no significant impact on hemoglobin levels. Sick-cell disease is unlikely to represent a major contributor, as low hemoglobin S allele frequencies (1.8%–2.1%) have been found in Amazonia.<sup>12</sup> To our knowledge, no other hemoglobinopathies have been investigated in Amazonian populations. Malaria and geohelminth infections affect iron status indicators either because of true iron deficiency<sup>13</sup> or increased erythropoiesis following hemolysis,<sup>2</sup> but the contribution of malaria and geohelminth to anemia appear to be less marked in rural Amazonians than in African<sup>5</sup> and Asian<sup>3</sup> populations. ■

### About the Authors

Marcelo U. Ferreira and Mônica da Silva-Nunes are with the Departamento de Parasitologia, Instituto de Ciências Biomédicas da Universidade de São Paulo, São Paulo, Brazil. Carla N. Bertolino and Marly A. Cardoso are with the Departamento de Nutrição, Faculdade de Saúde Pública da Universidade de São Paulo, São Paulo. Rosely S. Malafronte is with the Laboratório de Protozoologia, Instituto de Medicina Tropical de São Paulo, São Paulo. Pascoal T. Muniz is with the Departamento de Ciências da Saúde, Universidade Federal do Acre, Rio Branco, Brazil.

Requests for reprints should be sent to Marly A. Cardoso, Department of Nutrition, School of Public Health, University of São Paulo, Av. Dr. Arnaldo, 715, 01246–904, São Paulo, Brazil (e-mail: marlyac@usp.br).

This article was accepted March 3, 2006.

### Contributors

M. U. Ferreira and M. A. Cardoso conceptualized the study and supervised all aspects of its implementation. M. da Silva-Nunes assisted with the study and completed the analyses. C. N. Bertolino performed laboratory analyses. R. S. Malafronte and P. T. Muniz assisted with the field work. M. U. Ferreira synthesized analyses and led the writing. M. A. Cardoso completed the analyses and reviewed drafts of the article.

### Acknowledgments

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; grant 05/51988-0) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant 504332/2004-0, 470067/2004-7). M. da Silva-Nunes is supported by a FAPESP scholarship; M. U. Ferreira, C. N. Bertolino, and M. A. Cardoso are recipients of CNPq scholarships.

We thank the inhabitants of Ramal do Granada for their enthusiastic participation in the study; Sebastião Bocalom Rodrigues (Mayor of Acrelândia), Damaris de Oliveira, and Nésio M. de Carvalho for their overall support; and Adamilson L. de Souza, Erika H. E. Hoffmann, Estéfano A. de Souza, and Bruna de A. Luz for help in field work, enzyme-linked immunosorbent assay experiments, and data handling, respectively. We also thank Dr. Fumihiko Kawamoto (Oita University, Oita, Japan) for glucose-6-phosphate dehydrogenase screening reagents, and Cesar G. Victora for critical reading of the article.

### Human Participant Protection

This study was approved by the ethical review board of the Institute of Biomedical Sciences of the University of São Paulo, São Paulo, Brazil.

**TABLE 2—Multiple Linear Regression Analysis of Covariates Associated with Iron Status Indicators in Rural Amazonians: Brazil, 2004**

Dependent and Independent Variables <sup>a</sup>	$\beta$ Coefficient (95% CI)	<i>P</i>	<i>R</i> <sup>2</sup>	No.
Hemoglobin <sup>b</sup>			0.190	327
Age in years	0.252 (0.150, 0.354)	<.001		
Gender	-9.676 (-13.226, -6.086)	<.001		
Pregnancy <sup>c</sup>	-15.490 (-30.016, -0.963)	.037		
G6PD deficiency	-7.091 (-15.947, -1.772)	.116		
Constant	134.688 (129.439, 139.767)	<.001		
Log SF <sup>b</sup>			0.233	323
Age in years	0.020 (0.015, 0.025)	<.001		
Gender	-0.419 (-0.597, -0.242)	<.001		
Geohelminth infection <sup>d</sup>	-0.326 (-0.624, -0.027)	.033		
Recent malaria <sup>e</sup>	0.142 (-0.049, -0.322)	.145		
Constant	3.797 (3.542, 4.052)	<.001		
sTfR <sup>b</sup>			0.074	323
Age in years	-0.018 (-0.030, -0.006)	.004		
Recent malaria	0.671 (0.215, 1.127)	.004		
Constant	5.852 (5.241, 6.463)	<.001		

Note. CI = confidence interval; G6PD = glucose-6-phosphate dehydrogenase; SF = serum ferritin; sTfR = soluble transferrin receptor.

<sup>a</sup>The independent variables used in the multiple linear regression analysis were as follows: age (years; continuous variable); gender (1 = female); pregnancy (1 = yes); education of household head (0 = no schooling; 1 = 1-4 years of schooling; 2 = 5-8 years of schooling; 3 = > 8 years of schooling); wealth index (continuous variable); G6PD deficiency (1 = yes); current geohelminth infection (1 = yes); and recent or current malaria (1 = yes). Only variables associated with *P* values < .15 are shown.

<sup>b</sup>Dependent variable.

<sup>c</sup>A separate model was built to include only women (*n* = 155), with similar results: *B* = -16.061 (95% CI = -31.100, -0.971); *P* = .037.

<sup>d</sup>Geohelminths (overall prevalence, 11.6%) found in this population included hookworm (prevalence, 7.2%), *Ascaris lumbricoides* (4.3%), *Strongyloides stercoralis* (3.2%), and *Trichuris trichiura* (2.3%); Participants may be coinfecting with more than one species.

<sup>e</sup>Malaria in the past 6 months (prevalence, 32.2%) or current malaria (prevalence, 2.1%).

## References

- DeMaeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. *World Health Stat Q*. 1985;38:302-316.
- Cook JD. Diagnosis and management of iron-deficiency anaemia. *Best Pract Res Clin Haematol*. 2005;18:319-332.
- Dreyfuss ML, Stoltzfus RJ, Shrestha JB, et al. Hookworms, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. *J Nutr*. 2000;130:2527-2536.
- Lewis DK, Whitty CJM, Walsh AL, et al. Treatable factors associated with severe anaemia in adults admitted to medical wards in Blantyre, Malawi, an area of high HIV seroprevalence. *Trans R Soc Trop Med Hyg*. 2005;99:561-567.
- Stoltzfus RJ, Chwaya HM, Montresor A, Albonico M, Savioli L, Tielsch JM. Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0- to 5-y old Zanzibari children and these relationships change with age. *J Nutr*. 2000;130:1724-1733.
- Iron Deficiency Anaemia. Assessment, Prevention and Control. A Guide for Programme Managers*. Geneva: World Health Organization; 2001.
- Tantular IS, Kawamoto F. An improved, simple screening method for detection of glucose-6-phosphate dehydrogenase deficiency. *Trop Med Int Health*. 2003;8:569-574.
- Hoffman WA, Pons JA, Janer JL. The sedimentation concentration method in *Schistosomiasis mansoni*. *PR J Public Health Trop Med*. 1934;9:283-291.
- Filmer D, Pritchett LH. Estimating wealth effects without expenditure data-or tear: an application to educational enrolments in states of India. *Demography* 2001;38:115-132.
- Osborn J, Cattaruzza MS. Odds ratio and relative risk for cross-sectional data. *Int J Epidemiol*. 1995;24:464-465.
- Katsuragawa TH, Gil LHS, Stabile RG, Pires MG, Bonini-Domingos CR. Evaluation of the prevalence of glucose-6-phosphate dehydrogenase deficiency and hematologic profile of a population from Rondônia. *Rev Bras Hematol Hemoter* 2004;26:268-273.
- Ferreira RGM, Moura MM, Engracia V, et al. Ethnic admixture composition of two Western Amazonian populations. *Hum Biol*. 2002;74:607-613.
- Brabin BJ. The role of malaria in nutritional anaemias. In: Fornon SJ, Slotkin S, eds. *Nutritional Anaemias*. New York: Raven Press; 1992:65-80.