MAJOR ARTICLE

Dynamics of Anemia in Relation to Parasitic Infections, Micronutrient Status, and Increasing Age in South-Central Côte d'Ivoire

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Background. Parasitic diseases (eg, malaria and helminthiases) exert enormous burdens on public health and social well-being. Moreover, parasitic infections are important causes of anemia in tropical Africa, exacerbated by lack of a diversified diet and inflammatory and genetic diseases. There is a paucity of longitudinal studies monitoring the dynamics of anemia in relation to the aforementioned parameters.

Methods. We designed a 14-month prospective longitudinal study in 3 cohorts (ie, infants aged 6–23 months, children aged 6–8 years, and women aged 15–25 years) in the Taabo health demographic surveillance system located in south-central Côte d'Ivoire. Parasitological, hematological, and micronutrient data were obtained from repeated cross-sectional surveys, utilizing standardized, quality-controlled methods.

Results. We found that young age, *Plasmodium* and *Schistosoma* infections, cellular iron deficiency, and stunting were significantly negatively associated with hemoglobin concentration. Moreover, iron status biomarkers (ie, ferritin and soluble transferrin receptor) were significantly associated with inflammatory parameters.

Conclusions. Based on our results, effective prevention and control measures that target parasitic diseases and iron deficiency are needed. These measures might include the distribution of long-lasting insecticidal nets, intermittent preventive treatment for malaria, regular anthelmintic drug administration, and improved intake of bioavailable iron, coupled with health and nutritional education and improved hygiene, water, and sanitation.

Keywords. anemia; hemoglobin; malaria; helminth; inflammation; iron; micronutrient; longitudinal study; Côte d'Ivoire.

Parasitic diseases such as malaria and helminthiases drain the social and economic development of a country and exert an enormous burden on public health and social well-being [1–3]. Malaria and helminthiases are important causes of morbidity and mortality, particularly in rural communities that often lack access to clean water, sanitation, hygiene, and health systems [4]. A common feature shared by malaria and helminthiases is their association with anemia. Moreover, the lack of a diversified diet with an adequate intake of bioavailable iron, inflammatory diseases, and hemoglobinopathies also significantly contribute to the high burden of anemia in the tropics [5, 6]. Anemia in newborns may also be the result of poor iron status of the mother prior to or during pregnancy, although this relation lacks evidence [7].

The etiology of anemia is multifactorial, and hence there are different preventive and curative measures for its control. For example, iron fortification or supplementation, sleeping under long-lasting insecticidal nets (LLINs), intermittent preventive treatment (IPT) of malaria, and preventive chemotherapy using albendazole or mebendazole against soil-transmitted

Received 18 June 2012; accepted 11 December 2012; electronically published 18 February 2013.

Some data included here were presented at the Annual Meeting of the Swiss Society of Tropical Medicine and Parasitology, Basel, Switzerland, October 2011.

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The Journal of Infectious Diseases 2013;207:1604–15

DOI: 10.1093/infdis/jit066

helminthiases and praziquantel against schistosomiasis have been suggested [6, 8, 9].

Previous studies suggest that >40% of the population in Côte d'Ivoire is anemic, and iron deficiency (ID) was identified as a moderate cause [10, 11]. However, a recent study conducted in south-central Côte d'Ivoire showed that, among iron fortification with electrolytic iron, IPT using sulfadoxine–pyrimethamine, and preventive chemotherapy using albendazole and praziquantel in school-aged children, preventive chemotherapy was the only intervention that had a significant, although minimal, effect on improving hemoglobin (Hb) concentration [8].

The etiology of anemia has been investigated in various population groups throughout Africa using cross-sectional designs, whereas only a handful of longitudinal studies assessed the dynamics of Hb over time in relation to specific variables [12–15]. We designed a 14-month prospective longitudinal study, with repeated cross-sectional surveys conducted every 3–4 months, to assess the dynamics of Hb in relation to parasitic infections, micronutrient status, and increasing age in 3 cohorts in south-central Côte d'Ivoire.

METHODS

Ethical Considerations

The study was approved by the institutional research commissions of the Swiss Tropical and Public Health Institute and the Eidgenössische Technische Hochschule (ETH) Zurich. The ethics committees of Basel and Côte d'Ivoire approved the study. Investigators were covered by liability insurance. The study is registered at controlled-trials.com (identifier ISRCTN02181959). Village authorities, participants, and parents/guardians of minors were informed about the purpose, procedures, and potential risks and benefits of the study. Written informed consent (or fingerprints of illiterate people) was obtained from study participants and parents/guardians of infants and children aged 6–8 years. Participation was voluntary; hence one could withdraw from the study at any time without further obligations.

Clinical malaria cases (defined by a positive rapid diagnostic test [RDT] and tympanic temperature >38°C) were treated with artesunate–amodiaquine (Maphar, Sanofi-Aventis, Casablanca, Morocco). Soil-transmitted helminth and schistosome infections were treated with albendazole (GlaxoSmithKline) and praziquantel (Bayer), respectively. Severely anemic participants (ie, Hb <8 g/dL, according to national guidelines of Côte d'Ivoire) were referred to healthcare centers.

Study Design and Procedures

The setting, selection of study participants, and field and laboratory procedures have been described elsewhere [16, 17]. In brief, a 14-month prospective longitudinal study was performed between April 2010 and June 2011 in 3 settings of the Taabo health demographic surveillance system (HDSS) in south-central Côte d'Ivoire. The 3 settings were Taabo Cité, a small district town where the only hospital is located; Ahondo, a village in close proximity to the Bandama River; and Katchénou, a small hamlet with no health facility. We set up the following 3 cohorts: infants aged 6–23 months; children of early school age (6–8 years); and young women aged 15–25 years. A total of 732 individuals were invited to participate.

At each cross-sectional survey, people were asked to provide stool and urine samples. Participants' height (to the nearest cm), weight (to the nearest 0.5 kg), and tympanic temperature (to the nearest 0.1°C) were measured. Finger-prick blood was collected and Hb concentration determined using a HemoCue 301 (HemoCue AB; Ängelholm, Sweden). Infection with *Plasmodium falciparum* was assessed using an RDT (ICT ML01 malaria Pf kit; ICT Diagnostics, Cape Town, South Africa). Additionally, thick and thin blood films were made to determine parasitemia and species-specific *Plasmodium* infection. At the baseline and end-of-study surveys, venous blood samples (5–10 mL) were drawn from each participant directly into heparin-coated tubes and put in a cooler containing ice.

Duplicate Kato-Katz thick smears were prepared from each stool sample using 41.7-mg templates [18]. The slides were allowed to clear for at least 30 minutes before microscopic examination by experienced laboratory technicians who recorded the number of eggs of soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) and *Schistosoma mansoni*. Urine samples were subjected to a filtration method [19]; all *Schistosoma haematobium* eggs in a filtrate of 10 mL were counted. For quality control, a senior laboratory technician reexamined 10% of Kato-Katz and urine filtration slides.

Pregnancies and deliveries known by the participating women or by the community health workers were recorded during the study in order to adapt the cut-off used for anemia in women.

Venous Blood Examination

Riboflavin was measured by an erythrocyte glutathione reductase activity coefficient (EGRAC) assay in whole blood [20]. Ferritin, soluble transferrin receptor (sTfR), retinol binding protein (RBP), α1-acid glycoprotein (AGP), and C-reactive protein (CRP) were measured with a sandwich enzyme-linked immunosorbent assay [21]. Serum retinol (SR) was measured by high-pressure liquid chromatography (Merck-Hitachi; Tokyo, Japan) [22].

Statistical Analysis

Parasitological and hematological data were entered twice in Microsoft Access version 10.0 (2007; Microsoft Corporation)

and cross-checked using EpiInfo version 3.4.1 (Centers for Disease Control and Prevention; Atlanta, GA). Anemia and storage iron depletion were defined according to World Health Organization (WHO) guidelines [23]. Acute and chronic inflammation were defined as CRP >5 mg/L and AGP >1 g/L, respectively. Cellular ID was defined as sTfR >8.5 mg/L [24]. Vitamin A deficiency was defined as RBP <0.825 μmol/L [25].

Household socioeconomic status was calculated using an asset-based index [26]. Data on household assets, housing characteristics, and number of people per room were obtained from the readily available Taabo HDSS database. Using principal component analysis to weigh the binary data of these variables, we subsequently divided the households into socioeconomic groups (wealth tertiles); namely, very poor, poor, and least poor.

Height-for-age and weight-for-height Z-scores were calculated with WHO AnthroPlus version 1.0.3 (WHO; Geneva, Switzerland). Stunting and underweight were defined as having a height-for-age or weight-for-age, respectively, of more than 2 standard deviations below the median of the National Center for Health Statistics (NCHS)/WHO growth reference [27].

We employed logistic regressions to identify predictors of anemia for those individuals who had complete parasitological, hematological, and micronutrients data at baseline or at the end-of-study survey. Crude and adjusted odds ratios were calculated, including 95% confidence intervals and *P* value using a Wald test. Setting was included as random effect in multivariate models, and covariates were removed until the best fitting model was identified, based on the results of a likelihood ratio test.

We used linear regression analyses with 2 clustering effects (ie, individual and setting level) to identify independent significant predictors of Hb, natural log sTfR, and natural log AGP. Individuals who had complete parasitological and hematological data for at least 1 of the cross-sectional surveys were considered. For the models with sTfR and AGP as the outcome, we included AGP only as a marker of inflammation to prevent colinearity with CRP and because AGP was better correlated to the outcome variables. For each outcome, we report the results of the best predictive model, based on Akaike's information criterion. McNemar test and Wilcoxon signed rank test were used to compare matched prevalence data and matched mean ranks, respectively.

RESULTS

Attrition, Participation, and Socioeconomic Parameters

Overall, 732 individuals were invited and, during the baseline cross-sectional survey, 407 (55.6%) were available and agreed

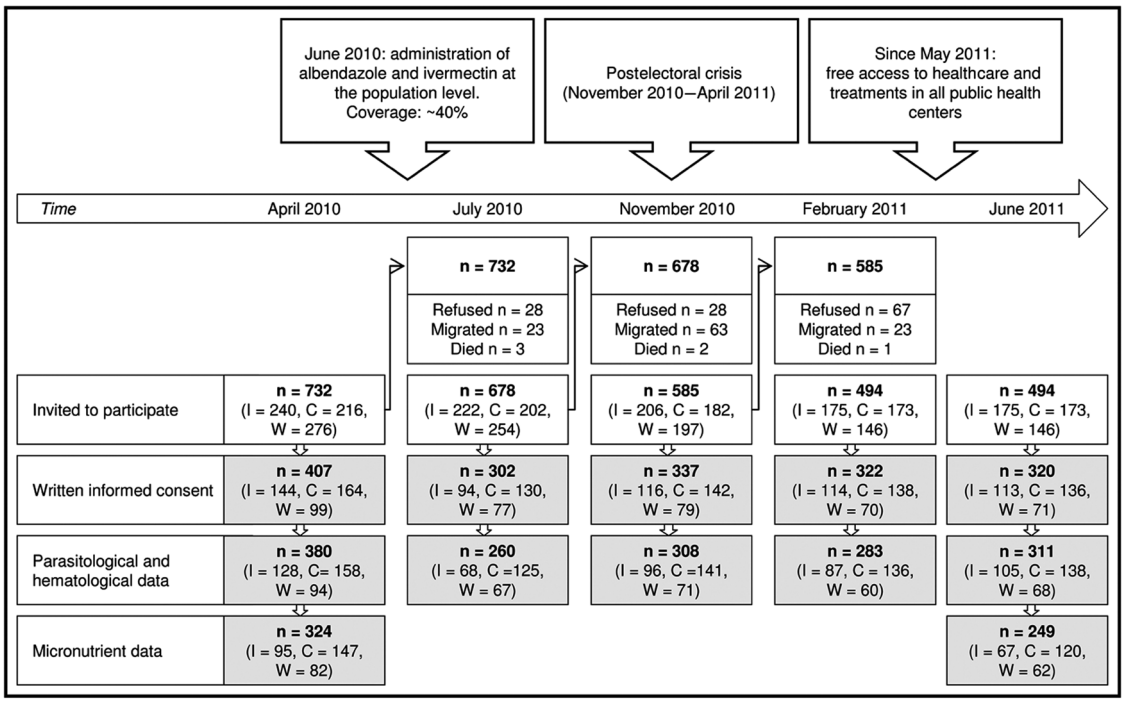

Figure 1. Participation, adherence, and major events during the 14-month longitudinal study. In April 2010, 732 people were invited to participate, of whom 407 provided written informed consent for longitudinal monitoring. All 732 people were given the opportunity to participate in the second cross-sectional survey in July 2010. For the following rounds, only those who did not refuse to participate and did not move were asked to participate. Abbreviations: I, infants; C, school-aged children; W, women.

Table 1. Sociodemographic Parameters, Household Assets, and Other Characteristics of Infants, School-Aged Children, and Women

	Taabo Cité		Ahondo		Katchénou		Difference	
Characteristic	n	%	n	%	n	%	χ^2	*P* Value
Sex (female)[a]	38	44.7	40	47.6	60	51.3	0.87	.647
Education (binary)								
Maternal education[b]	15	28.9	7	18.4	4	13.8	2.06	.358
Attending school[c]	20	43.5	23	45.1	17	27.9	4.34	.114
Ever attended school[d]	27	77.1	18	58.1	8	28.6	14.98	.001
Mean number of people per room (categorical)								
1–1.9	33	21.7	43	37.1	55	49.1		
2–2.9	50	32.9	33	28.5	38	33.9		
≥3	69	45.4	40	34.5	19	17.0	30.50	<.001
Household assets (binary)								
Ventilator	69	45.4	23	19.8	0	0.0	[e]	<.001
Cell phone	136	89.5	88	75.9	82	73.2	13.19	.001
Television	106	69.7	65	56.0	8	7.1	106.74	<.001
Refrigerator	19	12.5	2	1.7	0	0.0	[e]	<.001
Bicycle	98	64.5	93	80.2	99	88.4	21.79	<.001
Car	3	2.0	0	0.0	0	0.0	[e]	.116
Source of light (categorical)								
Candle	1	0.7	0	0.0	0	0.0		
Oil lamp	3	2.0	14	12.1	111	99.1		
Plugged lamp	46	96.1	102	87.9	0	0.0		
Other	2	1.3	0	0.0	1	0.9	[e]	<.001
Source of water (categorical)								
Tap water	151	99.3	12	10.3	0	0.0		
Pump, well	1	0.7	59	50.9	112	100.0		
River	0	0.0	45	38.8	0	0.0	[e]	<.001
Sanitation (categorical)								
Water closet	67	44.1	1	0.9	0	0.0		
Latrine	53	34.9	58	50.0	0	0.0		
No sanitation	32	21.1	57	49.1	112	100.0	[e]	<.001

Participants with complete parasitological data at the baseline cross-sectional survey (April 2010) were included in the analyses, stratified by study setting (n = 380).

[a] For infants and school-aged children only (n = 286).

[b] For mothers of infants who answered the questionnaire (n = 105).

[c] For school-aged children only (n = 158).

[d] For young women only (n = 94).

[e] Fisher's exact test.

to participate in the longitudinal monitoring (Figure 1). The participation rate decreased considerably from July 2010 onward. An awareness campaign conducted in October 2010 stabilized the number of participants in the subsequent surveys despite a presidential postelectoral crisis that lasted until April 2011. An attrition analysis revealed that there were no differences in gender composition, median age, and socioeconomic status between participants with and without complete parasitological, hematological, and micronutrient data in April 2010 and in June 2011, regardless of the age group investigated. Moreover, median age and sex ratio of the 108 participants who had complete data for all 5 surveys did not significantly differ from the initial cohort of 732 individuals. The 3 settings were very different in terms of sociodemographic and economic parameters (Table 1).

Dynamics of Parasitic Infections, Micronutrient Status, and Anemia

Hb concentration was higher in June 2011 compared with April 2010 in the 3 cohorts (Figure 2*A*), and the change was significant for infants and school-aged children (Table 2). The intensity of *Plasmodium* parasitemia slightly decreased during the long dry season (from October until March) in the 3 cohorts but increased with the start of the rainy season (Figure 2*C*). The

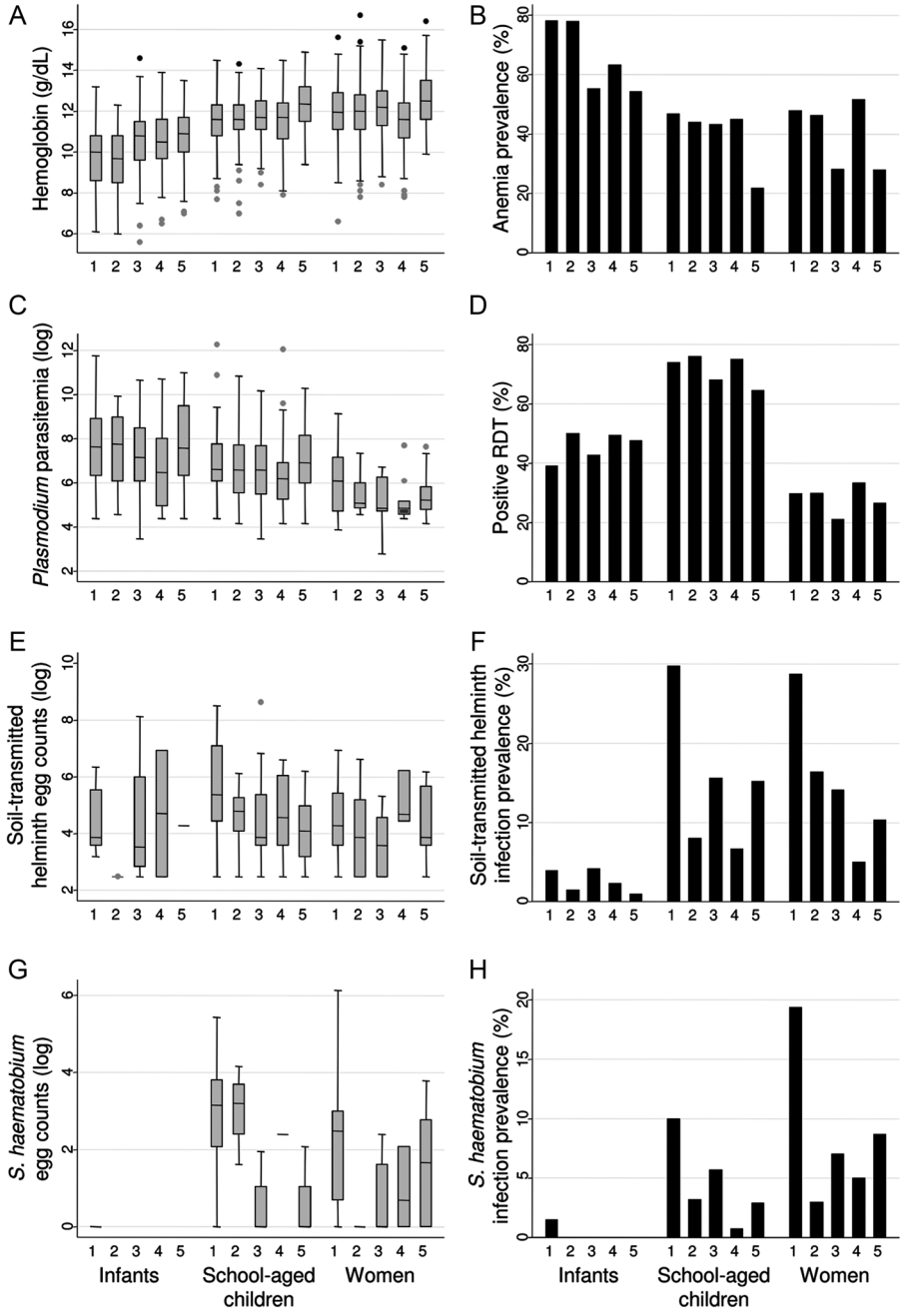

Figure 2. Anemia and parasitic infection profiles over the 14-month longitudinal study, stratified by age group. *A*, Dynamics of hemoglobin concentration in each age group. *B*, Prevalence of anemia. *C*, Dynamics of *Plasmodium* parasitemia in each age group. *D*, Prevalence of *Plasmodium* infection. *E*, Dynamics of soil-transmitted helminth infection intensity in each age group. *F*, Prevalence of soil-transmitted helminth infection. *G*, Dynamics of *Schistosoma haematobium* infection intensity in each age group. *H*, Prevalence of *S. haematobium* infection. For each survey, all participants with complete parasitological and hematological data were included. April 2010, n = 380; July 2010, n = 260; November 2010, n = 308; February 2011, n = 283; June 2011, n = 311. Box plot: The ends of the box represent the 75th and 25th percentiles; the middle line represents the median; the upper whisker represents the upper quartile +1.5* (interquartile range); the lower whisker represents the lower quartile −1.5* (interquartile range). Abbreviation: log, naturally log-transformed, RDT, rapid diagnostic test.

geometric mean of soil-transmitted helminth and *Schistosoma* infections in school-aged children was significantly lower in June 2011 compared with the baseline cross-sectional survey done in April 2010 (Table 2). The geometric mean of *Schistosoma* infection at the end of the study was significantly lower among women compared with baseline prevalence data

Table 2. Comparison of Hematological, Infection, Inflammation, and Micronutrient Parameters at Baseline (April 2010) and at the End-of-Study Survey (June 2011), Stratified by Age Group

	Infants			School-Aged Children			Women		
Variable	April 2010	June 2011	*P*	April 2010	June 2011	*P*	April 2010	June 2011	*P*
Hemoglobin (g/dL)[a,b]	9.82 ± 0.17	10.76 ± 0.17	**<.001**	11.53 ± 0.11	12.40 ± 0.11	**<.001**	12.17 ± 0.26	12.60 ± 0.22	.065
Plasmodium falciparum[c,d]	9.57 (3.42–24.27)	16.17 (5.97–41.34)	.630	143.09 (78.93–258.74)	42.34 (21.71–81.72)	.069	7.33 (1.99–22.21)	7.26 (2.48–18.61)	.548
Soil-transmitted helminth[c,d]	0.19 (0.00–0.46)	0	.083	3.21 (1.67–5.64)	0.87 (0.40–1.50)	**.002**	2.66 (0.77–6.60)	0.47 (0.00–1.17)	.081
Schistosoma haematobium[c,d]	0.00 (0.00–0.03)	0	.317	0.46 (0.19–0.80)	0.04 (0.00–0.08)	**.006**	0.90 (0.24–1.90)	0.02 (0.00–0.05)	**.003**
sTfR (mg/L)[d,e]	11.50 (8.47–16.37)	8.95 (7.12–13.10)	**.001**	7.88 (6.50–9.49)	7.50 (5.56–9.55)	.100	7.22 (5.54–10.45)	6.54 (4.65–8.70)	.233
Ferritin (μg/L)[d,e]	32.80 (15.00–69.20)	60.82 (29.92–96.84)	**.046**	63.50 (45.80–103.80)	77.28 (47.20–105.36)	.220	44.25 (27.35–60.60)	50.21 (24.70–69.61)	.900
Serum retinol (μg/dL)[d,e]	19.06 (15.14–26.90)	22.38 (15.35–24.46)	.602	21.20 (16.00–27.91)	23.18 (18.96–28.80)	**.003**	34.53 (25.41–44.32)	37.31 (28.09–46.83)	.635
EGRAC[d,e]	1.35 (1.18–1.50)	1.42 (1.32–1.62)	**.002**	1.47 (1.29–1.71)	1.50 (1.39–1.67)	.163	1.45 (1.28–1.65)	1.54 (1.41–1.66)	.952
AGP (g/L)[d,e]	0.99 (0.84–1.33)	1.08 (0.90–1.33)	.566	0.92 (0.77–1.06)	0.93 (0.66–1.05)	.110	0.80 (0.70–0.94)	0.67 (0.52–0.89)	**.013**
CRP (mg/L)[d,e]	2.18 (0.69–5.80)	1.20 (0.46–4.51)	.650	1.57 (0.51–4.55)	1.72 (0.61–4.16)	.514	1.04 (0.50–3.54)	1.06 (0.57–1.92)	.456

Matched parasitological and hematological data (n = 231), iron status and inflammation data (n = 189), serum retinol concentration (n = 163), and EGRAC (n = 211) are compared.

Abbreviations: AGP, α1-acid glycoprotein; CRP, C-reactive protein; EGRAC, erythrocyte glutathione reductase activity coefficients; sTfR, soluble transferrin receptor.

[a] Mean ± standard error.

[b] Bilateral paired *t* test.

[c] Geometric mean of natural log-transformed values (95% CI).

[d] Wilcoxon signed rank test.

[e] Median (95% CI).

Table 3. Association of Anemia With Sociodemographic, Parasitic, and Micronutrient Status Parameters

	Infants				School-Aged Children				Women			
	April 2010 (n = 95)		June 2011 (n = 67)		April 2010 (n = 147)		June 2011 (n = 120)		April 2010 (n = 82)		June 2011 (n = 62)	
Exploratory variable	OR	Adjusted OR (95% CI)	OR	Adjusted OR (95% CI)	OR	Adjusted OR (95% CI)	OR	Adjusted OR (95% CI)	OR	Adjusted OR (95% CI)	OR	Adjusted OR (95% CI)
Demographic variables												
Age class: middle[a]	1.3		1.3		1.3		1.4		0.4		0.9	
Age class: older[a]	1.3		0.6		0.8		1.1		N/A		N/A	
Sex (female)	0.9		0.7		1.1		0.7		N/A		N/A	
Occupation: student[b]	N/A		N/A		N/A		N/A		1.2	1.4 (0.4–4.9)	1.1	
Occupation: housekeeper[b]	N/A		N/A		N/A		N/A		**0.3**	**.2 (.0–.8)**	1.4	
Attending school	N/A		N/A		0.5	.5 (.2–1.0)	0.5		N/A		N/A	
Socioeconomic status												
Poor	0.8		0.8		0.9		0.7		2.3		1.5	
Least poor	0.2		0.8		0.5		0.4		0.9		0.6	
Parasitic infection												
Plasmodium falciparum	**3.8**	**3.8 (1.0–14.5)**	2.8		2.8		1.8		1.2		0.9	
Soil-transmitted helminths	0.4		N/A		0.7		0.7		1.5		1.1	
Schistosoma	N/A		N/A		0.9		N/A		1.6		1.9	
Micronutrient deficiency												
Stunted (HAZ < 2)	1.1		2.0		2.5		**5.9**	**5.8 (2.0–17.0)**	N/A		N/A	
Underweight (WAZ < 2)	4.4		2.1		2.1		4.4		N/A		N/A	
Iron storage depletion	1.5		2.5		2.4		N/A		2.3		0.4	
Cellular iron deficiency	0.8		**3.4**	**3.2 (1.0–9.7)**	**2.9**	**2.3 (1.1–4.9)**	1.7		**4.5**	**6.1 (1.9–19.6)**	1.1	
Vitamin A deficiency (RBP)	1.2		3.1	3.3 (1.0–10.9)	1.4		1.9		N/A		N/A	
Riboflavin deficiency	0.7		0.5		0.6		0.8		**0.4**	**.3 (.1–.9)**	1.9	
Other parameters												
Chronic inflammation	0.9		**4.3**	**3.6 (1.2–10.9)**	**4.4**	**3.8 (1.8–8.3)**	2.0		1.1		1.7	
Acute inflammation	1.4		2.2		3.2		**1.6**	**3.0 (1.0–8.5)**	1.9		**4.7**	**5.8 (1.2–28.0)**

Logistic regression was used in each age group to identify variables significantly associated with anemia. Potential explanatory variables were: sex (binomial, where applicable), age (categorical), socioeconomic status (categorical), occupation (for women only, categorical), school attendance (for children, binomial), *Plasmodium falciparum* infection (binomial), soil-transmitted helminth infection (binomial), *Schistosoma* infection (binomial), iron storage depletion (binomial), cellular iron deficiency (binomial), vitamin A deficiency (RBP, binomial), riboflavin deficiency (binomial), chronic inflammation (binomial), acute inflammation (binomial). Multivariate regression model was computed with all potential covariates, with setting included as random effect. In a first step, covariates were removed by stepwise backward procedure, keeping only explanatory variables with *P* values <.2. In a second step, likelihood ratio test was applied to identify the best predictive model. We report adjusted ORs of those explanatory variables kept in the final model. Explanatory variables with crude and adjusted *P* values <.05 are reported in bold.

Abbreviations: CI, confidence interval; HAZ, height-for-age Z score; N/A, not applicable; OR, odds ratio; RBP, retinol-binding protein; WAZ, weight-for-age Z score.

[a] Reference age class: youngest class. Subclasses: infants: 6-month ranges; children: 1-year ranges; women: 5-year ranges.

[b] Reference occupation: working out of the family house.

(Table 2). Iron status of infants significantly improved during the study, as reported by higher ferritin concentration and lower sTfR concentration in June 2011 compared with concentrations in April 2010 (Table 2).

Logistic regression revealed that variables significantly associated with anemia in June 2011 differed from those identified in April 2010 in each age group (Table 3). At the end-of-study survey, the variables significantly associated with anemia among infants were identical to those identified for young school-aged children at the beginning of the study. In June 2011, cellular ID and chronic inflammation were significantly associated with anemia in infants, while chronic malnutrition, defined as stunting, and acute inflammation were significant predictors of anemia in school-aged children. At the end-of-

Table 4. Association Between Age, Parasitic Infections, Micronutrient Status, and Hemoglobin Concentration

Exploratory variable	Coefficient	95% CI	*P* Value
Infants			
2nd survey	−0.09	−.45, .27	.615
3rd survey	0.61	.24, .98	.001
4th survey	0.59	.16, 1.03	.008
5th survey	0.68	.17, 1.19	.009
Age (months)	0.03	.00, .06	.026
Plasmodium parasitemia	−0.15	−.18, −.12	<.001
Stunted	−0.23	−.48, .01	.063
Intercept	9.76	9.26, 10.30	<.001
School-aged children			
2nd survey	−0.03	−.26, .19	.770
3rd survey	0.16	−.06, .38	.150
4th survey	−0.07	−.29, .15	.527
5th survey	0.75	.53, .97	<.001
Plasmodium parasitemia	−0.03	−.06, −.01	.014
Schistosoma haematobium egg counts	−0.15	−.28, −.01	.040
Stunted	−0.48	−.80, −.16	.003
Intercept	11.81	11.58, 12.00	<.001
Women			
2nd survey	0.05	−.32, .42	.798
3rd survey	0.34	−.03, .72	.072
4th survey	−0.28	−.68, .12	.166
5th survey	0.59	.22, .96	.002
Pregnancy	−0.99	−1.40, −.58	<.001
Plasmodium parasitemia	−0.04	−.09, .01	.112
Intercept	12.12	11.82, 12.40	<.001

A mixed-effect linear regression was applied for each age class, with hemoglobin concentration (g/dL) as outcome and 2 levels of clustering (individual and setting). Potential explanatory variables were: survey (1–5), age (longitudinal), sex, *Plasmodium* parasitemia (natural log-transformed), *Schistosoma haematobium* egg counts (natural log-transformed), soil-transmitted helminth egg counts (natural log-transformed), stunting, and underweight for infants and school-aged children and pregnancy status and weight for women. The coefficients from the best predictive model, as defined by the Akaike's information criterion, are reported with their 95% CI and respective *P* value.

Abbreviation: CI, confidence interval.

study survey, acute inflammation was the only parameter significantly associated with anemia in women.

Mixed-effect linear regression with 2 levels of clustering (individual and setting) revealed significant relationships between Hb concentration and age, *Plasmodium* parasitemia, and time, and Hb concentration in infants (Table 4). *Plasmodium* parasitemia, *S. haematobium* egg counts, and chronic malnutrition (stunting) were significantly associated with Hb concentration in school-aged children. Among women, pregnancy status was significantly associated with lower Hb concentration. While mean Hb steadily increased over time in the infant cohort, this trend was less obvious in school-aged children and women (Table 4). The investigation of potential associations between parasitic infections and micronutrient biomarkers revealed that *Plasmodium* parasitemia and AGP both significantly contributed to higher ferritin concentration in all age groups (Supplementary Table 5). Furthermore, *S. haematobium* egg counts and EGRAC were significantly associated with lower ferritin concentration in the school-aged child cohort. The inflammatory marker AGP was significantly positively associated with sTfR concentration in the 3 age groups investigated (Supplementary Table 5). Natural log sTfR was significantly correlated with natural log AGP (Spearman ρ: 0.43; $P < .001$) and log CRP (Spearman ρ: 0.22; $P < .001$; Figure 3*A* and 3*B*). Natural log sTfR was correlated to natural log AGP (Spearman ρ: 0.40; $P < .001$) and CRP concentration (Spearman ρ: 0.19; $P = .001$) in participants free of *Plasmodium* infection as well (data not shown). At baseline, participants with chronic or acute inflammation had significantly higher sTfR concentration than their counterparts without inflammation (Figure 3*C* and 3*D*). Natural log sTfR was significantly lower in June 2011 compared with that in April 2010 in participants with inflammation at baseline and no inflammation at the end-of-study survey (Figure 3*E* and 3*F*). Our data indicate that serum retinol concentration was inversely correlated with sTfR in school-aged children and women (Supplementary Table 5). *Plasmodium* parasitemia was associated with higher AGP values in the 3 cohorts.

DISCUSSION

To our knowledge, this is the first prospective longitudinal survey to investigate the dynamics of anemia and putative associated factors over a 14-month period in 3 cohorts in tropical West Africa. Our data indicate that infection with *Plasmodium* and *Schistosoma*, cellular ID, chronic malnutrition, and inflammation are significantly associated with lower Hb concentration in the current, primarily rural, setting of south-central Côte d'Ivoire. The observation that sTfR concentration correlated with both AGP and CRP concentration in the 3 cohorts investigated challenges the robustness of this marker to assess the prevalence of ID in areas where inflammatory diseases are common.

Our longitudinal survey revealed higher Hb concentration among infants and school-aged children at the end-of-study survey in June 2011 compared with the concentration at the baseline cross-sectional survey in April 2010. This difference can be explained, at least partially, by lower *S. haematobium* and *P. falciparum* infection intensities in school-aged children and by older age and improved iron status in infants at the end-of-study survey. However, some survey time points were significantly associated with hemoglobin concentration (see

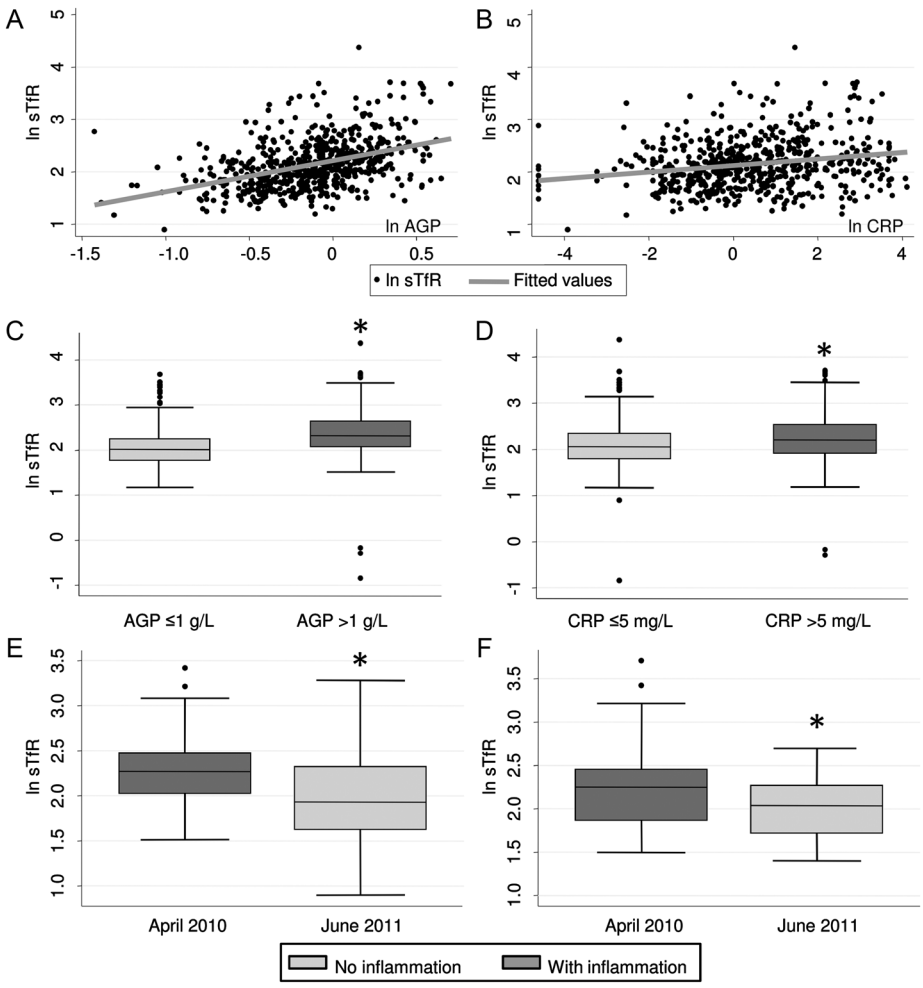

Figure 3. Association between inflammatory markers and soluble transferrin receptor (sTfR). *A*, Scatter and fitted plot of natural log-transformed sTfR concentration and natural log-transformed AGP concentration (n = 610; 3 outliers with log sTfR <0 not shown). *B*, Scatter and fitted plot of natural log-transformed sTfR concentration and natural log-transformed CRP concentration (n = 610; 3 outliers with natural log-transformed sTfR <0 not shown). *C*, Comparison of natural log-transformed sTfR concentration between individuals with and without chronic inflammation (n = 344; April 2010). *D*, Comparison of natural log-transformed sTfR concentration between individuals with and without acute inflammation (n = 344; April 2010). *E*, Comparison of natural log-transformed sTfR concentration between individuals with and without chronic inflammation (n = 38). *F*, Comparison of natural log-transformed sTfR concentration between individuals with and without acute inflammation (n = 33). Acute inflammation is defined as CRP >5 mg/L. Chronic inflammation is defined as AGP >1 g/L. Asterisks indicate Wilcoxon rank-sum or signed-rank (for paired data) *P* value <.05. Abbreviations: AGP, α1-acid glycoprotein; CRP, C-reactive protein; sTfR, soluble transferrin receptor.

Table 4), indicating the influence of other factors not investigated here or that the study itself contributed to a decline in anemia prevalence. We observed a slight decrease in Hb concentration in February 2011, which might be a consequence of 1 or several factors. First, this survey was done during the primary dry season, a period characterized by restricted food supply and poor diet diversification. Second, the sociopolitical unrest and armed conflict that Côte d'Ivoire went through in connection with the presidential postelection turmoil between November 2010 and April 2011 had an impact on population diet as well [28]. Indeed, access to a diversified diet not only became more difficult due to the cessation of food exchanges between rural and urban areas, but many residents from the economic capital Abidjan sought shelter in their home villages at the height of the conflict, possibly decreasing the relative amount of food available per person. Moreover, access to healthcare was compromised during this period. Third, among women, infections with *Plasmodium* were more prevalent in the February 2011 cross-sectional survey than in the preceding survey in November 2010 and the end-of-study survey in June 2011.

The identification of variables significantly associated with anemia and parameters significantly associated with lower Hb concentration in each age group confirms the multifactorial

etiology of anemia in the current setting of rural West Africa. Importantly, our data indicate that the variables associated with anemia in infants and school-aged children shifted during the study. Indeed, we found the same risk factors for anemia as infants grew older (ie, 20–38 months) as observed in young school-aged children (6–8 years).

Our data indicate that, for the infant cohort at the baseline cross-sectional survey, the result of an RDT for malaria is the single most accurate predictor for the odds of anemia. The relationship between *Plasmodium* infection and parasitemia, on the one hand, and Hb and iron status parameters (ie, ferritin and sTfR), on the other hand, was marked in each of the 3 cohorts and confirms the important burden of malarial anemia in these age groups in areas where malaria is highly endemic [29–31]. However, the partial immunity that develops against *Plasmodium* in people living in malaria-endemic areas is reflected in our data by the significant relationship between *Plasmodium* infection and anemia in infants only and the weak association between *Plasmodium* parasitemia and Hb concentration in school-aged children and women [32]. Of note, at the time of our study, coverage rates with LLINs in Côte d'Ivoire were very low (ie, <10% of children aged <5 years slept under LLINs) [33].

Our data confirm that ID contributes to the burden of anemia in sub-Saharan Africa as well. Indeed, cellular ID was significantly associated with anemia in infants, school-aged children, and women, although infants showed markedly higher prevalence of ID in our study. Other studies have reported a low prevalence of ID in school-aged children in this region of Côte d'Ivoire [8, 10, 11], which could be due to a diet largely based on cassava and yams and thus with a low phytate content. Furthermore, among infants, iron status was the only parameter that significantly improved during the study, coupled to increased Hb concentration. Considering that Plasmodium infection decreases iron absorption, and in view of adverse events that may result from mass iron supplementation in malaria-endemic settings, high LLIN coverage, improved access to prompt diagnosis, and quality malaria treatment should precede potential iron fortification campaigns in Côte d'Ivoire. [34–36].

Our prospective longitudinal monitoring highlights the fact that, in addition to *Plasmodium* infection and ID, chronic malnutrition and inflammation are significantly associated with anemia in infants and young school-aged children in this area of West Africa [31]. The inflammation induced by *Plasmodium* parasites was obvious in all cohorts studied, as reflected by the association between *Plasmodium* parasitemia and AGP concentration, illustrating one of the mechanisms implicated in malarial anemia [37]. The overall low prevalence and intensities of helminth infections in the study area might explain why we did not find any significant association between soil-transmitted helminth infection and Hb concentration in any age group. Indeed, although soil-transmitted helminth infection prevalence significantly decreased in school-aged children, most likely explained by the administration of anthelmintic drugs at the individual and population levels, this parameter was not significantly associated with Hb concentration in this age group. However, our data confirm the negative association between *S. haematobium* egg counts, on the one hand, and iron stores and Hb, on the other hand, in school-aged children and women [38, 39].

In addition, we found that *Plasmodium* parasitemia and inflammation were significantly correlated with ferritin and sTfR concentration, respectively. Ferritin, which is a positive acute-phase protein, has been associated with *Plasmodium* infection in previous studies, confirming the current observation [40]. However, the regulation of sTfR is less well understood, particularly in the context of inflammation. During the last decade, soluble transferrin receptor became a popular proxy for assessing iron status at the population level, particularly in regions where inflammatory diseases are prevalent [10, 41]. However, 2 studies carried out in Kenya reported higher sTfR concentration in *Plasmodium*-infected children [42, 43]. Interestingly, in a study conducted in Benin, *Plasmodium*-infected women showed higher concentrations of sTfR after having received a malarial treatment, most likely reflecting the suppression of erythropoiesis, which occurs during chronic *Plasmodium* infection [36]. Our results indicate that, while the inflammatory marker AGP showed a significant positive correlation with sTfR concentration in all cohorts, *Plasmodium* parasitemia was negatively correlated with sTfR concentration in women, most likely due to a decreased erythropoiesis rate in this age group. Moreover, our results show that sTfR concentration significantly differs between and among people with and without inflammation. These findings suggest that sTfR is also influenced by inflammation. Hence, it is likely that, in our setting, high sTfR may, in part, reflect functional ID that results from the prevention of intestinal iron absorption and iron recycling from macrophages by hepcidin [44], rather than inadequate dietary iron supply. Future studies should include hepcidin measures, weighed food record, and quantitative food recalls in order to better understand the regulation of sTfR expression and the source of cellular ID.

During the April 2010 baseline cross-sectional survey done, we found that women with riboflavin deficiency had lower odds of anemia [16]. However, the negative correlation between ferritin concentration and EGRAC observed in school-aged children emphasizes the association of iron handling and riboflavin status. Our findings are in line with previous results from a study done in the same area that reported a detrimental effect of riboflavin deficiency on iron status among school-aged children [45, 46]. Although previous laboratory investigations and clinical trials showed a positive effect of vitamin A on Hb concentration, iron mobilization, and iron absorption,

the effect on ferritin and sTfR concentration was less well characterized [47–50]. The observation that vitamin A and sTfR are inversely correlated in children and women reflects this uncertain interaction and emphasizes the need of further research in order to gain a better understanding of nutrient–nutrient interactions and the interplay between micronutrients and parasitic infections [51].

The current prospective longitudinal study sheds light on the complex etiology of anemia in rural, tropical West Africa. Most importantly, our data show that parasitic infections (eg, malaria and schistosomiasis), inflammatory diseases, cellular ID, and chronic malnutrition are associated with lower Hb concentration. These considerations emphasize the urgent need to implement efficient prevention and control programs that target these parasitic diseases. Concerted efforts must include distribution of LLINs, anthelmintic drug administration, and collaborative efforts to facilitate more equitable access to a diversified diet. This diet should include an adequate intake of bioavailable iron, especially for infants, coupled with health and nutritional education, which is mandatory to achieve a sustainable impact on anemia in sub-Saharan Africa.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank the study participants for their commitment and the 5 field workers, without whom this study would not have been possible. We are grateful to Prof Bassirou Bonfoh, director-general of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, and to all Taabo HDSS collaborators for their support during the study. Many thanks go to the team from the Laboratory of Zoology and Animal Biology at the Unité de Formation et de Recherche Biosciences, Université Félix Houphouët-Boigny, for their efforts and dedicated work in the field and at the bench. We thank Christophe Zeder, Adam Krzystek, and Jasmin Tajeri for their expertise and advice regarding the laboratory work and Prof Christian Schindler and Jan Hattendorf for their assistance in statistical analyses.

Financial support. This work was supported by the Swiss National Science Foundation (project no. IZ70Z0_123900) and the Eremitage Fund from the Rudolf Geigy-Foundation. Start-up funding for the establishment and running of the Taabo HDSS was provided by Fairmed.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Sachs J, Malaney P. The economic and social burden of malaria. Nature **2002**; 415:680–5.
2. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. J Clin Invest **2008**; 118:1311–21.
3. Utzinger J, Raso G, Brooker S, et al. Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. Parasitology **2009**; 136:1859–74.
4. Bartram J, Cairncross S. Hygiene, sanitation, and water: forgotten foundations of health. PLoS Med **2010**; 7:e1000367.
5. Crawley J. Reducing the burden of anemia in infants and young children in malaria-endemic countries of Africa: from evidence to action. Am J Trop Med Hyg **2004**; 71(Suppl 2):25–34.
6. Tolentino K, Friedman JF. An update on anemia in less developed countries. Am J Trop Med Hyg **2007**; 77:44–51.
7. Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? J Nutr **2001**; 131:590S–601.
8. Rohner F, Zimmermann MB, Amon RJ, et al. In a randomized controlled trial of iron fortification, anthelmintic treatment, and intermittent preventive treatment of malaria for anemia control in Ivorian children, only anthelmintic treatment shows modest benefit. J Nutr **2010**; 140:635–41.
9. ter Kuile FO, Terlouw DJ, Kariuki SK, et al. Impact of permethrin-treated bed nets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya. Am J Trop Med Hyg **2003**; 68:68–77.
10. Staubli Asobayire F, Adou P, Davidsson L, Cook JD, Hurrell RF. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Côte d'Ivoire. Am J Clin Nutr **2001**; 74:776–82.
11. Wegmüller R, Camara F, Zimmermann MB, Adou P, Hurrell RF. Salt dual-fortified with iodine and micronized ground ferric pyrophosphate affects iron status but not hemoglobin in children in Côte d'Ivoire. J Nutr **2006**; 136:1814–20.
12. Cornet M, Le Hesran JY, Fievet N, et al. Prevalence of and risk factors for anemia in young children in southern Cameroon. Am J Trop Med Hyg **1998**; 58:606–11.
13. McElroy PD, Lal AA, Hawley WA, et al. Analysis of repeated hemoglobin measures in full-term, normal birth weight Kenyan children between birth and four years of age. III. The Asemobo Bay Cohort Project. Am J Trop Med Hyg **1999**; 61:932–40.
14. Zimmermann MB, Chaouki N, Hurrell RF. Iron deficiency due to consumption of a habitual diet low in bioavailable iron: a longitudinal cohort study in Moroccan children. Am J Clin Nutr **2005**; 81: 115–21.
15. Bloland PB, Boriga DA, Ruebush TK, et al. Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission II. Descriptive epidemiology of malaria infection and disease among children. Am J Trop Med Hyg **1999**; 60:641–8.
16. Righetti AA, Koua AYG, Adiossan LG, et al. Etiology of anemia among infants, school-aged children, and young non-pregnant women in different settings of south-central Côte d'Ivoire. Am J Trop Med Hyg **2012**; 87:425–34.
17. Righetti AA, Glinz D, Adiossan LG, et al. Interactions and potential implications of *Plasmodium falciparum*-hookworm coinfection in different age groups in south-central Côte d'Ivoire. PLoS Negl Trop Dis **2012**; 6:e1889.
18. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev Inst Med Trop São Paulo **1972**; 14:397–400.
19. WHO. Basic laboratory methods in medical parasitology. Geneva: World Health Organization, **1991**.
20. Dror Y, Stern F, Komarnitsky M. Optimal and stable conditions for the determination of erythrocyte glutathione reductase activation coefficient to evaluate riboflavin status. Int J Vitam Nutr Res **1994**; 64:257–62.
21. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding

protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr **2004**; 134:3127–32.
22. Tanumihardjo SA, Permaesih D, Muhilal. Vitamin A status and hemoglobin concentrations are improved in Indonesian children with vitamin A and deworming interventions. Eur J Clin Nutr **2004**; 58: 1223–30.
23. WHO/UNICEF/UNU. Iron deficiency anemia: assessment, prevention and control. Geneva: World Health Organization, **2001**; WHO/NHD/01.3.
24. Cook JD, Skikne BS, Baynes RD. Serum transferrin receptor. Annu Rev Med **1993**; 44:63–74.
25. Gorstein JL, Dary O, Pongtorn, Shell-Duncan B, Quick T, Wasanwisut E. Feasibility of using retinol-binding protein from capillary blood specimens to estimate serum retinol concentrations and the prevalence of vitamin A deficiency in low-resource settings. Public Health Nutr **2008**; 11:513–20.
26. Filmer D, Pritchett LH. Estimating wealth effects without expenditure data—or tears: an application to educational enrollments in states of India. Demography **2001**; 38:115–32.
27. WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO expert committee. WHO Tech Rep Ser **1995**; 854: 1–452.
28. Bonfoh B, Raso G, Koné I, et al. Research in a war zone. Nature **2011**; 474:569–71.
29. Desai M, ter Kuile FO, Nosten F, et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis **2007**; 7:93–104.
30. Snow RW, Craig M, Deichmann U, Marsh K. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. Bull World Health Organ **1999**; 77:624–40.
31. Magalhães RJ, Clements ACA. Mapping the risk of anaemia in preschool-age children: the contribution of malnutrition, malaria, and helminth infections in West Africa. PLoS Med **2011**; 8:e1000438.
32. Langhorne J, Ndungu FM, Sponaas AM, Marsh K. Immunity to malaria: more questions than answers. Nat Immunol **2008**; 9:725–32.
33. Noor AM, Mutheu JJ, Tatem AJ, Hay SI, Snow RW. Insecticide-treated net coverage in Africa: mapping progress in 2000–07. Lancet **2009**; 373:58–67.
34. Menendez C, Kahigwa E, Hirt R, et al. Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. Lancet **1997**; 350:844–50.
35. Sazawal S, Black RE, Ramsan M, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet **2006**; 367:133–43.
36. Cercamondi CI, Egli IM, Ahouandjinou E, et al. Afebrile *Plasmodium falciparum* parasitemia decreases absorption of fortification iron but does not affect systemic iron utilization: a double stable-isotope study in young Beninese women. Am J Clin Nutr **2010**; 92:1385–92.
37. Chang KH, Stevenson MM. Malarial anaemia: mechanisms and implications of insufficient erythropoiesis during blood-stage malaria. Int J Parasitol **2004**; 34:1501–16.
38. Prual A, Daouda H, Develoux M, Sellin B, Galan P, Hercberg S. Consequences of *Schistosoma haematobium* infection on the iron status of schoolchildren in Niger. Am J Trop Med Hyg **1992**; 47: 291–7.
39. Olsen A, Magnussen P, Ouma JH, Andreassen J, Friis H. The contribution of hookworm and other parasitic infections to haemoglobin and iron status among children and adults in western Kenya. Trans R Soc Trop Med Hyg **1998**; 92:643–9.
40. Stoltzfus RJ, Chwaya HM, Albonico M, Schulze KJ, Savioli L, Tielsch JM. Serum ferritin, erythrocyte protoporphyrin and hemoglobin are valid indicators of iron status of school children in a malaria-holoendemic population. J Nutr **1997**; 127:293–8.
41. Koulaouzidis A, Said E, Cottier R, Saeed AA. Soluble transferrin receptors and iron deficiency, a step beyond ferritin. A systematic review. J Gastrointestin Liver Dis **2009**; 18:345–52.
42. Verhoef H, West CE, Ndeto P, Burema J, Beguin Y, Kok FJ. Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria. Am J Clin Nutr **2001**; 74:767–75.
43. Grant FK, Suchdev PS, Flores-Ayala R, et al. Correcting for inflammation changes estimates of iron deficiency among rural Kenyan preschool children. J Nutr **2012**; 142:105–11.
44. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta **2012**; 1823:1434–43.
45. Powers HJ. Riboflavin (vitamin B-2) and health. Am J Clin Nutr **2003**; 77:1352–60.
46. Rohner F, Zimmermann MB, Wegmüller R, Tschannen AB, Hurrell RF. Mild riboflavin deficiency is highly prevalent in school-age children but does not increase risk for anaemia in Côte d'Ivoire. Br J Nutr **2007**; 97:970–6.
47. Mejia LA, Chew F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. Am J Clin Nutr **1988**; 48:595–600.
48. Garcia-Casal MN, Layrisse M, Solano L, et al. Vitamin A and beta-carotene can improve nonheme iron absorption from rice, wheat and corn by humans. J Nutr **1998**; 128:646–50.
49. Walczyk T, Davidsson L, Rossander-Hulthen L, Hallberg L, Hurrell RF. No enhancing effect of vitamin A on iron absorption in humans. Am J Clin Nutr **2003**; 77:144–9.
50. Jiang S, Wang CX, Lan L, Zhao D. Vitamin A deficiency aggravates iron deficiency by upregulating the expression of iron regulatory protein-2. Nutrition **2012**; 28:281–7.
51. Taylor CE, Higgs ES. Micronutrients and infectious diseases: thoughts on integration of mechanistic approaches into micronutrient research. J Infect Dis **2000**; 182(Suppl 1):S1–4.