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Iron Deficiency and Anemia Predict Mortality in Patients with Tuberculosis[1–3]

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Abstract

Many studies have documented a high prevalence of anemia among tuberculosis (TB) patients and anemia at TB diagnosis has been associated with an increased risk of death. However, little is known about the factors contributing to the development of TB-associated anemia and their importance in TB disease progression. Data from a randomized clinical trial of micronutrient supplementation in patients with pulmonary TB in Tanzania were analyzed. Repeated measures of anemia with iron deficiency, anemia without iron deficiency, and iron deficiency without anemia were assessed as risk factors for treatment failure, TB recurrence, and mortality. The prevalence of anemia (hemoglobin $<$ 110 g/L) at baseline was 64%, more than one-half of which was related to iron deficiency (mean corpuscular volume $<$ 80 fL). We found no evidence of an association between anemia (with or without iron deficiency) or iron deficiency without anemia at baseline and the risk of treatment failure at 1 mo after initiation. Anemia without iron deficiency was associated with an independent, 4-fold increased risk of TB recurrence [adjusted RR = 4.10 (95% CI = 1.88, 8.91); $P < 0.001$]. Iron deficiency and anemia (with and without iron deficiency) were associated with a 2- to nearly 3-fold independent increase in the risk of death [adjusted RR for iron deficiency without anemia = 2.89 (95% CI = 1.53, 5.47); $P = 0.001$; anemia without iron deficiency = 2.72 (95% CI = 1.50, 4.93); $P = 0.001$; iron deficiency anemia = 2.13 (95% CI = 1.10, 4.11); $P = 0.02$]. Efforts to identify and address the conditions contributing to TB-associated anemia, including iron deficiency, could play an important role in reducing morbidity and mortality in areas heavily affected by TB. J. Nutr. 142: 350–357, 2012.

Introduction

Major achievements have been made in reducing the global burden of TB with expansion of efforts to improve TB care and control, but TB remains an important global health concern. In 2009, there were 9 million new cases of TB and nearly 2 million associated deaths (1). The greatest burden of TB, in terms of incidence and mortality, is found in sub-Saharan Africa, in part due to the high prevalence of HIV in this region, because HIV-infected individuals are more likely to develop active TB due to immunosuppression (2).

An estimated 1.6 billion people, or nearly one-quarter of the world's population, are anemic, a condition characterized by a lower than normal hemoglobin concentration in the blood (3). In the context of infectious disease, particularly HIV/AIDS, anemia has been found to be an independent predictor of disease progression and mortality (4–6). Many studies have documented a high prevalence of anemia among TB patients [32–86% (7–11)] and there is some evidence to suggest that anemia at TB diagnosis is associated with an increased risk of death (12–14). Given the high burden and potential consequences of TB-associated anemia, further studies to clarify its role in TB disease progression are needed.

To guide clinical decision-making and provide a basis for treatment recommendations, it is also important to characterize the factors contributing to TB-associated anemia. Iron deficiency is considered the most important contributor to the development of anemia worldwide, but other causes often coexist. If iron deficiency were established as an important contributor to TB-associated anemia, the targeted provision of supplemental iron may be used to increase blood hemoglobin concentrations and improve clinical outcomes in TB patients. The contribution of

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[3] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[13] Abbreviations used: AFB, acid-fast bacilli; MCV, mean corpuscular volume; TB, tuberculosis.
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iron deficiency without anemia to TB disease progression may also be of direct importance, because iron deficiency has been associated with impaired immune function and reduced capacity to control infection (15,16).

Using prospectively collected data from a micronutrient supplementation trial conducted in Dar es Salaam, Tanzania (17), we evaluated the presence and intersection of anemia and iron deficiency among adult TB patients. To understand the independent and joint contributions of anemia and iron deficiency to clinical outcomes in TB, we examined the associations of anemia (with or without iron deficiency) and iron deficiency without anemia with the risk of treatment failure, TB recurrence, and mortality.

Methods

Study population. Participants in this study were participants in the Nutrition, Immunology and Epidemiology of Tuberculosis Study in Dar es Salaam, Tanzania, the details of which have been previously published (17). In brief, between April 2000 and April 2005, 887 adults (471 HIV infected and 416 HIV uninfected) with pulmonary TB were enrolled in a randomized, placebo-controlled trial to examine the effect of micronutrient supplementation on sputum conversion (defined as the finding of an AFB-negative sputum culture) at 1 mo, TB recurrence, and mortality. At enrollment in the parent trial, at least 2 positive sputum smears for AFB in direct microscopy were considered as evidence of pulmonary TB infection. Patients with hemoglobin at baseline $\leq$ 70 g/L or a history of >1 mo of TB treatment in the previous year were excluded. Patients were randomized, stratified by HIV status, to receive a daily oral dose of multiple micronutrients [1500 μg of retinol, 20 mg of thiamin, 20 mg of riboflavin, 25 mg of vitamin B-6, 100 mg of niacin, 50 μg of vitamin B-12, 500 mg of vitamin C (niacinamide ascorbate and ascorbic acid), 200 mg of vitamin E (racemic-α-tocopheryl acetate), 0.8 mg of folic acid, and 100 μg of selenium] or placebo, and all patients received an 8-mo short course of anti-TB treatment in accordance with the Tanzania National Tuberculosis and Leprosy Program guidelines. Micronutrient supplementation in the parent trial decreased the risk of TB recurrence among HIV-infected patients, increased CD3 and CD4 cell counts, and decreased the risk of extrapulmonary TB and genital ulcers among HIV-uninfected patients, and decreased the risk of peripheral neuropathy overall (17). Resistance to anti-TB drugs in this population was low [5% resistance to any anti-TB drug and 0.4% multidrug resistance (18)]. Antiretroviral therapy was not available to the majority of HIV-infected individuals in Tanzania at the time of study, including participants in this trial. The study protocol was approved by the institutional review boards of Muhimbili University of Health and Allied Sciences, the Tanzanian National AIDS Control Program of the Tanzanian Ministry of Health, and Harvard School of Public Health.

Enrollment and follow-up. Detailed background information was collected at enrollment from all patients by research nurses using standardized questionnaires. Monthly follow-up was conducted at the study clinics and included anthropometric assessment and recording of any illness in the previous month. Physician visits were scheduled every 3 mo, during which the study physicians recorded a detailed medical history and performed a complete physical examination. Home visits were made in the event that a scheduled clinic visit was missed, and neighbors and relatives were contacted to obtain information on the survival status of those who had left Dar es Salaam. Participants were followed until death, loss to follow-up, or study closure in August 2005.

Sputum specimens were collected for AFB smear and culture at baseline, 1, 2, 5, 8, and 12 mo after treatment initiation and every 6 mo thereafter until the end of follow-up. Blood specimens were collected for a complete blood count and measurement of T-lymphocyte subtypes at baseline, 1, 2, 5, 8, and 12 mo after treatment initiation, and every 6 mo thereafter until the end of follow-up. Blood collected at baseline, 2, 8, and 14 mo was also examined for malaria parasites and measured for viral load (HIV RNA levels) among HIV-infected patients. HIV disease stage was assessed every 3 mo at physician visits according to the WHO staging system for HIV disease (19).

Laboratory procedures. Sputum smears were prepared and stained using the Ziehl-Neelsen technique and examined for AFB using direct microscopy by trained technicians. AFB-positive specimens were cultured for *M. tuberculosis* using Lowestein Jensen medium and examined weekly for typical growth for a maximum of 8 wk. Hemoglobin and MCV were measured using a CBC5 Coulter Counter (Coulter) and T-lymphocyte subtypes were quantified using the FACScount and FACScan systems (Becton Dickinson). Thick blood films were microscopically examined for the presence of malaria parasites. HIV RNA in samples from HIV-infected patients was determined using the Roche Amplicor assay version 1.5 (Roche Diagnostic Systems). In 2010, CRP was measured in plasma specimens collected at baseline and stored at -80°C using a particle-enhanced immunoturbidimetric assay (Roche Diagnostics) in the Roche Cobas Integra 400 Plus analyzer.

Classification of exposure. We used a hemoglobin cutoff of <110 g/L to define anemia and a MCV cutoff of <80 fL to define iron deficiency (20). We then defined individual hematological status by cross-classifying the presence of anemia and iron deficiency, creating 4 groups for analysis: no anemia or iron deficiency (hemoglobin $\geq$110 g/L and MCV $\geq$80 fL); iron deficiency without anemia (hemoglobin $\geq$110 g/L and MCV <80 fL); anemia without iron deficiency (hemoglobin <110 g/L and MCV $\geq$80 fL); and anemia with iron deficiency (hemoglobin <110 g/L and MCV <80 fL).

Outcome measures. We considered two TB-related endpoints: treatment failure at 1 mo and TB recurrence. Treatment failure was defined as an AFB-positive sputum culture at 1 mo from treatment initiation. Recurrence was defined as a positive culture after 1 mo among those who were culture negative by 1 mo after treatment initiation. Recurrence included both endogenous reactivation and exogenous reinfection, because the two could not be distinguished in this study. We also evaluated death from any cause, and among HIV-infected patients, HIV disease progression from WHO stage 3 to stage 4 and the combined endpoint of death or HIV progression.

Statistical analysis. We used the χ^2 and Kruskal-Wallis tests to compare participant characteristics by baseline levels of hematological status. Log-binomial regression was used to assess the associations of baseline hematological status with treatment failure at 1 mo (21). We used proportional hazards regression to examine the relationship between serial measurements of hematological status and time to TB recurrence and to death (22). Among HIV-infected patients, we similarly examined the relationship between serial measurements of hematological status and time to HIV disease progression from WHO stage 3 to stage 4 and to the combined endpoint of death or HIV progression. Follow-up for the recurrence analysis was calculated as the time from the negative culture at 1 mo until the outcome, loss-to-follow-up, or study closure, whichever occurred first. Follow-up time for the mortality endpoint was calculated as the time from treatment initiation until the outcome, loss to follow-up, or study closure. The end of follow-up for the HIV disease progression endpoints was the date when HIV stage was last assessed. Serial measurements of hematological status used in the proportional hazards regression analyses were lagged such that each outcome was related to the most proximate preceding measurement in order to maintain the prospective nature of the analyses. The median duration between the lagged measurement of hematological status and assessment of the mortality outcome was 5.5 mo.

We accounted for potential confounders, including sex, age (years), money spent on food (<500, $\geq$500 Tanzanian shillings/person/d, equivalent to US $0.63 at the time of the start of the study), number of AFB colonies in sputum culture (0, 1–100, >100 colonies), Karnfosky score (<70%, $\geq$70%), previous TB disease (yes/no), HIV infection status (infected/uninfected), malaria infection (yes/no), BMI (kg/m^2), CD4 cell count (cells/mm^3), and log HIV RNA (copies/mm^3) as well as randomized trial regimen (micronutrient treatment/placebo). In analyses relating baseline hematological status with treatment failure at 1 mo, we

used multivariate regression to account for baseline values of each potential confounder. In analyses relating serial measurements of hematological status with the risk of TB recurrence, death, or HIV disease progression, we used the marginal structural model approach of inverse probability weighting to account for the baseline value of each potential confounder as well as serial measures of BMI, CD4 cell count, log HIV RNA, and malaria infection (23,24). To allow for nonlinearity, continuous covariates were modeled with restricted cubic splines; knots were chosen through stepwise selection (25,26). We assessed the association of hematological status with treatment failure at 1 mo after treatment initiation, TB recurrence, and mortality in both the entire cohort and by HIV infection status. Analyses were performed using SAS version 9.1 (SAS Institute). *P* values were 2-sided and considered significant at $P \leq 0.05$.

Results

Of the 887 patients enrolled in the supplementation trial, 96% ($n = 855$, 456 HIV infected and 399 HIV uninfected) had at least one assessment of hemoglobin and MCV and were included in the present analysis. Patient enrollment and follow-up are shown in **Supplemental Figure 1**. At baseline, information on hemoglobin and MCV was available for 684 patients, for whom a comparison of participant characteristics by levels of baseline hematological status is presented in **Table 1**. The median (IQR) hemoglobin concentration of patients at baseline was 104 g/L (92 g/L, 116 g/L). Nearly two-thirds of patients (64%) were anemic at baseline, more than one-half of whom (58%) also showed evidence of iron deficiency. Iron deficiency was present in 53% of patients at baseline, with iron deficiency anemia being more common than iron deficiency without anemia (37 vs. 16% overall). Patients with anemia, with or without iron deficiency, were more likely to be female, whereas patients with iron deficiency, with or without anemia, were more likely to be younger. Anemia and iron deficiency status did not differ by socioeconomic status (e.g., education, household size, money spent on food), but anemic patients, with or without iron deficiency, were more likely to be HIV infected, have had previous TB disease, and be in poorer clinical condition, as measured by low Karnofsky score, BMI, and CD4 cell count.

Information on hematological status at baseline and sputum culture status at 1 mo after treatment initiation was available for 475 patients. Of these, 16% of patients were culture positive 1 mo after treatment initiation, including 13% of HIV-infected patients and 20% of HIV-uninfected patients. We found no evidence for an association between iron deficiency or anemia at baseline and the risk of treatment failure at 1 mo (**Table 2**).

TABLE 1 Patient characteristics by levels of baseline hematological status ($n = 684$)[1]

	Baseline hematological status[2]				
	No anemia or iron deficiency	Iron deficiency without anemia	Anemia without iron deficiency	Iron deficiency anemia	P[3]
All, *n (%)*	135 (20)	111 (16)	182 (27)	256 (37)	
Socio-demographic characteristics					
Sex, *n (% F)*	16 (12)	22 (20)	56 (31)	142 (55)	<0.001
Age, *y*	34 ± 10	30 ± 9	35 ± 9	31 ± 8	<0.001
Highest education, *n (%)*					0.15
None	14 (10)	5 (5)	18 (10)	31 (12)	
Incomplete primary	9 (7)	9 (8)	22 (12)	32 (13)	
Incomplete secondary	95 (70)	83 (75)	116 (64)	171 (67)	
Secondary or higher	17 (13)	14 (13)	26 (14)	22 (9)	
Household size,[4] *n*	4 ± 3	5 ± 3	4 ± 3	5 ± 3	0.33
Amount spent on food[5]	667 ± 946	566 ± 399	561 ± 377	533 ± 405	0.34
Clinical characteristics					
HIV infected, *n (%)*	67 (50)	34 (31)	137 (75)	175 (68)	<0.001
History of previous TB disease, *n (%)*	81 (60)	73 (66)	90 (49)	130 (51)	0.02
Colonies in AFB culture, *n (%)*					0.13
None	6 (4)	9 (8)	5 (3)	13 (5)	
1–100	69 (51)	55 (50)	103 (57)	156 (61)	
>100	59 (44)	47 (42)	74 (41)	86 (34)	
Karnofsky score <70%, *n (%)*	7 (5)	5 (5)	20 (11)	42 (16)	<0.001
CRP,[6] *mg/L*	18 (9,36)	18 (9,35)	23 (10,42)	25 (14,41)	0.03
BMI, *kg/m²*	19 ± 3	20 ± 3	19 ± 3	19 ± 3	0.01
CD4 count, *cells/mm³*	588 ± 340	611 ± 318	412 ± 281	401 ± 274	<0.001
WHO clinical stage,[7] *n (%)*					0.84
Stage 3	46 (94)	24 (92)	89 (90)	107 (90)	
Stage 4	3 (6)	2 (8)	10 (10)	12 (10)	
HIV RNA >50,000 copies/mm³,[7] *n (%)*	29 (48)	10 (44)	68 (60)	75 (54)	0.35

[1] Values are mean ± SD unless otherwise noted. Totals may be <684 due to missing values. AFB, acid-fast bacilli; Hb, hemoglobin; MCV, mean corpuscular volume.
[2] Hematological status was categorized as no anemia or iron deficiency: Hb ≥110 g/L and MCV ≥80 fL; iron deficiency without anemia: Hb ≥110 g/L and MCV <80 fL; anemia without iron deficiency: Hb <110 g/L and MCV ≥80 fL; and iron deficiency anemia: Hb <110 g/L and MCV <80 fL.
[3] *P* is from the χ^2 test for proportions and the Kruskal-Wallis test for continuous measures.
[4] Household size was defined as the number of people eating in the household.
[5] Amount spent on food is in Tanzanian shillings per person per day. At the time of the start of the study in 2000, the mean exchange rate was 1 USD = 799 Tanzanian shillings.
[6] Values are median (IQR).
[7] WHO clinical stage and HIV RNA assessed only in HIV-infected patients.

TABLE 2 Association of hematological status at baseline with treatment failure at 1 mo among TB-infected patients in Tanzania[1]

Hematological status[2]	Events/at risk, *n/n*	Unadjusted model RR (95% CI)	Multivariate model RR (95% CI)[3]
All patients			
No anemia or iron deficiency	12/92	1.00	1.00
Iron deficiency without anemia	15/81	1.42 (0.71, 2.85)	1.28 (0.64, 2.57)
Anemia without iron deficiency	21/125	1.29 (0.67, 2.48)	1.76 (0.91, 3.40)
Iron deficiency anemia	28/177	1.21 (0.65, 2.27)	1.41 (0.73, 2.72)
HIV-infected patients[4]			
No anemia or iron deficiency	3/43	1.00	1.00
Iron deficiency without anemia	6/28	3.07 (0.84, 11.29)	3.28 (0.91, 11.86)
Anemia without iron deficiency	14/92	2.18 (0.66, 7.19)	2.51 (0.78, 8.05)
Iron deficiency anemia	15/120	1.79 (0.54, 5.89)	1.78 (0.52, 6.13)
HIV-uninfected patients[4]			
No anemia or iron deficiency	9/49	1.00	1.00
Iron deficiency without anemia	9/53	0.92 (0.40, 2.14)	0.85 (0.38, 1.89)
Anemia without iron deficiency	7/33	1.15 (0.48, 2.79)	1.48 (0.63, 3.46)
Iron deficiency anemia	13/57	1.24 (0.58, 2.65)	1.39 (0.68, 2.85)

[1] AFB, acid-fast bacilli; Hb, hemoglobin; MCV, mean corpuscular volume; TB, tuberculosis.
[2] Hematological status was categorized as no anemia or iron deficiency: Hb ≥110 g/L and MCV ≥80 fL; iron deficiency without anemia: Hb ≥110 g/L and MCV <80 fL; anemia without iron deficiency: Hb <110 g/L and MCV ≥80 fL; and iron deficiency anemia: Hb <110 g/L and MCV <80 fL.
[3] Adjusted RR from a log-binomial regression model adjusting for sex, age (years via cubic splines), money spent on food (<500, ≥500 Tanzanian shillings spent/person/d), number of colonies in AFB culture (0, 1–100, >100 colonies), Karnfosky score (<70%, ≥70%), BMI (kg/m^2 via cubic splines), previous TB disease (yes/no), HIV status, CD4 cell count (cells/mm^3 via cubic splines), log HIV RNA (copies/mm^3 via cubic splines), malaria infection (yes/no), and trial regimen.
[4] *P*, test for interaction by HIV status = 0.32.

We next assessed the association between iron deficiency and anemia with TB recurrence. Among the 456 patients who were culture negative at 1 mo, 16% had a subsequent positive culture with a median (IQR) time to positive culture of 5 (2–13) mo. Neither form of iron deficiency was significantly associated with the risk of TB recurrence, but anemia without iron deficiency was associated with a 4-fold increased risk of TB recurrence [adjusted RR = 4.10 (95% CI = 1.88, 8.91); $P < 0.001$] (**Table 3**).

The median (IQR) duration of follow-up for the mortality endpoint was 43 mo (28–53). Seventeen percent of patients (n = 146/855) died during follow-up, 90% of whom were HIV-infected patients. We found a 2- to nearly 3-fold independent increase in risk associated with iron deficiency and anemia (with and without iron deficiency) [adjusted RR for iron deficiency without anemia = 2.89 (95% CI = 1.53, 5.47); $P = 0.001$; anemia without iron deficiency = 2.72 (95% CI = 1.50, 4.93); $P = 0.001$; iron deficiency anemia = 2.13 (95% CI = 1.10, 4.11); $P = 0.02$] (**Table 4**). Among the HIV-infected patients, iron deficiency and anemia (with or without iron deficiency) predicted a 3-fold increased risk of HIV disease progression from WHO stage 3 to 4 and a 3- to 4-fold increased risk of the combined endpoint of HIV disease progression or death [adjusted RR for iron deficiency without anemia = 3.51 (95% CI = 1.62, 7.59); $P = 0.001$; anemia without iron deficiency = 4.13 (95% CI = 2.07, 8.25); $P < 0.001$; and iron deficiency anemia = 3.40 (95% CI = 1.69, 6.86); $P < 0.001$]. We found no evidence that the relationship between anemia and iron deficiency status was modified by HIV status for any clinical endpoint (all P-interaction > 0.05).

Discussion

We found iron deficiency and anemia were highly prevalent and coexisted in our cohort of adult TB patients. Iron deficiency without anemia and anemia with and without iron deficiency were positively associated with an increased risk of mortality and HIV disease progression. Anemia without iron deficiency was associated with an increased risk of TB recurrence.

Similar to other studies, we found a high burden of anemia in our cohort of TB patients (7–11), supporting the clinical importance of this routinely measured indicator. We also show that iron deficiency, defined by low MCV, is common in this population. A lower prevalence of iron deficiency has been reported in other cohorts of TB patients [0–12% (11,27,28)]. These studies, however, assessed the presence of iron deficiency using concentrations of the iron storage protein, ferritin. Ferritin is an acute-phase reactant that increases in concentration during conditions of inflammation or infection, and the true burden of iron deficiency may have been underestimated.

Our data also suggest that, in this population, iron deficiency is an important contributor to anemia, with iron deficiency occurring in more than one-half of anemic patients at baseline. Previous reports have suggested a smaller contribution of iron deficiency to TB-associated anemia, indicated by a high proportion of patients with a normochromic, normocytic blood picture (RBC morphology inconsistent with iron deficiency) and the post-TB treatment improvement in hematological status without the provision of iron (8,9,28,29). The relative importance of iron deficiency in TB-associated anemia is likely to depend on contextual factors, including the prevalence of other infections and dietary intake, and will vary across populations.

We found that the risk of treatment failure at 1 mo was not related to any class of anemia or iron deficiency at baseline. Few studies have examined hematological status in relation to clinical recovery in TB. Hemoglobin concentration was positively associated with sputum conversion among TB patients in South Africa (28), but the resolution of anemia during anti-TB treatment was not significantly associated with good response to

TABLE 3 Association of hematological status with TB recurrence among TB-infected patients in Tanzania[1]

Hematological status[2]	Events, *n/person-mo*	Unadjusted model RR (95% CI)	Multivariate model RR (95% CI)[3]
All patients			
No anemia or iron deficiency	24/5638	1.00	1.00
Iron deficiency without anemia	19/1646	2.00 (1.08, 3.67)	2.02 (0.96, 4.23)
Anemia without iron deficiency	14/871	2.71 (1.39, 5.27)	4.10 (1.88, 8.91)
Iron deficiency anemia	15/1044	2.20 (1.13, 4.25)	1.70 (0.71, 4.05)
HIV-infected patients[4]			
No anemia or iron deficiency	7/2339	1.00	1.00
Iron deficiency without anemia	2/487	1.11 (0.23, 5.35)	2.11 (0.36, 12.46)
Anemia without iron deficiency	12/664	4.65 (1.82, 11.87)	6.68 (2.50, 17.83)
Iron deficiency anemia	11/704	3.61 (1.39, 9.38)	3.81 (1.26, 11.57)
HIV-uninfected patients[4]			
No anemia or iron deficiency	17/3298	1.00	1.00
Iron deficiency without anemia	17/1159	1.92 (0.97, 3.81)	1.82 (0.80, 4.12)
Anemia without iron deficiency	2/207	1.43 (0.33, 6.24)	2.45 (0.50, 12.13)
Iron deficiency anemia	4/341	1.54 (0.51, 4.62)	0.68 (0.13, 3.44)

[1] Hb, hemoglobin; MCV, mean corpuscular volume; TB, tuberculosis.
[2] Hematological status was categorized as no anemia or iron deficiency: Hb ≥110 g/L and MCV ≥80 fL; iron deficiency without anemia: Hb ≥110 g/L and MCV <80 fL; anemia without iron deficiency: Hb <110 g/L and MCV ≥80 fL; and iron deficiency anemia: Hb <110 g/L and MCV < 80 fL.
[3] Adjusted RR from a log-binomial regression model adjusting for sex, age (years via cubic splines), money spent on food (<500, ≥500 Tanzanian shillings spent/person/d), number of colonies in AFB culture (0, 1–100, >100 colonies), Karnfosky score (<70%, ≥70%), BMI (kg/m^2 via cubic splines), previous TB disease (yes/no), HIV status, CD4 cell count (cells/mm^3 via cubic splines), log HIV RNA (copies/mm^3 via cubic splines), malaria infection (yes/no), and trial regimen.
[4] *P*, test for interaction by HIV status = 0.24.

treatment (defined as sputum conversion and no evidence of relapse) among TB patients in South Korea (8). The cross-sectional design of both analyses, however, precludes any determination of temporality in the potential association between anemia status and treatment response. In a small randomized study of iron supplementation among mild to moderately anemic TB patients in India (n = 43/group), 75 mg elemental iron twice daily for 2 mo did not decrease the severity of chest lesions, increase BMI, nor improve hematological status (29). Further prospective studies with greater power may be warranted to confirm the negative finding reported in the present analysis.

Experimental and epidemiological evidence suggests that iron is required for proper immune function. Iron deficiency has been shown to compromise cell-mediated immunity, decreasing T-cell numbers and proliferative response and potentially reducing macrophage activity (15,16), which may reduce host capacity to control infection. Iron status may also modulate the type of immune response mounted through its influence on the body's cytokine profile. Experimental evidence has shown that iron deficiency alters the balance between Th1 and Th2 cytokines, promoting a dominant Th2 response that has been associated with clinical TB disease and suggested to play a role in HIV progression (30–32). Consistent with these mechanisms, we found iron deficiency to be associated with an increased risk of mortality and HIV disease progression.

We found a strong, positive association of anemia without iron deficiency with TB recurrence, mortality, and HIV disease progression, suggesting that factors other than iron deficiency also contribute to the association of anemia with poor clinical outcomes. One possible explanation for this finding is that anemia without iron deficiency in this cohort is due to factors associated with poor health status or advanced disease. We do not, however, expect that this fully explains the association, because our analysis accounts for health status over time through detailed statistical adjustment for 3 well-known indicators of health status: BMI, CD4 cell count, and viral load. An alternative explanation may relate to the consequences of the redistribution of iron known to occur in anemia of inflammation. The sequestration and loading of iron in macrophages where *M. tuberculosis* resides and replicates may both facilitate its acquisition of iron required for growth and inhibit cellular defense systems; evidence from in vitro and animal models suggests that iron loading can increase bacterial replication (33–35), promote a shift from a Th1 to Th2 cytokine response (36,37), and reduce the cytotoxic activity of macrophages, preventing IFNγ-mediated defense mechanisms and blocking NO-dependent bactericidal activity (38–40). Anemia at TB diagnosis was previously associated with an increased risk of mortality in a small number of studies (12–14), and conditions consistent with macrophage iron loading have been associated with increased risk of TB (41) and death from TB (42). In the context of HIV infection, high iron storage may accelerate disease progression by facilitating the production of reactive oxygen species, increasing activity of iron-dependent ribonucleotide reductase required for viral replication, or promoting the growth of invading pathogens (43,44). The positive association between elevated iron and the risk of HIV disease progression was previously reported (45–47).

Iron supplementation is recommended for the treatment of iron deficiency anemia. Experimental and epidemiological evidence that excess iron may be harmful to TB patients cautions against the universal use of iron therapy (33,41,42,45,48), but the meaningful contribution of iron deficiency to anemia in this population suggests that targeted iron supplementation may be used to prevent and treat a proportion of TB-associated anemia.

TABLE 4 Association of hematological status with mortality and HIV disease progression among TB-infected patients in Tanzania[1]

Hematological status[2]	Events, n/person-mo	Unadjusted model RR (95% CI)	Multivariate model RR (95% CI)[3]
Mortality			
All patients			
No anemia or iron deficiency	38/18,483	1.00	1.00
Iron deficiency without anemia	21/6126	1.52 (0.89, 2.60)	2.89 (1.53, 5.47)
Anemia without iron deficiency	52/3194	6.98 (4.55, 10.72)	2.72 (1.50, 4.93)
Iron deficiency anemia	35/4657	3.40 (2.13, 5.41)	2.13 (1.10, 4.11)
HIV-infected patients[4]			
No anemia or iron deficiency	34/6419	1.00	1.00
Iron deficiency without anemia	15/1686	1.64 (0.89, 3.00)	2.78 (1.33, 5.81)
Anemia without iron deficiency	50/2479	3.75 (2.41, 5.83)	2.53 (1.36, 4.68)
Iron deficiency anemia	33/2689	2.34 (1.44, 3.80)	1.99 (1.01, 3.93)
HIV-uninfected patients[4]			
No anemia or iron deficiency	4/12,064	1.00	1.00
Iron deficiency without anemia	6/4441	3.82 (1.07, 13.55)	3.79 (1.04, 13.79)
Anemia without iron deficiency	2/714	8.77 (1.60, 48.07)	6.19 (1.03, 37.08)
Iron deficiency anemia	2/1969	3.06 (0.56, 16.71)	3.81 (0.64, 22.57)
HIV progression[5]			
No anemia or iron deficiency	26/4698	1.00	1.00
Iron deficiency without anemia	13/959	2.50 (1.28, 4.91)	3.72 (1.71, 8.10)
Anemia without iron deficiency	26/1671	3.11 (1.78, 5.44)	3.55 (1.73, 7.31)
Iron deficiency anemia	22/2094	1.93 (1.08, 3.45)	2.99 (1.44, 6.23)
HIV progression or death[5]			
No anemia or iron deficiency	37/4273	1.00	1.00
Iron deficiency without anemia	16/824	2.07 (1.15, 3.73)	3.51 (1.62, 7.59)
Anemia without iron deficiency	46/1495	3.77 (2.42, 5.89)	4.13 (2.07, 8.25)
Iron deficiency anemia	37/1927	2.38 (1.49, 3.79)	3.40 (1.69, 6.86)

[1] Hb, hemoglobin; MCV, mean corpuscular volume; TB, tuberculosis.
[2] Hematological status was categorized as no anemia or iron deficiency: Hb ≥110 g/L and MCV ≥80 fL; iron deficiency without anemia: Hb ≥110 g/L and MCV <80 fL; anemia without iron deficiency: Hb <110 g/L and MCV ≥80 fL; and iron deficiency anemia: Hb <110 g/L and MCV <80 fL.
[3] Adjusted RR from a log-binomial regression model adjusting for sex, age (years via cubic splines), money spent on food (<500, ≥500 Tanzanian shillings spent/person/d), number of colonies in AFB culture (0, 1–100, >100 colonies), Karnfosky score (<70%, ≥70%), BMI (kg/m^2 via cubic splines), previous TB disease (yes/no), HIV status, CD4 cell count (cells/mm^3 via cubic splines), log HIV RNA (copies/mm^4 via cubic splines), malaria infection (yes/no), and trial regimen.
[4] *P*, test for interaction by HIV status for mortality endpoint = 0.83.
[5] Among patients classified as WHO stage 3 at baseline.

Recommendations for the use of supplemental iron would require randomized studies to confirm that the benefits of supplementation outweigh any potential risks in this population. The finding that >40% of anemia in our study cohort was not related to iron deficiency highlights that other causes of anemia, including inflammation, parasitic infection, hemoglobinopathy, and other nutritional deficiencies, are also important in adults with TB. Studies to identify and address these other contributing factors are necessary to reduce the burden of TB-associated anemia.

To our knowledge, this is the first study to relate serial measures of anemia and iron deficiency with the risk of poor clinical outcomes in TB. Estimation of risk based on the cross-classification of anemia and iron deficiency status provides a clear basis to guide clinical decision-making and the development of treatment recommendations, while utilization of serial measurements accounts for changes in hematological status over time and may reflect a more clinically relevant measure than a single assessment of hematological status at TB diagnosis. This study applied marginal structural models implemented with inverse probability weighting methods to control for time-dependent confounding by a number of measured factors, including BMI, CD4 cell count, log HIV RNA, and malaria infection. Application of these methods, relatively novel in the field of nutrition but used in other areas of research, allows for fine control for potential confounding by changing health status over time. Finally, this study presents all analyses by HIV infection status, providing greater detail to our examination of how hematological status may relate to TB disease progression, specifically among HIV-infected and HIV-uninfected populations.

This study, however, has several limitations. First, we defined iron deficiency in this population using MCV. MCV reflects the mean RBC volume and has been the most widely used index for the evaluation of nutritional iron deficiency (49). Low MCV, however, is not specific to iron deficiency and can result from other causes, including thalassemia and, less commonly, anemia of inflammation (50). Low MCV in this population may be well correlated with iron deficiency, but this cannot be confirmed with the data available. Biochemical measures more specific to iron deficiency, such as ferritin or soluble transferrin receptor, should be considered in future studies. Second, we did not have information on several factors that may be associated with both iron imbalance and TB infection, including smoking, alcohol

history, and diabetes; we therefore cannot exclude the possibility that such factors may contribute to the associations observed. Finally, at the time of this study, antiretroviral treatment for HIV was not available in Tanzania and the generalizability of our findings among HIV-infected patients may be limited as treatment becomes more available. The potential impact of anemia and iron deficiency will need to be studied further among TB and HIV co-infected patients receiving antiretroviral therapy.

In this study of TB patients in Dar es Salaam, we found that both iron deficiency and anemia strongly predicted mortality and HIV disease progression and that anemia without iron deficiency was associated with increased risk of TB recurrence. Despite the availability of effective treatment, TB infection still carries a high rate of recurrence and mortality. Advances are therefore urgently needed in the identification and prevention of individual risk factors for disease progression. A better understanding of the conditions contributing to TB-associated anemia could motivate more focused clinical management of selected patients and result in important improvements in their overall health and survival.

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