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Iron supplementation and paediatric HIV disease progression: a cohort study among children receiving routine HIV care in Dar es Salaam, Tanzania

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Abstract

Background: Anaemia is common among HIV-infected children and iron supplementation is prescribed routinely for the prevention and management of anaemia among children. Limited evidence suggests iron supplementation may have adverse effects among HIV-infected populations. We aimed to estimate the effect of iron supplement use on mortality, disease progression and haematological outcomes among HIV-infected children in Dar es Salaam, Tanzania.

Methods: A prospective cohort study was conducted among HIV-infected children (aged 0–14 years) receiving antiretroviral treatment or supportive care between October 2004 and September 2014. Clinical data were recorded on morbidity and vital status, haematological status and prescriptions at each clinical visit. Cox proportional hazards models adjusted for time-varying covariates were used to estimate the association of time-varying iron supplementation on the hazard rate of mortality, HIV disease stage progression, tuberculosis incidence and anaemia and microcytosis persistence.

Results: In all, 4229 children were observed during 149 260 clinic visits for a mean follow-up of 2.9 years. After adjustment for time-varying clinical covariates, time-varying iron supplementation was associated with a 2.87 times higher hazard rate of mortality (95%

CI: 1.70, 4.87) and a 1.48 times higher hazard rate of HIV disease stage progression (95% CI: 1.10, 1.98). Iron supplementation was also associated with a lower rate of anaemia persistence (HR = 0.47; 95% CI: 0.37, 0.61). No differences in the association between iron supplementation and clinical outcomes were observed by antiretroviral therapy or anaemia status.

Conclusions: Iron supplementation may increase the risk of HIV disease stage progression and mortality among HIV-infected children, while reducing the risk of anaemia.

Key words: HIV, children, iron, cohort, mortality, antiretroviral therapy

Key Messages

- This is the largest study to date on the subject of iron supplementation among HIV-infected children, and the first to investigate mortality outcomes.
- After control for time-varying clinical covariates, the hazard rate of mortality associated with iron supplementation was 2.87 (95% CI: 1.70, 4.87) times higher than for children not receiving iron supplementation; these results did not differ according to antiretroviral therapy use or anaemia status.
- Clinicians, particularly in contexts where anaemia is presumptively treated with iron supplementation, may use the findings of this study to consider the relative risks and benefits of prescribing iron to HIV-infected children.

Introduction

Iron deficiency and human immunodeficiency virus (HIV) infection are each an important contributor to morbidity and mortality among children in low- and middle-income countries. Iron deficiency is thought to be responsible for 25–50% of the 273 million cases of anaemia among children aged 6–59 months in the year 2011, making it among the most common causes of disability in this age group.[1–4] To address this challenge, the World Health Organization (WHO) recommends daily oral iron supplementation for all children 6 months to 12 years of age in regions with a prevalence of anaemia >40% and adequate malaria control.[5] With respect to HIV, 1.7 million children <15 years were estimated to be living with an HIV infection in the year 2018.[6] Although immediate initiation of antiretroviral therapy (ART) is now recommended for all children diagnosed with HIV, only slightly more than half of children receive treatment.[7,8] A meta-analysis estimated that 34% of HIV-infected children are estimated to be iron-deficient (though this may be an underestimate, as some iron status biomarkers were not corrected for inflammation) and iron supplementation is sometimes given in conjunction with ART.[9]

However, the safety of iron supplementation among HIV-infected children is called into question by evidence from molecular biology which indicates that iron facilitates HIV replication and the virulence of opportunistic pathogens. Reverse transcription of HIV RNA, transcription of HIV genes, and assembly of HIV capsid proteins are iron-dependent or iron-regulated processes.[10] In vitro studies have suggested that these processes are sensitive to changes in iron availability, with viral replication increasing with the addition of cellular iron and decreasing with iron chelation.[11–14] Furthermore, *Mycobacterium tuberculosis*—the most common cause of death among AIDS patients—competes for iron stores within host macrophages, and down-regulation of macrophage iron uptake is thought to be part of innate immune defence.[15]

A large, long-term observational cohort study of HIV-infected adults initiating antiretroviral therapy in Tanzania ($n = 40\,657$) between the years 2004 and 2012 found a starkly increased rate of mortality among those who were prescribed iron-folic acid supplements. The risk of mortality associated with iron supplement use was higher among patients with no anaemia (HR = 3.8) than those with severe anaemia (HR = 1.6).[16] These findings raise the question of whether similar risks are apparent for children, as the symptoms and treatment of HIV infection among children differ substantially from those of adults.[17] Additionally, iron supplements are more widely recommended for children than adults.[5]

Two prior studies of iron supplementation among HIV-infected children were done in small populations over short periods of follow-up and do not examine mortality as an outcome.[18,19] The present study aims to address these gaps. We present a longitudinal cohort study of HIV-infected Tanzanian children who received supportive care

or antiretroviral therapy over 2004–2012. We aim to estimate the effect of iron supplement use on disease progression and haematological outcomes, as well as potential effect modification by antiretroviral therapy.

Methods

Study population

A prospective cohort study was prepared using medical record data from HIV-infected children aged 0–14 years in Dar es Salaam, Tanzania. Data on patient visits were available from October 2004 to September 2014. Study participants were enrolled in supportive care and antiretroviral treatment services at Management and Development for Health (MDH), an HIV care provider supported by the U.S. President's Emergency Plan for AIDS Relief. Patients received treatment free of charge. The start of follow-up for this study was defined as the date that HIV-infected children had their first clinical visit at an MDH facility.

Clinical care and data collection

Patients were scheduled for monthly visits with a physician where they received regular evaluations, were given counselling on nutrition and ART adherence, and obtained ART refills. When patients missed a visit or had abnormal laboratory results, they were contacted in person or by phone to encourage them to return to the clinic. A team of community health workers and volunteers worked with the treatment clinics to trace and ascertain the vital status of individuals lost to follow-up.

Blood specimens were scheduled to be drawn every 4 months by trained phlebotomists. Haemoglobin and mean corpuscular volume (MCV; using ACT5 Diff haematology analyzer; Beckman Coulter, Brea, CA) as well as CD4 cell counts or percent (using FACS Calibur; Becton Dickinson, San Jose, CA) were assessed. Children were eligible for inclusion in the study from the time they had a blood specimen with complete haemoglobin, MCV and CD4 data. Iron supplements (ferrous sulphate alone, ferrous sulphate plus folic acid, or ferrous sulphate-B12 complex) were prescribed by the physician on the basis of haemoglobin levels or severe clinical pallor. In some cases, iron may have been prescribed to non-anaemic children based on clinical indications in the absence of a recent haemoglobin assay, or in order to replenish iron stores following the resolution of anaemia. Iron supplement prescriptions were typically for a course of 1-2 months. The amount of elemental iron contained in the supplements, according to the age of the child, is listed in Supplementary Table S1, available as Supplementary data at *IJE* online. No monitoring of adherence to supplementation was performed in this routine clinical context.

The presence of active pulmonary tuberculosis was assessed via chest X-ray and sputum smear for acid-fast bacilli at the first visit and subsequently if suggestive symptoms appeared. HIV disease stage progression (stage I-IV, defined based on clinical symptoms) was assessed using WHO criteria.[20] Viral load testing was not routinely performed in this clinical setting. Antiretroviral care was provided per the Tanzania National Guidelines for the Management of HIV and AIDS, which changed three times over the course of the study.[21–24] Antiretroviral therapy consisted of three drugs: two nucleoside reverse transcriptase inhibitors (zidovudine, lamivudine, abacavir, stavudine) plus one non-nucleoside reverse transcriptase inhibitor (nevirapine, efavirenz). Stavudine replaced zidovudine in cases of anaemia, and efavirenz replaced nevirapine in the case of rifampin-based tuberculosis treatment.

Nurses and physicians collected clinical data on standard case report forms using unique patient identifiers. Data reviewers at each clinic ensured completeness of records and conducted quality assurance checks before entering data into a secure computerized database.

Variable definitions

Exposure to iron supplements was defined as a patient having been prescribed iron alone, iron plus folic acid, or iron-B12 complex. For the main analysis, the iron exposure period was considered to continue for 3 months from the date of prescription. Sensitivity analyses were performed by alternatively defining the exposure period to be 6 months, until the end of follow-up and until the next visit date.

Time to mortality was defined as the number of days from the time of exposure status (i.e. either receiving or not receiving iron supplements) to the date of death as confirmed by clinical records. Other time-to-event outcomes included diagnosis of incident tuberculosis, HIV disease stage progression, anaemia persistence and microcytosis persistence. Incident tuberculosis was defined as the absence of tuberculosis at or prior to the start of follow-up followed by a tuberculosis diagnosis during follow-up. HIV disease stage progression was defined as an increase of at least one stage from the one recorded at the start of follow-up. Categories of anaemia (none, mild, moderate and severe) were defined using age-specific haemoglobin cut-offs as per the WHO.[25] As no WHO guidance exists for defining anaemia in children 0–5 months of age, the definitions for children aged 6–59 months were applied. Microcytosis (MCV <–2 standard deviations), a marker of iron deficiency, was defined using age-specific cut-offs

according to the WHO.[26] As a majority of children (82.9%) were anaemic at baseline, we examined the effect of iron supplementation on the probability of reduction in anaemia severity or microcytosis, defined as a change in status to a less severe category than the one observed at the start of follow-up (conversely, persistence was defined as a lack of reduction in severity).

Covariates were assessed at every clinical visit and carried forward in the case of missing data for a given visit. Covariates included age (0–5 months, 6–23 months, 24–59 months, 5–11 years 12–14 years), sex, Tanzania HIV treatment guideline under effect (1st edition April 2002—March 2005, 2nd edition April 2005—January 2009, 3rd edition February 2009—March 2012, 4th edition April 2012—April 2015), body mass index for age z score (BMIz; BMIz >1, 1≥BMIz≥–1, –1>BMIz≥–2, BMIz<–2), district of Dar es Salaam (Ilala, Kinondoni, Temeke), health facility type (hospital, health centre, dispensary), immune deficiency (as defined by CD4 count or percent per WHO criteria[20]; none, mild, advanced, severe), HIV disease stage (I, II, III, IV) and ART status (on ART, not on ART).

Statistical methods

The data were structured in Andersen-Gill format, with each row representing exposure, outcome, and covariate data for a given patient on a given date of observation.[27] Covariate values were carried forward until the next date when a new measurement was available. Missing indicators were used when covariate values were not available.[28] Outlier values of haemoglobin and mean corpuscular volume were defined according to Tukey's criteria (using cutoffs that are a multiple of three times the interquartile range below the first quartile or above the third quartile) and dropped from analysis.[29] Cox proportional hazards regression models with time-varying exposure and covariate data were used to estimate the association between iron supplementation and time-to-event outcomes. Interaction terms were tested for time-varying ART status and anaemia severity. Marginal structural models, which account for potential time-varying exposure and covariate feedback, were used as a sensitivity analysis.[30] The degree of unmeasured confounding needed to explain the observed hazard ratios was calculated.[31] Two-sided statistical tests were used, with statistical significance defined as $p < 0.05$. Statistical analyses were performed using Stata software (version 13, College Station, TX).

Results

There were 12 248 HIV-infected children, 0-14 years of age, who visited a study health centre between 2004 and 2014, for a total of 312 429 patient-visits (Figure 1). Among these children, 7816 were excluded due to a lack of blood specimens that contained CD4, haemoglobin or MCV values. After these exclusions, there were an additional 203 patients excluded because they did not have any follow-up visits after their first complete blood specimen. The final sample size is 4229 children observed over 149 269 patient-visits and a mean follow-up time of 2.90 years.

The plurality of children (41.5%) began follow-up between the ages of 5 and 11 years, with very few enrolled in the first 6 months of life (7.4%; Table 1). Approximately half of the study population was female (50.9%). Anaemia was observed in 82.9% of children and microcytosis in 51.5% of children at the start of follow-up; 44.5% of children had both anaemia and microcytosis at the start of follow-up. However, only 64.1% of follow-up time was among children with anaemia and 39.1% among children with microcytosis. Substantial improvements were seen for immune status, with nearly half of children (44.9%) experiencing some degree of immunodeficiency at the start of follow-up, but only 5.5% of follow-up time spent with immunodeficiency. The plurality of children were observed to have HIV disease stage 3 at both baseline and throughout follow-up. Nearly eight in 10 children (78.5%) were on ART at some point during follow-up. The most common drug regimen was lamivudine and nevirapine along with either stavudine or zidovudine.

Iron supplementation was prescribed to 827 (19.6%) children (Table 2). Over half of children who received a prescription were prescribed iron only once; 23% of iron supplements were prescribed in the first 30 days of treatment; the median time until supplementation was 6.5 months. Iron supplementation was more frequently prescribed to children

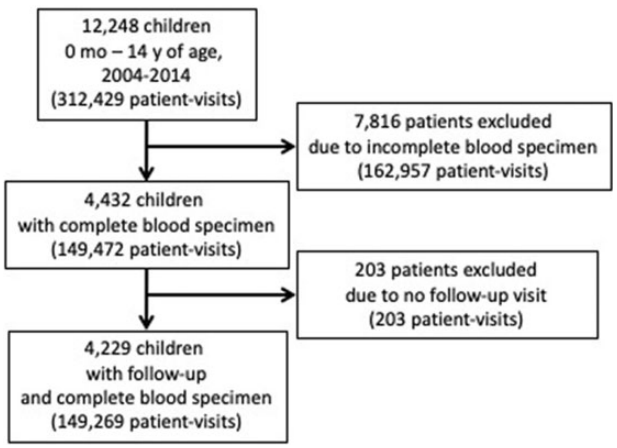

Figure 1 Flowchart of exclusions from clinical database

Table 1 Clinical characteristics among study population

	Baseline		Follow-up time in category
	n	%	%
Age			
0–5 months	314	7.4	0.8
6–23 months	769	18.2	6.8
24–59 months	835	19.7	21.1
5–11 years	1757	41.5	52.9
12–14 years	554	13.1	18.3
Sex			
Female	2151	50.9	50.3
Male	2078	49.1	49.7
Tanzania HIV treatment guideline			
1st edition (April 2002–March 2005)	75	1.8	0.2
2nd edition (April 2005–January 2009)	2993	70.8	35.1
3rd edition (February 2009–March 2012)	1160	27.4	50.5
4th edition (April 2012–April 2015)	1	0.0	14.1
BMIz category[a]			
BMIz >1	221	13.5	17.1
BMIz ≤1 & BMIz≥–1	703	42.8	54.2
BMIz≤–1 & BMIz≥–2	303	18.5	15.8
BMIz<–2	414	25.2	12.9
District of Dar es Salaam[a]			
Ilala	1935	46.9	47.8
Kinondoni	1078	26.1	25.4
Temeke	1114	27.0	26.8
Health facility type[a]			
Hospital	3655	87.9	87.9
Health centre	288	6.9	7.6
Dispensary	215	5.2	4.5
Anaemia status			
No anaemia	722	17.1	35.9
Mild anaemia	693	16.4	21.0
Moderate anaemia	2393	56.6	40.0
Severe anaemia	421	10.0	3.1
Mean corpuscular volume			
Normocytosis	2050	48.5	60.8
Microcytosis	2179	51.5	39.1
Immune status			
No immune deficiency	2331	55.1	94.5
Mild deficiency	353	8.3	2.1
Advanced deficiency	479	11.3	1.7
Severe deficiency	1066	25.2	1.7
HIV disease stage[a]			
Stage 1	693	17.2	11.6
Stage 2	1195	29.7	28.4
Stage 3	1893	47	55.4
Stage 4	246	6.1	4.6
ART start relative to inclusion in study			
Started ART prior to study inclusion	253	6.0	n/a
Started ART in first month	1548	36.6	n/a
Started ART after first month	1520	35.9	n/a
Did not start ART	908	21.5	n/a

(Continued)

Table 1 Continued

	Baseline		Follow-up time in category
	n	%	%
Antiretroviral drugs prescribed to children on ART[b]			
Nucleoside reverse transcriptase inhibitors			
Lamivudine	2635	79.3	82.1
Stavudine	1196	36.0	42.8
Zidovudine	1435	43.2	39.3
Non-nucleoside reverse transcriptase inhibitors			
Efavirenz	508	15.3	11.5
Nevirapine	2110	63.5	70.2
Other or unspecified drug	623	18.8	15.4

ART, antiretroviral therapy; BMIz, body mass index for age z score; HIV, human immunodeficiency virus; WHO, World Health Organization.

[a]No data were available on the following variables for some children: BMIz, $n = 2588$ missing (61.2%); district of Dar es Salaam, $n = 102$ missing (2.4%); health facility type, $n = 71$ missing (1.7%); HIV disease stage, $n = 202$ missing (2.8%).

[b]Percent values do not sum to 100 because children were prescribed multiple drugs.

Table 2 Iron supplementation and outcomes among paediatric HIV-infected patients in Dar es Salaam, Tanzania ($n = 4229$)

	Prescribed any during follow-up		Number received during follow-up	
	n	%	Median	1st, 3rd quartile
Iron supplement	827	19.6	1	(1, 2)
	At least one event		Time to first event (years)	
	n	%	median	1st, 3rd quartile
Mortality	247	5.8	0.41	(0.14, 1.67)
Incident pulmonary tuberculosis	615	14.5	1.17	(0.52, 2.46)
HIV disease stage progression	1671	39.5	0.57	(0.15, 1.77)
Reduction in anaemia severity	2246	53.1	0.45	(0.20, 0.94)
Resolution of microcytosis	691	16.3	0.96	(0.45, 1.86)

HIV, human immunodeficiency virus.

with moderate and severe anaemia than to children with no anaemia (Supplementary Table S2, available as Supplementary data at *IJE* online). Mortality occurred for 247 children at a median time of 0.41 years after the start of follow-up. Mortality rates were higher among younger children (Supplementary Table S3, available as Supplementary data at *IJE* online). Incident tuberculosis occurred for approximately one in seven children after a median time of 1.17 years. HIV disease stage progression was the most commonly occurring adverse outcome, affecting four in 10 children. Over half of children experienced a reduction in anaemia severity from baseline after a median time of 0.45 years. Transitioning from microcytosis to normocytosis was observed among only 16.3% of children.

Iron supplementation was associated with a higher rate of mortality in both unadjusted and adjusted models (Table 3). In a model examining the association between time-varying iron supplementation adjusted for time-varying covariates, the rate of mortality was nearly three times higher in children who were prescribed iron supplements (HR = 2.87; 95% CI: 1.70, 4.87). An association of lesser magnitude was observed for iron supplementation and HIV disease stage progression (HR = 1.48; 95% CI: 1.10, 1.98). No association was seen between iron supplementation and incident tuberculosis. Iron supplementation was also significantly associated with reductions in anaemia (HR = 0.47; 95% CI: 0.37, 0.61) but not with microcytosis (HR = 0.63; 95% CI: 0.35, 1.15). The hazard rate of mortality among children not on ART (HR = 3.04; 95% CI: 1.14, 8.10) did not differ from that among children on ART (HR = 2.83; 95% CI: 1.55, 5.15; *P* for heterogeneity = 0.90; Table 4). Similar findings by ART status were seen for all other analysed outcomes. Furthermore, no significant differences in the association of iron supplements with clinical outcomes was observed by time-varying anaemia status.

To assess whether the association between iron supplementation and mortality was sensitive to the assumed 3-month period of iron effect, additional models

were run specifying alternative effect periods. Models assuming immediate effects (i.e. effects within the next recorded clinical visit), 6-month periods and enduring effects (i.e. from the time of supplementation to the end of follow-up) all found statistically significant and qualitatively similar associations of iron supplementation with higher rates of mortality (Supplementary Table S4, available as Supplementary data at *IJE* online). A marginal structural model, which accounts for time-varying treatment and covariate feedback, found similar estimates of elevated mortality associated with iron supplementation (HR = 2.40; 95% CI: 1.35, 3.16). A model which excluded deaths that occurred within 2 weeks of iron supplementation ($n = 13$ out of 34 total deaths among children who received iron in the prior 3 months) found an attenuated but qualitatively similar association (HR = 1.81; 95% CI: 0.98, 3.35).

We assessed the possibility whether unmeasured confounding may explain the observed increased hazard ratios for mortality. A 50% point disparity in prevalence of an unmeasured confounder between the iron supplemented and non-supplemented children, along with a hazard ratio between the confounder and mortality of three or more, would be needed to render the observed hazard ratio between iron supplementation and mortality as statistically insignificant (Supplementary Table S5, available as Supplementary data at *IJE* online).

Table 3 Estimated effects of time-varying iron supplementation on health among HIV-infected children ($n = 4229$)[a]

Model	Mortality	HIV disease stage progression	Tuberculosis incidence[b]	Anaemia persistence	Microcytosis persistence
Bivariate	5.43 (3.26, 9.04)	1.51 (1.14, 2.01)	0.92 (0.36, 2.34)	0.68 (0.53, 0.88)	0.72 (0.42, 1.24)
Baseline covariate adjustment[c]	3.00 (1.79, 5.02)	1.86 (1.40, 2.48)	0.98 (0.39, 2.52)	0.85 (0.65, 1.10)	0.64 (0.36, 1.15)
Time-varying covariate adjustment[d]	2.87 (1.70, 4.87)	1.48 (1.10, 1.98)	0.93 (0.36, 2.40)	0.47 (0.37, 0.61)	0.63 (0.35, 1.15)

HIV, human immunodeficiency virus; ART, antiretroviral therapy; BMIz, body mass index for age z score.

[a]Values are hazard ratio (95% confidence interval).

[b]Children with tuberculosis at or prior to the start of study follow-up were excluded, resulting in a sample size of 3420.

[c]Controlling for baseline age category, sex, HIV treatment guidelines, BMIz, facility level, district of Dar es Salaam, anaemia, mean corpuscular volume, immune status, HIV disease stage and ART use.

[d]Controlling for time-varying age category, sex, HIV treatment guidelines, BMIz, facility level, district of Dar es Salaam, anaemia, mean corpuscular volume, immune status, HIV disease stage and ART use.

Table 4 Estimated effects of time-varying iron supplementation on health among HIV-infected children ($n = 4229$), stratified by time-varying antiretroviral therapy use and anaemia[a,b]

	Mortality	HIV disease stage progression	Tuberculosis incidence	Anaemia persistence	Microcytosis persistence
ART use					
Not on ART	3.04 (1.14, 8.10)	1.49 (0.95, 2.32)	1.21 (0.43, 3.42)	0.38 (0.21, 0.67)	1.01 (0.15, 6.58)
On ART	2.83 (1.55, 5.15)	1.49 (1.03, 2.17)	0.65 (0.39, 1.08)	0.46 (0.35, 0.62)	0.67 (0.34, 1.30)
P-value for heterogeneity	0.90	0.99	0.29	0.52	0.69
Anaemia					
No anaemia	4.43 (1.39, 14.11)	0.73 (0.27, 1.96)	0.19 (0.02, 2.33)	n/e	0.67 (0.20, 2.21)
Mild anaemia	2.66 (0.28, 24.87)	1.97 (1.01, 3.85)	0.78 (0.24, 2.53)	1.17 (0.46, 2.95)	0.40 (0.12, 1.40)
Moderate anaemia	2.09 (0.92, 4.75)	1.79 (1.21, 2.64)	0.70 (0.37, 1.30)	0.81 (0.58, 1.13)	0.99 (0.35, 2.78)
Severe anaemia	4.65 (1.89, 11.47)	1.15 (0.54, 2.45)	1.01 (0.46, 2.22)	0.29 (0.20, 0.44)	0.57 (0.09, 3.70)
P-value for heterogeneity	0.62	0.19	n/e	n/e	n/e

ART, anti-retroviral therapy; BMIz, body mass index for age z score; HIV, human immunodeficiency virus; n/e, not estimable.

[a]Values are hazard ratio (95% confidence interval).

[b]Controlling for time-varying age category, sex, HIV treatment guidelines, BMIz, facility level, district of Dar es Salaam, anaemia, mean corpuscular volume, immune status, HIV disease stage and ART use.

Discussion

In this cohort study of paediatric HIV-infected patients in Tanzania, we found that iron supplementation was associated with a significant increase in the hazard rate of mortality and HIV disease stage progression. The effects observed for iron did not differ by ART use or anaemia status. These associations persisted even after control for a broad set of clinical morbidity and demographic indicators. The observed associations were also robust to alternative model specifications and the possibility of substantial unmeasured confounding. Prior studies, with smaller sample sizes and shorter periods of follow-up, have not investigated the relationship between iron supplementation and mortality among HIV-infected children.

Prior studies among adults have found mixed results. Two trials and one observational cohort suggest that HIV viral loads are not affected by iron supplementation. However, their small size, limited use of antiretroviral therapy among participants and unique study populations prevent a strong generalized conclusion of no evidence for harm. The earliest of these studies randomized ART-naïve adults ($n = 32$) in Kenya to twice-weekly parenteral iron (60 mg), and did not find a difference in the change in viral load over the 4-month intervention period.[32] Another study randomized HIV and hepatitis C co-infected female injection drug users in the USA ($n = 138$) to micronutrients with iron (18 mg daily) versus micronutrients alone for 12 months. CD4 counts and viral loads did not differ at the end of the study.[33] The third study, among a cohort of anaemic pregnant women in Zambia ($n = 59$), examined the influence of iron supplementation at 2 weeks postpartum on breast milk viral load. At 6 weeks postpartum there was no statistically significant difference in milk viral loads between those who used iron supplements and those who did not.[34] However, a large study of adult patients ($n = 40\,657$) receiving HIV care and treatment (conducted at the same facilities as for the children analysed in this study) found an increased risk of mortality associated with iron supplements.[16]

Two small studies of iron supplementation among HIV-infected children provide some evidence among paediatric populations. One cohort study followed HIV-infected Indian children aged 2–12 years for 12 months ($n = 194$). The analysis compared children who were prescribed iron supplements with those who were not, and found that haemoglobin, CD4% and HIV disease stage were similar after 1 year.[18] However, the analysis did not control for any potentially confounding variables, making questionable any claims of causality with respect to iron supplementation. The strongest evidence comes from a trial in Malawi that randomized 209 HIV-infected children aged 6–59 months with moderate anaemia to 3 months of iron (3 mg/kg daily) plus multivitamins (vitamins A, C and D) versus multivitamins alone. Approximately one-third of enrolled children were receiving antiretroviral therapy at baseline. At the conclusion of the iron therapy period, iron-supplemented children had a decreased prevalence of anaemia and increased CD4%, but the incidence rate of malaria more than doubled.[19] The present study followed a larger cohort of children for a longer period of time and assessed mortality as an outcome.

It is unclear from this study by what mechanism iron supplements would induce higher rates of mortality and HIV disease stage progression. No association was found between iron supplementation and incident tuberculosis, which suggests that this may not be the primary pathway. An alternative explanation is that impacts are mediated by changes in viral load, which make the immune system susceptible to a broad range of opportunistic infections, including not just tuberculosis but also *Pneumocystis jiroveci* pneumonia, oesophageal candidiasis and recurrent bacterial infections.[35] Viral load data were not available for this study, but the increase in HIV disease stage associated with iron supplementation would support the viral load hypothesis. A final hypothesis is malaria infection, as iron supplementation is associated with an increased risk of clinical malaria in areas without effective malaria prevention and control.[36] Data on malaria infections were not available for study participants, but malaria is endemic at a low prevalence in Dar es Salaam (among children aged 6–59 months, 1.2% in 2007 and 3.6% in 2011), and therefore may be a driver of increased mortality associated with iron supplementation.[37,38] A sensitivity analysis which excluded deaths that occurred within 2 weeks of iron supplementation found a somewhat attenuated association with mortality, which suggests that the biological mechanism may act more strongly soon after supplementation. Further research is needed to illuminate the potential aetiological pathway from iron supplementation to mortality among HIV-infected children.

Improvements in anaemia were observed, with children receiving iron supplements being over twice as likely to improve their anaemia status. However, the haematological benefits accrued to some children were not enough to result in improved mortality outcomes in the iron-supplemented group. It is important to note there are not sufficient data available for this cohort to ascertain the aetiology of anaemia for participants. Other studies of hospitalized children in Tanzania have found that half or more of anaemia cases were not associated with iron deficiency.[39,40] It is likely that anaemia of inflammation is an important contributor, but this cannot be confirmed directly. However, in our models of iron supplementation,

we included time-varying antiretroviral therapy as a covariate and found it was associated with a 49% reduction in the hazard of anaemia persistence. This suggests that inflammation is an important cause of anaemia in the study population. Whether the effects of iron may differ depending on anaemia aetiology is an important consideration for future research.

As with all observational research, there is a risk of residual confounding by unmeasured determinants of iron supplementation and clinical outcomes. In this study, we have controlled for a broad set of time-varying clinical indicators. These are the key clinical variables available to study clinicians which could be used for decision making regarding the prescription of iron supplements, which suggests that important unmeasured confounding is unlikely. Even in the case that an important confounder was omitted from this analysis, sensitivity analyses show that only confounders with a very strong association with mortality (HR $\geq$3) and a large difference in prevalence between iron-supplemented and non-supplemented children (prevalence difference >50%) would be sufficient to explain the observed associations. Another potential source of bias is inaccurate measurement of iron supplementation. This could occur if children were prescribed iron supplements but failed to consume them. Iron supplementation trials among children have found that adherence to supplementation is often above 80% in these study contexts.[41] However, adherence may be lower in a routine clinical setting, such as the present study. If adherence were below 100%, then the estimated hazard ratios would be closer to the null, and therefore an underestimate of the true effect of iron supplementation.

Clinicians may use the findings of this study to consider the relative risks and benefits of prescribing iron to HIV-infected children. Given that this study found that there is an increased hazard of mortality and HIV disease stage progression among anaemic children with HIV, even a 'test and treat' strategy of iron supplementation for anaemic children could pose risks. The findings are also relevant to public health policies, such as the WHO's current recommendation that iron supplements be provided to all children in areas with a high burden of anaemia. Currently, a caveat is made in this guidance for malaria-endemic regions, and it may be prudent to consider a caveat for HIV-infected children as well. Evidence from one observational study—even if strongly designed—is rarely a sufficient basis for changes in clinical or public health policy. Additional observational studies of iron supplementation in HIV-infected populations could include those receiving different ART regimens and contexts with differing burdens of comorbidities and risk factors. Consideration should also be given to randomized trials that assess the safety and efficacy of varying doses and timing of supplementation, although the requirement of equipoise may require close scrutiny, given the risks observed in this study.

Ethics approval

Ethical approval for this study was granted by the institutional review boards of the Harvard T.H. Chan School of Public Health and the Tanzanian National Institute of Medical Research at the Muhimbili University of Health and Allied Sciences (IRB 17-1998).

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary data

Supplementary data are available at *IJE* online.

Author contributions

C.T.A., C.P.D., G.R.S., D.S. and W.W.F. desiged the study; K.M., D.S., N.U. and W.W.F.oversaw data collection; C.T.A. and N.P. performed statistical analysis; C.T.A. wrote the first draft of the manuscript; all authors revised the manuscript for important intellectual content.

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Conflict of interest

None declared.

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Commentary: Time for precision in iron supplementation in children

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Iron is a Janus-faced nutrient, presenting faces of protection and of risk for those who are supplemented with it. One face is protective, preventing and curing iron-deficiency anaemia which is associated with physical and cognitive functional deficits and mortality risk in children. This has led to dedicated iron supplementation programmes to address anaemia through improvements of iron status. The World Health Organization[1] (WHO) recommends universal daily iron supplementation for children 6 months to 12 years of age in regions with a prevalence of anaemia >40% and adequate malaria control. This 'push to push' iron has expanded, as a variety of methods of improving iron intake, such as fortification of different staple foods, have been tried and occasionally layered on top of pharmacological supplementation, in an attempt to reduce anaemia prevalence rapidly.[2]

The other face of iron, particularly as a supplemented nutrient, is one of potential adversity. Therefore, its administration should be performed with precision, and not as a 'one size fits the entire population' approach. A dated systematic review concluded that iron supplementation had no apparent harmful effect on the overall incidence of infectious illnesses in children, although it slightly increased the risk of developing diarrhoea.[3] Subsequently, a large study in Zanzibar found an increased risk of malaria-associated morbidity and mortality in iron-sufficient children receiving iron supplements, leading to the recognition that universal iron supplementation of children who were not iron deficient could be harmful.[4] This marked the beginning of a policy shift towards safety and precision in iron supplementation programmes in areas with high malarial morbidity, wherein 'adequate malaria control' was coupled with iron supplementation programmes.[1] The most recent Cochrane review, on 35 randomized trials in 31 955 children in malaria-endemic areas, reaffirms the wisdom of adopting this approach.[5] Notably, the review omitted an analysis of iron administration during proven malaria episodes. Iron supplementation improved haemoglobin and reduced anaemia, but did not increase the risk of severe or clinical malaria when regular malaria prevention or management services were provided. However, malarial parasite density was higher in four of the six trials reporting such data. Further, in areas where such services were unavailable, iron (with or without folic acid) increased the incidence of malaria (risk ratio 1.16, 95% CI 1.02, 1.31; 19 086 participants). There is still uncertainty whether iron supplementation alone increases the risk of malaria in the subset of iron-replete, non-anaemic children living in malaria-endemic areas.

Iron supplementation also probably facilitates the replication of human immunodeficiency virus (HIV).[6] In a large study of HIV-infected adults ($n = 40\,657$), a higher risk of mortality was documented in those who were iron supplemented, particularly if they were non-anaemic.[7] Two small studies in children with HIV infection provide conflicting evidence. In a cohort study from India ($n = 194$), without