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Practical Management of HIV-Associated Anemia in Resource-Limited Settings: Prospective Observational Evaluation of a New Mozambican Guideline

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Abstract

Mozambique's updated guideline for management of HIV-associated anemia prompts clinicians to consider opportunistic conditions, adverse drug reactions, and untreated immunosuppression in addition to iron deficiency, intestinal helminthes, and malaria. We prospectively evaluated this guideline in rural Zambézia Province. Likely cause(s) of anemia were determined through prespecified history, physical examination, and laboratory testing. Diagnoses were ''etiologic'' if laboratory confirmed (sputum microscopy, blood culture, *Plasmodium falciparum* malaria rapid test) or ''syndromic'' if not. To assess hemoglobin response, we used serial point-of-care measurements. We studied 324 ambulatory, anemic (hemoglobin <10 g/dl) HIV-infected adults. Study clinicians treated nearly all [315 (97.2%)] for suspected iron deficiency and/or helminthes; 56 (17.3%) had laboratory-confirmed malaria. Other assigned diagnoses included tuberculosis [30 (9.3%)], adverse drug reactions [26 (8.0%)], and bacteremia [13 (4.1%)]. Etiologic diagnosis was achieved in 79 (24.4%). Of 169 (52.2%) subjects who improved (hemoglobin increase of ≥1 g/dl without indications for hospitalization), only 65 (38.5%) received conventional management (iron supplementation, deworming, and/or antimalarials) alone. Thirty (9.3%) died and/or were hospitalized, and 125 (38.6%) were lost to follow-up. Multivariable linear and logistic regression models described better hemoglobin responses and/or outcomes in subjects with higher $CD4^+$ T-lymphocyte counts, pre-enrollment antiretroviral therapy and/or co-trimoxazole prophylaxis, discontinuation of zidovudine for suspected adverse reaction, and smear-positive tuberculosis. Adverse outcomes were associated with fever, low body mass index, bacteremia, esophageal candidiasis, and low or missing $CD4^+$ T cell counts. In this severely resource-limited setting, successful anemia management often required interventions other than conventional presumptive treatment, thus supporting Mozambique's guideline revision.

Introduction

ANEMIA, ONE OF THE ''MOST intractable public health problems in Africa,'' has historically been attributed to three principal causes: nutritional deficiencies, helminth infections, and malaria.[1] Children and women of child-bearing age have comprised the two main target populations for standard anemia control initiatives. The advent of the human immunodeficiency virus (HIV) epidemic has now yielded a third population with a very high anemia burden, but standard international guidelines for first-level health workers in resource-constrained settings have not yet reflected the relevant differences in anemia etiology for HIV-infected vs. uninfected populations.

HIV-infected persons are vulnerable to the common causes of anemia: iron deficiency, intestinal parasites, and malaria.[2–4] Malaria incidence and severity are greater in the presence of HIV, and the prognosis of HIV infection may be altered by concurrent helminth infection and/or deworming.[5–8] Opportunistic and other complications of HIV infection further increase anemia risk. HIV viremia impairs hematopoiesis.[9] Advanced HIV/AIDS disease is associated with lower hemoglobin (Hb) levels.[10–12] HIV-associated immunosuppression increases the incidence of opportunistic conditions associated with anemia, including tuberculosis (TB), non-Hodgkin's lymphoma, oral candidiasis, chronic diarrhea, and Kaposi's sarcoma.[9,13–17] HIV-associated anemia confers an

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increased risk of mortality.[10,18–22] Though combination antiretroviral therapy (ART) generally improves anemia, specific antiretroviral drugs may exacerbate it, and persistent anemia after ART initiation is also associated with higher mortality. [19,23–26]

In HIV-infected persons, HIV-associated causes of anemia may be more prevalent than the ''usual'' causes of anemia.[12,27] In hospitalized Malawian adults with severe anemia, for example, mycobacterial infections and bacteremia were the predominant causes in HIV-infected patients, while iron deficiency and hookworm prevailed in HIV-uninfected people.[28] Thus, in the presence of HIV, the differential diagnosis of anemia may be broader, and prior probabilities of possible causes may differ. In resource-limited settings characterized by large caseloads and scarce diagnostics, identification of the etiology of anemia in an HIV-infected individual may be difficult. Diagnosis is further complicated by the possibility of multiple concurrent conditions that simultaneously depress hemoglobin levels.

Mozambique is a low-income nation with a high prevalence of anemia in its HIV-infected population.[29] The median Hb level was 9.9 g/dl in adults upon enrollment in HIV care in rural Zambézia Province, and Hb was <8.0 g/dl in 25% of TB/HIV patients at ART initiation.[30,31] Malaria and intestinal helminthes are prevalent, and iron-deficiency anemia is thought to be common.[32–34]

During Mozambique's initial response to the HIV epidemic, first-level health workers used a 2005 Mozambican guideline adapted from a standard World Health Organization guideline recommending that anemia (or pallor) be treated with presumptive iron supplementation, anthelminthics, and antimalarials.[35] In 2009, the Mozambican Ministry of Health (MISAU) updated its anemia guideline to address several concerns: overprescription of antimalarials, underdetection of treatable HIV-associated causes of anemia, and possible increases in malaria and mortality risk resulting from overprescription of iron.[36–39] The new anemia guideline (Fig. 1), designed for nonphysician clinicians providing HIV/AIDS care in resource-limited peripheral health centers, required hemoglobin and malaria testing and consideration of an expanded differential diagnosis that included TB, adverse drug reactions (ADRs), and HIV/AIDS Stage III anemia.[11] Presumptive therapy with ferrous sulfate, folic acid, and anthelminthics was permitted. The anemia guideline focused only on initial management.

In 2012, we conducted a prospective observational study (trial registration NCT 01681914, www.clinicaltrials.gov) in rural Zambézia Province (the estimated HIV prevalence based on 2009 survey data was 12.6% in adults 15–49 years old),[40,41] with the goal of describing the performance of the new Mozambican anemia guideline when applied to the management of ambulatory, HIV-infected anemic adults in MISAU peripheral health facilities. We sought to determine the proportion of anemia cases that could be ascribed to specific cause(s) and the proportion improving with guideline-driven management, and to describe correlates of response to therapy.

Materials and Methods

HIV-infected ambulatory patients ≥18 years of age presenting for scheduled (routine) or unscheduled care at three participating health centers, with current Hb <10 g/dl, were eligible (Fig. 2). All three health centers provided ongoing HIV/AIDS care, including ART. Random sampling was not logistically feasible. Clinic staff reviewed arriving patients' medical records in the outpatient HIV/AIDS clinic and in the prevention of mother-to-child transmission of HIV (PMTCT) clinic to identify those overdue for Hb testing. Records were reviewed approximately in the order in which patients arrived. Study laboratory technicians assisted site-based laboratories with Hb measurement, and study coordinators determined eligibility. Potential subjects were excluded if they presented with rising Hb, current ferrous sulfate treatment for anemia, inability to communicate in Portuguese or Echuabo (the major local language), or guideline-defined clinical ''danger signs'' (Hb <5 g/dl, shock, congestive heart failure, severe pallor, hemorrhage, acute abdomen, or obstetrical emergency) based on rapid preenrollment screening. Pregnant women and patients newly diagnosed with HIV were eligible. Study staff screened patients on weekdays, enrolling up to five patients per site per day. Recruitment began in April, during the peak malaria season, and ended in August, when malaria transmission still persisted but had declined from its seasonal peak.

Our general methods have been described previously in the context of a parallel study.[42,43] Study coordinators recorded subjects' demographic and medical information. Study clinicians reviewed medical histories (including TB screening questions) and laboratory data, conducted physical examinations, and determined the most likely cause(s) of each subject's anemia. Diagnoses assigned by study clinicians were defined as ''etiologic'' if confirmed by laboratory methods (smear-positive pulmonary TB, culture-confirmed bacteremia, and rapid-test confirmed *Plasmodium falciparum* malaria) or ''syndromic'' if not laboratory confirmed. The syndromic diagnosis of adverse drug reaction was based on Mozambican national guidelines, derived in turn from World Health Organization standards.[44,45] Study clinicians also addressed patients' other signs or symptoms, and managed ART and co-trimoxazole prophylaxis. The first follow-up Hb was usually measured 30 days after the enrollment visit and sooner if indicated. Community outreach workers visited subjects at home if they defaulted for scheduled visits or if blood cultures were positive, unless subjects specifically declined home visits.

Preplanned study endpoints were improvement (defined as a 1 g/dl increase in Hb either from enrollment or from Hb nadir), hospitalization, death, or loss to follow-up (LTFU). Subjects who were hospitalized with increasing Hb levels were classified as hospitalized. Hospitalization was defined as an endpoint because nonphysician clinicians were not authorized to provide in-patient care, and the anemia guideline was not meant for in-hospital use.

All subjects were tested for anemia and malaria at enrollment and retested at subsequent visits when indicated. Hemoglobin was measured using the HemoCue™ Hb 201+analyzer (HemoCue Inc., Lake Forest, CA). We conducted quality control using blood specimens with known hemoglobin concentrations as measured by a Sysmex KX21N™ (Sysmex Corporation, Kobe, Japan) automated hematology analyzer because of global stockouts of HemoCue quality-control reagents. Eightcheck-3WP/X-Tra™ controls (Sysmex America, Lincolnshire, IL) were run daily to ensure the function of the Sysmex KX21N, except when Sysmex autoanalyzers were not

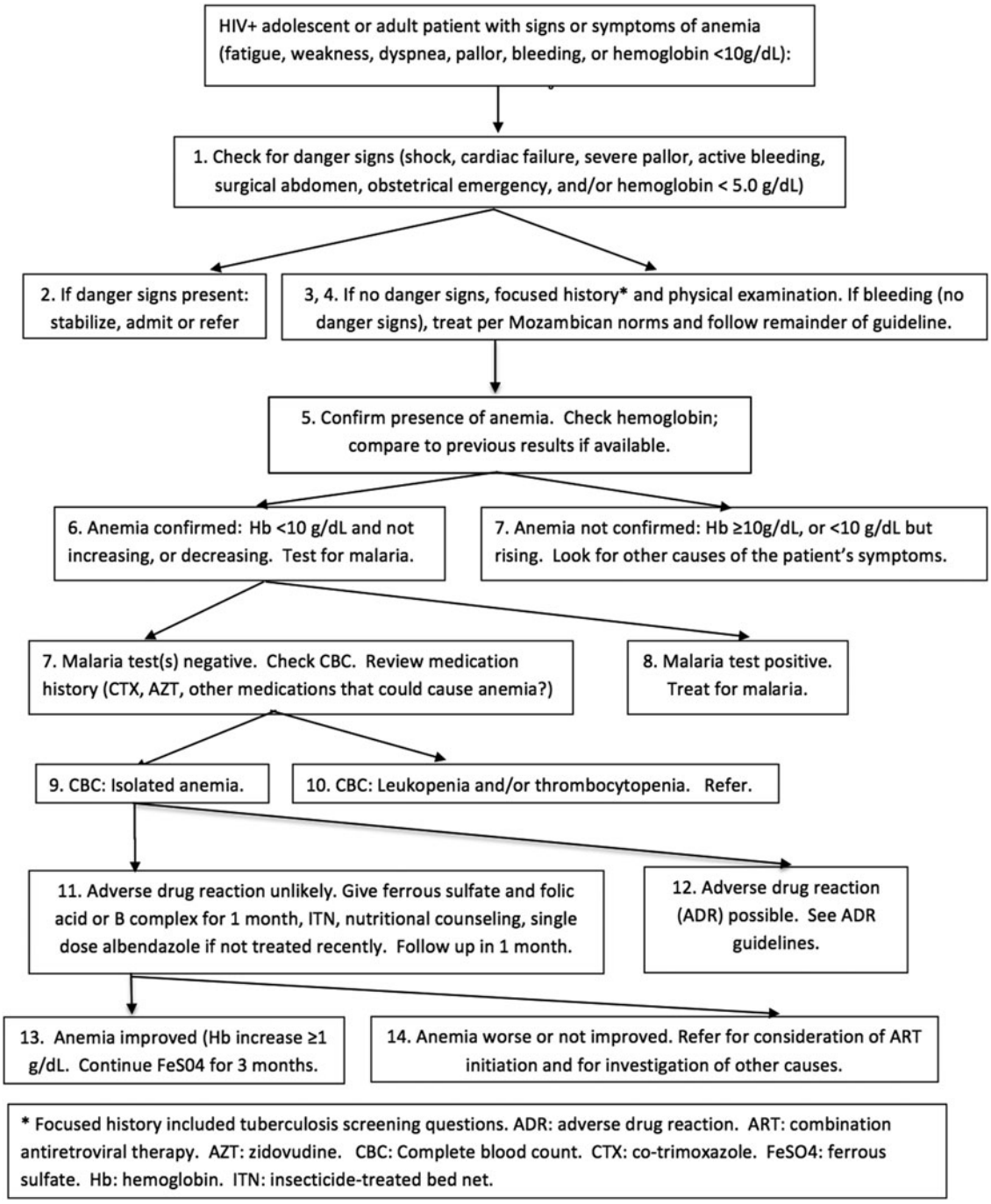

FIG. 1. English translation (by the authors) of current Mozambican HIV/anemia guidelines.

functioning (for example, during reagent stockouts). Peripheral blood was tested for malaria antigenemia using the rapid ICT Malaria *Plasmodium falciparum* assay (ICT Diagnostics, Sydney, Australia). Positive tests were followed by light microscopy examination of thick and thin Giemsa-stained smears; three microscopists read each smear, although only the first reading was available at the time of the patient encounter.

For patients concurrently eligible for a parallel study of Mozambican fever guidelines,[43] blood culture specimens (one set per subject) were collected at the first fever-study visit. We have described our methods for pathogen identification, antibiotic susceptibility testing, and quality control of blood cultures elsewhere.[42]

$CD4^+$ T-lymphocyte (CD4) testing, complete blood counts (CBCs), and sputum microscopy for acid-fast bacilli (AFB) could also be ordered by study clinicians. These were not study procedures, although the study team provided quality assurance for CBCs and sputum AFB smears read by health center staff. Laboratory tests for iron and other micronutrient deficiencies, and for hemoglobinopathy, were not available.

Our target sample size was 324 subjects. We estimated that a cause of anemia would be identifiable in 60%. With 20%

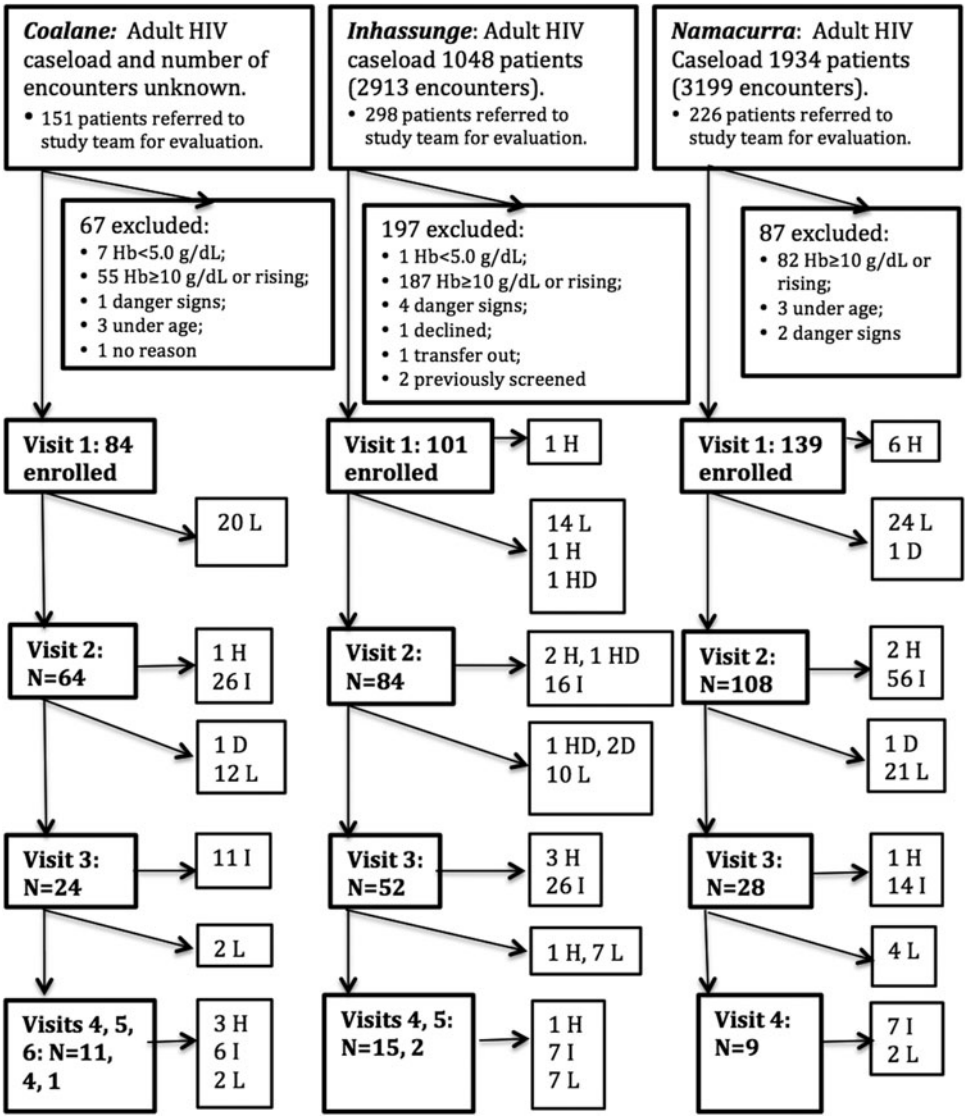

FIG. 2. Screening, eligibility, enrollment, and outcomes, by study site. Adult HIV caseload is the number of HIV-infected adults known to be in care at each site during the study period. Number of encounters is the estimated number of outpatient encounters for HIV-infected adults at each site during the period of recruitment. H, hospitalized; D, died; HD, died after hospitalization; L, lost to follow-up; I, improved. *Diagonal lines*: events between scheduled study visits.

LTFU, if the true proportion of anemia cases with diagnosable/classifiable causes was 0.60, the 95% confidence interval (CI) would be 0.54, 0.66, and the lower limit of the CI would exclude 0.50.

We used proportions and medians to describe subject characteristics at enrollment. We evaluated correlates of Hb response with linear regression, and correlates of adverse outcomes with logistic regression models. To evaluate the first primary outcome (proportion of patients who were assigned an etiologic or syndromic diagnosis of anemia cause), we reviewed study instruments to identify diagnoses assigned by study clinicians. To evaluate the second primary outcome (proportion improving), we calculated the proportion of subjects whose Hb rose by ≥1 g/dl and who were discharged from the study. To characterize associations between subject and treatment characteristics and Hb evolution, we used bivariate and multivariable linear regression with Hb change from enrollment to first follow-up Hb measurement as the dependent variable to assess response to initial management. Because over 100 subjects remained in the study and often acquired additional diagnoses after the first follow-up Hb, we repeated these analyses using Hb change from enrollment (or nadir Hb) to final study Hb to assess response to overall management. Finally, we used bivariate and multivariable logistic regression to describe associations between subject or treatment characteristics and adverse outcomes.

The Mozambican National Bioethics Committee and Vanderbilt's Institutional Review Board approved the study protocol. Literate subjects gave written informed consent; illiterate subjects gave witnessed oral consent.

Results

We screened 675 ambulatory HIV-infected adults for enrollment (Fig. 2) and enrolled 324 (48.0%). Our subjects (Table 1) were predominantly female; the majority were on co-trimoxazole prophylaxis but were not receiving ART in spite of low $CD4^+$ cell counts. Most who had already initiated ART [88/96 (91.7%)] were receiving zidovudine (ZDV)-based regimens.

Table 2 describes diagnoses assigned by the study clinicians (our first primary outcome). Syndromic diagnoses predominated. Three hundred and fifteen subjects (97.2%) were assigned the syndromic diagnosis of iron deficiency (with or

Table 1. Subject Characteristics at Study Entry, Overall and by Study Outcome

Subject characteristics (n = *324*)	*All subjects* (n = *324*) N *(%) or median (IQR)*	*Improved* (*n = 169; 52.2%*) N *(%) or median (IQR)*	*Hospitalized or died* (n = *30; 9.3%*) N *(%) or median (IQR)*	*Lost to follow-up, by hemoglobin trend at last study visit* (n = *125; 38.6%*)		
				LTFU: No second Hb or no change in Hb (n = *70; 21.6%*) N *(%) or median (IQR)*	*LTFU: rising Hb* (n = *37; 11.4%*) N *(%) or median (IQR)*	*LTFU: falling Hb* (n = *18; 5.6%*) N *(%) or median (IQR)*
Demographics						
Study site						
Coalane	84 (25.9)	43 (25.4)	5 (16.7)	27 (38.6)	5 (13.5)	4 (22.2)
Inhassunge	101 (31.2)	49 (29.0)	14 (46.7)	15 (21.4)	16 (43.2)	7 (38.9)
Namacurra	139 (42.9)	77 (45.6)	11 (36.7)	28 (40.0)	16 (43.2)	7 (38.9)
Gender, pregnancy						
Male	62 (19.1)	39 (23.1)	10 (33.3)	8 (11.4)	4 (10.8)	1 (5.6)
Female, not pregnant	193 (59.6)	98 (58.0)	19 (63.3)	35 (50.0)	28 (75.7)	13 (72.2)
Pregnant	69 (21.3)	32 (18.9)	1 (3.3)	27 (38.6)	5 (13.5)	4 (22.2)
Age (years)	29 (23, 36)	29 (23, 37)	31 (25, 36)	25 (21, 34)	28 (23, 38)	30 (22, 40)
Illiterate	148 (45.7)	71 (42.1)	13 (43.3)	38 (54.3)	16 (43.2)	10 (55.6)
Did not accept home visits	48 (14.8)	20 (11.8)	2 (6.7)	18 (25.7)	8 (21.6)	0
HIV/AIDS status						
Most recent CD4 count (cells/μl) (73 missing)	249 (112, 436)	300 (150, 509)	153 (86, 235)	229 (85, 419)	190 (121, 469)	189 (70, 324)
Most recent CD4 count (cells/μl), categories						
<200	106 (32.7)	50 (29.6)	15 (50)	19 (27.4)	16 (43.2)	6 (33.3)
200–349	51 (15.7)	26 (15.4)	7 (23.3)	9 (12.9)	5 (13.5)	4 (22.2)
350–499	43 (13.3)	28 (16.6)	1 (3.3)	9 (12.9)	4 (10.8)	1 (5.6)
≥500	51 (15.7)	37 (21.9)	1 (3.3)	6 (8.6)	6 (16.2)	1 (5.6)
None available	73 (22.5)	28 (16.6)	6 (20)	27 (38.6)	6 (16.2)	6 (33.3)
On CTX	237 (73.2)	128 (75.7)	18 (60)	44 (62.9)	32 (86.5)	15 (83.3)
On ART	96 (29.6)	62 (36.7)	8 (26.7)	11 (15.7)	11 (29.7)	4 (22.2)
On ZDV (ART or PMTCT)	114 (35.2)	67 (39.6)	8 (26.7)	25 (35.7)	10 (27.0)	4 (22.2)
Malaria prevention						
Used bed net on night before visit 1 (15 missing)	155 (47.8)	82 (50.6)	13 (46.4)	38 (58.5)	16 (43.2)	6 (35.3)
Indoor residual spraying (7 missing)	100 (30.9)	58 (34.3)	7 (23.3)	20 (28.6)	9 (24.3)	6 (33.3)
Clinical characteristics at enrollment (study visit one)						
Temperature (axillary, °C) (4 missing)	36.7 (36.2, 37.2)	36.6 (36.2, 37.0)	37.5 (37.0, 38.5)	36.7 (36.2, 37.3)	36.6 (36.1, 37.4)	36.6 (36.2, 38.0)
Body mass index (kg/m^2) (9 missing)	19.0 (17.3, 21.2)	19.2 (17.8, 21.1)	17.3 (16.0, 18.3)	19.8 (17.6, 22.0)	19.5 (17.7, 21.5)	17.0 (15.2, 21.1)
Hemoglobin (g/dl) at baseline	8.8 (7.9, 9.6)	8.8 (7.9, 9.4)	8.2 (7.0, 9.0)	8.7 (7.4, 9.6)	9.5 (9.0, 9.8)	9.1 (8.1, 9.7)
Key diagnoses and comorbidities at visit 1 (enrollment visit)						
Smear-positive pulmonary TB	2 (0.6)	1 (0.6)	0	1 (1.4)	0	0
Smear-negative pulmonary TB	13 (4.0)	8 (4.7)	2 (6.7)	3 (4.3)	0	0
TB suspect	26 (8.0)	10 (5.9)	9 (30)	3 (4.3)	4 (10.8)	0
Bacteremia[a]	14 (4.3)	4 (2.4)	5 (16.7)	2 (2.9)	2 (5.4)	1 (5.6)
Malaria (rapid test positive)	50 (15.4)	30 (17.8)	3 (10)	12 (17.1)	3 (8.1)	2 (11.1)
Malaria (slide-confirmed) (4 missing)	39 (12.2)	23 (13.6)	2 (6.7)	9 (12.9)	3 (8.1)	2 (11.1)
Oral candida	12 (3.7)	6 (3.6)	4 (13.3)	0	0	2 (11.1)
Esophageal candida	4 (1.2)	0	3 (10.0)	0	0	1 (5.6)
Any bleeding	4 (1.2)	3 (1.8)	0	1 (1.4)	0	0
Suspected Stage III anemia	4 (1.2)	2 (1.2)	0	2 (2.9)	0	0
Suspected ADR, CTX	5 (1.5)	4 (2.4)	0	0	1 (2.7)	0
Suspected ADR, ZDV	9 (2.8)	7 (4.1)	1 (3.3)	1 (1.4)	0	0

[a]Bacteremia could not be confirmed until 2 to 4 days after culture specimens were obtained.

ADR, adverse drug reaction; ART, combination antiretroviral therapy; CD4, CD4+ T-lymphocyte count; CTX, co-trimoxazole prophylaxis; Hb, hemoglobin (g/dl); IQR, interquartile range; LTFU, lost to follow-up; PMTCT, prevention of mother-to-child transmission of HIV, TB, tuberculosis; ZDV, zidovudine.

Table 2. Assigned Diagnoses and Clinical Management at Enrollment and Follow-up Visits

	Subjects not hospitalized at enrollment visit (n = 317)[a]		
	At enrollment visit: N (%) subjects not hospitalized at this visit	*After enrollment but before final Hb measurement*	*After enrollment, any visit (includes subjects who had no follow-up hemoglobins)*
Key diagnoses and comorbidities			
Iron deficiency (presumed; 1 missing)	315 (99.4)	0	0
Intestinal helminth infection (presumed)	293 (92.4)	1 (0.3)	2 (0.6)
Bleeding	1 (0.3)	1 (0.3)	3 (0.9)
Suspected HIV/AIDS Stage III anemia, ART initiated	4 (1.3)	0	0
Suspected ADR, ZDV discontinued	9 (2.8)	6 (1.9)	6 (1.9)
Suspected ADR, CTX discontinued	5 (1.6)	12 (3.8)	14 (4.4)
Malaria (rapid test positive)	50 (15.8)	2 (0.6)	6 (1.9)
Malaria (slide-confirmed)	38 (12.0)	1 (0.3)	4 (1.3)
Syndromically diagnosed malaria (rapid test negative)	3 (0.9)	0	1 (0.3)
Smear-positive pulmonary TB	2 (0.6)	1 (0.3)	4 (1.3)
Smear-negative pulmonary TB	14 (4.4)	5 (1.6)	10 (3.2)
TB suspect	21 (6.7)	3 (0.9)	10 (3.2)
Bacteremia (confirmed)[b]	12 (3.8)	0	1 (0.3)
Oral candida	9 (2.8)	3 (0.9)	7 (2.2)
Esophageal candida	3 (0.9)	0	2 (0.6)
Kaposi's sarcoma	6 (1.9)	1 (0.3)	4 (1.3)
Management			
Start ferrous sulfate/folic acid (1 missing)	315 (99.4)	0	0
Start mebendazole or albendazole	293 (92.4)	1 (0.3)	2 (0.6)
Start ART	13 (4.1)	10 (3.2)	25 (7.9)
Start ZDV (ART or PMTCT regimen)	15 (4.8)	6 (1.9)	17 (5.4)
Start CTX	70 (22.2)	1 (0.3)	8 (2.5)
Stop ZDV	6 (1.9)	7 (2.2)	8 (2.5)
Stop CTX	5 (1.6)	12 (3.8)	14 (4.4)
Start antimalarial	52 (16.5)	2 (0.6)	6 (1.9)
Start TB treatment	4 (1.3)	6 (1.9)	12 (3.8)
Continue TB treatment	12 (3.8)	N/A	N/A
Start antibiotics	115 (36.4)	9 (2.8)	17 (5.4)
Start antifungals	30 (9.5)	1 (0.3)	8 (2.5)
No recorded treatment other than ferrous sulfate, anthelmintics, and/or antimalarials	120 (37.9)	49 (15.5)	106 (33.4)

[a]Seven additional subjects were hospitalized at the enrollment visit; the full lists of diagnoses acquired during these hospitalizations were unavailable and so these subjects have been excluded from the table.

[b]Bacteremia could not be confirmed until 2 to 4 days after obtaining blood culture specimens. Two of the subjects hospitalized at enrollment were subsequently found to be bacteremic.

ADR, adverse drug reaction; ART, combination antiretroviral therapy; CTX, co-trimoxazole prophylaxis; Hb, hemoglobin; TB, tuberculosis; ZDV, zidovudine.

without presumed helminth infection). Only 79 (24.4%) subjects were assigned etiologic diagnoses (laboratory-confirmed malaria, tuberculosis, or bacteremia). Fifty-nine (18.2%) acquired the syndromic diagnoses of TB, malaria, ADR, stage III anemia, and/or bleeding (specifically contemplated in the current guideline). One hundred and seven (33.0%) never acquired diagnoses other than those emphasized in the previous anemia guideline: malaria, iron deficiency, and/or helminth infection.

Slightly more than half of the subjects [169 (52.2%)] attained the study endpoint of ''improvement'' (the second primary outcome); 30 (9.3%) died or were hospitalized and 125 (38.6%) were LTFU. All adverse outcomes were associated with declining Hb, bacterial bloodstream infection [predominantly non-typhoid *Salmonella* (NTS)], low CD4 count in the absence of ART, suspected TB, febrile illness, and/or a recent diagnosis of HIV. Of 169 subjects who improved, 65 (38.5%) were managed only with conventional treatment (iron supplements, anthelminthics, and/or antimalarials) throughout the study, while the other patients required antibiotics, antifungals, TB treatment, or initiation or revision of ART. Fifty-two (41.6%) of those LTFU were known to be alive after the final study visit, based on chart review and active defaulter tracing.

Most subjects reached study endpoints between enrollment and the first follow-up Hb measurement. At enrollment, seven subjects (2.2%) were hospitalized; nearly all (99.4%) of the 317 not hospitalized were presumptively treated for iron deficiency, usually in combination with presumptive deworming. Antimalarials were prescribed for 49 subjects with positive malaria rapid tests (84.8% confirmed by microscopy). Fewer than half (120; 37.9%) were treated exclusively with conventional treatment at the first study visit.

Second Hb measurements were available for 240 subjects at a mean interval of 34 days after enrollment. The median initial Hb change was +1.0 g/dl (IQR 0.10, 1.95; range −5.3,

Table 3. Association of Subject Characteristics at Study Entry and Hemoglobin Change at Second Hemoglobin Measurement: Results of Bivariate and Multivariable Linear Regression

Subject characteristics	*Bivariate coefficient for hemoglobin change from baseline to measurement 2 (g/dl), with 95% CI* N = *240*	p	*Multivariable coefficient for hemoglobin change from baseline to measurement 2 (g/dl), with 95% CI* N = *232*	p
Demographics				
Study site				
Coalane	−0.50 (−1.04, 0.03)	0.064	−0.62 (−1.15, −0.09)	0.022
Inhassunge	−0.88 (−1.35, −0.40)	<0.001	−0.98 (−1.45, −0.52)	<0.001
Namacurra	Reference	Reference	Reference	
Gender, pregnancy status				
Male	0.53 (0.00, 1.07)	0.05	—	
Female, not pregnant	Ref	Reference	—	
Pregnant	0.39 (−0.18, 0.97)	0.175	—	
Age (years)	0.005 (−0.02, 0.03)	0.680		
Illiterate	−0.17 (−0.60, 0.26)	0.437		
Did not accept home visits	0.61 (−0.03, 1.25)	0.061	—	
HIV/AIDS status				
CD4 count adjusted for interaction with ART status at enrollment[a]				
CD4 <350, no ART	−0.11 (−0.70, 0.48)	0.719	0.10 (−0.46, 0.65)	0.729
CD4 <350, started ART	−1.51 (−2.62, −0.40)	0.008	−1.43 (−2.50, −0.36)	0.009
CD4 <350, on ART	−0.59 (−1.28, −0.10)	0.095	−0.40 (−1.04, 0.25)	0.229
CD4 >350	Reference		Reference	
No CD4 available	−0.38 (−0.25, +1.02)	0.231	0.17 (−0.43, +0.77)	0.586
On zidovudine (ART or PMTCT)	0.01 (−0.45, 0.43)	0.964		
On co-trimoxazole prophylaxis	−0.05 (−0.56, 0.45)	0.842		
Clinical characteristics at enrollment				
Temperature (axillary, °C) (4 missing)	−0.26 (−0.51, −0.02)	0.038	—	
Body mass index (kg/m^2) (9 missing)	0.08 (0.01, 0.15)	0.017	0.08 (0.02, 0.14)	0.010
Hemoglobin (g/dl) at baseline	−0.25 (−0.44, −0.06)	0.009	−0.38 (−0.57, −0.20)	<0.001
Smear-positive pulmonary TB	1.22 (−2.08, 4.52)	0.467		
Smear-negative pulmonary TB	−0.08 (−1.10, 0.94)	0.876		
TB suspect	−0.35 (−1.20, 0.51)	0.424		
Active TB, not diagnosed until after enrollment visit	−1.31 (−2.18, −0.45)	0.003	—	
Bacteremia (detected on blood culture drawn at enrollment visit)	0.09 (−1.09, 1.28)	0.878		
Malaria (rapid test positive)	−0.17 (−0.76, 0.41)	0.557		
Malaria (slide-confirmed)	−0.11 (−0.77, 0.54)	0.731		
Oral candida	0.20 (−0.92, 1.32)	0.726		
Esophageal candida	−3.06 (−4.93, −1.18)	0.001	−2.63 (−4.38, −0.87)	0.003
Suspected Stage III anemia	0.57 (−1.77, 2.91)	0.632		
Suspected ADR, CTX	1.04 (−0.45, 2.52)	0.170		
Suspected ADR, ZDV	0.49 (−0.69, 1.68)	0.413		
Management at visit 1				
Start ART	−0.90 (−1.83, 0.04)	0.060	See above	
Start ZDV (for ART or PMTCT)	0.38 (−0.73, 1.50)	0.499		
Stop ZDV	0.49 (−0.69, 1.68)	0.413		
Start CTX	−0.20 (−0.74, 0.35)	0.473		
Stop CTX	1.04 (−0.48, 2.52)	0.170		
Start antimalarial	−0.31 (−0.88, 0.26)	0.280		
Start TB treatment	−0.22 (−2.14, 1.69)	0.820		
Continue TB treatment	0.12 (−1.00, 1.24)	0.835		
Start antibiotics	−0.07 (−0.52, 0.37)	0.743		
Start antifungals	0.42 (−0.24, 1.08)	0.211		
Start ferrous sulfate[b]	Undefined			
Start anthelminthics	−0.60 (−1.48, 0.27)	0.174		
No interventions aside from ferrous sulfate, anthelminthics, and/or antimalarials before hemoglobin 2	0.09 (−0.34, 0.52)	0.685		
Constant			3.28 (1.29, 5.26)	0.001

[a]Antiretroviral use was very infrequent in patients with CD4 ≥350 or missing.

[b]100% ferrous sulfate prescription in subjects with nonmissing data.

ADR, adverse drug reaction; ART, combination antiretroviral therapy; CD4, CD4+ T-lymphocyte count, cells/μL; CI, confidence interval; CTX, co-trimoxazole prophylaxis; PMTCT, prevention of mother-to-child transmission of HIV; TB, tuberculosis; ZDV, zidovudine.

+6.4). One hundred and twenty-four subjects (51.7%) achieved a ≥1.0 g/dl increase and therefore reached a study endpoint. Of the 116 subjects who did not achieve the target Hb increase and remained in the study, 51 (44.0%) had experienced Hb declines [median decline 0.90 g/dl (IQR −1.3, −0.3, range −5.8, −0.10)].

Table 3 describes linear associations between subject characteristics at enrollment and Hb evolution from enrollment to the second Hb measurement. In multivariable models, higher BMI was significantly and positively associated with Hb trend [+0.08 g/dl per 1 kg/m^2 increase [0.02, 0.14)]. Covariates significantly but negatively associated with Hb trend were study sites [Coalane, coefficient: −0.62, (95% CI −1.15, −0.09) and Inhassunge, −0.98 (−1.45, −0.52)], higher baseline Hb [−0.38 per 1 g/dl increase (−0.57, −0.20)], esophageal candidiasis [−2.63 (−4.38, −0.87)], and ART initiation [predominantly zidovudine (ZDT) based] at enrollment [−1.43 (−2.50, −0.35)]. In bivariate models, higher temperature and the presence of undetected TB were also associated with Hb decline, and male sex with Hb increase, but these associations did not persist in multivariable analysis. In this and other models (see Table 5, discussed below), CD4-ART interactions could be assessed only for subjects with CD4 <350 cells/μl, owing to infrequent ART use in other subjects.

After the first follow-up Hb measurement, subjects who remained in the study had up to three subsequent Hb measurements before reaching endpoints. The evolution of anemia in individual subjects was heterogeneous throughout (Fig. 3). For example, one subject's Hb rose by 3.4 g/dl after iron supplementation and discontinuation of ZDV. Another's Hb fell by 2.3 g/dl after an episode of NTS bacteremia, then rose 5.4 g/dl after initiation of TB treatment, ART, and iron.

Anemia management after the first follow-up Hb differed from initial management. Initiation of TB treatment and ART regimen changes to address suspected ADR were more common after the first study visit, while new malaria treatment was less common (Table 2). Table 4 describes linear correlates of Hb response from enrollment or nadir (for those with hemoglobin improvement after decline) to final Hb. The multivariable model resembled that for initial Hb response, but detected three additional factors significantly and positively associated with Hb trend: continuation of ART from enrollment through study endpoint [coefficient 0.49 (0.11, 0.88)], discontinuation of ZDV for suspected ADR [1.08 (0.26, 1.89)], and diagnosis of smear-positive pulmonary TB [1.66 (0.13, 3.19)]. CD4 <350 was significantly associated with a negative Hb trend [−0.59 (−0.98, −0.20)].

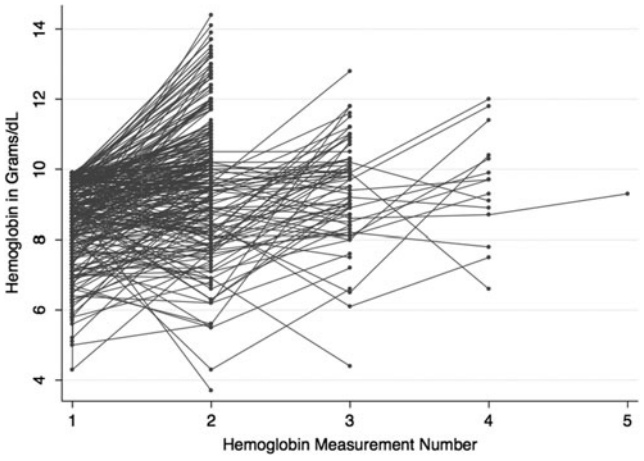

FIG. 3. Hemoglobin Evolution in Study Subjects. Each line links the serial hemoglobin values for an individual study subject from study entry (hemoglobin measurement 1) to the final measurement before study endpoint (range: 1 to 5 measurements; *x*-axis). The *y*-axis describes measured hemoglobin values in grams per deciliter.

Logistic regression models (Table 5) assessing associations with the composite adverse outcome (hospitalization or death at any point in the study) echoed the linear regression findings, in that adverse outcomes were significantly associated with lower or missing CD4, lower BMI, and bleeding. They also described significant positive associations between adverse outcomes and increased axillary temperature on enrollment [OR 2.21 (1.26, 3.87 per 1°C elevation)] and significant negative associations with reported use of cotrimoxazole prophylaxis at enrollment [0.16 (0.04, 0.62)]. However, the odds ratios for association with adverse outcome were undefined for smear-positive pulmonary TB and suspected stage III anemia, prescription of ferrous sulfate, and esophageal candidiasis, because of small cell sizes.

Discussion

We recruited HIV-infected Mozambican adults with measured Hb <10 g/dl and no current ferrous sulfate supplementation as determined by chart review and subject interview. Because chart notes were usually quite brief, it is possible that some subjects had recently been given treatment doses of iron supplements, but it is not possible to quantify the effect of this potential misclassification based on available information.

Using a new Mozambican guideline, non-physician clinicians assigned etiologic or syndromic diagnoses of the presumed cause of anemia in all subjects not hospitalized upon enrollment. Although the syndromic diagnosis of iron deficiency (with or without helminth infection) was assigned almost universally, as it would have been using Mozambique's earlier guideline, fewer than one in five patients received antimalarials (seldom without confirmatory testing) and nearly one-third of subjects also received other HIV-associated diagnoses (e.g., TB, ADR, stage III anemia, oroesophageal candidiasis) that were not contemplated in the previous anemia guideline. This suggests that the new guideline did result in decreased overprescription of antimalarials and increased consideration of HIV-associated anemia, as hoped. However, barely half of subjects achieved the modest 1 g/dl Hb increase that defined ''improvement.'' Nearly 1 in 10 arrived at the composite adverse endpoint of hospitalization and/or death.

Subjects treated only with conventional anemia treatment (presumptive iron supplementation, deworming, and/or antimalarials) comprised fewer than half of those who improved. But improvement of a substantial minority with conventional treatment alone does suggest that the historic diagnoses of iron deficiency, intestinal helminthes, and malaria still contribute importantly to anemia burden in HIV-infected Mozambican adults, as anticipated, and must be retained in standard guidelines. It is possible that variability in treatment outcomes across study sites might be the result of

Table 4. Association of Subject and Treatment Characteristics at Any Study Visit with Hemoglobin Response from Hemoglobin Nadir to Last Hemoglobin Measurement: Results of Bivariate and Multivariable Linear Regression

Subject characteristics (n = *240 subjects with at least 2 hemoglobin measurements*)[a]	*Bivariate coefficient, 95% CI*	p	*Multivariable coefficient, 95% CI*	p
Demographics				
Study site				
Coalane	−0.42 (−0.92, 0.08)	0.098	−0.77 (−1.24, −0.31)	0.001
Inhassunge	−0.94 (−1.39, −0.49)	0.000	−1.06 (−1.46, −0.66)	<0.001
Namacurra	Reference		Reference	
Gender, pregnancy status				
Male	0.41 (−0.09, 0.91)	0.109		
Female, not pregnant	Reference			
Pregnant	0.32 (−0.22, 0.85)	0.250		
Age (years)	0.003 (−0.02, 0.02)	0.741		
Illiterate	−0.01 (−0.42, 0.39)	0.950		
Did not accept home visits	0.50 (−0.09, 1.11)	0.097	—	
HIV/AIDS status				
Most recent CD4 count (cells/μl), categories				
<350	−0.60 (−1.04,-0.16)	0.008	−0.59 (−0.98, −0.20)	0.003
≥350	Reference		Reference	
None available	−0.04 (−0.62, 0.55)	0.898	−0.22 (−0.74, 0.29)	0.393
On ART at enrollment	0.53 (0.11, 0.94)	0.013	—	
On CTX at enrollment	0.11 (−0.38, 0.60)	0.655		
On ZDV (ART or monotherapy for PMTCT) at enrollment	0.45 (0.04, 0.86)	0.032	—	
Clinical characteristics at visit 1				
Temperature (axillary, °C) (2 missing)	−0.27 (−0.50, −0.04)	0.024	—	
Body mass index (kg/m^2) (7 missing)	0.04 (−0.02, 0.10)	0.237		
Hemoglobin (g/dl) at baseline	−0.33 (−0.50, −0.15)	<0.001	−0.41 (−0.58, −0.24)	<0.001
Key Diagnoses and comorbidities at any visit				
Smear-positive pulmonary TB	1.92 (0.15, 3.70)	0.034	1.66 (0.13, 3.19)	0.034
Smear-negative pulmonary TB	0.38 (−0.42, 1.17)	0.355		
Bacteremia (detected on blood culture drawn at this visit)	0.04 (−0.96, 1.04)	0.934		
Malaria (rapid test positive)	−0.28 (−0.80, 0.23)	0.284		
Oral candida	−0.87 (−1.69, −0.06)	0.036	—	
Esophageal candida	−3.02 (−4.37, −1.68)	<0.001	−2.39 (−3.60, −1.17)	<0.001
Any bleeding	−1.84 (−3.22, −0.46)	0.009	−1.69 (−2.89, −0.50)	0.006
Suspected Stage III Anemia	0.05 (−2.14, 2.25)	0.964		
Management at any visit prior to endpoint visit				
Start antibiotics	−0.34 (−0.75, 0.07)	0.101		
Start antimalarials	−0.43 (−0.95, 0.09)	0.107		
Start antifungals	0.19 (−0.42, 0.80)	0.536		
Any TB treatment	0.26 (−0.46, 0.98)	0.474		
Start ART	−0.60 (−1.27, 0.08)	0.083	—	
Start PMTCT	0.02 (−0.98, 1.02)	0.967		
Start cotrimoxazole	−0.38 (−0.88, 0.12)	0.133		
Interaction: ART/ZDV discontinuation				
On ART at study entry, no discontinuation of ZDV	0.44 (−.004, 0.88)	0.048	0.49 (0.11, 0.88)	0.012
On ART at study entry, ZDV discontinued	1.09 (0.18, 2.01)	0.019	1.08 (0.26, 1.90)	0.010
No ART	Reference		Reference	
Interaction: CTX/CTX discontinuation				
On CTX at study entry, no discontinuation of CTX	0.05 (−0.45, 0.54)	0.858	—	
On CTX at study entry, CTX discontinued	1.01 (0.08, 1.94)	0.033	—	
No CTX	Reference		—	
No management aside from iron supplements, anthelminthics, antimalarials	0.32 (−0.10, 0.74)	0.132		
Constant			5.86 (4.29, 7.43)	<0.001

[a]Subjects without second hemoglobin measurements either died, were hospitalized, or were lost to follow-up before the second measurement could be obtained.

ADR, adverse drug reaction; ART, combination antiretroviral therapy; CD4, CD4$^+$ T-lymphocyte count; CI, confidence interval; CTX, co-trimoxazole prophylaxis; PMTCT, prevention of mother-to-child transmission; TB, tuberculosis; ZDV, zidovudine.

Table 5. Association of Subject Characteristics at Study Entry with Adverse Outcomes (Hospitalization or Death): Results of Bivariate and Multivariable Linear Regression

Subject characteristics (n = 324)	*Bivariate OR for poor outcome (hospitalization or death), 95% CI*	p	*Multivariable OR for poor outcome (hospitalization or death), 95% CI*	p
Demographics				
Study site				
Coalane	0.81 (0.27, 2.49)	0.719	0.15 (0.02, 0.99)	0.049
Inhassunge	2.00 (0.84, 4.76)	0.117	1.78 (0.52, 6.15)	0.361
Namacurra	Reference		Reference	
Gender, pregnancy status				
Male	1.32 (0.56, 3.10)	0.520	—	
Female, not pregnant	Reference		—	
Pregnant	0.16 (0.02, 1.25)	0.081	—	
Age (years)	1.00 (0.96, 1.04)	0.990		
Illiterate	1.05 (0.48, 2.31)	0.893		
Did not accept home visits	0.53 (0.12, 2.41)	0.412		
HIV/AIDS status				
Most recent CD4 count (cells/μl), categories with adjustment for 3 categories of ART at enrollment				
<350, no ART at enrollment	8.77 (1.70, 45.36)	0.010	8.94 (1.18, 67.49)	0.034
<350, start ART at enrollment	5.38 (0.38, 75.38)	0.212	3.29 (0.06, 181.82)	0.561
<350, already on ART at enrollment	6.45 (1.09, 38.13)	0.040	13.66 (1.31, 141.46)	0.028
>=350	Reference		Reference	
No CD4 available	6.92 (1.29, 37.17)	0.024	12.39 (1.37, 111.82)	0.025
On co-trimoxazole prophylaxis at enrollment	0.48 (0.21, 1.08)	0.076	0.13 (0.03, 0.57)	0.007
On zidovudine (ART or prevention of mother-to-child transmission) at enrollment	0.55 (0.23, 1.32)	0.181		
Clinical characteristics at visit 1				
Temperature (axillary, °C) (2 missing)	2.91 (1.84, 4.62)	<0.001	2.21 (1.26, 3.87)	0.005
Body mass index (kg/m^2) (7 missing)	0.75 (0.64, 0.88)	<0.001	0.76 (0.61, 0.95)	0.014
Hemoglobin (g/dl) at baseline	0.73 (0.53, 0.996)	0.047	—	
Key diagnoses and comorbidities at first or any study visit				
Lowest Hb recorded during study (Hb nadir)	0.62 (0.47, 0.83)	0.001	0.43 (0.25, 0.73)	0.002
Smear-positive pulmonary TB, first or any visit[a]	Undefined	—		
Smear-negative pulmonary TB, visit 1	6.81 (2.48, 18.69)	<0.001	—	
TB suspect at visit 1	6.81 (2.48, 18.69)	<0.001	—	
Active TB, not diagnosed until after enrollment visit (enrollment visit only)	4.03 (1.22, 13.29)	0.022	—	—
Bacteremia (detected on blood culture drawn at this visit), visit 1	8.25 (2.07, 32.81)	0.003	5.89 (0.85, 40.58)	0.072
Malaria (rapid test positive), visit 1	0.51 (0.15, 1.81)	0.300		
Oral candida, any visit	4.03 (1.22, 13.29)	0.022	—	
Esophageal candida (any visit)[b]	Undefined.	—		
Any bleeding, any study visit	5.96 (0.81, 44.09)	0.080	110.21 (4.31, 2819.10)	0.002
Suspected Stage III Anemia, visit 1 or any visit[a]	Undefined.			
Suspected ADR CTX, any visit	0.93 (0.20, 4.40)	0.932		
Suspected ADR ZDV, any visit Hb	2.74 (0.78, 9.53)	0.114		
Management at any study visit				
Start ART, visit 1	0.69 (0.08, 5.76)	0.735		
Start ZDV (ART or PMTCT), any visit	0.31 (0.04, 2.40)	0.262		
Stop ZDV, any study visit	2.74 (0.78, 9.53)	0.114		
Start CTX, any study visit	0.89 (0.34, 2.34)	0.816		
Stop CTX, any study visit	0.93 (0.20, 4.40)	0.932		
Start antimalarial, visit 1	0.71 (0.23, 2.19)	0.555		
Start TB treatment, any study visit	1.27 (0.26, 6.19)	0.768		
Start antibiotics, any study visit	1.97 (0.90, 4.31)	0.089	—	
Start antifungals, any study visit	1.84 (0.71, 4.75)	0.209		
Start iron supplements, first or any study visit[c]	Undefined.			
Start anthelminthics, any study visit	0.45 (0.13, 1.53)	0.202		
No new interventions other than iron, anthelmintics, and/or antimalarial, all visits	0.32 (0.17, 0.88)	0.027	—	

[a]Undefined: no adverse outcomes in this category.

[b]Undefined: all subjects in this category had adverse outcomes.

[c]Undefined: all subjects with known outcomes and non-missing data were started on iron at study visit 1.

ADR, adverse drug reaction; ART, combination antiretroviral therapy; CD4, CD4+ T-lymphocyte count, cells/μL; CI, confidence interval; CTX, co-trimoxazole prophylaxis; Hb, hemoglobin, g/dL; LTFU, lost to follow-up; OR, odds ratio; PMTCT, prevention of mother-to-child transmission of HIV; TB, tuberculosis; ZDV, zidovudine.

differing malaria transmission intensities; malaria transmission was least intense in the site with the best outcomes.

Nearly all of those who failed to improve also received presumptive treatment with iron supplements and anthelminthics (with or without other interventions). These observed failures have several possible explanations. First, the subjects who failed to improve in spite of iron supplementation and deworming may not have had iron deficiency or helminth infection. Iron deficiency may be a less prominent contributor to anemia in HIV-infected patients. In Malawians with severe anemia, iron deficiency (assessed by complete blood count, serum ferritin, serum transferrin, and bone marrow aspirate) was present in only 16% of HIV-infected patients vs. 59% of HIV-uninfected patients.[46] In pregnant HIV-infected Tanzanian women, although 48.3% were anemic, fewer than half of the anemic women were iron deficient (based on measurement of hemoglobin, serum ferritin, serum transferrin receptor, and C-reactive protein).[4] Recent mass deworming campaigns may have decreased the historically high prevalence of helminth infections in Mozambique.

Second, our subjects may not have been adherent. In pregnant Pakistani women with severe anemia thought to be caused primarily by iron deficiency, better than 85% adherence was required to achieve a 65% response to therapy,[47] but adherence to iron supplementation in pregnant Mozambican women has been reported as 67%.[48] Lack of adherence could be caused by both patient factors and increasingly frequent pharmacy stockouts in the study environment.

Third, imprecision in point-of-care Hb testing may have failed to detect significant Hb increases. When used by trained laboratory personnel, the precision of the HemoCue is very good.[49] The mean difference between our study HemoCue measurements and Sysmex analyzer readings was –0.29 g/dl (SD 0.03 g/dl). Thus, 95% of misclassification of Hb changes from enrollment to second measurement should lie in the range of 1.0 ± 0.12 g/dl. Nineteen (7.9%) of our subjects' responses at Hb measurement two lay within this range. But in our study subjects, variability in HemoCue performance would be more likely to result in overestimation than underestimation of improvement, because 14 (73.7%) of these 19 observed Hb changes were ≥1.0 g/dl, and could only have been failures misclassified as successes.

Fourth, the 1 g/dl Hb improvement threshold (attained at 1 month) may not have been ideal. We could not locate a published standard for Hb evolution with iron supplementation (with or without deworming and/or malaria treatment) in HIV-infected adults. In HIV-uninfected adults with uncomplicated iron deficiency anemia, expected Hb increases in response to oral iron have been variously stated as 0.04–0.05 g/dl/day, 2.0 g/dl every 3 weeks, 2.0–3.0 g/dl within 4 weeks, and 2.0 g/dl within 3 months.[47,50–52] Because HIV-infected adults are unlikely to have ''uncomplicated'' iron deficiency, the higher targets may not be attainable in this rural Mozambican population. Also, normal day-to-day within-individual fluctuations in measured Hb have been found to have an SD of 0.82 g/dl.[49] About half [129 (53.8%)] of subjects who had at least two Hb readings had Hb changes within the range of 1.0 ± 1.6 g/dl between enrollment and the second Hb. Thus, normal Hb fluctuations could have caused substantial nondifferential misclassification of individual responses to treatment. Though a higher threshold for Hb improvement would have alleviated concern about misclassification caused by normal fluctuations and/or the imprecision of HemoCue devices, it might have required a much longer period of observation to attain, and the longer wait could have caused avoidable delays in detection of other contributing causes of anemia.

Finally, subjects who did not respond to conventional therapy alone may have had other, undetected causes of anemia. Within the study, we believe that there was considerable underascertainment of many anemia-associated clinical entities: (1) advanced immunosuppression, because of slow CD4 turnaround times and the difficulty of clinical staging in the study environment;[46] (2) adverse drug reactions, given low standard Hb thresholds for discontinuation of ZDV in the setting of ADR and frequent absence of baseline pre-ART Hb measurements; (3) TB, with long turnaround times for AFB smears, frequent stockouts of AFB reagents, and the scant availability of chest radiography; (4) other micronutrient deficiencies (e.g., vitamin A) that could not be evaluated in the study setting but were likely prevalent in this malnourished population; and/or (5) most Stage IV conditions, given the limited availability of diagnostics. Failure to diagnose and treat these possible causes of anemia may have led to anemia treatment failures.

Subjects who were treated with nonconventional anemia interventions, particularly those addressing HIV/AIDS itself, adverse drug reactions, and opportunistic infections, often improved. As anticipated within the Mozambican guidelines, ongoing ART, discontinuation of ZDV in the setting of suspected ADR, and treatment of smear-positive pulmonary TB were significantly associated with Hb improvement in our multivariable models. The Mozambican guideline did not anticipate the observed contribution of bacterial bloodstream infections to anemia burden, though this has been reported elsewhere and is associated with untreated AIDS.[28,53] The observed association between active bleeding and hemoglobin decline is difficult to interpret because few subjects reported overt bleeding, and tests for occult bleeding were unavailable at study sites.

Anemia management in HIV-infected individuals should routinely consider ART initiation or regimen change and include TB evaluation. In Malawi, for example, 91% of HIV-infected patients with Hb ≤10 g/dl were reported to have CD4 <350 cells/μl and were eligible for ART.[12] In South Africa, the prevalence of culture-confirmed TB was 26% and 40% in HIV-infected patients with moderate and severe anemia, respectively.[27] Our study is limited by nonrandom selection, a paucity of local diagnostic capacity, and high dropout rates. Nonetheless, our findings support these recommendations to consider ART status and TB screening in anemic HIV-infected adults, and also support the Mozambican Ministry of Health's decision to update its previous guidelines for use in the context of HIV/AIDS care.

Further improvements to non-physician clinician management of anemic HIV-infected adults in Mozambique might include improved access to point-of-care CD4 and TB testing and to blood cultures; more specific instructions for management of Hb that is very low, falling, or slow to rise; the eventual introduction of laboratory tests that can accurately detect iron deficiency in the setting of HIV infection[46]; and the re-evaluation of thresholds for the definition of Hb ''improvement'' and for discontinuation of specific medications when ADR is suspected. More aggressive initiation of ART in

eligible patients is critical. Given the high prevalence of anemia and the association between anemia and poor HIV/AIDS outcomes, we believe that further refinement of the approach to anemia management at first-level health centers is a high priority in resource-constrained settings.

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