Relative Contributions of Malaria, Inflammation, and Deficiencies of Iron and Vitamin A to the Burden of Anemia during Low and High Malaria Seasons in Rural Zambian Children

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Objective To estimate the burden of anemia attributable to malaria, inflammation, and deficiency of iron or vitamin A during low and high malaria seasons among Zambian children.

Study design From a cohort of children (n = 820), 4-8 years of age participating in a randomized controlled trial of pro-vitamin A, we estimated attributable fractions for anemia (hemoglobin of <110 or 115 g/L, by age) owing to current malaria or inflammation (C-reactive protein of >5 mg/L, or α-1 acid glycoprotein of >1 g/L, or both), and current or prior iron deficiency (ID; defined as low ferritin [<12 or 15 μg/L for age <5 or >5 years] or functional ID [soluble transferrin receptor of >8.3 mg/L] or both) and vitamin A deficiency (retinol of <0.7 μmol/L), during low and high malaria seasons, using multivariate logistic regression. Serum ferritin, soluble transferrin receptor, and retinol were adjusted for inflammation.

Results The burden of anemia independently associated with current malaria, inflammation, ID, and vitamin A deficiency in the low malaria season were 12% ($P < .001$), 6% ($P = .005$), 14% ($P = .001$), and 2% ($P = .07$), respectively, and 32% ($P < .001$), 15% ($P < .001$), 10% ($P = .06$), and 2% ($P = .06$), respectively, in the high malaria season. In both seasons, functional ID was independently associated with more anemia (approximately 11%) than low ferritin (approximately 4%). Anemia and ID in the low malaria season, accounted for 20% ($P < .001$) and 4% ($P = .095$) of the anemia in the subsequent high malaria season.

Conclusions Anemia in this population is strongly linked to malaria, inflammation, and functional ID, and to a lesser extent, low iron stores. Integrated control strategies are needed. *(J Pediatr 2019;213:74-81)*.

Despite increased global commitment to anemia control in the last 2 decades, the global burden of anemia in children has remained largely unchanged, decreasing only slightly from 47% to 43% in the last 2 decades.[1,2] The burden of anemia is especially high among children in South East Asia and sub-Saharan Africa. The proximal causes of anemia in these regions include infections, deficiencies of iron, and to a lesser extent, vitamin A, and hemoglobinopathies.[2,3] Unfortunately, evidence is lacking regarding the relative contributions of these causes to the burden of anemia, challenging efforts to prevent and alleviate it. Historically, global policies and programs have been guided by a premise that approximately 50%-60% of the burden of anemia is attributable to iron deficiency (ID).[2,4,5] However, the evidence base for this assumption is inconsistent[6-8] and the emerging evidence from controlled trials and observational studies suggests that the contribution of ID to the overall burden of anemia may be lower than previously assumed.[7,9,10] In addition, evidence on the burden of other nutritional anemias is lacking. In particularly, the anemia of vitamin A deficiency (VAD) is of interest in malaria endemic regions because marginal or deficient vitamin A status is associated with impaired erythropoiesis, and may exacerbate anemia risk through its adverse effect on clinical malaria outcomes.[11-13]

Anemia associated with malaria is a well-documented public health problem in sub-Saharan Africa,[14] where >90% of malaria deaths are concentrated.[15] Acute malaria episodes are characterized by the destruction of large numbers of both parasitized and nonparasitized red blood cells during the erythrocyte phase of

AGP	α1-Acid glycoprotein
BRINDA	Biomarkers Reflecting Inflammation and Nutritional Determinant of Anemia
CRP	C-reactive protein
ID	Iron deficiency
PAF	Population attributable fraction
sTfR	Soluble transferrin receptor
RDT	Rapid diagnostic test
TDRC	Tropical Disease Research Center
VAD	Vitamin A deficiency

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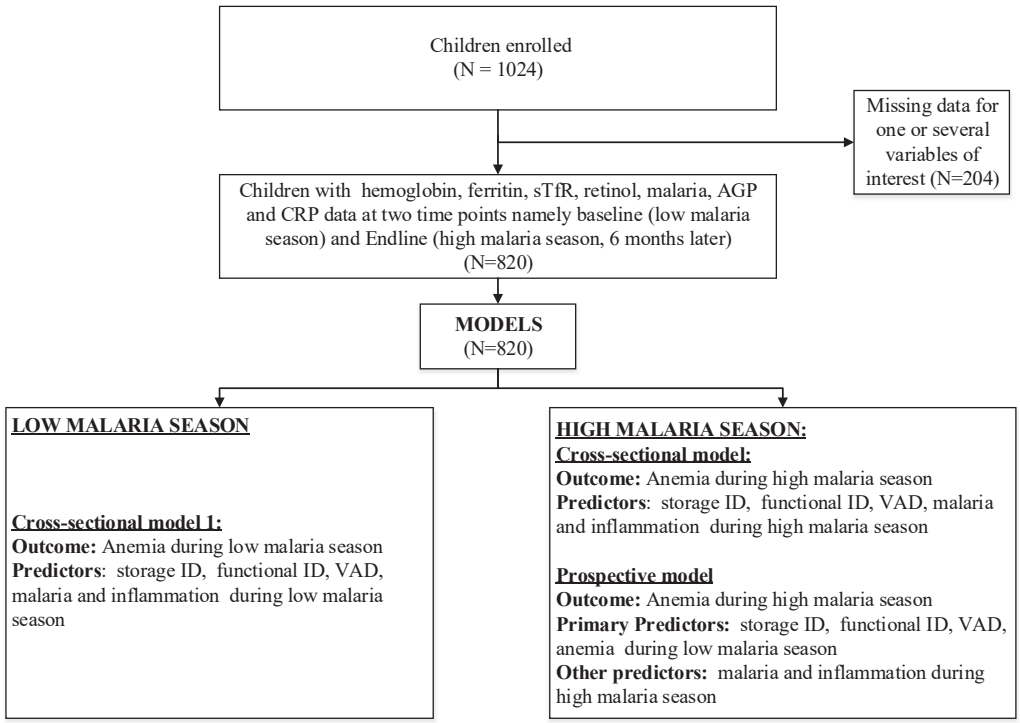

Figure. Flow chart showing the selection of study participants and the models used in estimating cause-specific PAFs for anemia. Anemia is defined as a hemoglobin of <110 g/L for children <60 months and <115 g/L in older children. Storage ID is defined as a ferritin of <12 μg/L in children <5 years and a ferritin of <15 μg/L in older children. Functional ID (elevated sTfR) is defined as an sTfR of >8.3 mg/L.[30] VAD is defined as serum retinol concentration of <0.7 μmol/L. Inflammation categories were defined as reference (AGP ≤ 1 g/L and CRP ≤ 5 mg/L), incubation (AGP ≤ 1 g/L and CRP > 5 mg/L), early convalescence (AGP > 1 g/L and CRP ≤ 5 mg/L), and late convalescence (AGP ≥ 1 g/L and CRP ≥ 5 mg/L), as proposed by Thurnham et al.[30]

the parasite's life cycle.[16-21] Malaria may also contribute indirectly to the burden of anemia through accompanying systemic inflammation.[20-22] Inflammation disrupts the process of erythropoiesis and it is associated with functional ID, characterized by iron sequestration in hepatocytes and macrophages.[23] Hence, in malaria endemic regions in particular, additional evidence is needed to determine how much of the anemia burden may be decreased by iron supplementation programs compared with malaria control. This information is especially important in light of evidence suggesting that universal iron supplementation, without concurrent control of malaria infections, may have adverse consequences for child health.[24]

Part of the challenge in generating reliable cause-specific population attributable fractions (PAF) for anemia is the lack of quality data on etiologic factors. Global burden of anemia estimates are often generated from cross-sectional national surveys,[2,5,10] which typically do not collect biochemical data on risk factors. In addition, cause-specific PAFs estimated from cross-sectional surveys, without appropriate adjustments for the other known risk factors, may be inappropriate. We report estimates of the cause-specific PAF for childhood anemia with respect to malaria, inflammation, and prior or current ID and VAD, using both cross-section and prospective models, across 2 malaria seasons in rural Zambia.

Methods

Ethical Approval

Ethical approval was obtained from the Institutional Review Board of the Johns Hopkins University Bloomberg School of Public Health (Baltimore, Maryland), and the Ethics Review Committee of the Tropical Disease Research Center (TDRC), Ndola, Zambia.

Study Design

This study was designed to estimate the PAFs for anemia with respect to current or prior ID or VAD (**Figure**). In addition, we also estimated the cause-specific attribution fractions for anemia owing to current malaria or inflammation. In the current status approach, both anemia and predictors were assessed at the same time (ie, either in the low malaria season or the high malaria season). In the prior status approach, cause-specific PAF for anemia in the high malaria season was estimated using ID and VAD status determined in the low malaria season (ie, 6 months prior).

Sample Size and Power Considerations

Analyses was restricted to children (n = 820) who had complete baseline and endline data for hemoglobin, ferritin, soluble transferrin receptor (sTfR), malaria, C-reactive protein (CRP), and α1-acid glycoprotein (AGP). Our sample size of 820 enabled the detection of a difference in anemia prevalence of approximately 5 percentage points between children with and without malaria or between children with ID and without ID with power of 80% and 5% type I error rate assuming an anemia burden of 50% among malaria cases and an equal proportion among children with ID.

Subjects and Data Collection

This study included 4- to 8-year-old children from rural communities in Mkushi District, Central Province, Zambia, an area with a high burden of malaria and anemia. The data used in this study were collected as part of a cluster-randomized, controlled trial designed to evaluate the efficacy of provitamin A carotenoid biofortified maize meal consumption in improving the vitamin A status of children (ClinicalTrials.gov: NCT01695148). We included in this analysis the cohort of children (n = 820) who had hemoglobin, ferritin, sTfR, malaria, CRP, and AGP assessed at 2 time points representing the low and high malaria seasons. Details of the parent study have been published elsewhere.[25] At both time points, approximately 7 mL of venous blood was collected (Monoject sterile tubes with no additives; Covidien, Mansfield, Massachusetts). Capillary hemoglobin was assessed using a Hemocue 201 Photometer (Angelholm, Sweden). A malaria rapid diagnostic test (RDT; SD Bioline Malaria Ag P.f, Standard Diagnostics, Yongin, South Korea; 05FK50) was used to test for *Plasmodium falciparum* parasitemia in the field. Children who tested positive by RDT were treated with Coartem in accordance with Zambian guidelines. All children were given an insecticide-treated bed net at the baseline visits. In addition, thick and thin venous blood films were prepared using approximately 2 μL and 10 μL of blood, respectively. Blood collection tubes were immediately put into cooler boxes containing ice packs, allowed to clot, and transported to field laboratories for separation into serum by centrifugation. Serum was aliquoted into prelabeled cryovials and stored in liquid nitrogen until transported to the TDRC laboratory in Ndola, Zambia (approximately 120 km away), where samples were kept at −80°C until analyzed. We also collected socioeconomic and morbidity data, and measured anthropometric status, including height to the nearest 0.1 cm using a Shorr board (Weight and Measure, Olney, Maryland) and weight to the nearest 0.1 kg with a SECA 874 digital scale (SECA, Hamburg, Germany). We measured axillary temperature with a digital thermometer and referred children with high fever (axillary temperature >39.5°C) to the nearest health center.

Laboratory Analyses

Malaria microscopy was performed at the TDRC laboratory. Slides were stained with 3% Giemsa, washed, dried, and read by 2 independent microscopists. Where necessary, a third independent reading was done to resolve discordant results. Parasite density was estimated using thick films by counting the number of parasites per 200 white blood cells. A slide was ruled negative if no parasites were detected after counting 200 oil immersion fields. The number of parasites per 1 μL of blood was calculated by assuming 8000 white blood cells/μL of blood. For each positive reading, the corresponding thin film was read to determine the *Plasmodium* species. Commercial enzyme-linked immunosorbent assay kits were used to determine serum concentrations of AGP (Abcam, Cambridge, Massachusetts; catalog # ab108854), ferritin (Ramco Laboratories, Inc, Stafford, Texas; catalog # S-22), and sTfR (Ramco Laboratories Inc; catalog # TFC-94) at TDRC. Reversed-phase high-performance liquid chromatography was used in the determination of serum retinol concentration (Craft Technologies Inc, Wilson, North Carolina). CRP was measured on an Immulite analyzer (Immulite 1000; Siemens Medical Solutions Diagnostics, Tarrytown, New York; LKCRP1).

Definitions

Anemia was defined as a hemoglobin of <110 g/L for children <5 years and <115 g/L in older children.[26] Low iron store was defined as a ferritin of <12 μg/L in children <5 years and a ferritin of <15 μg/L in older children.[26] Functional ID was defined as an sTfR of >8.3 mg/L.[27] Children were considered to have malaria if they had *P falciparum* parasitemia of any density as defined by either microscopy or RDT or both, and malaria negative if both RDT and microscopy were negative. This combined malaria definition was implemented because we observed that both RDT-defined and microscopy-defined malaria were associated with anemia. VAD was defined as a serum retinol of <0.7 μmol/L. Four inflammation categories were defined: reference (AGP of ≤1 g/L and CRP of ≤5 mg/L), incubation (AGP of ≤1 g/L and CRP of >5 mg/L), early convalescence (AGP of >1 g/L and CRP of >5 mg/L), and late convalescence (AGP of >1 g/L and CRP ≤ of 5 mg/L), as proposed by Thurnham et al.[28,29] We defined stunting and underweight as height-for-age and weight-for-age z-scores below −2 SD of the World Health Organization Growth Reference.[30,31]

Adjustment of Ferritin, sTfR, and Retinol for Inflammation

We previously showed that biomarkers of iron and vitamin A status were affected by systematic inflammation in this population.[32,33] In defining iron or vitamin A status, the measured and inflammation-adjusted biomarker concentrations were used separately. The correction for ferritin and sTfR concentration was based on a linear regression approach [(Y (adjusted biomarker) = Measured biomarkers − β1 (CRPobs − CRPref) − β2 (AGPobs − AGPref)] as proposed by the Biomarkers Reflecting Inflammation and Nutritional Determinant of Anemia (BRINDA)[34] collaborative group. CRP and AGP reference concentrations of 0.59 mg/L and 0.1 g/L, respectively, were adopted as proposed by the BRINDA[7,35,36] initiative were adopted. STfR at the

endline (high malaria season) was not adjusted for inflammation because its association with both and CRP was not statistically significant. For retinol, correction for inflammation was done using a previously determined best fit model for this population.[32] This model used only CRP, instead of both CRP and AGP, to estimate the regression coefficients. In this CRP-only model, 2 levels of inflammation were defined (namely, CRP of <5-15 mg/L and CRP of >15 mg/L). The estimated coefficient for each level was subsequently applied to the measured retinol concentration to generate the inflammation-adjusted retinol concentrations.

Statistical Analyses

Summary baseline characteristics were presented as means and prevalence estimates (Table I). To test the changes in anemia and predictor variables, between the low and high malaria season, paired *t* test (for continuous variables) and the McNemar test (for categorical outcomes) were used (Tables II and III). We used the marginal homogeneity test to determine the difference in inflammatory categories across the 2 malaria seasons.

To estimate the population attribution fractions, 3 multivariate logistic regression models, including 2 cross-sectional models and 1 prospective model, were used to estimate the burden of anemia attributable to malaria, inflammation, low ferritin, functional ID (based on sTfR) and VAD (Figure). In the cross-sectional models, anemia and the predictor variables were assessed at the same time. Separate cross-sectional models were constructed for the low and high malaria seasons respectively. In the prospective model, we assessed the burden of anemia in the high malaria season that was attributable prior to the status of predictor variables (ie, low ferritin, functional ID, and VAD) as assessed 6 months earlier (in the low malaria season). In the prospective models, we also included prior anemia (ie, anemia in the low malaria season), current malaria, and current inflammation as additional covariates. In all models, the nutritional status markers (ie, ferritin, sTfR, and retinol) were adjusted for inflammation using the procedures as described.

Following each logistic regression model, we used the PUNAF function in Stata (StataCorp, College Station, Texas) to estimate the population unattributable fractions and, then, the PAF for anemia with respect to each covariate (ie, log (population unattributable fraction) = log [1 – logPAF]). The PAF estimations are based on the recommendation of Greenland and Drescher for estimating PAFs in cohort and cross-sectional studies[37] (PAF = [(P × (RR – 1)/(P × (RR – 1) + 1]), where P is the prevalence of the particular exposure or predictor variable and RR is the relative risk or OR comparing the risk of the outcome (ie, anemia) in the exposed to unexposed. The estimate the combined PAF for iron status, ID, was redefined as low ferritin and/or elevated sTfR and then rerunning the models. A positive PAF is interpreted as the proportion of anemia that would be prevented if a particular exposure (eg, malaria or ID) is eliminated. We assessed the presence of collinearity among the variables included in the regression model and found no evidence of

Table I. Baseline characteristics of Zambian children assessed for etiology of anemia

Descriptions	No.	Value
Household characteristics		
Literate household head	789	658 (83.4)
Household with electricity	809	40 (4.9)
Child characteristics		
Age, months	820	68.3 ± 15.0
Age <60 months	820	285 (34.8)
Female	820	408 (49.8)
Nutritional status		
Weight, kg	797	17.7 ± 3.3
Height, cm	796	107.4 ± 9.3
Stunted*	797	230 (28.9)
Morbidity history		
Fever in past 2 weeks	808	231 (28.6)
Cough in the past 2 weeks	810	461 (56.9)
Diarrhea in the past 2 weeks	809	47 (5.8)

Values are arithmetic number (%) or mean ± SD.
*Stunting defined as height-for-age of <−2 SDs of the World Health Organization Growth Reference (World Health Organization, 2006). Analyses restricted to children (n = 820) who had complete baseline and endline data for hemoglobin, ferritin, sTfR, malaria, CRP, and AGP.

Table II. Prevalence of anemia, malaria, inflammation and iron or vitamin deficiencies in Zambian children aged 4-8 years during low- and high-malaria transmission seasons (n = 820)

Indicators	Low malaria season (September 2012)	High malaria season (March 2013)	*P* value
Hemoglobin, g/dL	11.7 ± 1.3	11.8 ± 3.7	.413
Anemia	274 (33.4)	330 (40.2)	<.001
Malaria	174 (21.2)	414 (50.5)	<.001
RDT positive	161 (19.6)	401 (48.9)	<.001
Microscopy positive	110 (13.7)	197 (24.0)	<.001
Inflammation			
AGP > 1.0 g/L	363 (44.2)	604 (73.7)	<.001
CRP > 5.0 mg/L	140 (17.1)	263 (32.1)	<.001
Stage of inflammation			
Reference	417 (50.9)	205 (25.0)	
Incubation	40 (4.9)	11 (1.3)	<.001
Early convalescence	263 (32.1)	352 (42.9)	
Late convalescence	100 (12.2)	252 (30.7)	
Ferritin, μg/L	43 ± 3	88 ± 3	<.001
Low ferritin	59 (7.2)	37 (4.5)	.004
Low Ferritin adjusted	97 (11.8)	147 (17.9)	
sTfR, mg/L	7.0 ± 1.5	9.2 ± 1.9	<.001
Functional ID	201 (27.0)	399 (53.6)	<.001
Functional ID adjusted	156 (19.0)	–	–
Vitamin A Status			
VAD	86 (10.5)	130 (15.9)	<.001
Adjusted VAD	48 (5.9)	50 (6.1)	.8137

Data are number (%) or mean (geometric for ferritin and sTfR, arithmetic for hemoglobin) ± SD, unless otherwise specified.
Statistical test of difference across season done with paired *t* test for continuous variables and McNemars or marginal homogeneity test for categorical variables. Anemia is defined as a hemoglobin of <110 g/L for children <60 months and <115 g/L in older children.[28] Storage ID is defined as a ferritin of <12 μg/L in children <5 years and a ferritin of <15 μg/L in older children.[28] Functional ID (elevated sTfR) is defined as an sTfR of >8.3 mg/L.[29] VAD is defined as a serum retinol concentration of <0.7 μmol/L. Inflammation categories were defined as reference (AGP of ≤1 g/L and CRP of ≤5 mg/L), incubation (AGP of ≤1 g/L and CRP of >5 mg/L), early convalescence (AGP of >1 g/L and CRP of >5 mg/L), and late convalescence (AGP of >1 g/L and CRP of ≤5 mg/L), as proposed by Thurnham et al.[30] Endline sTfr was not significantly associated with inflammation, and hence was not adjusted. Adjusted ID and VAD were based on ferritin, sTfR, and retinol concentrations adjusted for inflammation. Adjustment of ferritin and sTfR was based on linear regression model approach involving both AGP and CRP as proposed by the BRINDA project. Adjustment of retinol was based on CRP alone.[36]

Table III. Anemia prevalence stratified by malaria, iron or vitamin A status, and inflammation in the low and high malaria among rural Zambian children (n = 820)

	Low malaria season			High malaria season		
Predictors*	n	Anemia	*P* value	n	Anemia	*P* value
Malaria						
Negative	646	183 (28.3)		406	102 (25.1)	
Positive	174	91 (52.3)	<.001	414	228 (55.1)	<.001
Ferritin status						
Normal	723	229 (31.2)		673	283 (42.0)	
Low	97	45 (46.4)	.004	147	47 (32.0)	.024
Functional iron status						
Normal	664	193 (29.0)		379	131 (34.6)	
Deficient	156	81 (51.9)	<.001	441	199 (45.1)	.002
Vitamin A status						
Normal	772	251 (32.5)	–	768	308 (39.9)	–
Deficient	48	23 (48.0)	.03	5048	22 (45.8)	.080
Inflammation						
Reference	417	126 (30.2)	–	205	58 (28.3)	–
Incubation	40	20 (50.0)	.012	11	5 (45.5)	.232
Early convalescence	263	47 (47.0)	.002	252	149 (59.1)	<.001
Late convalescence	100	81 (30.8)	.872	352	118 (33.5)	.201

Values are number (%) unless otherwise indicated.
*Anemia is defined as a hemoglobin of <110 g/L for children <60 months and <115 g/L in older children.[28] Low ferritin is defined as a ferritin of <12 μg/L in children <5 years and a ferritin of <15 μg/L in older children.[28] Functional ID (elevated sTfR) is defined as an sTfR of >8.3 mg/L.[29] VAD is defined as a serum retinol concentration of <0.7 μmol/L. Inflammation categories were defined as reference (AGP of ≤1 g/L and CRP of ≤5 mg/L), incubation (AGP of ≤1 g/L and CRP of >5 mg/L), early convalescence (AGP of >1 g/L and CRP of >5 mg/L), and late convalescence (AGP of >1 g/L and CRP of ≤5 mg/L), as proposed by Thurnham et al.[30] Nutritional status indicators are adjusted for inflammation. Endline (high malaria season) sTfR was not significantly associated with inflammation, and hence was not adjusted.

collinearity (**Table IV**; available at www.jpesd.com). Statistical significance was set at a *P* value of <.05. All analyses were conducted with STATA 13 software (StataCorp).

Results

Our analytic dataset included 820 children with a median age of 65 months (IQR, 23 months), and approximately 35% below the age of 5 years. The reported prevalence of fever, diarrhea, and cough at baseline (low malaria season) were 29%, 57%, and 6%, respectively. The prevalence of stunting was 29%, consistent with significant undernutrition in this population.

Table II shows the distribution of anemia, malaria, inflammation, and deficiencies of iron and vitamin A in the low and high malaria seasons. The prevalence of anemia increased from 33% in the low malaria season to 40% during the high malaria season. The prevalence of malaria more than doubled from 21% (low malaria season) to 51% in the high malaria season. Similarly, the proportion of children with systemic inflammation (elevated CRP or AGP) increased from 50% in the low malaria season to 75% in the high malaria season, largely driven by an increase in the proportion of children in the early and late convalescent stages of inflammation. The inflammation adjusted storage ID remained low, increasing only slightly from 11.8% to 17.9% between the 2 seasons, whereas functional ID remained high, doubling from 27% to 54%. The prevalence of VAD was 6% in in both seasons, lower than expected.

The distribution of anemia by the different predictors is presented in **Table III**. In both seasons, more than one-half of children with malaria were anemic, whereas only one-quarter of children without malaria were anemic. After correction for inflammation, the prevalence of anemia was higher in children with low ferritin (48% vs 31%; $P = .01$) in the low malaria season. In the high malaria season, however, the prevalence of anemia in children with low ferritin (32%) was significantly lower ($P = .02$) compared with those with normal ferritin (42%). Functional ID was associated with an approximately 20-percentage point increase in anemia in both seasons ($P < .01$). The association between anemia and inflammation was dependent on the stage of inflammation. In particular, the anemia risk was highest in the early convalescent stage, in both the low (47%) and high (59%) malaria seasons. VAD was associated with a 10- to 13-percentage point increase in anemia.

Malaria, functional ID, inflammation, and to a lesser extent VAD emerged as the determinants of anemia based on the cross-sectional models (**Table V** and **Table VI**; **Table VI** available at www.jpeds.com). Current malaria was the single most important determinant of anemia in this population, independently associated with approximately 12% and approximately 30% of anemia in the low and high malaria seasons, respectively. Current functional ID was independently associated with approximately 11% of the anemia burden in both the low and high malaria season, whereas current low ferritin was independently associated with 4% of the burden of anemia risk in both season. The early convalescence phase of inflammation was independently associated with 6% and 14% of anemia in the low and high malaria seasons, respectively. The contribution from current VAD was

Table V. Estimated population attributable risks for anemia with respect to malaria, inflammation, and iron or vitamin A status during low and high malaria seasons among rural Zambia children

Factors	Cross-sectional	*P* value	Prospective	*P* value
Low malaria season				
Malaria positive	12.4 (6.8, 17.6)	<.001	–	–
Low ferritin	4.8 (1.2, 8.4)	.051	–	–
Functional ID	10.5 (5.5, 15.3)		–	–
Vitamin A deficient	2.3 (0.0, 5.9)	.07	–	–
Inflammation			–	–
Incubation	1.3 (−0.1, 3.6)	.255	–	–
Early convalescence	5.6 (1.7, 9.3)	.005	–	–
Late convalescence	−3.0 (−9.9, 3.4)	.366	–	–
Prior anemia	–	–	–	–
ID combined	14.6 (8.3, 20.5)	<.001		
High malaria season				
Malaria positive	28.7 (18.5, 37.6)	<.001	31.7 (22.2, 40.1)	<.001
Low ferritin	−2.0 (−5.3, 19)	.371	3.5 (0.6, 6.3)	.018
Functional ID	11.5 (3.2, 19.0)	.007	2.0 (−2.0, 5.6)	.326
Vitamin A deficient	2.0 (0.0, 4.0)	.062	−0.5 (−2.5, 1.4)	.588
Inflammation				
Incubation	0.5 (−0.5, 1.5)	.307	0.5 (−0.5, 1.4)	.330
Early convalescence	14.0 (5.8, 21.5)	.001	15.3 (7.3, 22.6)	<.001
Late convalescence	0.7 (−8.3, 9.0)	.859	1.8 (−6.9, 9.7)	.679
Prior anemia	–	–	19.1 (13.2, 24.7)	<.001
ID combined	9.6 (0.0, 18.6)	.06	4.2 (0.0, 8.9)	.095

*Nutritional status indicators are adjusted for inflammation; Endline (high malaria season) sTfr was not significantly associated with inflammation, and hence was not adjusted; In the cross-sectional models, anemia and risk factors were assessed at the same time. In the prospective model, anemia, malaria and inflammation were assessed in the high malaria season, whereas the nutritional risk factors were assessed in the low malaria season (6 months prior). The PAF estimations are based on the recommendation of Greenland and Drescher for estimating population attributable fractions in cohort and cross-sectional studies.[39] (PAF= ((P X (RR-1)/((P X (RR-1)+1)), where P is the prevalence of the particular exposure or predictor variable, and RR is the relative risk or odds ratio comparing the risk of the outcome (i.e. anemia) in the exposed to unexposed. P-values represent significance of PAF estimates. A positive PAF is interpreted as the proportion of anemia that would be prevented if a particular exposure (e.g. malaria or iron deficiency) is eliminated. A negative PAF would imply that eliminating the risk factor would increase the anemia burden. Anemia defined as hemoglobin <110 g/L for children <60 months and <115 g/L in older children.[28] Low ferritin defined as ferritin <12 μg/L in children <5 years and ferritin <15 μg/L in older children.[28] Functional iron deficiency (elevated sTfR) defined as sTfR > 8.3 mg/L.[29] Vitamin A deficiency defined as serum retinol concentration <0.7 μmol/L. Inflammation categories were defined as reference (AGP≤1 g/L and CRP ≤5mg/L), incubation (AGP≤1 g/L and CRP >5mg/L), early convalescence (AGP>1 g/L and CRP >5mg/L) and late convalescence (AGP>1 g/L and CRP ≤5mg/L), as proposed by Thurnham et al.[30]

marginal (only 2%-3%) in either season. In the prospective approach, prior low ferritin and prior anemia status emerged as the only predictors of long-term anemia risk (Table V), accounting for approximately 4% and 20% of the anemia burden in the high malaria seasons, respectively.

Discussion

Our data suggest that malaria (which accounted for ≤30% of anemia) and inflammation (which accounted for ≤14% of anemia) are important determinants of anemia in this population. Although ID accounted for approximately 15% of anemia in both seasons, much of this (approximately 11%) was attributable to functional ID, assessed as elevated sTfR, indicative of a reduction in the availability of iron for erythropoiesis. Low ferritin, current or prior, accounted for only 4% of the burden of anemia, and VAD accounted for an additional 2%-3% in this population. Anemia in the low malaria season accounted for approximately 20% of the anemia burden in the high malaria season, demonstrating the persistence of anemia over time in this population.

The etiology of malarial anemia is complex, emanating primarily from the destruction of red blood cells (both parasitized and nonparasitized) and inflammation-induced dyserythropoiesis, and likely exacerbated by underling ID.[16-19] In this population, more than one-half of malaria cases presented with anemia in both seasons. The importance of malaria and other infections in the etiology of anemia is consistent with the observation of a strong association between anemia and inflammation. The early convalescent phase, the most intense stage of inflammation, had the highest burden of anemia, independent of malaria status. Although our data did not permit the assessment of the specific causes of inflammation, the increase in inflammation-specific PAF, from 6% in the low malaria season to 14% in the high malaria season, suggests that much of the inflammation was driven by current or recent malaria exposure. In light of these findings, the management of malaria in this population, regardless of season, must be designed to also ensure hematologic recovery.

Our data suggest that the relative contribution from micronutrient deficiencies to the overall burden of anemia may be lower than generally perceived. ID, from both low ferritin and functional deficiency accounted for only approximately 15% of anemia in the low malaria season, and only 10% in the high malaria season. Plausible explanations for this finding may include the age of the study population and the definition of iron status. In children, the burden of iron-deficiency anemia typically decreases with age, with a peak prevalence at 6-23 months of age. Hence, the iron-deficiency anemia risk in our study population, which included children 4-8 years of age, was relatively lower than reported in other childhood population.[5,7] Second, defining iron status in the context of infections is challenging, because of the associations between inflammation and the conventional biomarkers of iron status. We have

previously shown in this population that both ferritin and sTfR are substantially increased by inflammation.[33] Hence, storage ID (based on ferritin) may be underestimated, whereas the functional ID (based on sTfR) may be overestimated, without the appropriate adjustment for inflammation. In this study, we adjusted for inflammation by implementing guidelines recommended by the BRINDA project.[7,35,36]

Finally, although VAD increased the risk of anemia, it did not contribute significantly to the population burden of anemia, likely because of the low prevalence (6%). However, considering that VAD was associated with approximately a 10-percentage point increase in the anemia risk, it is plausible that in populations where the prevalence of VAD is higher, VAD may contribute significantly to the burden of anemia.

This study is limited by the lack of data on other known causes of anemia such as helminthes and hemoglobinopathies.[38-40] Evidence from Asia suggests that, in some contexts, hemoglobinopathies may be more relevant to anemia than ID.[9] Additional evidence is needed to assess the contribution of prevalent hemoglobin variants, specifically hemoglobin S, in this region. Another limitation of this study is that the PAF was in part estimated with cross-sectional models. Because the predictors are linked via several biologic pathways, the estimated anemia burden attributable to any one predictor may in fact be driven by another predictor. For instance, because malaria transiently alters the concentrations of ferritin, retinol, and sTfR through the acute phase phenomenon, the proportion of anemia attributable to ID and VAD may be wholly or partially explained by concurrent malaria, as opposed to a state of low dietary intake. This problem was addressed in 2 ways: first, all predictors were added to the models concurrently, ensuring that the PAFs for each predictor were statistically independent of the other predictors; and second, ferritin, sTfR, and retinol were adjusted for inflammation using recommended procedures. However, these corrective measures are based on assumptions deemed generally appropriate for large population surveys, but may not necessarily improve the diagnosis of iron or vitamin A status at the individual level. Because the malaria-specific effect on anemia is largely an acute, direct phenomenon, it is less likely that the estimated malaria-specific PAF is biased by the use of cross-sectional models.[17,19] In addition, the estimated PAF for functional iron status likely reflects an independent effect because sTfR was either minimally affected or unaffected by inflammation.

Our data suggest that anemia in this population may be persistent. About 60% of children who had anemia at baseline also had anemia during the follow-up, compared with only 40% children who were without anemia at baseline. Preexisting anemia (ie, in the low malaria season) accounted for 20% of the overall anemia burden in the high malaria transmission season, suggesting a need to scale up anemia control efforts. The current World Health Organization recommendation for managing anemia in the context of malaria is to couple iron supplementation with measures to prevent and treat the underlying infections. Our findings suggest a greater focus on prompt diagnosis and treatment of infections may be optimal in reducing the burden of anemia in this age group. ■

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Table IV. Test of multicollinearity among variables included in the logistic regression models used for estimating PAFs for anemia

Malaria season	Anemia	Malaria	Inflammation	Low ferritin	Elevated sTfR	VAD	Mean VIF
Low							
Anemia	1.000						
Malaria	0.2078	1.0000					
Inflammation	0.0781	0.1159	1.0000				
Low ferritin	0.1880	0.1138	0.0348	1.0000			1.59
Elevated sTfR	0.1902	0.1664	0.0527	0.7858	1.0000		
VAD	0.0767	0.1374	0.0375	0.0090	−0.0282	1.0000	
High							
Anemia							
Malaria	0.3053	1.0000					
Inflammation	0.2397	0.4244	1.0000				
Low ferritin	0.0709	0.0379	0.0323	1.0000			1.77
Elevated sTfR	0.1235	0.1125	0.0358	0.8132	1.0000		
VAD	0.0611	−0.0127	0.0175	−0.0040	−0.0027	1.0000	

The values represents the correlation coefficients between the covariates and the Variance Inflation Factor (VIF) for each cross sectional model. The correlation coefficients were estimated separately for the low and high malaria transmission seasons. The mean VIF is specific to each season. A VIF of 10 is suggestive of potential collinearity.

Table VI. Malaria, inflammation, ID, and VAD as predictors of anemia during low and high malaria seasons among rural Zambia children

Season	Cross-sectional	Prospective
Low malaria season*		
Malaria positive	2.4 (1.7, 3.4)	–
Low ferritin	1.9 (1.2, 3.0)	–
Functional ID	2.3 (1.6, 3.3)	–
VAD	1.8 (1.0, 3.4)	–
Inflammation		–
Incubation	1.5 (0.7, 3.0)	–
Early convalescence	0.9 (0.6, 1.2)	–
Late convalescence	2.0 (1.3, 3.2)	–
Prior anemia	–	–
High malaria season*		
Malaria positive	2.7 (1.9, 3.8)	3.3 (2.3, 4.6)
Low ferritin	0.8 (0.6, 1.3)	1.8 (1.1, 2.9)
Functional ID	1.6 (1.1, 2.1)	1.2 (0.8, 1.8)
VAD	1.8 (1.0, 2.4)	0.8 (0.4, 1.6)
Inflammation		
Incubation	2.0 (0.6, 7.1)	2.0 (0.5, 7.5)
Early convalescence	1.0 (0.7, 1.5)	1.1 (0.7, 1.6)
Late convalescence	2.2 (1.4, 3.4)	2.5 (1.6, 3.9)
Prior anemia	–	3.1 (2.2, 4.3)

*Values represent odds ratio of the association between anemia (outcome) and predictor variables. Nutritional status indicators are adjusted for inflammation. Endline (high malaria season) sTfr was not significantly associated with inflammation and was not adjusted. In the cross-sectional models, anemia and risk factors were assessed at the same time. In the prospective model, anemia, malaria, and inflammation were assessed in the high malaria season, whereas the nutritional risk factors were assessed in the low malaria season (6 months prior). Anemia is defined as a hemoglobin of <110 g/L for children <60 months and <115 g/L in older children.[28] Low ferritin is defined as a ferritin of <12 mg/L in children <5 years and a ferritin of <15 mg/L in older children.[28] Functional ID (elevated sTfR) is defined as an sTfR of >8.3 mg/L.[29] VAD is defined as a serum retinol concentration of <0.7 mmol/L. Inflammation categories were defined as reference (AGP of ≤1 g/L and CRP of ≤5 mg/L), incubation (AGP of ≤1 g/L and CRP of >5 mg/L), early convalescence (AGP of >1 g/L and CRP of >5 mg/L), and late convalescence (AGP of >1 g/L and CRP of ≤5 mg/L), as proposed by Thurnham et a.[30]